THE BEHAVIOURAL CONSEQUENCES OF CEREBRAL SMALL VESSEL DISEASE

An MRI approach

Ingeborg W.M. van Uden

The behavioural consequences of cerebral small vessel disease
an MRI approach

Author: Ingeborg Wilhelmina Maria van Uden
Cover: Ingeborg Wilhelmina Maria van Uden
Lay-out and printing by: ProefschriftMaken
ISBN: 978-94-6284087-4

The studies in this thesis were carried out at the Department of Neurology of the Donders Institute for Brain, Cognition and Behaviour, Centre for Neuroscience, Radboud university medical centre, Nijmegen, the Netherlands with financial support by a VIDI innovational grant from the Netherlands Organisation for Scientific Research (NWO, grant 016.126.351; prof.dr. FE de Leeuw) and from the MIRA Institute for Biomedical Technology and Technical Medicine, University of Twente.

THE BEHAVIOURAL CONSEQUENCES OF CEREBRAL SMALL VESSEL DISEASE

An MRI approach

Proefschrift

ter verkrijging van de graad van doctor
aan de Radboud Universiteit Nijmegen
op gezag van de rector magnificus
prof. dr. J.H.J.M. van Krieken,
volgens besluit van het college van decanen
in het openbaar te verdedigen
op dinsdag 14 februari 2017 om 12.30 uur precies

door
Ingeborg Wilhelmina Maria van Uden
geboren op 23 januari 1985
te ‘s-Hertogenbosch

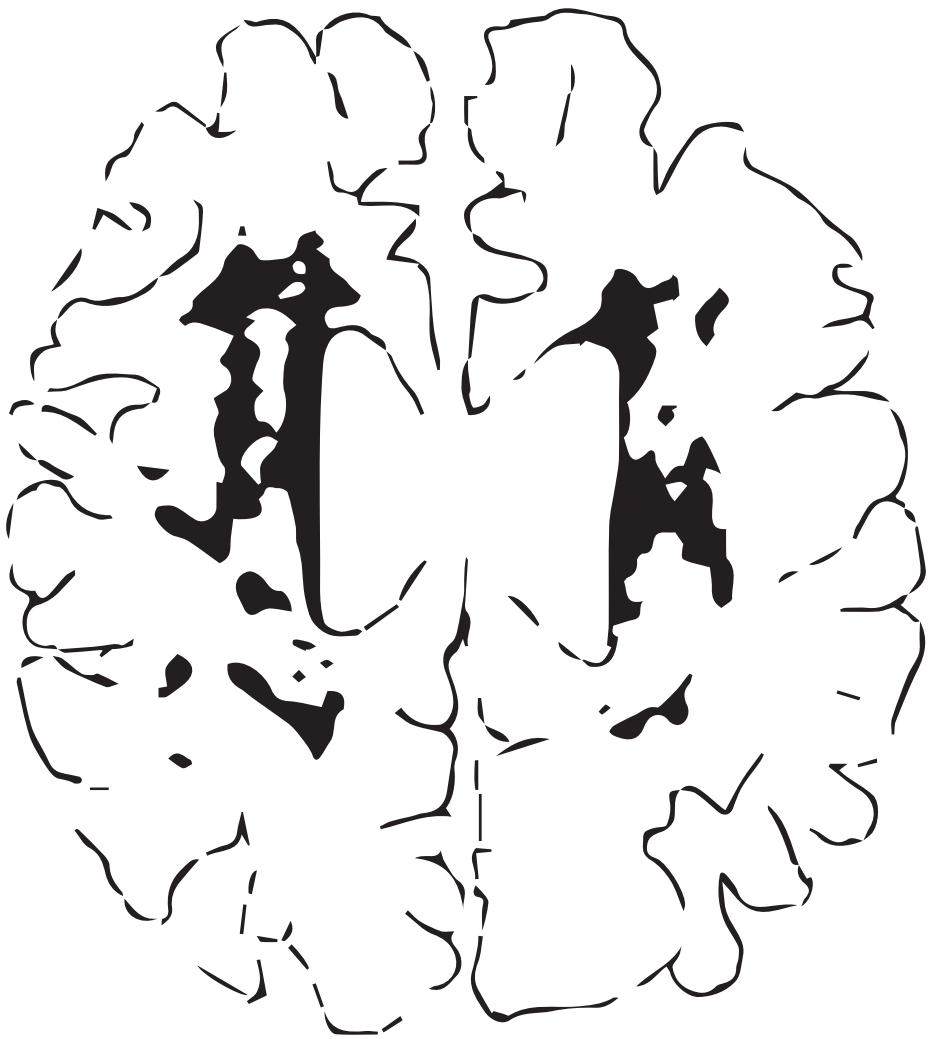
Promotoren
Prof. dr. H.F. de Leeuw
Prof. dr. C.J.M. Klijn

Copromotor
dr. E.J. van Dijk

Manuscriptcommissie
Prof. dr. M.G.M. Olde Rikkert
Prof. dr. W.M. van der Flier (Alzheimercentrum VUmc , Amsterdam)
dr. F.J.A. Meijer

voor mijn ouders

Table of contents



1.
General introduction

Introduction

Cerebral Small Vessel Disease

Cerebral small vessel disease (cSVD) has first been described in pathology studies in patients with a history of dementia and cardiovascular disease in the 19th century.[1-4] Much progress in our understanding came with the introduction of CT scans in the 70's followed by the Magnetic Resonance Imaging (MRI) scans in the 80's.[5] The frequently observed white matter hyperintensities (WMH) and lacunes,[6,7] together with prominent perivascular spaces (EPVS), were among the first to be recognized as part of the small vessel disease spectrum, followed by cerebral microbleeds (MB),[8] (sub)cortical atrophy[9] and recent small subcortical infarcts. More recently cortical superficial siderosis (cSS),[10] microinfarcts,[11] and intra-cerebral haemorrhage were added to the cSVD spectrum. Standards for nomenclature and imaging of these cSVD markers were recently published as the STRIVE-criteria[12] by an international working group from the Centres of Excellence in Neurodegeneration. **(PANEL 1)**

cSVD encompasses all pathological processes that affect the small vessels of the brain supplying the white matter, the brainstem, the deep grey nuclei and the cortex. These small vessels cannot be visualized on cerebral imaging yet, therefore these parenchymal lesions have been adopted as imaging markers of cSVD. Vascular risk factors, probably via the development of arterio(lo)sclerosis are thought to be most important in the aetiology of cSVD, and this type of cSVD will therefore be the focus of this thesis. The other types (hereditary cerebral amyloïd angiopathy, genetic, inflammatory or venous collagenosis) will not be discussed.[13]

Prevalence and progression of cSVD

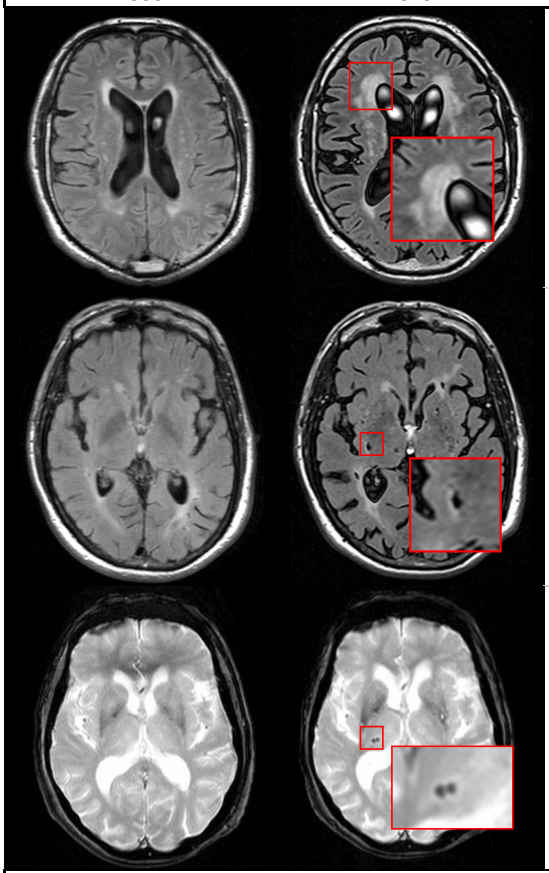
WMH are very common in persons aged above 60 years[6] and progression of WMH is estimated between 0.2 and 2.5 mL/year.[14-16] Lacunes are present in a fifth of individuals over 60 years of age,[7] with a yearly incidence of 0.7 to 6 percent.[17,18] Microbleeds are less frequent with a prevalence of about 5% in this elderly population[8] with a yearly incidence between 3 and 9 percent.[19,20] Progression of these characteristics however are suggested to be non-linear, and related to baseline cSVD load, age and vascular risk factors, [21] however actual proof for these assumptions is lacking.

WMH can be categorized into mild, moderate and severe. **(PANEL 2)** These different categories of WMH volume possibly are of non-uniform origin, ranging from non-ischemic to almost complete infarction. [22,23] For this reason different WMH volumes might have different clinical correlates, and therefore might differ in incidence of clinical syndromes such as dementia.

Macrostructural versus microstructural cSVD

With the advent of the development of MRI techniques, including diffusion tensor imaging (DTI), the radiologic spectrum of cSVD has extended beyond lesions visible on conventional

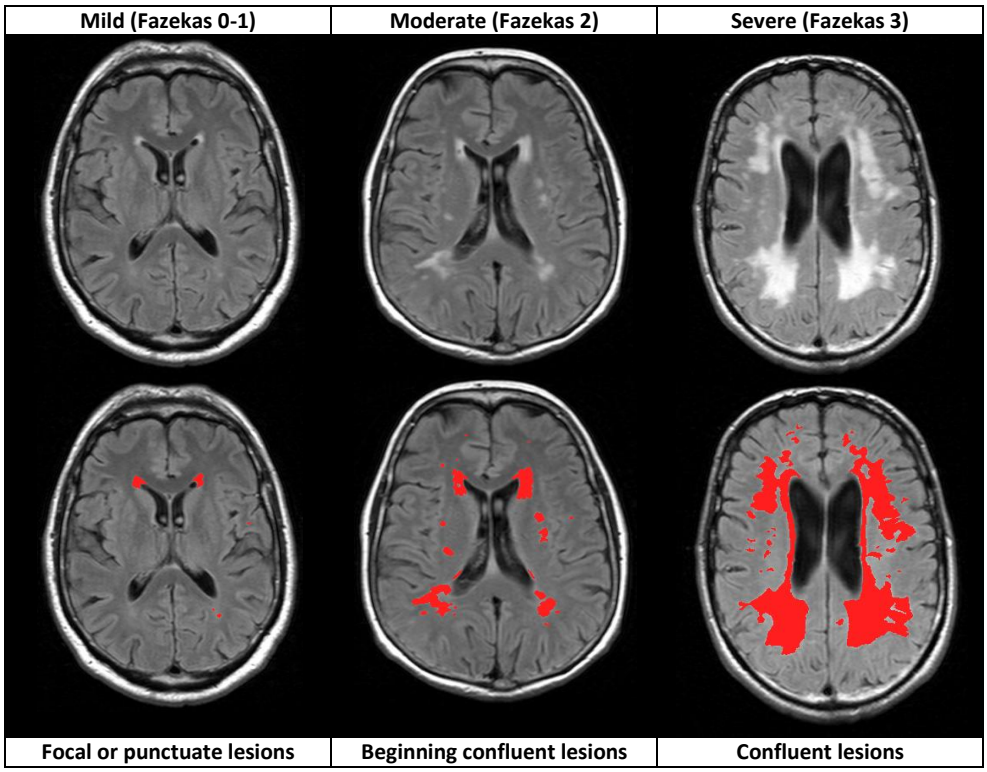
PANEL 1: SVD markers according to the STRIVE criteria

2006	2015	SVD marker; STRIVE	Numbers
		**White matter hyperintensity of presumed vascular origin** • *WMH* • Located in WM • No cavitation • Variable size • FLAIR MRI	Prevalence: 90% > 60 yrs Progression: 0.2 to 2.5 mL/yr
		Lacune of presumed vascular origin • *Lacune* • Usually hyperintense rim • Round or ovoid • Subcortical • Fluid-filled cavity • 3-15 mm • FLAIR MRI and T1	Prevalence: 20% > 60 yrs Progression: 0.7 to 6 %/yr
		Cerebral microbleed • *MB* • Round or ovoid, blooming • 2-5 mm, ≤ 10 mm • T2*	Prevalence: 5% > 60 yrs Progression: 3 to 9 %/yr

- (sub)cortical atrophy (lower brain volume not related to a specific macroscopic focal injury such as trauma or infarction) can also be observed in the FLAIR sequence
- Perivascular spaces (PVR) can also be observed in the FLAIR sequence
- In our study in the WMH volume, we included hypertintensities in the deep grey matter (also called subcortical hyperintensities)

MRI, including impaired WM microstructural integrity.[24] DTI measures the movement of water (both the ***magnitude*** and the ***direction***) within the cerebral white matter. Two main measures provide information on this WM microstructure: the Fractional Anisotropy (FA) and the Mean Diffusivity (MD). A lower FA and corresponding higher MD are generally believed to reflect lower microstructural integrity.[25] The opportunity to assess not only the WMH but also the normal appearing white matter (NAWM) surrounding the on FLAIR MRI visible WMH is interesting, because the initial stages of cSVD may already develop beyond the detection limits of conventional MRI.[26] Knowledge on the additional value of these DTI parameters in the early identification of those at risk of dementia or depressive symptoms, especially taking into account the macrostructural MRI characteristics, thus far is not unravelled yet.

PANEL 2: Mild, Moderate and Severe WMH



Clinical consequences of cSVD

The clinical spectrum and the prognosis of cSVD is highly heterogeneous and ranges from very mild symptoms to full blown stages of dementia or parkinsonism. Thus far it is very difficult to predict which patients with cSVD are at highest risk to develop these severe clinical symptoms, and who will not. As brain changes, measured by MRI, are thought to exist long before the clinical symptoms become apparent, the investigation of these imaging characteristics in relation to the presence of clinical symptoms or its progression seems a plausible next step.

There is a thin line between 'neurodegenerative' and 'vascular' dementias, especially in the late onset dementias.[27-32] Therefore both neurodegenerative (hippocampal and cortical atrophy)[33] and cSVD MRI characteristics should be investigated together in their role on incident dementia at older age. The added clinical value of DTI characteristics on top of the macrostructural MRI characteristics in explaining the clinical variance has not been studied very often, and longitudinal studies investigating microstructural integrity and incident dementia as an outcome measure are lacking. By investigating these imaging characteristics, macrostructural (both cSVD characteristics as brain volumes) and microstructural combined,

this might provide further insight in which MRI characteristics (combined) explain most of the clinical variance in older adults with cSVD.

Hippocampal volume **(PANEL 3)** is known to be related to incident dementia and progression of cSVD has previously been related with hippocampal atrophy.[31,32] This hippocampal atrophy, however, is visible on conventional MRI at a relatively late stage of dementia, most of the time when the cognitive disturbances have already become apparent. For this reason early detection of hippocampal damage on a microstructural level might provide earlier insight in the aetiology and course of dementia, and provide a better correlation with clinical symptoms. Prospective studies however, investigating hippocampal microstructural integrity with incident dementia as an outcome measure are lacking.

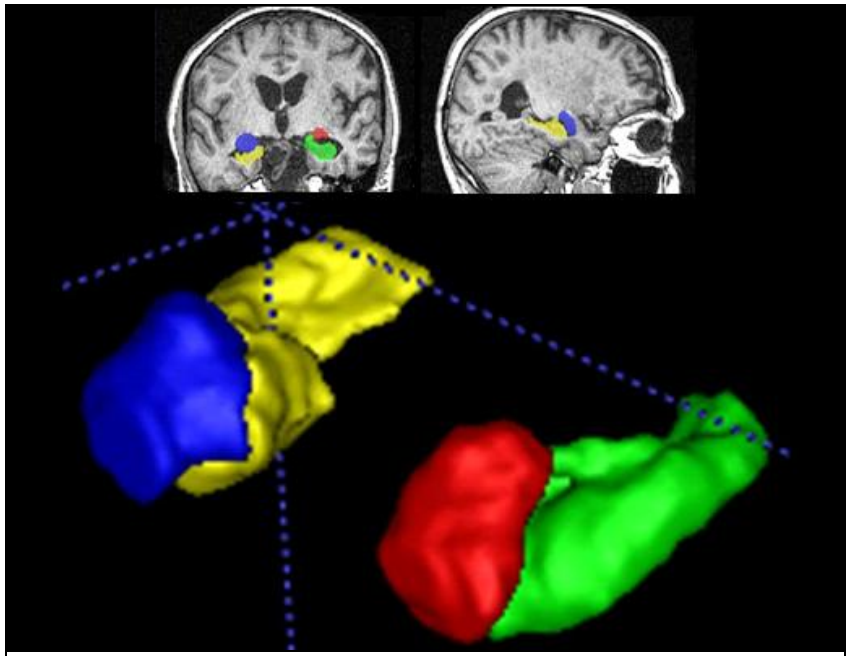
Cognitive disturbances in patients with cSVD, (usually consisting of a characteristic 'subcortical' pattern including psychomotor slowing due to impaired executive function, problems in attention and planning)[34] are thought to be caused by a disruption of the white matter tracts (both on a macro- and microstructural level), resulting in a loss of connectivity; a 'disconnection-syndrome', and the attendant clinical function.[35] As associations with traditional markers of cSVD and cognitive decline are weak, possibly DTI parameters might provide a better clinical correlate with cognitive decline at follow-up.

Traditional cSVD markers in relation to depressive symptoms are well investigated.[36] Especially for depressive symptoms that occur for the first time in life after the age of 60 years, an important role for structural abnormalities (such as cSVD) of the brain are hypothesized.[36-38] The dominant view is that cSVD disrupts the white matter tracts of the neural circuit that mediates emotion perception and mood regulation,[39] which might lead to the same 'disconnection-syndrome', resulting in depressive symptoms. Because not every individual with cSVD experiences depressive symptoms, other factors than the 'traditional cSVD markers' might play a role. Such factors could be a lower amygdala volume (known to be involved in mood regulation), **(PANEL 3)** or white matter microstructural integrity. Thus far, the limited studies investigating amygdala volume and WM microstructural integrity in relation to depressive symptoms, have small sample sizes or did not take the confounding role of cSVD into account.

Furthermore, the exact relation between depressive symptoms and cognitive problems has not been elucidated yet. It is unclear whether depressive symptoms increase the dementia risk by itself or whether this is mediated by the same underlying cSVD.[36,38,40,41] Therefore the role of depressive symptoms in the development of cognitive decline must be taken into account and vice versa.

More knowledge on the role of cSVD (both macro and microstructural) and its progression in relation to clinical symptoms is important for several reasons. First the pathophysiology of cSVD might be better understood, and it could lead to a better understanding of the clinical correlates in patients with cSVD. The detection of those who develop dementia (or other clinical consequences) early in the disease, might offer possible intervention targets

PANEL 3: Segmented hippocampus and amygdala



Right amygdala is presented in blue, left amygdala is presented in red
Right hippocampus is presented in yellow, left hippocampus is presented in green

before the symptoms progress to a clinical end stage, and those who are not at risk can be comforted. Possibly these MRI markers could help us to identify those at risk to deteriorate early in the disease. Finally these imaging characteristics might offer more sensitive outcome measures in therapeutic trials.[42]

Aim en outline of the thesis

The aim of this thesis was therefore to investigate the relation between both macro and microstructural aspects cSVD, brain volumes and the behavioural consequences (cognitive decline, dementia and depressive symptoms). Furthermore the temporal aspects of cSVD was investigated because of the availability of 3 MRI assessments, giving us the opportunity to get more insight into the aetiology and development of cSVD, and a better understanding of the clinical correlates in the future.

This was done within the Radboud University Nijmegen Diffusion Tensor and Magnetic resonance Cohort (RUN DMC) study, a prospective cohort study among 503 non-demented, older adults with cerebral cSVD on MRI, and investigates risk factors and clinical consequences of brain changes during aging. Participants were between 50 and 85 years old at baseline (2006). The first follow-up was completed in 2012 and the second follow-up was completed in 2015. This thesis is based on cross-sectional data and foremost the first follow-up moment.

In **Part I (chapter 2)** we report at the cross-sectional level on the possible (additional) clinical value of DTI over conventional MRI measures of cSVD with respect to cognitive performance *(van Norden*, van Uden* et al. JAD, 2012)*[43]

Part II prospectively describes the relation between baseline brain structures, both assessed with MRI (FLAIR, T1 and DTI) and the development of incident dementia or cognitive decline after 5 years. In **chapter 3** we report on both micro and macrostructural imaging characteristics on the risk of dementia *(van Uden et al. JAD, 2015)*[44]. In **chapter 4** we additionally investigated the relation between diffusion parameters of the hippocampus and the risk of dementia risk, independent of hippocampal volume, brain volume and cSVD markers *(van Uden*, Tuladhar* et al, HBM, 2016)*[45] In **chapter 5** we report on baseline global WM DTI parameters and cognitive decline (different cognitive domains) after 5 years, taking into account demographics and previous described imaging parameters (both volumetric and cSVD). *(van Uden et al. BBA Clin, 2015)*[46]

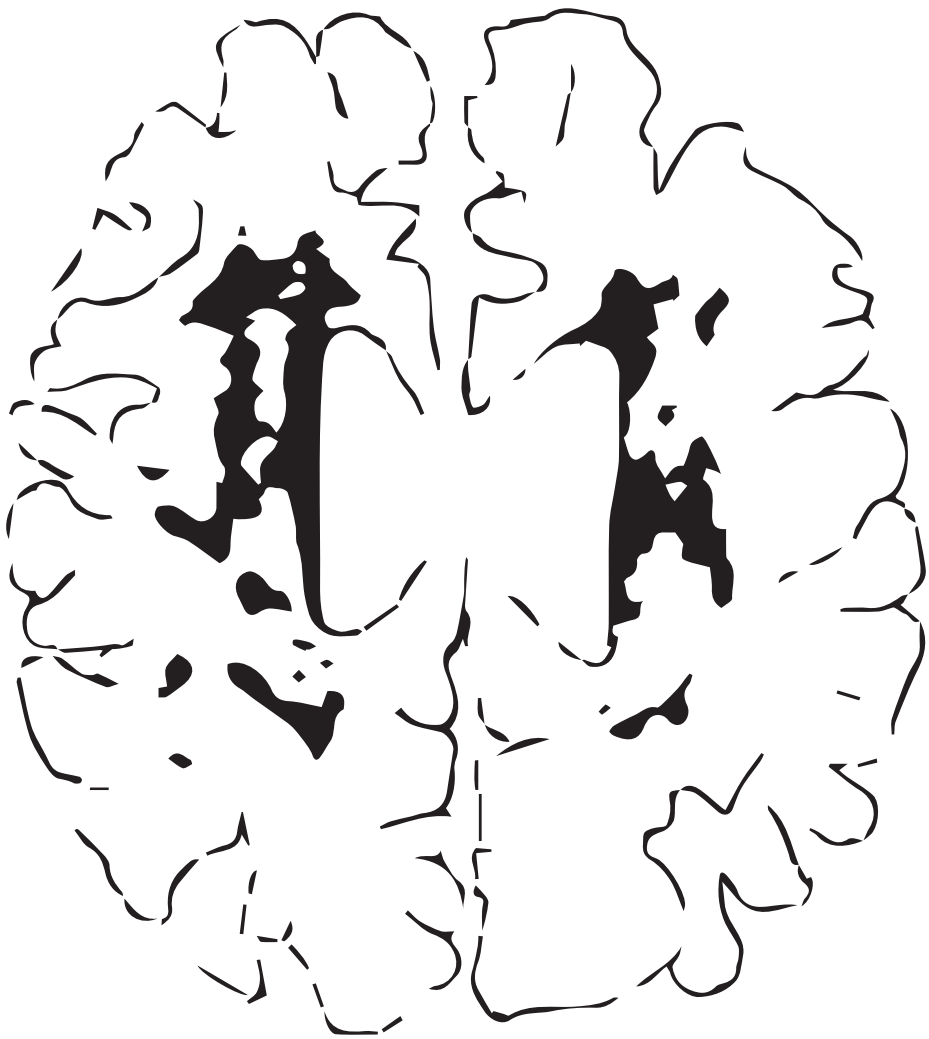
In **Part III** we report on depressive symptoms, and the relation between depressive symptoms and cognition in older adults with cSVD. The association between amygdala volume and depressive symptoms is described in **chapter 6** on a cross-sectional level. *(van Uden et al. JAR, 2011)*[47] In **chapter 7** we investigated the white matter integrity of older adults with and without depressive symptoms, using Tract-Based-Spatial-Statistics (TBSS), on a cross-sectional level *(van Uden*, Tuladhar* et al. AJGP, 2014)*[48] In **chapter 8** we prospectively report on the role of depressive symptoms on incident all-cause dementia after 5 years follow-up, taking both the age of onset and baseline cognitive performance into account *(van Uden et al. Neurology, 2016)*[49]

In **part IV (chapter 9)** we review the clinical consequences; (cognitive, motor, mood) and the risk factors of microstructural damage of the white matter, as assessed with diffusion tensor imaging. *(Pasi, van Uden et al. Stroke, 2016)*[50]

Finally in **part V** we describe the 9-year change of cSVD markers, using two follow-up assessments (2011 and 2015). In this **chapter 10** we elaborate on the dynamics of cSVD, taking both newly developed as well as apparent decline of the traditional cSVD markers into account. We calculated incidence rates of cSVD markers (WMH, lacunes, microbleeds) using 2 follow-up moments, allowing us to report on the non-linear progression of cSVD. *(van Uden*, van Leijsen* et al. submitted)*

Part I:

‘Conventional MRI’ compared to Diffusion Tensor Imaging (DTI)



2.

Cognitive function in cerebral small vessel disease: the additional value of Diffusion Tensor Imaging to Conventional Magnetic Resonance Imaging

Anouk G.W. van Norden*, Ingeborg W.M. van Uden*, Karlijn F. de Laat,
Ewoud J. van Dijk and Frank-Erik de Leeuw

Journal of Alzheimer's disease 2012;32(3):667-76

* Both authors contributed equally

Abstract

The structural integrity of the cerebral white matter, including that of the white matter lesions (WML) and of the surrounding normal appearing white matter (NAWM), can be assessed with diffusion tensor imaging (DTI), which is suggested to be of added value in the explanation of cognitive dysfunction in cerebral small vessel disease (cSVD). We investigated the value of DTI of NAWM and WML in addition to conventional magnetic resonance imaging (MRI) parameters in the variance of cognitive performance in subjects with cSVD. 499 individuals with cSVD, 50–85 years, without dementia, underwent MRI scanning, including a DTI sequence. Grey matter (GM), white matter (WM), and WML volume, number of microbleeds, lacunar and territorial infracts, and mean diffusivity (MD) and fractional anisotropy (FA) in NAWM, WML, and total WM were related to cognitive performance in multivariate regression analyses, after adjustment for age, gender, and education. All MRI parameters together accounted for 1–6% of the variance in cognitive function on top of 22–36% already explained by age, gender, and level of education. Both mean MD and FA of the NAWM, WML, and total WM did not substantially contribute to the explained variance of cognitive function, to that already explained by conventional MRI parameters. When considered separately, the MD of the (NA)WM had the strongest association with cognitive performance. In conclusion, DTI of NAWM and WML has limited additional value to conventional MRI parameters in the etiological explanation of the variance in cognitive function among individuals with cSVD.

Introduction

Conventional magnetic resonance imaging (MRI) plays an essential role in the diagnosis of cerebral small vessel disease (cSVD), including white matter lesions (WML), lacunar infarcts, and microbleeds.

Both hospital- and population-based studies have shown that severe cerebral SVD is an important cause of cognitive impairment and may ultimately contribute to the development of dementia, including Alzheimer's disease.[51-53] Despite the high prevalence of cSVD in the population over 60 years of age only few individuals with cSVD ultimately develop dementia.[6,8,54]

Apparently, factors other than cSVD as seen on conventional MRI play a role in this transition to cognitive disturbances that, in some, will eventually lead to cognitive decline and dementia. With respect to cSVD, conventional MRI differentiates normal appearing white matter (NAWM) from WML and lacunar infarcts. Within WML, the extent of white matter (WM) tract disruption substantially varies.[55] The integrity of NAWM surrounding the cSVD related lesions may be another potential factor contributing differential cognitive decline. cSVD on conventional MRI usually affects only a few percent of the total WM, leaving the remainder of the WM appearing normal.[26]

Diffusion tensor imaging (DTI) has proven beneficial in assessing the microstructural integrity of both the WML and the NAWM.[25,56] DTI provides quantitative information about the structural integrity of the WM.[57,58] This technique is based on the principle of molecular diffusion of water and provides, amongst others, two parameters: fractional anisotropy (FA) and mean diffusivity (MD). FA reflects the directionality of diffusion, whereas MD reflects the degree of diffusivity. FA decreases and MD increases are typical indications of impaired structural integrity.[59] However, the relative importance of each of these MRI and DTI parameters in explaining the variance of cognitive performance in patients with cSVD, and with that the value of DTI in clinical practice, is not clear. It is unknown which (combination of) conventional MRI parameter(s) explains most of the variance in cognitive function and to what extent the microstructural integrity of the WML and NAWM contributes to cognitive disturbances, on top of the abnormalities already seen on conventional MRI.

We therefore investigated which (combination of) conventional MRI parameter(s) accounted for the largest portion of variance explained in cognitive function. Furthermore, we determined the additional variance in cognitive function explained by the microstructural integrity of the WML and the NAWM to that explained by cSVD, and other brain lesions, including territorial infracts and grey matter (GM) and WM volume, visible on conventional MRI.

Methods

Study population

The Radboud University Nijmegen Diffusion tensor and Magnetic resonance imaging Cohort (RUN DMC) study prospectively investigates the risk factors and clinical consequences of brain changes among 503, 50-85 years old non-demented elderly individuals with cerebral SVD. The selection procedure of the participants and the study rationale and protocol were described in detail previously.[60]

In short, on the basis of established research criteria cSVD was defined as the presence of lacunar infarcts and/or WML on neuroimaging.[61] Accordingly, in 2006, consecutive patients referred to the Department of Neurology between October 2002 and November 2006, were selected for participation and investigated from 2006 to 2007. MRI scanning was performed in this same time window. Inclusion criteria were: (a) age between 50 and 85 years; (b) cerebral SVD on neuroimaging (WML and/or lacunar infarcts). The main exclusion criteria were dementia,[62] (psychiatric) disease interfering with cognitive testing or follow-up, WML or cSVD mimics and MRI contraindications or known claustrophobia.

From 1,004 invited individuals by letter, 727 were eligible after contact by phone of whom 525 agreed to participate. In 22 individuals, exclusion criteria were found during their visit to our research centre, yielding a response of 71.3% (503/705). All participants signed informed consent. The local Medical Review Ethics Committee approved the study. For the present study four subjects were excluded because of MRI artefacts, yielding a sample of 499 subjects.

MRI scanning protocol

The complete scanning protocol on a single 1.5 Tesla scanner was described in detail previously.[60] The scanning protocol included whole brain 3D T1 magnetization-prepared rapid gradient-echo (MPRAGE) sequence, FLAIR pulse sequences, transversal T2* weighted gradient echo sequence and a DTI sequence.

Conventional MRI analysis

WML were manually segmented, and the number of lacunar infarcts, territorial infarcts (diameter >15 mm in known arterial territories) and microbleeds were rated according to a standardized protocol.[60]

GM, WM and cerebrospinal fluid (CSF) probability maps were obtained by automated segmentation using Statistical Parametric Mapping 5 unified segmentation routines on the T1-weighted MPRAGE images (SPM5; Wellcome Department of Cognitive Neurology, University College London, UK).[63] Total GM, WM, and CSF volumes were calculated by summing all voxel volumes that had a $p > 0.5$ for belonging to the tissue class. Total brain volume was taken as the sum of total GM and WM. Intracranial volume (ICV) was a summation of all tissue classes, i.e., total GM, total WM, and CSF volume. To normalize for head size, total brain volume was expressed as percentage of total ICV. Mutual information co-registration (SPM5) was used to align WML maps to the T1 image and to yield a NAWM map (the complement of WML in WM).[60]

Measurement of DTI parameters

The diffusion weighted images of each subject were realigned on the unweighted image using mutual information based co-registration routines from SPM5. Then, the diffusion tensor and its eigenvalues were computed using an SPM5 add-on (http://sourceforge.net/projects/spmtools). Unphysical spurious negative eigenvalues of the diffusion tensor were set to zero, after which the tensor derivatives the MD and FA were calculated.[64] The mean unweighted image was used to compute the co-registration parameters to the anatomical reference T1 image (SPM5 mutual information co-registration), which were then applied to all diffusion weighted images and derivates. All images were visually checked for motion artefacts and co registration errors. The mean MD and FA were then calculated in the WML, NAWM, and total WM.

Measurement of cognitive function

Cognitive function was assessed by a standardized neuropsychological test battery that has proven to be sensitive and suitable for this purpose in other large epidemiological studies[53] and has been described in detail elsewhere.[60] In short, we calculated compound scores for seven cognitive domains; 1) Global cognitive function was evaluated by the a)Mini Mental State Examination (MMSE) and b) the Cognitive Index. The Cognitive Index is a compound score that was calculated as the mean of the z-scores of the 1-letter subtask of the Paper-Pencil Memory Scanning Task, the mean of the reading subtask of the Stroop test, the mean of the Symbol-Digit Substitution Task and the mean of the added score on the three learning trials of the Rey Auditory Verbal Learning Test (RAVLT) and the delayed recall of this last test.[53] 2) Verbal memory was assessed as the compound score of the mean of two z-scores from the RALVT; one for the added scores of the three learning trials of this test, and one for the delayed recall of this test. 3) Visuospatial memory was assessed as a compound score of the mean of the z-scores of the immediate recall trial and the delayed recall trial of the Rey Complex Figure Test. 4) Psychomotor speed was calculated as the mean of the z-scores of the 1-letter subtask of the Paper-Pencil Memory Scanning Task, the reading subtask of the Stroop test, and the Symbol-Digit Substitution Task.[53] 5) Fluency was calculated from the mean of the z-scores of both verbal fluency tasks. 6) Concept shifting was calculated as the z-score of the third subtask of the Stroop. 7) Attention was assessed as a compound score of the z-score of the total time of the verbal series attention test.[53]

Other measurements

Level of education was assessed (classified using 7 categories; 1 being less than primary school and 7 reflecting an academic degree).[65] Cardiovascular risk factors were assessed by structured questionnaires and an experienced research nurse measured blood pressure (3 times in the supine position after 5 minutes of rest). Cardiovascular risk factors were defined; hypertension as mean blood pressure ≥ 140/90 mmHg and/or use of antihypertensive medications,[66] diabetes as treatment with anti-diabetic medications, hypercholesterolemia

as treatment with lipid lowering medications, and smoking as current, former, or never smoker.

Statistical analysis

The baseline characteristics are presented as mean ± standard deviation (SD) and for the positively skewed WML volume distribution, the median and inter quartile range were calculated. WML volume distribution was normalized by log-transformation.

First, we used Pearson's correlations to assess the associations between age and MRI parameters. Second, to establish which MRI parameters (conventional MRI or DTI parameter) explained most of the variance in different cognitive domains, we performed a multiple linear regression analysis (with age, gender, and education included in every model), for each MRI parameter separately. Territorial infarcts and brain atrophy were taken into account, because these abnormalities often co-exist with cSVD-related MRI abnormalities[26] and are related to cognition as well. Third, we assessed which combination of conventional MRI parameters (only those conventional MRI parameters that showed significant correlations when analyzed separately, as described above in the second step) and DTI parameters (MD and FA of both WML and NAWM) accounted for the largest variance in cognitive performance. For each cognitive domain, a multiple regression analysis was performed with the introduction of age, gender, and education prior to the MRI parameters. The MRI parameters were subsequently added in an automated (forward) stepwise fashion to optimize the model fit.

Finally, we assessed the increase of variance explained in cognitive performance by DTI on top of conventional MRI parameters in order to assess the added value of DTI over conventional MRI. We did so by using a multiple linear regression model in which age, gender, and level of education were introduced first, followed by all conventional MRI parameters (those that showed significant correlations when analyzed separately in step two), and subsequently the DTI parameters (MD of NAWM or WML or total WM).

Results

Demographics and neuroimaging characteristics of the 499 subjects are shown in Table 1. Mean age was 65.6 years (SD 8.8) and 56.5% were male. Mean WM volume was 464.4 ml (SD 66.5), the largest part of the WM consisted of NAWM, with a median percentage of 98.5 (IQR 96.0–99.2).

Table 2 represents the Pearson's correlations between the different MRI and DTI parameters. Almost all MRI parameters were significantly correlated to each other, apart from some correlations with territorial infarcts and microbleeds. The correlations with the mean FA and MD in the NAWM were almost similar for both the NAWM and total WM. Mean FA and MD of the WML, NAWM, and total WM strongly correlated with WML volume (correlation coefficients for FA between –0.42 and –0.54, $p < 0.001$; for MD between 0.62 and 0.66, $p < 0.001$) and age.

Second, we determined the additional amount of variance in cognitive performance explained by each MRI parameter separately on top of age, gender, and education (Table 3). Most of the

Table 1: characteristics of the study population

Characteristics	n=499
Demographic and clinical characteristics	
Age, yrs.	65.6 (8.8)
Male, no.	282 (56.5)
Subjects with hypertension, no.	367 (73.5)
Subjects with diabetes, no.	73 (14.6)
Subjects with hypercholesterolemia, no.	234 (46.9)
Current smokers, no.	75 (15.0)
Former smokers, no.	275 (55.1)
MMSE	28.1 (1.6)
Subjects who finished primary school, no.	450 (90.2)
Neuroimaging characteristics	
Grey matter volume, ml	628.8 (67.0)
White matter volume, ml	464.4 (66.5)
Intracranial volume, ml	1676.3 (156.3)
WML volume, ml†	7.1 (3.4; 18.1)
NAWM volume, ml	450.3 (70.3)
Subjects with microbleeds, no.	52 (10.4)
Subjects with lacunar infarcts, no.	171 (34.3)
Subjects with territorial infarcts, no.	58 (11.6)
Mean MD in NAWM * 10^-3 mm2/s	0.89 (0.04)
Mean MD in WML * 10^-3 mm2/s	0.10 (0.67)
Mean MD in total WM * 10^-3 mm2/s	0.89 (0.05)
Mean FA in NAWM mm2/s	0.33 (0.02)
Mean FA in WML mm2/s	0.34 (0.03)
Mean FA in total WM mm2/s	0.33 (0.02)

Data represent numbers (%), mean (Standard deviation) or median † (inter quartile range). WML = white matter lesions; NAWM = normal appearing white matter; WM: white matter; MD = mean diffusivity; FA = fractional anisotropy.

MRI parameters were significantly associated with all cognitive domains, except for FA and MD in the WML, WM volume, and territorial infarcts. Mean MD in the NAWM and in the total WM explained most variance of the MMSE, cognitive index, verbal memory performance, and attention. In addition to age, gender, and education, it explained another 1.5–2.4% of the performance in the different cognitive domains, resulting in explained variance between 23% and 38% for the different cognitive domains. WM volume explained most of the variance in psychomotor speed, followed by the mean MD in the NAWM and total WM. In all cognitive domains, DTI parameters accounted for only slightly

Table 2: Pearson correlation coefficients for age and MRI parameters

	age	Grey matter volume	White matter volume	WML† volume	Micro-bleeds, no.	Lacunar infarcts, no.	Territorial infarcts, no.	NAWM, mean MD	WML, mean MD	total WM, mean MD	NAWM, mean FA	WML, mean FA
age	…											
Grey matter volume	-0.42**	…										
White matter volume	-0.44**	0.65**	…									
WML volume†	0.48**	-0.15**	-0.13*	…								
Microbleeds, no.	0.13*	0.05	-0.04	0.30**	…							
Lacunar infarcts, no.	0.21**	-0.13*	-0.12*	0.44**	0.32**	…						
Territorial infarcts, no.	0.11*	-0.05	-0.12*	0.11*	-0.03	0.12*	…					
NAWM, mean MD	0.66**	-0.39**	-0.41**	0.62**	0.32**	0.42**	0.18**	…				
WML, mean MD	0.56**	-0.16**	-0.19**	0.62**	0.36**	0.42**	0.17**	0.78**	…			
Total WM, mean MD	0.66**	-0.38**	-0.40**	0.66**	0.35**	0.45**	0.17**	1.00**	0.16**	…		
NAWM, mean FA	-0.29**	0.33**	0.24**	-0.54**	-0.32**	-0.37**	-0.02	-0.69**	-0.51**	-0.70**	…	
WML, mean FA	-0.30**	0.17**	0.13*	-0.42**	-0.29**	-0.34**	0.01	-0.52**	-0.54**	0.02	0.58**	
Total WM, mean FA	0.29**	0.33**	0.24**	-0.54**	-0.33**	-0.37**	-0.02	-0.69**	-0.70**	0.67**	1.00**	0.54**

†log transformed*correlation is significant at 2-tailed p<0.05; **p<0.001; WML= white matter lesions; NAWM= normal-appearing white matter; MD= mean diffusivity; FA = fractional anisotropy

Table 3: Variance explained and standardized beta of each MRI parameter separately by cognitive domain

		MMSE		Cognitive index		Verbal memory	
Step in model	**MRI parameter**	total explained variance (%)	standardized beta	total explained variance (%)	standardized beta	total explained variance (%)	standardized beta
1	Age, sex, education	21.7		35.7		24.3	
	Conventional MRI parameters						
2	GM volume, ml	22.6	0.12*	36.6	0.12*	24.7	0.07
2	WM volume, ml	21.8	0.05	36.8	0.13*	24.6	0.07
2	WML volume, ml†	22.9	-0.13*	36.4	-0.10*	24.4	-0.04
2	Microbleeds, no.	22.1	-0.07	37.3	-0.13**	25.3	-0.10*
2	Lacunar infarcts, no	23.1	-0.12*	36.8	-0.11*	24.9	-0.08*
2	Territorial infarcts, no	21.7	-0.03	36.5	-0.09*	24.4	-0.04
	DTI parameters						
2	NAWM, mean MD	23.1	-0.16*	37.6	-0.19**	25.7	-0.15*
2	WML, mean MD	22.8	-0.13*	37.1	-0.14*	24.9	-0.09
2	Total WM, mean MD	23.2	-0.17*	37.8	-0.19**	25.7	-0.15*
2	NAWM, mean FA	22.7	0.11*	37.0	0.13*	25.2	0.10*
2	WML, mean FA	22.3	0.08	36.3	0.09*	24.6	0.06
2	Total WM, mean FA	22.7	0.11*	37.0	0.13*	25.2	0.10*

Age, sex and education were introduced in the model prior to the MRI parameters †log transformed; *<0.05; **
p<0.001; GM= grey matter; WM=white matter; WML= white matter lesions; NAWM = normal appearing white matter;
MD: mean diffusivity; FA= fractional anisotropy;

Table 3: Variance explained and standardized beta of each MRI parameter separately by cognitive domain CONTINUED

		Psychomotor speed		Attention	
Step in model	**MRI parameter**	total explained variance (%)	standardized beta	total explained variance (%)	Standardized beta
1	Age, sex, education	35.0		27.9	
2	GM volume, ml	36.2	0.14*	28.6	0.11*
2	WM volume, ml	37.6	0.20**	28.7	0.11*
2	WML volume, ml†	35.9	-0.11*	28.4	- 0.09*
2	Microbleeds, no.	36.7	-0.13**	29.5	- 0.16**
2	Lacunar infarcts, no	36.4	-0.12*	28.6	- 0.09*
2	Territorial infarcts, no	37.0	-0.14**	27.9	- 0.02
	DTI parameters				
2	NAWM, mean MD	37.2	-0.20**	30.3	- 0.13**
2	WML, mean MD	37.1	-0.18**	30.2	- 0.12*
2	Total WM, mean MD	37.4	-0.20**	30.3	- 0.14**
2	NAWM, mean FA	36.5	0.13**	28.6	0.12*
2	WML, mean FA	35.3	0.05	29.0	0.11*
2	Total WM, mean FA	36.5	0.13**	28.9	0.13*

Age, sex and education were introduced in the model prior to the MRI parameters †log transformed; *<0.05; **p<0.001;

GM= grey matter; WM=white matter; WML= white matter lesions; NAWM = normal appearing white matter;

MD: mean diffusivity; FA= fractional anisotropy

more variance in cognitive performance than the conventional cSVD parameters (WML volume, number of lacunar infarcts and number of microbleeds).

Third, we determined which combination of MRI parameters explained most variance in cognitive performance, on top of age, gender, and level of education, which already accounted for 21.7%–35.7% (Table 4). The mean MD of the NAWM explained most of the variance of the MMSE (1.4%), cognitive index (1.9%), verbal memory performance (1.4%), attention (2.4%), and visuospatial memory (0.7%; data not shown). WM volume was the most important determinant of psychomotor speed (2.6%) and concept shifting (2.2%; data not shown). GM volume explained most of the variance in fluency (1%; data not shown). The total explained variance of all MRI parameters (both conventional and DTI) together, on top of age, gender, and education ranged from 1–6%.

Finally, Table 5 shows the results of the added value of the mean MD of the NAWM, WML, and total WM over conventional MRI parameters in terms of explained variance of cognitive function. Conventional MRI parameters accounted for 1.0% to 4.2% on top of age, gender, and level of education in the variance of all cognitive domains. A significant increase in variance, on top of conventional MRI measures, was explained by MD in the NAWM and total WM for the cognitive index, verbal memory performance, psychomotor speed and attention, but no more than 1.0%.

This was not found for MMSE, visuospatial memory performance, fluency, and concept shifting (data not shown for latter three). The MD in the WML did not explain an increase in variance in any of the cognitive domains.

We performed the same analyses for FA in the NAWM, WML and total WM and all cognitive domains and found a similar amount of variance in cognitive domains explained by the mean FA.

Discussion

In this large cohort of individuals with cerebral SVD, all MRI parameters together explained 1–6% variance in performance on different cognitive domains, in addition to the 22–36% variance already explained by age, gender, and level of education. Both the mean MD and FA of the NAWM, WML, and total WM did not substantially contribute to the variance explained of cognitive function, to that already explained by conventional MRI parameters. Conversely, when each MRI parameter (conventional and DTI) was investigated separately, the MD of the (NA)WM had the strongest association with most cognitive domains.

Strengths of our study included the large sample size, the single centre design, with a high response, the extensive assessment of cognitive function, and all participants examined by only two investigators. In addition, we performed all MRI scans (conventional and DTI sequences) on a single scanner, and with quantitative assessment of WML, GM, and WM volume. Other strengths are the manual segmentation of the WML without prior knowledge of the clinical data and the assessment of microbleeds as another manifestation of cSVD. A limitation is the cross-sectional analysis of this part of the study, which prevents us from

Table 4 Combination of MRI and DTI parameters that best explains variance for the various cognitive domains

Cognitive domain and step in model	**MRI parameters** (independent variables remaining in model)	**Added variance explained**	**Total explained variance (%)**	**Stand. beta**	**p-value**
MMSE					
1			21.7		
2	NAWM, mean MD	1.4	23.1	-0.15	0.003
Cognitive index					
1			35.7		
2	NAWM, mean MD	1.9	37.6	-0.12	0.006
3	Microbleeds, no.	2.8	38.5	-0.11	0.023
4	White matter volume	3.6	39.3	0.11	0.025
5	Territorial infarcts, no.	4.1	39.8	-0.08	0.046
Verbal memory					
1			24.3		
2	NAWM, mean MD	1.4	25.7	-0.15	0.003
Psychomotor speed					
1			35.0		
2	White matter volume	2.6	37.6	0.19	<0.001
3	White matter lesions†	4.9	39.9	-0.13	0.004
4	Territorial infarcts, no.	5.6	40.6	-0.09	0.012
5	Microbleeds, no.	6.4	41.4	-0.10	0.012
Attention					
1			27.9		
2	NAWM, mean MD	2.4	30.3	-0.13	0.001
3	Microbleeds, no.	3.0	30.9	0.11	0.028

Age, gender, and education were introduced in the model prior to MRI parameters (model 1). MRI parameters (those significant in Table 3 and MD in the WML and NAWM and mean FA in WML and NAWM) were then added in a stepwise fashion. Total explained variance and standardized beta-value describe the values till that step as variables are progressively added to the model. NAWM: normal appearing white matter, WML: white matter lesions MD: mean diffusivity, FA: fractional anisotropy, †log transformed.

drawing conclusions about the predictive value of MRI parameters in cognitive decline and Alzheimer's dementia (AD). However, the RUN DMC study has a longitudinal design and follow-up is currently being executed.[60] Second, cognitive function is also influenced by several factors that intentionally were not taken into account in this study such as medial temporal lobe atrophy, as we wanted to investigate the effect of cSVD on cognitive function. Finally, we investigated a rather healthy study population with a relatively intact cognitive function (mean MMSE 28.1; SD 1.6) and most of them had mild to moderate white matter damage on conventional MRI. Consequently the integrity of the NAWM in most participants will probably be relatively intact, limiting the statistical power to detect an additional effect of DTI parameters in the NAWM to that of conventional MRI parameters.

There might be several reasons why only a small part of the variance in cognitive function was accounted for by conventional MRI parameters for cSVD (4%) and by the microstructural integrity of the (NA)WM (an additional 1%). Non-differential misclassification in both determinant and outcome could have played a role in the little contribution found. In addition, other imaging parameters, such as medial temporal lobe atrophy, and the presence of depressive symptoms might be other important factors in the variance of cognitive function in individuals with cSVD, as both have been related to cSVD and cognitive performance as well.[32,67] In addition, selective survival may have played a role in the relative minor contribution of MRI parameters to cognitive performance due to a decreased susceptibility of the effect of cSVD on the cerebral WM among survivors. Moreover there is probably a selective nonparticipation of survivors with a higher degree of cSVD and more cognitive disturbances. Another explanation for the relative small contribution of cSVD in the variance of cognitive performance may be due to so called 'competing risks'. Competing risks occur in an aging population where multiple risk factors for one single disease are present, thereby limiting each individual (relative) contribution to the disease.

Our findings furthermore showed that GM and WM volume play an important role in the explained variance of psychomotor speed, fluency, and concept shifting. These findings are consistent with previous studies in cSVD patients that have shown a relation between cognitive function and markers of cerebral atrophy,[68] such as atrophy of the corpus callosum,[69] GM volume, and hippocampal volume.[70]

Other cross-sectional studies investigated the value of DTI compared to conventional MRI in relation to cognitive function and found inconsistent results. While some small studies among individuals with WML showed that DTI parameters correlated stronger with cognition than conventional MRI parameters, [71,72] a diffusion weighted imaging study on 147 CADASIL patients found brain atrophy to have a stronger independent influence on cognitive function than the mean apparent diffusion coefficient of the whole WM.[73] However, it is difficult to compare our results with the results from these studies because of different study populations, the much larger sample size of our study, different methods in lesion quantification and different outcome measures. In addition we took more conventional MRI markers into

Table 5: Added value of DTI to conventional MRI parameters in the explained variance of the various cognitive domains

Step in model	MRI parameter	Total explained variance (%)	Standardized beta-value	p-value
MMSE				
1	Age, sex and education	21.7		
2	Conventional MRI parameters	23.1		
3a	NAWM, mean MD	23.5	-0.11	0.097
3b	WML, mean MD	23.3	-0.07	0.214
3c	Total WM, mean MD	23.6	-0.12	0.076
Cognitive index				
1	Age, sex and education	35.7		
2	Conventional MRI parameters	38.7		
3a	NAWM, mean MD	39.4	-0.13	0.020
3b	WML, mean MD	39.0	-0.08	0.150
3c	Total WM, mean MD	39.5	-0.15	0.015
Verbal Memory				
1	Age, sex and education	24.3		
2	Conventional MRI parameters	25.3		
3a	NAWM, mean MD	26.3	-0.13	0.017
3b	WML, mean MD	25.5	-0.04	0.262
3c	Total WM, mean MD	26.3	-0.13	0.018
Psychomotor speed				
1	Age, sex and education	35.0		
2	Conventional MRI parameters	39.2		
3a	NAWM, mean MD	39.7	-0.11	0.046
3b	WML, mean MD	39.4	-0.10	0.065
3c	Total WM, mean MD	39.7	-0.12	0.039
Attention				
1	Age, sex and education	27.9		
2	Conventional MRI parameters	30.6		
3a	NAWM, mean MD	31.2	-0.10	0.047
3b	WML, mean MD	31.0	-0.07	0.103
3c	Total WM, mean MD	31.2	-0.10	0.042

Age, gender, and education were introduced in the model prior to conventional MRI parameters (model 1). Conventional MRI parameters (those related to cognitive function in Table 3) were then added and subsequently the mean MD of the NAWM (3a) the WML (3b) or the total WM (3c). Total explained variance and standardized beta-value describe the values till that step as variables are progressively added to the model. NAWM: normal appearing white matter; WML, white matter lesions; WM, white matter; MD, mean diffusivity.

account, including brain atrophy, territorial infarcts, lacunar infarcts, and microbleeds, which may have led to the lower additional value of DTI parameters compared to other studies.

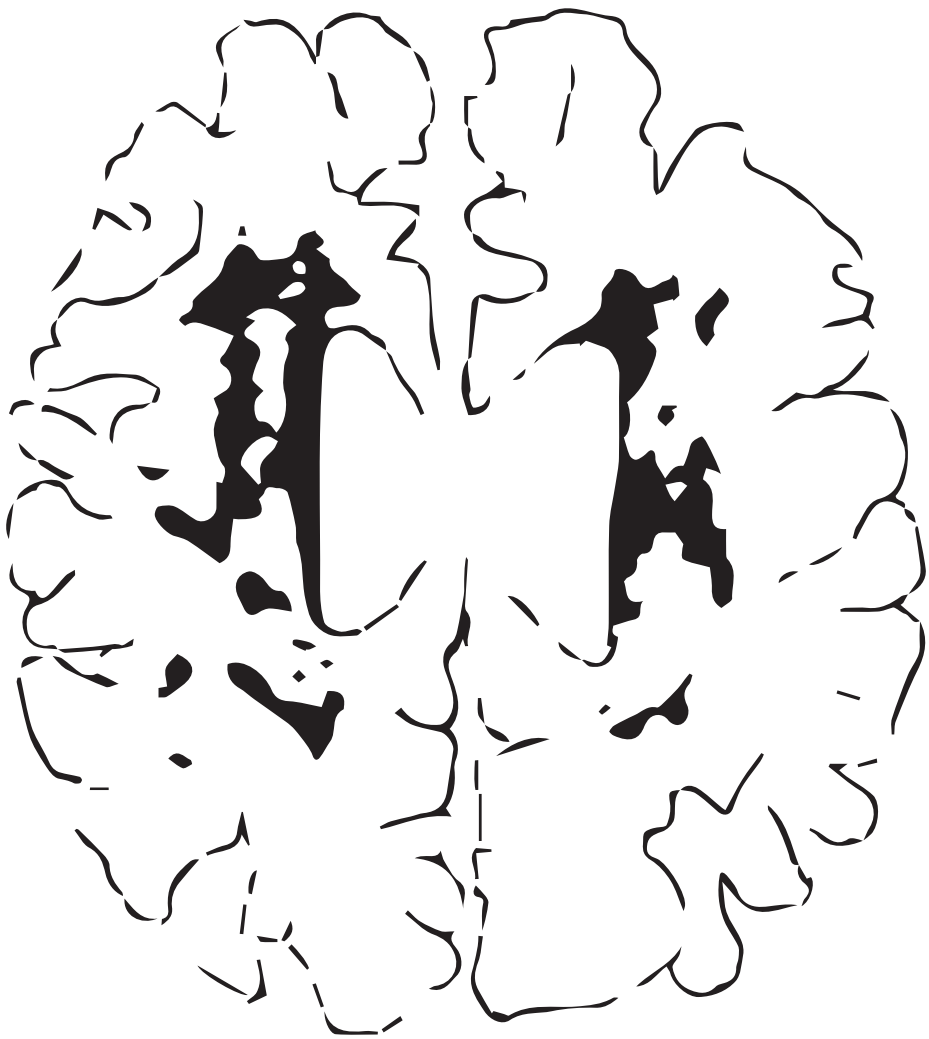
DTI parameters of the total WM and NAWM are highly correlated and explained the same variance in cognitive performance. This is probably due to the fact that the NAWM constitutes the largest part of the WM (median percentage of 98.5%). Damage to the structural integrity of the NAWM accounted for very little variance in cognitive performance in addition to the cSVD related lesions visible on conventional MRI. This might be due to the high correlation between WML volume and the mean FA and MD of the NAWM which we found (Table 2), which suggests that WML volume is a marker for the microstructural integrity of the NAWM. This is in line with findings in other studies.[74]

Our results show that DTI does not seem to have additive clinical value in understanding cognitive impairment in patients with cSVD. However, despite the small contribution of DTI parameters in the variance of cognitive performance at the cross-sectional level, DTI parameters may be of additional value in longitudinal studies and clinical trials in which there is interest in a surrogate marker for progression of cognitive disturbances, eventually resulting in dementia in some and in the evaluation of potential therapeutic effects of disease modifying drugs. This is of great interest as the rate of cognitive decline is slow, neuropsychology is time consuming, expensive and of limited value in repeated testing due to learning effects; these limitations require very large samples and/or long duration studies.[42] A few longitudinal studies showed that DTI is sensitive to change over a short period of time and correlated with change in cognitive test scores, while this is not the case for conventional MRI markers.[72,75,76] However, more longitudinal studies are needed to replicate these results with serial DTI analysis to establish the predictive value of DTI parameters in cognitive decline additional to conventional MRI parameters.

In conclusion, DTI of the WM (NAWM, WML or total WM) does not seem to be of added value to the conventional MRI parameters, in the etiological explanation of the variance of cognitive impairment in subjects with cSVD. Future longitudinal studies are needed to demonstrate the sensitivity of both conventional MRI and DTI markers to changes in lesion load over time and their association with cognitive performance and AD and to determine whether DTI measures of the (NA)WM or (a combination of) conventional MRI markers, such as WML volume, and atrophy could be a surrogate in therapeutic trials in cSVD.

Part II:

Baseline MRI characteristics and incident dementia or cognitive decline



3.

White matter and hippocampal volume predict the risk of dementia in patients with cerebral small vessel disease

Ingeborg W.M. van Uden, Helena M. van der Holst, Anil M. Tuladhar, Anouk G.W. van Norden, Karlijn F. de Laat, Loes C.A. Rutten-Jacobs, David G. Norris, Jurgen A.H.R Claassen, Ewoud J. van Dijk, Roy P.C. Kessels and Frank-Erik de Leeuw

Journal of Alzheimer's disease 2015; 49(3):863-73

Abstract:

Background: The relationship between cerebral small vessel disease (cSVD) and dementia has been studied without considering white matter (WM) volume, the microstructural integrity of the WM surrounding the cSVD, and grey matter (GM).

Objective: We prospectively investigated the relationship between these structures and the risk of dementia, and formed a prediction model to investigate which characteristics (macro- or microstructural) explained most of the variance.

Methods: The RUN DMC study is a prospective cohort study among 503 non-demented participants with an age between 50 and 85 years at baseline, with baseline assessment in 2006 and follow-up assessment in 2012. Two were lost to follow-up (yielding a 99.6% response-rate). Cox regression analysis was used, to calculate hazard ratios for dementia, of baseline MRI characteristics.
Tract-Based Spatial Statistics (TBSS) analysis was used to assess the added value of microstructural integrity of the WM.

Results: Mean age at baseline was 65.6 years (SD 8.8) and 56.8%was male. 43 participants developed dementia (8.6%), resulting in a 5.5-year cumulative risk of 11.1% (95%CI 7.7–14.6). Low WM and hippocampal volume are significant predictors for dementia. WM, white matter hyperintensities (WMH), and hippocampal volume (HV) explained most of the variance. TBSS analyses showed no additional value of diffusion parameters.

Conclusion: WM and hippocampal volume were the main predictors for the development of incident dementia at 5-year follow-up in elderly with cSVD. There was no additional diagnostic value of the diffusion tensor imaging parameters on top of the macrostructural characteristics.

Introduction

White matter hyper intensities (WMH) and lacunes occur in over 90% of all individuals aged 60 years and over. Expressions of cerebral small vessel disease (cSVD),[12] including white matter hyperintensities (WMH),[41] lacunes[51] and microbleeds[77] have always been studied in isolation, rather than in conjunction with respect to incident dementia, often without taking the surrounding (volume of) normal appearing white matter (NAWM) into account. The approach is usually similar for grey matter (GM) structures that have found to be related with dementia, such as neocortical or hippocampal volume (HV).[78] Obviously, the white matter (WM) connects and thereby influences the structure and function of these GM areas.[79] Given this, it seems plausible to also take these GM structures into consideration when assessing the relation between cSVD and cognitive performance or dementia and vice versa.

Apart from the with conventional MRI visible cSVD, the microstructural integrity of the WMH itself and that of the surrounding normal appearing white matter (NAWM), which can be assessed with DTI[25,80] has suggested to play a role in dementia in cross sectional studies.[81] However prospective studies are lacking.

We therefore investigated the relation between the total spectrum of cSVD, the GM (neocortex, basal ganglia, thalamus), the hippocampus, and the microstructural integrity of the WM altogether on the risk of dementia. With these macro- and microstructural parameters we constructed a prediction model to indicate which MRI- parameter accounts for the highest variance. We did so within the RUN DMC study, a prospective cohort study among 503 individuals with cSVD after a mean follow-up of over 5 years.

Materials and Methods:

Study population

The Radboud University Nijmegen Diffusion Tensor and Magnetic resonance Cohort (RUN DMC) study prospectively investigates risk factors and clinical consequences of brain changes during aging as assessed with MRI among 503 50-85 year old non-demented elderly with cerebral SVD. On the basis of established research criteria, cSVD was defined as the presence of lacunes and/or WMH on neuro-imaging. [61] Patients were referred either because of acute symptoms, such as transient ischemic attack (TIA) or lacunar syndromes, or subacute complaints such as cognitive, motor disturbances and/or depressive symptoms [61]. The baseline data collection was performed in 2006. Participants who underwent routine diagnostic brain imaging, (for amongst others: vascular causes (TIA, stroke), headache, mild traumatic brain injury and cognitive complaints) were eligible for participation. Inclusion criteria were: age between 50- and 85 and cerebral SVD on neuro-imaging (WMH and/or lacunes). Main exclusion criteria were dementia, (psychiatric) disease interfering with cognitive testing or follow-up; WMH or cSVD mimics (e.g., Multiple Sclerosis) and MRI contra-indications .[60]

Follow -up was completed in 2012. Of 503 baseline participants, two were lost to follow-up (but not deceased according to the Dutch Municipal Personal Records database), 49 had

died, and 54 refused in person follow-up, but clinical endpoints were available. (Figure 1) One was additionally excluded because of baseline MRI artefacts, yielding a final sample of 500 participants. This study was approved by the Medical Review Ethics Committee region Arnhem-Nijmegen and all participants gave written informed consent prior to inclusion.

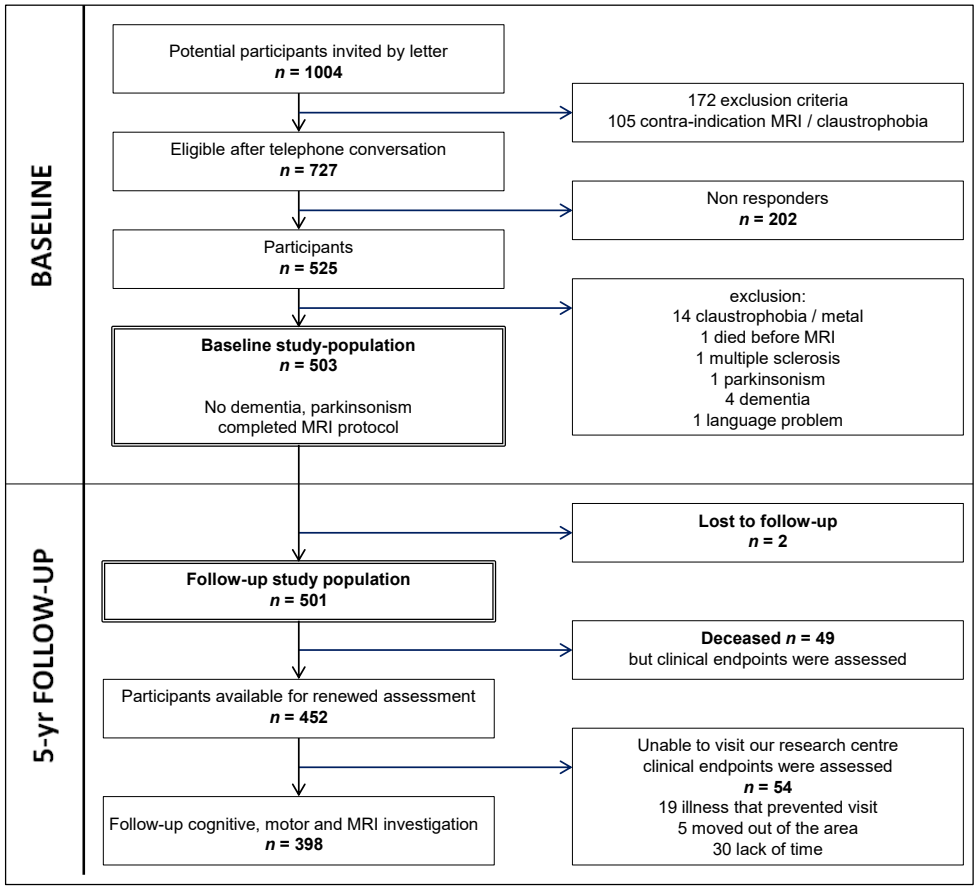


Figure 1: Flowchart study design baseline and follow–up.

Baseline and Follow-up study population are indicated by double-lined boxes. MRI: Magnetic Resonance Imaging

Dementia case finding

At baseline all participants were free of dementia. Dementia case finding was performed for those who participated in the face-to-face follow-up and for those who died or refused follow-up participation (they gave permission to perform follow-up based on available clinical data). The Mini–Mental State Examination (MMSE)[82] was used as a first screen among face-to-face participants. A score below 26 or a decline of three points or more from baseline was considered screen positive (n=34), of whom 20 were subsequently examined for dementia

at the Radboud Alzheimer Center (7 were diagnosed with dementia, and 13 were not). For the remaining 14, who refused additional analysis, a consensus diagnosis of dementia was made by a panel, consisting of a neurologist, clinical neuropsychologist and a geriatrician with expertise in dementia. They reviewed all available neuropsychological [60] and imaging information, which included (I) the difference in neuropsychological performance between baseline and follow-up, (II) outcome of the Mini International Neuropsychiatric Interview MINI,[83] (III) the follow-up MRI scan, or, if not available (in 7 cases), the baseline MRI-scan; and (IV) for the interpretation of these tests, age and level of education were taken into account ,[65] next to interference with daily living, confirmed by family or caregivers. Of these 14 participants, seven were diagnosed with dementia.

Medical records were reviewed from the participants who were not available for follow-up assessment (49 deceased, 54 follow-up data available, no center visit); in addition their general practitioners and medical specialists were contacted for information on their cognitive status. Dementia was mentioned in 37 participants. After review by members from the panel, 29 were diagnosed with dementia, and eight were not. In total, this resulted in 43 incident cases of dementia during a mean follow-up period of 5.2 (SD 0.7) years.

The diagnosis of dementia was based on the Diagnostic and Statistical Manual of Mental Disorders (IV)[62] criteria; probable Alzheimer's disease was based on the NIA-AA criteria,[84] and vascular dementia was based on NINDS-AIREN criteria.[85] Individuals not fulfilling these criteria were classified as possible Alzheimer's Dementia with etiologically mixed presentation [84] and fronto-temporal dementia (FTD). The onset of dementia was defined as the date on which the clinical symptoms allowed for the diagnosis.[51] When the date of diagnosis was not exactly known, we used the mid-point between the baseline visit and the first date the diagnosis was confirmed, [86] or the date someone was placed in a nursing home because of dementia.

MRI resonance imaging protocol and analysis

MRI scans of all participants were acquired on a single 1.5-Tesla MRI (Magnetom Sonata, Siemens Medical Solutions, Erlangen, Germany). The protocol included the following whole brain scans: a T1-weighted 3D magnetization-prepared rapid gradient-echo (MPRAGE) imaging (voxel size 1.0 x 1.0 x 1.0mm); Fluid-attenuated inversion recovery (FLAIR); voxel size 1.0 x 1.2 x 5.0mm, with an interslice gap of 1 mm); a transversal T2* weighted gradient echo s (voxel size 1.3 x 1.0 x 6.0mm, with an interslice gap of 1 mm) and a Diffusion Tensor Imaging (DTI) sequence (voxel size 2.5 x 2.5 x 2.5mm; 4 unweighted scans, 30 diffusion weighted scans with b-value = 900 $s \cdot mm^{-2}$) WMH were manually segmented on FLAIR images and the total WMH volume was calculated by summing the segmented areas multiplied by slice thickness. The rating of baseline lacunes and microbleeds were rated according to the recently published Standards for Reporting Vascular changes on neuro-imaging (STRIVE), by trained raters blinded to all clinical data[12] with good intra- and inter-rater variability (weighted kappa's of 0.87 and 0.95 for lacunes and 0.85 and 0.86 for microbleeds, calculated in 10 percent of the scans). To obtain GM (volume of the neocortex, basal ganglia and thalamus),

WM and cerebrospinal spinal fluid (CSF) volume, the T1 MPRAGE images were segmented using Statistical Parametric Mapping 5 unified segmentation routines.[60,63] Total GM, WM and CSF volumes were subsequently calculated by summing all voxel volumes that had a $p > 0.5$ for belonging to that tissue class. The intracranial volume (ICV) was a summation of total GM, total WM and CSF volume. Hippocampal volumes were manually segmented on the MPRAGE image using the interactive software program "ITK-SNAP" as described previously .[87 88] (http://www.itksnap.org). All volumes were normalized to total ICV.[89]

DTI analysis

Diffusion data were analyzed in the total WM, according to an earlier detailed procedure. [90] In short, diffusion data were pre-processed using an in-house developed algorithm for patching artifacts from cardiac and head motion.[91] Corrections of Eddy current and motion artifacts from affine misalignment were performed simultaneously, which was based on minimization of the residual diffusion tensor error.[92] FA and MD images were calculated using DTIFit within the FSL toolbox, which were fed into the TBSS pipeline.[93] A FA skeleton was created by conducting a thinning procedure on the mean FA image. This skeleton was thresholded at 0.3 and the skeleton projection vectors were then applied to the MD.

Other measurements

Education was classified using 7 categories (1 being less than primary school, 7 reflecting academic degree).[65] We then dichotomized in primary or less (level 1 and 2), or more than primary education (level 3 to 7). Depressive symptoms were assessed with the Centre of Epidemiologic Studies Depression Scale (CES-D); they were considered present with CES-D ≥ 16 and/or current use of anti-depressive medication, taken for depression.[48,60]

Statistical analysis

Person-years at risk were calculated from date of the baseline assessment, until onset of dementia, death, or date of the follow-up whichever came first. Patients who died or did not reach the endpoint were censored. WMH volume was log transformed because of the skewed distribution of the data. As frontotemporal dementia is basically genetic, we excluded this participant from the analysis.

The cumulative risk of incident dementia was estimated with Kaplan-Meier analysis, stratified by severity (in quartiles) of cSVD characteristics (WM and WMH volume, lacunes and microbleeds), GM characteristics and MD within WMH and NAWM. Subsequently, Kaplan-Meier curves were compared between the subgroups using a log-rank test.

Cox regression analysis was used, to calculate hazard ratios for dementia. Three models were constructed to predict dementia. First, age, sex education and baseline MMSE were forced into the model and a backward stepwise selection procedure was used to enter imaging characteristics into the model until all variables in the model had p values smaller than 0.15. Among the imaging characteristics, only cSVD characteristics were considered in model 1,

GM volumes were added in model 2 and DTI characteristics were added in model 3. C-statistic was used to assess the discriminatory performance of the three models. Two-sided p-values of less than 0.05 were considered to indicate statistical significance. For the TBSS analyses, we assessed voxel-wise correlations between the skeletal DTI parameters (FA and MD) and dementia, adjusting for age, sex, education, brain volume, MMSE and cSVD characteristics. Statistical analysis were performed using IBM SPSS Statistics version 20 and R version 2.15 (http://www.R-project.org) software packages.

Results

Baseline characteristics are shown in Table 1. 43 Participants developed dementia during a mean follow-up of 5.2 years (SD 0.7) resulting in a 5.5-year cumulative risk of dementia of 11.1% (95% CI 7.7-14.6).

The risk of dementia was highest in participants with the highest WMH volume compared with the lowest volume (14.4% vs. 0.8%, Log-Rank p< 0.001), whereas this risk did not differ by absence or presence of lacunes or microbleeds at baseline. Lower baseline WM, GM and HV were associated with a higher 5-year risk of dementia (risks for lower versus upper quartile of the volume 13.9% vs.0.8%, log-rank p< 0.001 for WM, 24.5% vs.4.0% Log-rank p<0.001 for GM, and 21.2% vs. 5.8%, log-rank p=0.003 for HV). Participants with the lowest quartile of microstructural integrity (both within WMH and NAWM), had a higher risk for dementia than participants in the highest quartile of structural integrity (Figure 2).

Low WM, CSF and HV at baseline were significant predictors of incident dementia, with adjustment for age, sex, education, baseline MMSE and territorial infarcts (Table 2).

After additional adjustment for hippocampal and GM volume, WM volume remained a significant predictor of incident dementia (HR 0.65 95%CI 0.44-0.96, p=0.034). After subsequent adjustment for all SVD characteristics separately, a lower HV also remained a risk factor of dementia (HR 0.68, 95%CI 0.47-0.99,p=0.045), but CSF volume did not. After additional adjustment for baseline depressive symptoms the strength of the associations did not markedly change.

Finally, in the prediction models of incident dementia, model 1 showed that WM volume, WMH volume and microbleeds retained in the model. After adding the volumes of GM structures (GM and HV) in model 2, HV was also retained in the model. Adding DTI characteristics in model 3 did not alter the predictive characteristics (Table 3). There were no indications that the proportional hazards assumption was violated. The performance of the three models was comparable for the three models (model 1: C-statistic 0.84; 95%CI 0.74-0.93, model 2:C-statistic 0.85; 95%CI 0.76-0.95) and for model 3:C-statistic 0.86; 95%CI 0.76-0.95). TBSS analysis revealed no significant differences between participants with and without incident dementia, for both FA and MD parameters (data not shown).

Table 1: Baseline characteristics of participants with and without incident dementia, and with Alzheimer's and Vascular dementia

	Total n=500	Dementia n=42	Non-dement =458	AD n=28	VaD n=11
Demographics					
Age at baseline (SD)	65.6 ± 8.8	74.6 ± 6.5	64.8 ± 8.5	74.2 ± 6.4	75.5 ± 7.6
Men, n (%)	284 (56.8)	24 (57.1)	260 (56.8)	15 (53.6)	7 (63.6)
Only primary education, n (%)	49 (9.8)	8 (19.0)	41 (9.0)	7 (25.0)	1 (9.1)
MMSE baseline (SD)	28.1 ± 1.6	27.1 ± 1.7	28.2 ± 1.6	27.0 ± 1.8	27.1 ± 1.6
Depressive symptoms baseline, n (%)	166 (33.2)	21 (51.2)	145 (31.7)	14 (50.0)	6 (54.5)
Baseline neuro-imaging characteristics					
Intracranial Volume, ml (SD)	1677.8 ± 156.2	1640.5 ± 157.3	1681.2 ± 155.8	1651.0 ± 170.1	1602.7 ± 136.1
White matter volume, ml (SD)	464.7 ± 51.9	424.2 ± 45.4	468.4 ± 50.9	436.3 ± 38.2	404.0 ± 55.3
WMH volume, ml (IQR)†	7.2 (3.6;18.4)†	14.7 (7.3;36.7)†	6.6 (3.3;17.5)†	10.6 (5.7;21.1)†	45.4 (23.9;86.3)†
NAWM volume, ml (SD)	450.7 ± 58.7	397.8 ± 59.5	455.6 ± 56.3	420.0 ± 46.8	350.2 ± 66.3
Lacunes, presence, n (%)	134 (26.8)	15 (35.7)	119 (26.0)	5 (17.9)	9 (81.8)
Microbleeds, presence, n (%)*	80 (16.1)	9 (21.4)	73 (15.9)	2 (7.1)	5 (45.5)
Territorial infarcts, presence, n (%)	56 (11.2)	7 (16.7)	47 (10.3)	3 (10.7)	3 (27.3)
Grey matter volume, ml (SD)	630.9 ± 53.9	596.8 ± 45.2	634.0 ± 53.6	596.4 ± 47.4	600.3 ± 46.6
Cerebro spinal fluid volume, ml (SD)	582.1 ±88.1	656.7 ± 65.7	575.3 ± 86.8	645.0 ± 58.5	673.5 ± 82.4
Hippocampal volume, ml (SD)**	6.8 ± 1.0	6.3 ± 1.0	6.9 ± 1.0	6.1 ± 1.0	6.7 ± 0.7
Baseline DTI-parameters	**n=497**	**n=42**	**n=455**	**n=26**	**n=11**
White matter, mean FA, (SD)	0.33 ± 0.02	0.32 ± 0.02	0.33 ± 0.02	0.33 ± 0.02	0.30 ± 0.02
WMH, mean FA, (SD)	0.34 ±0.03	0.33 ± 0.03	0.34 ± 0.03	0.34 ± 0.02	0.30 ± 0.03
NAWM, mean FA, (SD)	0.33 ± 0.02	0.32 ± 0.02	0.33 ± 0.02	0.32 ± 0.02	0.30 ± 0.02
White matter, mean MD 10^{-3} mm^2/s,(SD)	0.89 ± 0.05	0.93 ±0.04	0.89 ±0.04	0.91 ± 0.03	0.97 ± 0.04
WMH, mean MD 10^{-3} mm^2/s, (SD)	1.00 ± 0.07	1.05 ± 0.06	0.99 ± 0.07	1.02 ± 0.04	1.11 ± 0.06
NAWM, mean MD 10^{-3} mm^2/s, (SD)	0.89 ± 0.04	0.92 ± 0.04	0.88 ± 0.04	0.91 ± 0.03	0.96 ± 0.04

† log transformed. MMSE, Mini-Mental State Examination; SD, standard deviation; IQR, inter quartile range, AD, Alzheimer's dementia; VaD, vascular dementia; FU, follow-up; WMH, white matter hyperintensities; NAWM, normal appearing white matter; FA, fractional anisotropy; MD, mean diffusivity. Brain volumes represent normalized brain volumes; normalized to the total intracranial volume. ∗three participants were excluded because of missing values of microbleeds. ∗∗four participants were excluded because of missing values of hippocampal volume. Univariate analysis. 3 were additionally excluded for the DTI analysis because of baseline DTI-scan artifacts. Of the 43 dementia cases, one was additionally excluded because of baseline scan artifacts. Data of 39 incident probable AD and VaD cases are shown in the right side of Table 1. Data of the remaining 3 incident dementia cases: 2 participants with mixed dementia (AD/VaD) and one with frontotemporal dementia are not represented in this table separately.

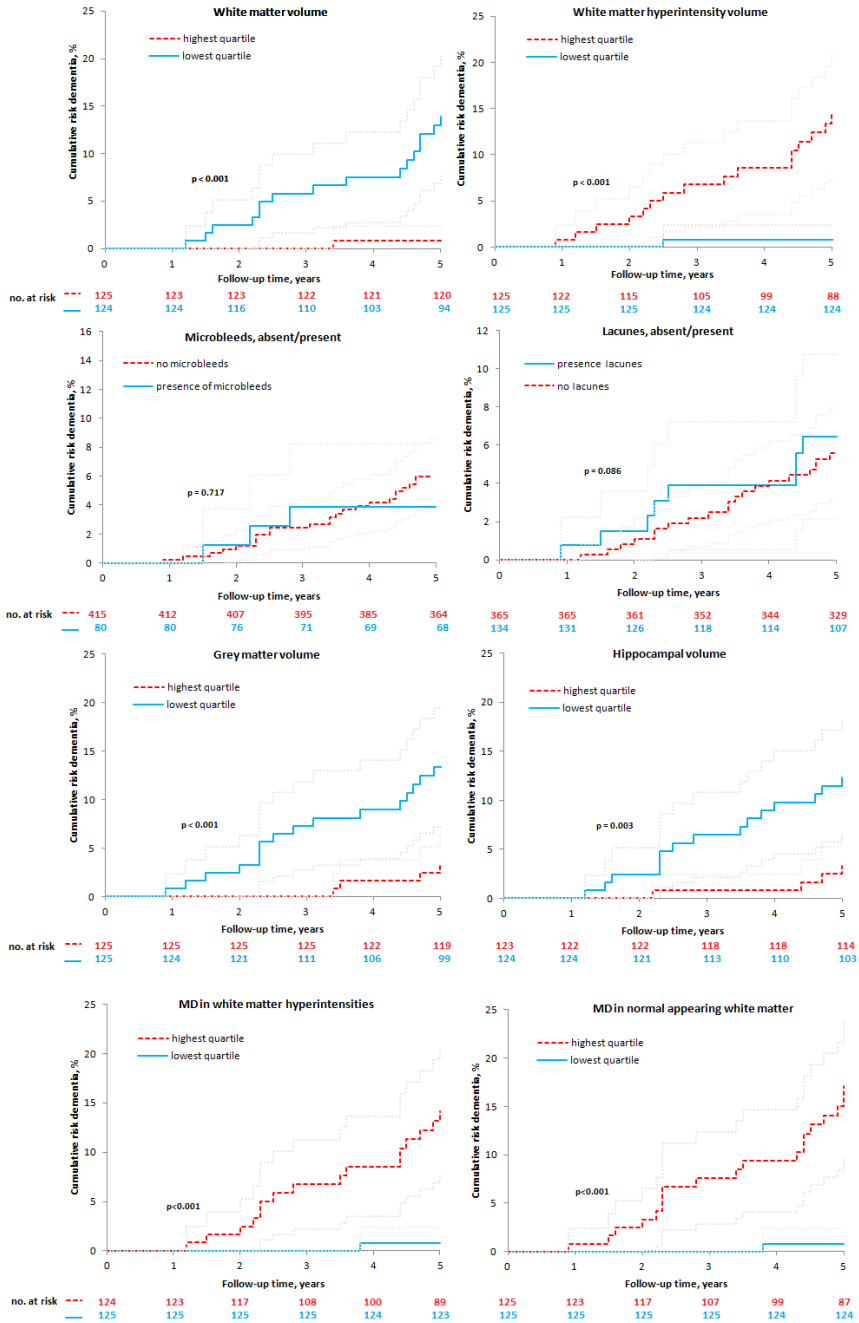


Figure 2: cumulative risk of dementia, stratified by MRI characteristics

Cumulative risk of dementia, stratified by MRI characteristics. Curves represent cumulative risk of dementia per 5-years follow-up, stratified by MRI characteristics; cerebral small vessel disease (white matter volume, white matter hyperintensity volume, lacunes, and microbleeds), grey matter characteristics (grey matter volume and hippocampal volume) and microstructural integrity (mean diffusivity in white matter hyperintensities and mean diffusivity in normal appearing white matter).

Table 2: Cox proportional hazards, relation between baseline and MRI characteristics and the risk of dementia at follow–up.

Baseline neuro-imaging characteristics (n=499)	**Hazard Ratio + 95% CI** Adjusted for age, sex, education, baseline MMSE and territorial infarcts	**significance**	**Hazard Ratio + 95% CI** Additionally adjusted for GM and Hippocampal volume **^** or all cSVD characteristics***	**significance**
White matter volume, SD (ml)	0.68 (0.47-0.99)	**p=0.045**	0.65 (0.44-0.96)^	**p=0.034**
White matter hyperintensity volume†, ml	1.78 (0.79-4.01)	p=0.167	1.83 (0.80-4.21)^	p=0.150
Lacunes, presence (y/n)	0.88 (0.44-1.76)	p=0.714	0.84 (0.42-1.70)^	p=0.630
Microbleeds, presence (y/n)*	0.60 (0.25-1.43)	p=0.252	0.58 (0.24-1.37)^	p=0.212
Grey matter volume, SD, (ml)	0.77 (0.51-1.15)	p=0.200	0.73 (0.49-1.08) ***	p=0.116
Cerebral spinal fluid volume, SD (ml)	1.80 (1.11-2.91)	**p=0.017**	1.68 (0.88-3.21)***	p=0.116
Hippocampal volume, SD (ml)**	0.68 (0.49-0.96)	**p=0.028**	**0.68 (0.47-0.99) *****	**p=0.045**
Baseline DTI-parameters (n=496)	**Hazard Ratio + 95% CI** Adjusted for age, sex, education baseline MMSE and territorial infarcts	**significance**	**Hazard Ratio + 95% CI** Additionally adjusted for all cSVD and GM characteristics	**significance**
White matter, mean FA, (SD)	0.92 (0.78-1.08)	p=0.300	0.98 (0.80-1.19)	p=0.840
WMH, mean FA, (SD)	0.99 (0.89-1.11)	p=0.915	1.03 (0.92-1.16)	p=0.608
NAWM, mean FA, (SD)	0.91 (0.77-1.08)	p=0.285	0.98 (0.80-1.19)	p=0.819
White matter, mean MD 10^{-3} mm^2/s,(SD)	1.54 (0.70-3.39)	p=0.280	0.96 (0.34-2.70)	P=0.935
WMH, mean MD 10^{-3} mm^2/s, (SD)	1.51 (0.87-2.64)	p=0.145	1.10 (0.51-2.36)	p=0.808
NAWM, mean MD 10^{-3} mm^2/s, (SD)	1.39 (0.58-3.30)	p=0.463	0.86 (0.30-2.48)	p=0.778

† log transformed. ∗one exclusion because of baseline T1/T2 scan artifacts, diagnosis frontotemporal dementia. ∗∗three participants were excluded because of missing values of microbleeds. ∗∗∗four participants were excluded because of missing values of hippocampal volume. Brain volumes, represent normalized brain volumes; normalized to the total intracranial volume. GM, grey matter; WM, white matter; WMH, white matter hyperintensities; NAWM, normal appearing white matter; FA, fractional anisotropy; MD, mean diffusivity (10−3 mm2/s). cSVD characteristics: normalized white matter volume, white matter hyperintensity volume, lacunes, and microbleeds. SD, hazard ratio per standard deviation difference from the mean. Significance level: $p < 0.05$.

Table 3: Indicator variables retained in Cox regression models for prediction of dementia

	Model 1 (n=495*) Hazard Ratio (95% CI)	Model 2 (n=491**) Hazard Ratio (95% CI)	Model 3 (n=489**) Hazard Ratio (95% CI)
Demographic characteristics			
Baseline age, (years)	1.13 (1.07-1.19), p<0.001	1.12 (1.06-1.19), p<0.001	1.12 (1.05-1.19), p<0.001
Sex, female	1.09 (0.58-2.04), p=0.797	1.12 (0.59-2.12), p=0.738	1.08 (0.57-2.06), p=0.810
Only primary education	0.66 (0.28-1.56), p=0.342	0.58 (0.23-1.44), p=0.239	0.55 (0.22-1.38), p=0.202
Baseline MMSE	0.81 (0.67-0.98), p=0.031	0.81 (0.67-0.98), p=0.030	0.80 (0.66-0.97), p=0.021
Baseline imaging characteristics; Small vessel disease			
WM volume, (SD)	0.69 (0.48-0.98), p=0.041	0.68 (0.47-0.98), p=0.039	0.64 (0.44-0.94), p=0.022
WMH volume†, (ml)	1.92 (0.85-4.36), p=0.119	2.04 (0.90-4.61), p=0.087	2.06 (0.93-4.60), p=0.077
Lacunes, presence	--	--	--
Microbleeds, presence	0.50 (0.21-1.18), p=0.115	0.48 (0.20-1.13), p=0.091	0.45 (0.19-1.06), p=0.069
Baseline imaging characteristics; grey matter brain volumes			
GM volume (SD)	x	--	--
Hippocampal volume (SD)	x	0.69 (0.48-0.99), p=0.047	0.68 (0.47-0.98), p=0.040
Baseline imaging characteristic; DTI parameters			
WMH, mean FA	x	x	--
NAWM, mean FA	x	x	--
WMH, mean MD 10^{-3} mm^2/s	x	x	--
NAWM, mean MD 10^{-3} mm^2/s	x	x	--
C-statistic (C-index)	0.84	0.85	0.86
95% CI	(0.74-0.93)	(0.76-0.95)	(0.76-0.95)

† Log transformed. ∗one exclusion because of baseline T1/T2 scan artifacts, one because of the diagnosis frontotemporal dementia and four because of missing microbleeds. ∗∗four additionally excluded because of missing hippocampal volumes. ∗∗∗two additionally excluded baseline DTI artifacts. Normalized brain volumes were normalized to the mean total ICV, hazard ratios of brain volume was calculated per standard deviation increase. Age was entered as a continuous variable; the increase in hazard is for every incremental year. Age, sex, education, and baseline MMSE entered the model by forced entry. To construct a prediction model, variables selected from the univariate analysis were entered into the model, using backward stepwise selection until all variables in the model had p values smaller than 0.15. For the consecutive models, we present imaging characteristics in three groups: cerebral small vessel disease (model 1), grey matter structures (model 2), and finally DTI parameters added (model 3). We used C statistic to assess the discriminatory performance of the models. For a clinical prediction rule, it is generally considered that a C statistic of less than 0.6 has no clinical value, 0.6–0.7 modest value and greater than 0.8 has discrimination adequate for genuine clinical utility. We used C statistic to assess the discriminatory performance of the models. MMSE, Mini-Mental State Examination; SD, standard deviation; WMH, white matter hyperintensities; NAWM, normal appearing white matter; FA, fractional anisotropy; MD, mean diffusivity.

Discussion

Lower WM and HV at baseline significantly increased the 5-year risk of dementia in individuals with cerebral SVD, but WMH, lacunes and microbleeds did not. The degree of microstructural integrity of the WM did not affect this risk. To the best of our knowledge, this is the first longitudinal study that assessed the relation between the whole spectrum of cSVD, GM structures and the microstructural integrity altogether and the risk of dementia in elderly with cSVD.

Other studies on dementia or cognitive performance were cross-sectional, did not include both GM and WM volume, did not include the whole cSVD spectrum, did not use TBSS analysis, or did not take dementia as a clinical endpoint [94-97].

Strengths of our study include its longitudinal assessment of a population that covers the whole spectrum of cerebral SVD and the large sample size, and the ascertainment of endpoints in 99.6% of our participants. Collection of our data in a single centre, allowed us to assemble baseline and follow-up data according to identical procedures, reducing the risk of procedural bias. Furthermore, we manually segmented the WMH and HV without prior knowledge of the clinical data. The relation between brain imaging characteristics and dementia was investigated with extensive adjustments for other brain imaging characteristics, reducing confounding. Finally, brain volumes were normalized to the total intracranial volume.

Several methodological issues need to be addressed. First, the nosological dementia diagnosis in our study was a clinical diagnosis, supported by MR imaging at the moment of diagnosis, and if not available, baseline MR imaging. In some cases, a distinction between Alzheimer's Dementia and Vascular Dementia (VaD) based on clinical data is hard to make, because neurodegeneration and vascular diseases often co-occur.[98,99] For this reason we investigated 'overall dementia' as outcome measure.[100] Second, it is possible that we missed some patients with incident dementia, because the cut-off point of 26 in the MMSE, although widely used, might not be sensitive enough, especially for early cases, Vascular Dementia, or dementia in higher educated participants. We think that if misclassification has occurred, it may have led to an underestimation of the effect. Third, we were not informed on the genetic APOE status, CSF biomarkers or positron emission tomography (PET) scan at baseline of our participants, which prevented us from further increasing the predictive value of the model. Finally, we feel that our study has a high generalizability to patients between 50 and 85 years presenting with cSVD on neuroimaging in a general neurology clinic due to comparable baseline complaints and baseline characteristics across studies. All our participants were functionally independent at baseline, with a mean MMSE of 28.1 (SD 1.6), reflecting estimates in the general population.[101] The median of WMH in our study is higher than found in population based studies. For this reason it is not surprising that our overall incidence rate of 16.4 per 1000 person years is higher than the overall incidence rate of 10.7 per 1000 person years found in a large population based study.[86]

We investigated both cSVD and GM structures on the risk of dementia, because the WM represents the structural connectivity between different brain structures, including the neocortex and hippocampus, which are altogether crucial for cognitive function. It might be that the loss of neocortical volume is, at least in part, secondary to cSVD, although the exact mechanisms remain to be elucidated. It could be that severe WMH represent areas of axonal loss that result in, reduced structural connectivity of the cortical areas once connected by these axons (with attendant loss of volume) for example by anterograde or Wallerian degeneration). [102,103] However, it might also be possible that neocortical atrophy leads to axonal damage as visualized by WMH on conventional FLAIR imaging. Irrespective of the actual mechanism, this may result in disconnection of the areas crucial in cognitive function, with attendant cognitive decline or even dementia.

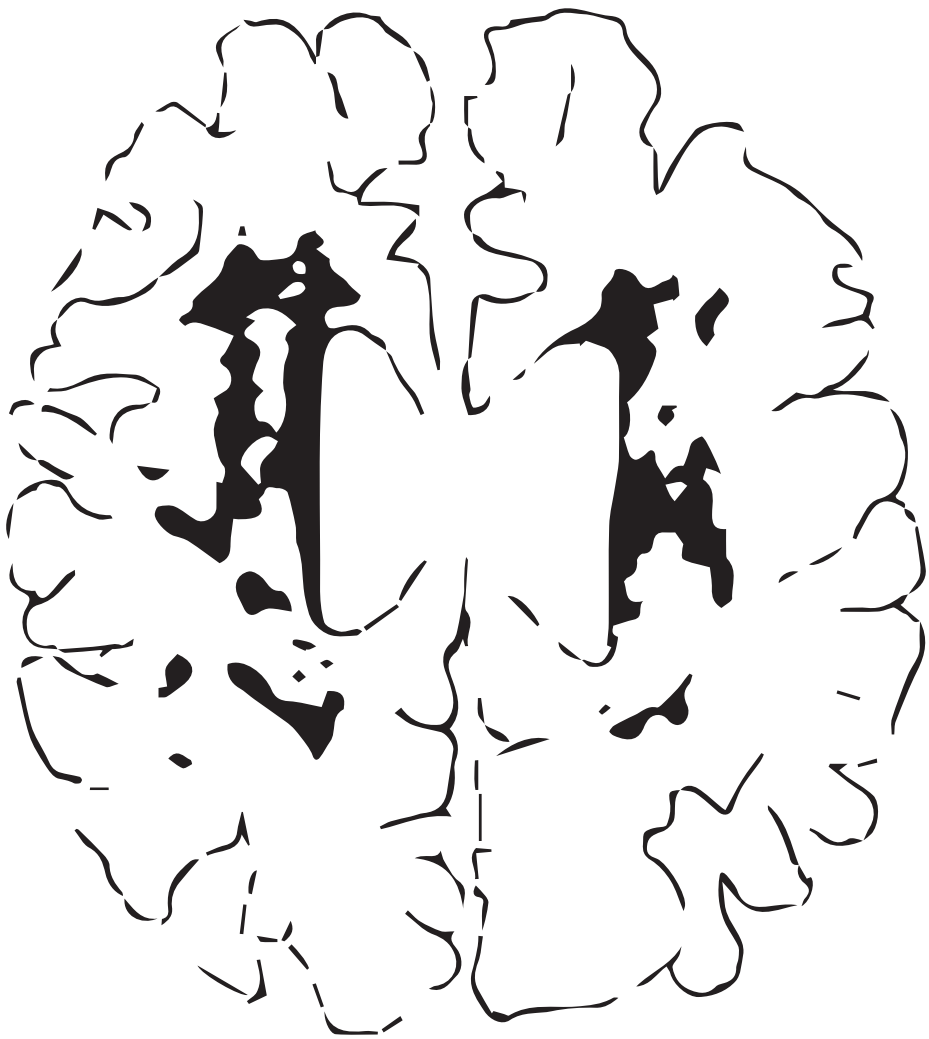
In contrast to another longitudinal study,[51] the risk of dementia was not significantly increased per increasing volumes of WMH, although the strength of the association was strikingly similar to that observed in the Rotterdam Study (HR 1.78, p=0.167, compared with an HR of 1.6, $p < 0.05$). However, similar to our study after additional adjustments for cSVD characteristics this association was no longer significant. Furthermore we have adjusted for additional confounders, including HV, which the other study did not.[100] In accordance with our findings, it may be that the relation between WMH and dementia in that study was confounded by hippocampal volume given the strong relation between WMH volume and hippocampal volume.[31]

Conflicting reports exist on impaired WM integrity in dementia compared to healthy controls.[81] Two recent longitudinal studies,[76,104] showed that microstructural changes in the WM predict a faster decline in several cognitive domains, but dementia has never been included as an outcome measure. We found no additional effect of microstructural integrity of the WM on the risk of dementia. Possibly the microstructural changes have a more subtle clinical correlate, such as lower scores on separate cognitive domains, but are not severe enough to result in a dementia syndrome; especially not in the presence of presumably more severe expressions of cSVD already visible on conventional imaging such as WMH and lacunes. Furthermore, at baseline, most participants had a relatively intact WM integrity, with only a mild to moderate WMH load. This might have limited the statistical power to detect additional value of the DTI parameters.

In our prognostic models, age is the foremost important predictor of dementia. The addition of WM, WMH and HV did only marginally increase the variance explained. We did not find any additional diagnostic value of the DTI parameters. We did not cross-validate the prediction model in our dataset, because of the relatively low number of outcome events, therefore validation in an independent dataset should be performed as a gold standard.

Our results imply a pre-clinical period, with lower WM and HV in those who might develop dementia in the future, where intervention might be possible before onset of clinical symptoms.

We demonstrated that in elderly with cerebral SVD, low WM volume, next to HV is significantly related to the development of incident dementia after 5 years. Baseline diffusion parameters (both the global parameters in the prediction model as well as assessed voxel-wise by TBSS analyses) were not related to incident dementia, and had no diagnostic value on top of the macrostructural characteristics. Possibly the microstructural changes have a more subtle clinical correlate, and are therefore not severe enough to result in a dementia syndrome in 5 years time. Possibly these novel markers of subtle damage need to be assessed years or even decades before the first clinical symptoms emerge. Future research is needed to investigate additional value of change in DTI parameters over time in relation to incident dementia.



4.

Diffusion Tensor Imaging of the hippocampus predicts the risk of dementia

Ingeborg W.M. van Uden*, Anil .M. Tuladhar*, Helena. M. van der Holst, Esther M.C. van Leijsen, Anouk .G.W. van Norden, Karlijn F. de Laat, Loes C.A. Rutten-Jacobs, David G. Norris, Jurgen A.H.R Claassen, Ewoud. J. van Dijk, Roy P.C. Kessels and Frank-Erik de Leeuw

Human brain mapping. 2016; Jan;37(1):327-37

** Both authors contributed equally*

Abstract

Introduction: Cerebral small vessel disease (cSVD) is one of the most important risk factors for dementia, and has been related to hippocampal atrophy, which is among the first observed changes on conventional MRI in patients with dementia. However, these volumetric changes might be preceded by loss of microstructural integrity of the hippocampus for which conventional MRI is not sensitive enough. Therefore we investigated the relation between the hippocampal diffusion parameters and the risk of incident dementia, using diffusion tensor imaging (DTI), independent of hippocampal volume (HV).

Methods: The RUN DMC study is a prospective study among 503 elderly with cerebral small vessel disease, without dementia, with 5 years follow-up in 2012 (99.6% response-rate). Cox regression analysis was performed to calculate hazard ratios for dementia, of fractional anisotropy (FA) and mean diffusivity (MD) within the hippocampus, adjusted for demographics, HV and white matter (WM). This was repeated in participants without evident hippocampal volume loss, because in these participants the visible damage might not yet have already started, whereas damage might have started on a microstructural level.

Results: 43 participants developed dementia (8.6%), resulting in a 5.5-year cumulative risk of 11.1% (95% CI 7.7-14.6). Higher mean diffusivity was associated with an increased 5-year risk of dementia. In the subgroup of participants with the upper half hippocampal volume, higher hippocampal mean diffusivity, more than doubled the 5-year risk of dementia.

Conclusion: This is the first prospective study showing a relation between a higher baseline hippocampal mean diffusivity and the risk of incident dementia in elderly with small vessel disease at 5-year follow-up, independent of hippocampal volume and white matter volume.

Introduction

The spectrum of cerebral small vessel disease (cSVD) includes white matter hyperintensities (WMH) and lacunes of presumed vascular origin (lacunes), microbleeds and subcortical atrophy.[12] cSVD increases the risk of cognitive decline, including memory loss, ultimately leading to dementia in some.[51,105] The presence and progression of cSVD has previously been related to the progression of hippocampal atrophy ,[31,32] which is thought to be a major cause of the profound deficit in memory consolidation in Alzheimer's dementia (AD).[33]

However this hippocampal atrophy, visible on conventional MRI, occurs at a relatively late stage of dementia when the cognitive disturbances have already become apparent. Earlier detection of hippocampal pathology may provide insights in the aetiology and course of dementia and offer opportunities for early treatment strategies, before irreversible degenerative damage of the hippocampus has occurred. Conceptually, there may be changes in the microstructural integrity of the hippocampus before macroscopic loss of volume occurs.

As conventional MRI is not sensitive to loss of microstructural integrity of the hippocampus, diffusion tensor imaging (DTI) might be of use to provide an early marker for dementia, using the diffusion properties of unbound water molecules.[25,80] Two DTI parameters are of special interest: mean diffusivity (MD) a measure water diffusion ***averaged*** in all spatial directions, and fractional anisotropy (FA), which provides information about the ***directionality*** of water diffusion. A low FA and high MD are believed to be an indication for lower microstructural integrity.[56] Cross-sectionally we showed that hippocampal diffusion parameters (especially high MD) were related to verbal memory performance, in participants with a volumetric intact appearing hippocampus.[106] One prospective study in 13 MCI patients showed higher left hippocampal MD at baseline in those who converted to AD after a follow up of 1.5 years, compared to those who did not convert .[107] To the best of our knowledge there is a lack of large longitudinal studies which predict dementia using diffusion data from the hippocampus, independent of hippocampal and grey matter (GM) volume.

We therefore aimed to investigate the role of the diffusion parameters FA and MD within the hippocampus in the development of incident dementia, independent of hippocampal volume, GM volume and cSVD. Additionally, we investigated the role of hippocampal diffusion parameters as an early marker for dementia in older adults without evident hippocampal volume loss, because in those the irreversible degenerative damage might not have started yet. The study was part of the RUN DMC study, a prospective cohort study of 503 individuals, with cSVD yet without dementia at the baseline assessment in 2006, with a subsequent follow-up assessment in 2012.

Methods:

Study population

The Radboud University Nijmegen Diffusion Tensor and Magnetic resonance Cohort (RUN DMC) study prospectively investigates risk factors and clinical consequences of brain changes as assessed by MRI among 503 50-85 year old non-demented elderly with cerebral SVD. On the basis of established research criteria, cSVD was defined as the presence of lacunes and/or WMH on neuro-imaging.[61] Symptoms of cSVD include acute symptoms, such as Transient Ischemic Attacks or lacunar syndromes, or sub acute manifestations such as cognitive, motor disturbances and/or depressive symptoms.[61] The baseline data collection was performed in 2006. Inclusion criteria were: age between 50 and 85 years and cerebral SVD on neuro-imaging. Main exclusion criteria were dementia, (psychiatric) disease interfering with cognitive testing or follow-up; WMH or cSVD mimics (e.g., MS) and MRI contra-indications or known claustrophobia.[60]

Follow-up was completed in 2012. Of 503 baseline participants, two were lost to follow-up (but not deceased according to the Dutch Municipal Personal Records database) and 49 had died. In person follow-up was performed in 398 participants (Figure 1), while 54 refused in person follow-up, but clinical endpoints were available for this group.

Dementia case finding

Dementia screening of participants was performed during a face-to-face follow-up examination (n=398) as follows. The Mini–Mental State Examination (MMSE)[82] was used as a first screening tool. A score below 26 or a decline of three points or more from baseline was considered screen positive (n=34). Of all screen-positives, 20 were subsequently examined for the presence of dementia at the Radboud Alzheimer Centre (7 were diagnosed with dementia, and 13 were not). The remaining 14 refused additional analysis. For them, a consensus diagnosis of dementia was made by a panel, consisting of a neurologist, clinical neuropsychologist and a geriatrician, all with expertise in dementia. They reviewed all available neuropsychological [60] and imaging information, which included (I) the difference in neuropsychological performance between baseline and follow-up, (II) outcome of the Mini International Neuropsychiatric Interview MINI,[83] (III) the follow-up MRI scan, or if not available, the baseline MRI-scan (in 7 cases) for classification. (IV) For the interpretation of these tests, age and level of education were taken into account,[65] next to interference with daily living, confirmed by family or caregivers. Of these 14 participants, 7 were diagnosed with dementia. Medical records were reviewed from the participants who were not available for follow-up assessment (49 deceased, from 54 follow-up data were available, but did not visit the centre). In addition their general practitioners and medical specialists were contacted for information on their cognitive status. Dementia was suspected in 37 participants. After review by panel members, 29 of these were classified as having dementia (Figure 2). In total, this resulted in 43 incident cases of dementia during a mean follow-up period of 5.2 years (SD 0.7).

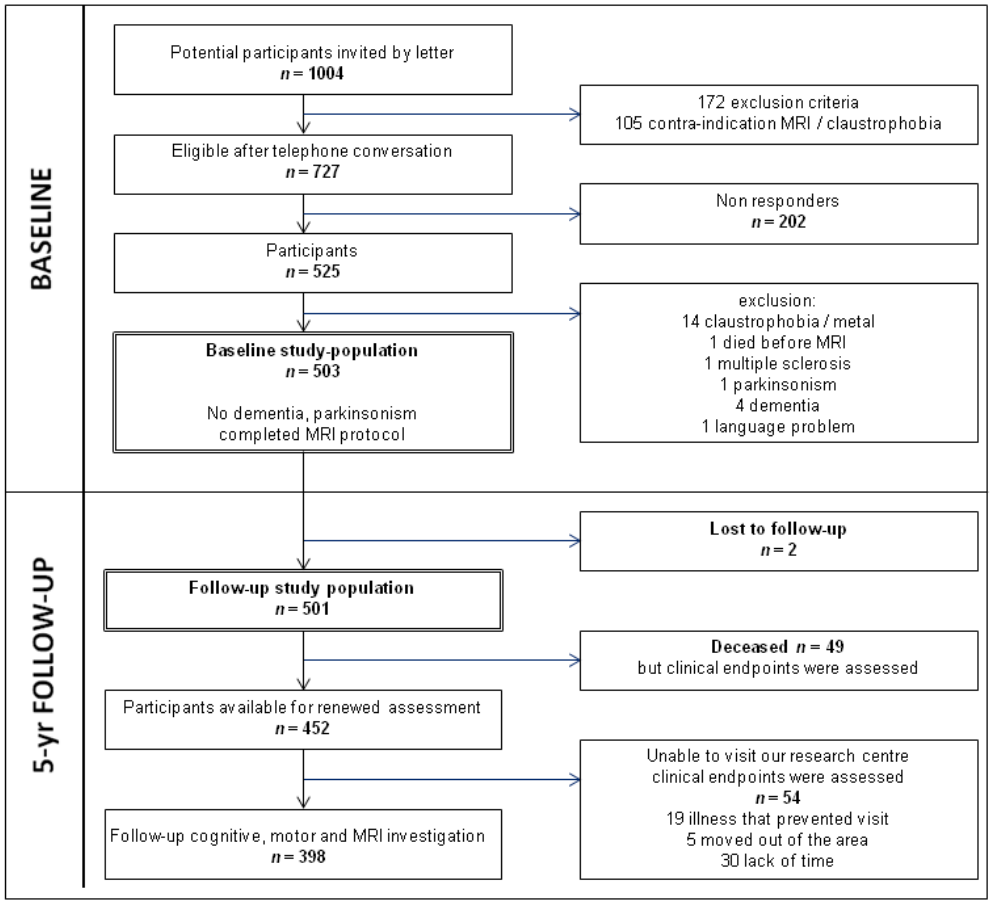


Figure 1: Flowchart study design baseline and follow–up

Baseline and Follow-up study population are indicated by double-lined boxes. MRI: Magnetic Resonance Imaging

The diagnosis of dementia was based on the Diagnostic and Statistical manual of mental disorders (IV)[62] criteria; probable Alzheimer's disease was based on the NIA-AA criteria (n=28), [84] and vascular dementia (VaD) was based on NINDS-AIREN criteria (n=11).[85] Individuals not fulfilling these criteria were classified as having possible AD with etiologically mixed presentation (n=3)[84] or frontotemporal dementia (n=1). The onset of dementia was defined as the date on which the clinical symptoms allowed for the diagnosis.[51] When the date of diagnosis was not exactly known, we used the mid-point between the baseline visit and the first date the diagnosis was confirmed,[86] or the date the participant was admitted to a nursing home because of dementia.

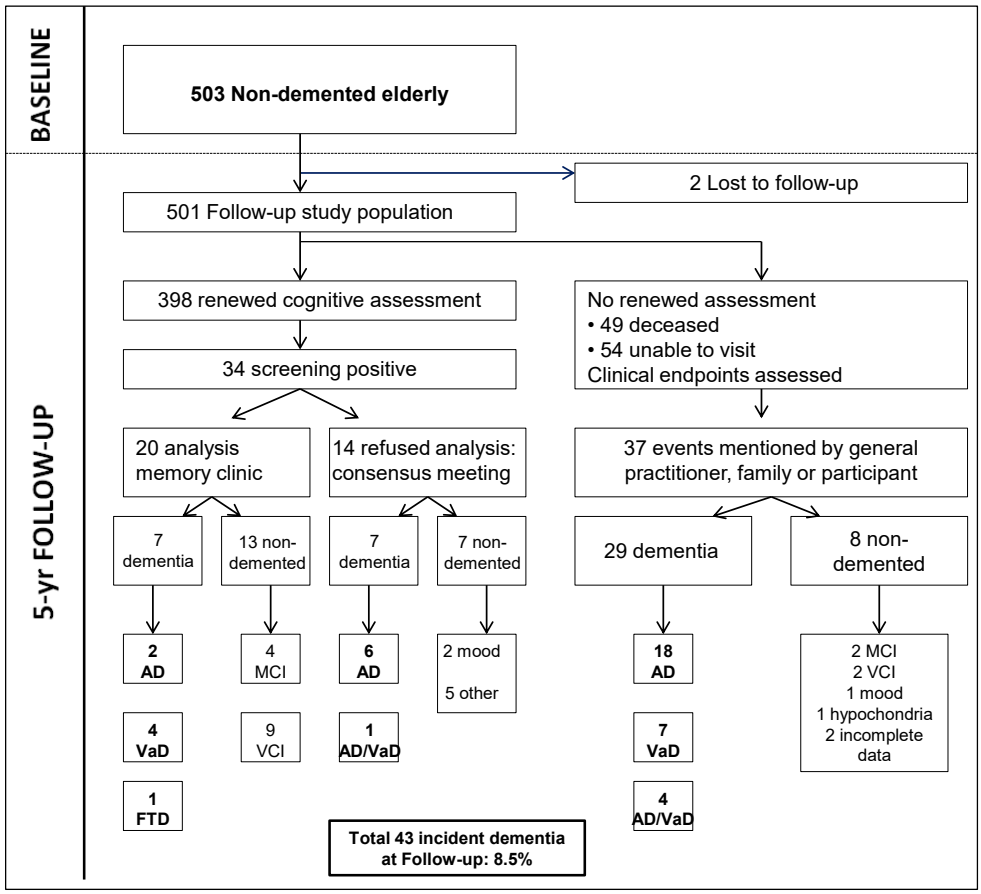


Figure 2: Flowchart diagnostic work–up dementia

AD: Alzheimer's dementia, VD: vascular dementia, FTD: fronto-temporal dementia, AD/VD: possible Alzheimer's Dementia with mixed aetiology, MCI: mild cognitive impairment, VCI: vascular cognitive impairment

MRI resonance scanning protocol

MRI scans of all participants were acquired on a single 1.5-Tesla MRI (Magnetom Sonata, Siemens Medical Solutions, Erlangen, Germany). The protocol included, the following whole brain scans: a T1-weighted 3D magnetization-prepared rapid gradient-echo (MPRAGE) imaging (TR/TE/TI 2250/3.68/850 ms; flip angle15°; voxel size 1.0 x 1.0 x 1.0mm); Fluid-attenuated inversion recovery (FLAIR) pulse sequences TR/TE/TI 9000/84/2200 ms; voxel size 1.0 x 1.2 x 5.0mm, with an interslice gap of 1 mm); a transversal T2*weighted gradient echo sequence (TR/ TE 800/26ms; voxel size 1.3 x 1.0 x 6.0mm, with an interslice gap of 1 mm) and a Diffusion Tensor Imaging (DTI) sequence (TR/TE 10100/93 ms; voxel size 2.5 x 2.5 x 2.5mm; 4 unweighted scans, 30 diffusion weighted scans with b-value = 900 $s \cdot mm^{-2}$).[60]

Conventional imaging analysis

WMH were manually segmented on FLAIR images and the total WMH volume was calculated by summing the segmented areas multiplied by slice thickness. The rating of lacunes, microbleeds and territorial infarcts were revised according to the recently published Standards for Reporting Vascular changes on neuro-imaging (STRIVE), by trained raters blinded to all clinical data.[12] There were good intra- and inter-rater variability's with weighted kappa of 0.87 and 0.95 respectively for the presence of lacunes and 0.85 and 0.86 for the presence of microbleeds, calculated in 10 percent of the scans. Inter-rater variability (assessed by intra-class correlation coefficient) for total WMH volume was 0.99.

The left and right hippocampus were manually segmented on the MPRAGE image, using the interactive software program "ITK-SNAP" version 2.1 [88] (http://www.itksnap.org). Segmentation was performed using a previously published protocol [108] in which segmentation was performed from posterior to anterior. Inter-rater studies showed an intraclass correlation coefficient of 0.73 and 0.79 for the left and right hippocampus respectively; intra-rater showed an intraclass correlation coefficient for the left and right hippocampus of 0.97 and 0.96. All imaging analyses were performed by raters blinded to clinical information.

To obtain the GM (which was composed of the volume of the cortex, basal ganglia and thalamus), WM and cerebrospinal fluid (CSF) volume, the T1 MPRAGE images were segmented using Statistical Parametric Mapping 12 unified segmentation routines (SPM12; Wellcome Department of Cognitive Neurology, University College London, UK (http://www.fil.ion.ucl.ac.uk/spm/software/spm12/). All images were visually checked for co-registration errors and for motion and/or segmentation artefacts. The GM, WM and CSF volumes were calculated by summing all voxels that had a $p > 0.5$ for belonging to that tissue class multiplied by the voxel volume in ml. The intracranial volume (ICV) was calculated by summing the volumes of GM, WM and CSF. All volumes were normalized to total ICV.[89]

DTI–analysis

The raw diffusion weighted images of each patient were first denoised using the Local Principal Component Analyses filter.[109] Diffusion data were then pre-processed using an in-house developed algorithm named 'patching artefacts from cardiac and head motion'.[110] In short, this iteratively reweighted-least-squares algorithm produces robust diffusion tensor estimates and provides weightings that are used to detect and correct head and cardiac motion artefacts in the diffusion-weighted data. Next, affine misalignments from eddy currents and subject motion were corrected simultaneously which is based on the minimization of the normalized mutual information measure.

We then unwarped echo planar imaging (EPI) distortions by normalizing the EPI-images to the T1-image only in the phase-encoding-direction. DTI calculations were generated using DTIFIT from FSL's FDT toolbox. The skull-stripped T1-images were then non-linearly registered to the FA map using FSL's Non-linear Image Registration Tool (FNIRT) with standard parameters. (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FNIRT) The transformation matrix derived from the non-

linear registration was then used to register the manually segmented hippocampus masks to the subject's DTI native space. The boundary voxels of these hippocampal masks were eroded to avoid potential partial volume effects. Mean FA and MD were then calculated within both hippocampi. All images were visually checked for motion artefacts and co-registration errors, especially for not including peri-hippocampal CSF.

Other measurements

Education was classified using 7 categories (1 being less than primary school, 7 reflecting academic degree) and then dichotomized in a group having only or less than primary school and a group having more than primary education.[65] Depressive symptoms were assessed with the Centre of Epidemiologic Studies Depression Scale (CES-D); they were considered present in participants with CES-D ≥ 16 and/or participants who currently used anti-depressive medication, taken for depression.[48,60]

Statistical analysis

Person-years at risk were calculated for each patient from date of the baseline assessment, until onset of dementia, death, or date of the follow-up assessment. Patients who died or did not reach the endpoint were censored. WMH volume was log transformed because of the skewed distribution of the data. In view of the fact that aetiology of frontotemporal dementia is basically genetic, as opposed to the vascular aetiology of vascular dementia and dementia due to Alzheimer's disease, we excluded this participant from the analysis. Cumulative risk of incident dementia was estimated with Kaplan-Meier analysis stratified by quartiles of total, left and right MD and FA within the hippocampus. Subsequently, Kaplan-Meier curves were compared between the subgroups using log-rank test.

We used Cox regression analyses to calculate hazard ratios (HRs) with 95% confidence intervals (CI's) for baseline GM, HV, and the DTI parameters within the hippocampus, adjusted for age, sex, education, baseline MMSE and territorial infarcts (no.). Second the HRs for the DTI parameters were calculated after additional adjustment for hippocampal and WM volume, because those were the only conventional MRI parameters independently related to the development of dementia, whereas WMH volume, GM volume, microbleeds and lacunes were not. Finally, in order to identify diffusion parameters of the hippocampus as an early marker for incident dementia, we investigated the mean hippocampal FA and MD in patients with a "normal" hippocampal volume (median split; cut-off of the normalized hippocampal volume 6.81 mL). In these participants the visible (volumetric) damage might not yet have started, but damage might possibly have started on a microstructural level. HRs for dementia were calculated for the DTI parameters within the hippocampus in that particular subgroup. After correction for age, sex, education, baseline MMSE and territorial infarcts (no.), these HRs were additionally adjusted for white matter volume. Two-sided P values of < 0.05 were considered to indicate statistical significance. Statistical analysis were performed using IBM SPSS Statistics version 20.

Results

The final sample consisted of 501 participants from which clinical endpoints could be obtained (two were lost to follow up). One participant was additionally excluded because of baseline T1 and T2 artefacts. Baseline demographic and neuro-imaging characteristics of 500 participants are shown in Table 1. Dementia developed in 43 participants during a mean follow-up of 5.2 years (SD 0.7). Mean age at follow-up was 70.8 years (SD 8.7) and 56.8% was male. Mean MMSE at baseline was 28.1 (SD 1.6). At baseline, median WMH volume was 7.2 ml (IQR 3.6;18.4), mean WM volume was 464.5 ml (SD 44.4 ml), 26.8% had lacunes and 16.1% had microbleeds. Mean normalized hippocampal volume was 6.8 ml (SD 0.9). Mean FA in the hippocampus was 0.11 (SD 0.02), and mean MD in the hippocampus was 0.99 10^{-3} mm2/s (SD 0.06). The 5.5-year cumulative risk of dementia was 11.1% (95%CI 7.7-14.6).

This risk of dementia was higher in participants with the lowest quartile hippocampal FA at baseline compared with participants with the highest quartile FA (lowest 19.1% vs. highest 10.0%, Log-Rank p< 0.022). The risk of dementia also was higher in participants with the highest quartile hippocampal MD at baseline compared with those with the lowest quartile MD (highest 24.7% vs. lowest 0.8% , Log-Rank p<0.001 (Figure 3). Baseline hippocampal diffusion parameters were a significant predictor of the risk of dementia, corrected for age, sex, education, baseline MMSE and territorial infarcts (no.) (Table 2). After additional adjustment for baseline WM and hippocampal volume, FA values of the hippocampus no longer were significant predictors. The mean MD of the hippocampus (left and right combined) (HR 1.44; 95%CI 1.10-1.88 per SD increase) remained a significant predictor of incident dementia at follow-up, as did the mean MD in the left hippocampus (HR 1.44; 95%CI 1.10-1.90 per SD increase). The analysis was rerun with an interaction term for hippocampal volume and mean hippocampal MD, which was however not significant. Furthermore, investigating whole brain FA and MD values as a predictor of dementia we found no significant relation with dementia (data not shown).

The mean hippocampal MD (of both hippocampi combined and the left hippocampus separately), was a significant predictor of the risk of dementia, corrected for age, sex, education, baseline MMSE and territorial infarcts in the subgroup of participants without evident hippocampal volume loss (Table 3). This association remained significant after additional correction for WM volume. This was not found for the mean hippocampal MD in participants with the lowest half of hippocampal volume (data not shown).

Discussion

The present study in elderly participants with cSVD initial free of dementia, demonstrated that higher hippocampal MD increased the risk of developing dementia after a 5-year follow-up. This was independent of demographics, WM and hippocampal volume and most pronounced in the left hippocampus, and the subgroup of elderly participants without evidence of hippocampal atrophy on conventional MRI. These findings are in line with the knowledge that the loss of function and structure of the hippocampus is a hallmark in the

Table I: Baseline characteristics of participants with and without incident dementia

	Total, n=500	Dementia, n=42	Non-dement, n=458
Demographics			
Age at baseline (SD)	65.6 ± 8.8	74.6 ± 6.5	64.8 ± 8.5
Men, n (%)	284 (56.8)	24 (57.1)	260 (56.8)
Only primary education, n (%)	49 (9.8)	8 (19.0)	41 (9.0)
MMSE baseline (SD)	28.1 ± 1.6	27.1 ± 1.7	28.2 ± 1.6
Depressive symptoms baseline, n (%)	166 (33.2)	21 (50.0)	145 (31.7)
Baseline neuro-imaging characteristics			
Intracranial Volume, mL (SD)	1456.8 ± 137.4	1438.6 ± 142.4	1458.4 ± 137.0
White matter volume, mL (SD)	464.5 ± 44.4	439.8 ± 40.4	466.7 ± 44.1
WMH volume, mL †	7.2 (3.5;17.9)†	15.3 (7.4;37.9)†	6.8 (3.3;17.4)†
NAWM volume, mL (SD)	450.4 ± 49.10	413.8 ± 45.1	453.8 ± 48.1
Lacunes, presence, n (%)	134 (26.8)	15 (35.7)	119 (26.0)
Microbleeds, presence, n (%)*	80 (16.1)	9 (21.4)	73 (15.9)
Territorial infarcts, presence, n (%)	56 (11.2)	7 (16.7)	47 (10.3)
Grey matter volume, mL (SD)	616.0 ± 50.8	576.7 ± 42.2	619.7 ± 50.0
Hippocampal volume, mL (SD)**	6.8 ± 0.9	6.2 ± 1.0	6.9 ± 0.9
Baseline DTI-parameters	**n=497**	**n=42**	**n=455**
Hippocampus, mean FA (SD)	0.11 ± 0.02	0.10 ± 0.02	0.11 ± 0.02
Left hippocampus, mean FA (SD)	0.11 ± 0.02	0.10 ± 0.02	0.11 ± 0.02
Right hippocampus, mean FA (SD)	0.10 ± 0.02	0.10 ± 0.02	0.11 ± 0.02
Hippocampus, mean MD (SD)	0.99 ± 0.06	1.04 ± 0.06	0.98 ± 0.06
Left hippocampus, mean MD SD)	1.00 ± 0.07	1.06 ± 0.07	0.99 ± 0.07
Right hippocampus, mean MD (SD)	0.98 ± 0.07	1.01 ± 0.07	0.97 ± 0.06

MMSE: Mini-mental State Examination, mL: millilitres, SD: standard deviation, FU: Follow-up, WMH: White Matter Hyperintensities, NAWM: Normal Appearing White Matter, FA: Fractional Anisotropy, MD: Mean Diffusivity (10^{-3} mm2/s). Brain volumes represent normalized brain volumes to the total ICV. Data shown are unadjusted values, and represent numbers (%), mean (SD) or median † (inter quartile range). *three were excluded because of missing values of microbleeds. ** four were excluded because of missing values of hippocampal volume. 3 were additionally excluded for the DTI analysis because of baseline DTI-scan artefacts.

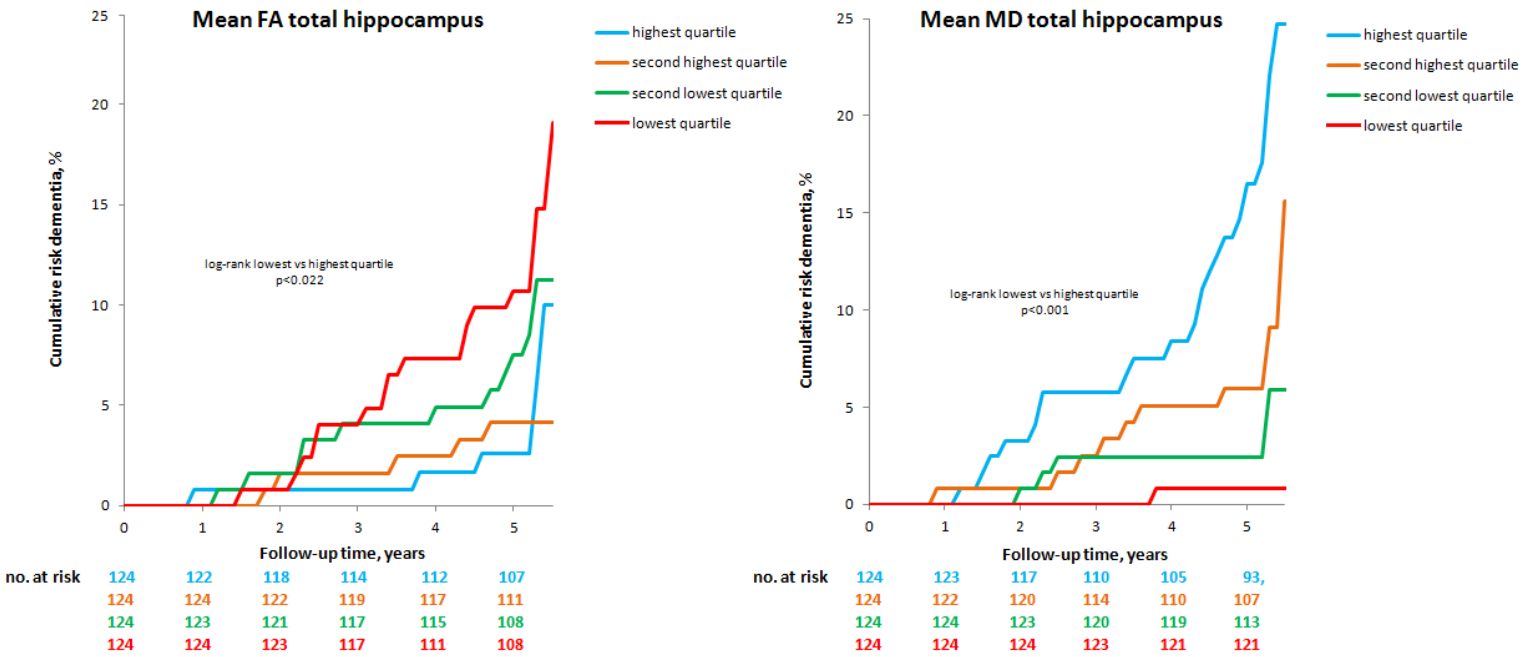


Figure 3: cumulative risk for dementia, stratified by quartiles structural integrity of the hippocampus

Cumulative risk of dementia in elderly with cerebral small vessel disease, stratified by quartiles structural integrity of the hippocampus. P-values were obtained by log-rank test, which was used to compare the curves of the highest and lowest quartile structural integrity. FA: Fractional Anisotropy, MD: Mean Diffusivity. The ranges of the Quartiles FA:0.060-0.093 for the first quartile; 0.093-0.104 for the second quartile; 0.105-0.115 for the third quartile and 0.115-0.165 for the fourth quartile. The ranges of the Quartiles MD are (10^{-4}mm2/s): 8.28-9.46 for the first quartile; 9.47-9.79 for the second quartile; 9.80- 10.22 for the third quartile and 10.22- 12.5 for the last quartile.

aetiology of AD. [111,112] Furthermore lower hippocampal microstructure, especially measured by MD in association to cognitive function, is consistent with previous cross-sectional studies in both non-demented elderly and elderly with MCI [113-116].

Strengths of our study include its longitudinal design in a population that covers the whole spectrum of cerebral SVD and the large sample size. Also the assessment of endpoints in 99.6% of our participants is a major strength. Collection of our data in a single centre allowed us to assemble baseline and follow-up data according to identical procedures, reducing the risk of procedural bias. Furthermore, we manually segmented the WMH and hippocampal volumes without prior knowledge of the clinical data. We performed a non-linear algorithm for the registration between the DTI and T1-image in order to deal with possible differential susceptibility effects. Furthermore the boundary voxels of the manually segmented hippocampal masks were eroded, to deal with potential partial volume effects. Finally, the relation between hippocampal diffusion parameters and dementia was investigated with adjustment for demographics, baseline global cognitive status and other brain imaging characteristics including WM and hippocampal volume, reducing confounds. Adjusting the associations between hippocampal diffusion parameters and dementia-risk for hippocampal volume, this remained a significant predictor for dementia, suggesting that this association was not accounted for by concomitant small hippocampal volumes. Due to the relatively small number of incident dementia cases in our analyses (n=41 in Table 2 and n=11 Table 3), the analyses could not be adjusted for all possible confounders (WMH, presence of lacunes , microbleeds and GM volume, on top of age, sex, education, baseline MMSE and territorial infarcts (no.), WM and Hippocampal volume), to avoid over-fitting. However, investigating this association between the hippocampal diffusion parameters and the risk of dementia for all above mentioned confounders, the results did not change.

Several methodological issues need to be addressed. First, the nosological dementia diagnosis in our study was a clinical diagnosis supported by MR imaging at the moment of diagnosis, and if not available, baseline MR imaging. In some cases, especially in the elderly, a distinction between AD and Vascular Dementia (VaD) is hard to make, because neurodegeneration and vascular diseases often co-occur.[30,98,99,117] For this reason we investigated 'overall dementia' as outcome measure. Second, it is possible that some patients with incident dementia were missed, because the cut-off point of 26 in the MMSE, although widely used, might not be sensitive enough, especially for cases of dementia in early stage of the disease, Vascular Dementia, or dementia in participants with higher education levels. We think that if misclassification has occurred, it may have led to an underestimation of the effect. Third, we were not informed on the APOE status of our participants, which prevented us from further increasing the predictive value of our analysis. Fourth, we measured WMH using the FLAIR images with a non-isotropic voxel size. For this reason some over or under-classification could not be excluded.

We think that our study has a high generalizability to patients between 50 and 85 years presenting with cSVD on neuro-imaging in a general neurology clinic. At baseline we included

Table 2: Cox proportional hazards for dementia at follow–up, derived from baseline hippocampal volume, GM volume and DTI–based Metrics

Baseline neuro-imaging characteristics (n=499)	**Hazard Ratio + 95% CI** Adjusted age, sex, education, baseline MMSE and territorial infarcts	**significance**	**Hazard Ratio + 95% CI** Additionally adjusted for normalized WM volume	**significance**
Grey matter volume, mL (SD)	0.75 (0.52-1.10)	p=0.138	0.73 (0.49-1.07)	p=0.102
Hippocampal volume, mL (SD)*	0.68 (0.49-0.96)	**p=0.028**	0.69 (0.49-0.97)	**p=0.032**
Baseline DTI-parameters **(n=496)	Adjusted for age, sex, education baseline MMSE and territorial infarcts		Additionally adjusted for normalized HV and WM volume	
Hippocampus, mean FA (SD)	0.70 (0.49-0.99)	**p=0.044**	0.72 (0.51-1.02)	p=0.067
Left hippocampus, mean FA (SD)	0.78 (0.56-1.10)	p=0.158	0.78 (0.55-1.11)	p=0.163
Right hippocampus, mean FA (SD)	0.68 (0.48-0.96)	**p=0.029**	0.73 (0.52-1.02)	p=0.064
Hippocampus, mean MD (SD)	1.54 (1.20-1.99)	**p=0.001**	1.44 (1.10-1.88)	**p=0.007**
Left hippocampus, mean MD (SD)	1.60 (1.23-2.09)	**p=0.001**	1.44 (1.10-1.90)	**p=0.009**
Right hippocampus, mean MD (SD)	1.29 (1.02-1.64)	**p=0.034**	1.26 (0.99-1.62)	p=0.066

One was additionally excluded because of the diagnosis Frontotemporal Dementia * four participants were excluded because of missing values of hippocampal volume. ** three participants were additionally excluded for the DTI analysis because of baseline DTI-scan artefacts. SD: Hazard ratio per standard deviation difference from the mean. Brain volumes, represent normalized brain volumes; normalized to the total ICV. WM: white matter, GM: grey matter, HV: hippocampus volume, FA: Fractional Anisotropy, MD: Mean Diffusivity (10^{-3}mm2/s).

Table 3: Cox proportional hazards; structural integrity of the volumetric intact hippocampi, and the risk of dementia at follow-up.

Baseline DTI-parameters (n=246)	**Hazard Ratio + 95% CI** Adjusted age, sex, education, baseline MMSE and territorial infarcts	**significance**	**Hazard Ratio + 95% CI** Additionally adjusted for normalized WM volume	**significance**
Hippocampus, mean FA (SD)	0.61 (0.28-1.30)	p=0.198	0.63 (0.29-1.37)	p=0.247
Left hippocampus, mean FA (SD)	0.75 (0.39-1.47)	p=0.409	0.79 (0.40-1.56)	p=0.501
Right hippocampus, mean FA (SD)	0.51 (0.22-1.20)	p=0.123	0.53 (0.23-1.25)	P=0.145
Hippocampus, mean MD (SD)	2.07 (1.11-3.88)	**p=0.022**	2.34 (1.17-4.68)	**p=0.016**
Left hippocampus, mean MD (SD)	2.44 (1.23-4.83)	**p=0.011**	2.66 (1.30-5.44)	**p=0.007**
Right hippocampus, mean MD (SD)	1.55 (0.82-2.92)	p=0.176	1.64 (0.83-3.23)	p=0.153

One was additionally excluded because of the diagnosis Frontotemporal Dementia. 11 cases of incident dementia were found in participants with the highest half hippocampal volume. Four participants were excluded for the DTI analysis because of baseline DTI-scan artefacts. SD: Hazard ratio per standard deviation difference from the mean. FA: Fractional Anisotropy, MD: Mean Diffusivity (10^{-3} mm2/s).

independent living, participants, with a mean MMSE of 28.1, corresponding with estimates in the general population.[53,101] All had some degree of cSVD on neuro-imaging, which has a prevalence of over 90% in the elderly population over 60 years of age.[6] However the median of WMH in our study is higher than found in population based studies. For this reason it is not surprising that our overall incidence rate of 16.4 per 1000 person years is higher than the overall incidence rate of 10.7 per 1000 person years found in a large population based study.[86] Comparing our cases of incident dementia with another study in participants with cerebral SVD[118] we had less cases of incident dementia (43/501 after 5 years of follow up vs. 90/588 after 3 years of follow up). This could be due to the fact that our population had a mean age which was approximately 10 years younger and has less severe WMH at baseline; both factors are known to be related with the incidence of dementia.[86,119]

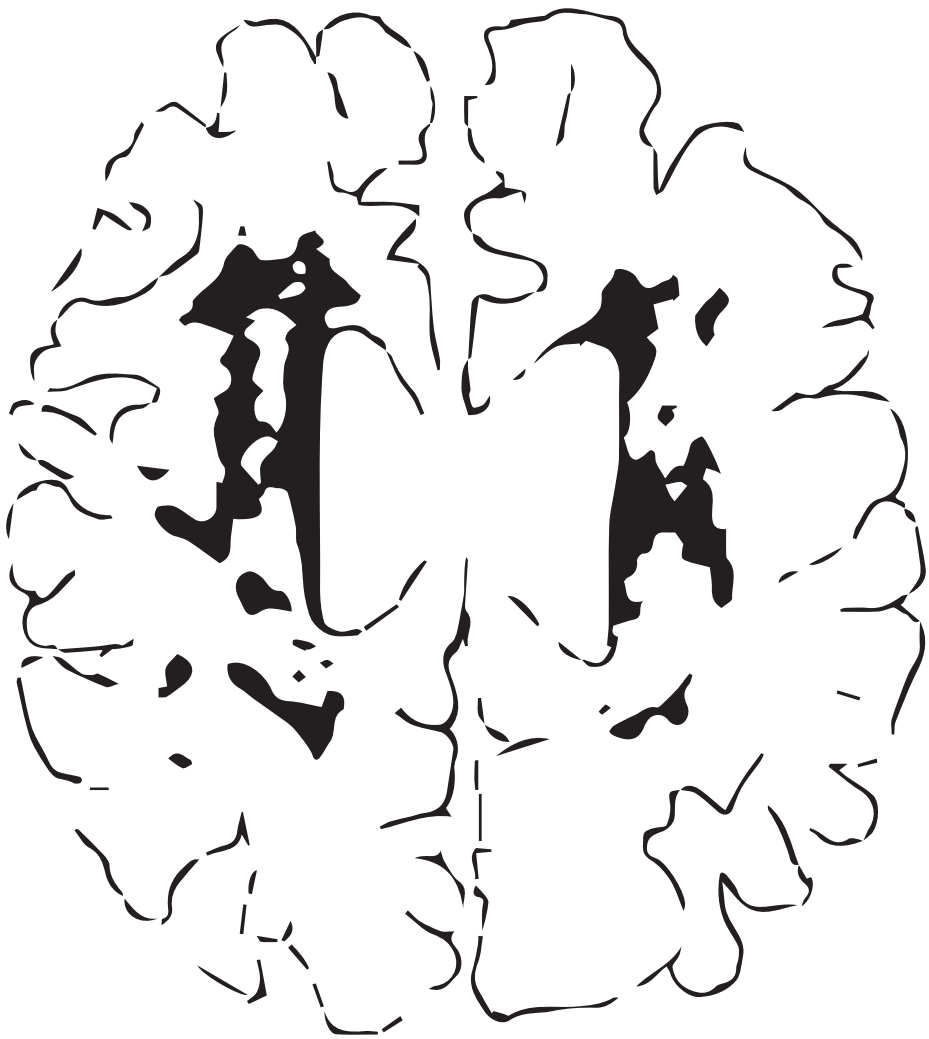
We found that the relation between the FA of the hippocampus and dementia was less prominent than for MD. This may be because FA mostly reflects the dominant directionality of the water diffusion. Multiple fibres present on the same location may all have different directions (crossing-fibres), influencing FA. It might be that because of this, low FA may not necessarily reflect a lower underlying structural integrity in the hippocampus.[25] In contrast, MD is less affected by fibre-crossing because it reflects the magnitude of water diffusion, which is not influenced by direction.[25] A recent meta-analysis comparing DTI parameters and volume measures in MCI and AD, indicated that MD values had more discriminative power than FA values,[120] which is in line with our findings.

It could be that the discriminative power of the DTI parameters, is higher in the group of elderly classified in the upper half of hippocampal volume, because the low microstructural integrity of the hippocampus may reflect changes that have not yet resulted in visible hippocampal volume loss. Possibly, once the hippocampal atrophy has started, this atrophy is of more predictive value for the development of dementia, than the diffusion changes, lowering the discriminative power of the DTI parameters. Furthermore, DTI parameters are less sensitive in a smaller hippocampus than in a volumetric intact one, which also might contribute to a stronger effect for the DTI parameters in our participants with the upper half of hippocampal volume. We did not find a significant relation between whole brain DTI metrics and dementia, which might indicate that hippocampal DTI parameters are more specific predictors for the development of dementia . In contrast, a recent longitudinal study[104] showed that microstructural changes in the WM predict a faster decline in several cognitive domains, but dementia has never been included as an outcome measure.

The underlying mechanisms of higher hippocampal MD (and lower FA), indicating a lower microstructural integrity still remains to be elucidated. Several mechanisms, whether or not related to cSVD, have been proposed. Possibly severe WMH lead to axonal loss by anterograde or Wallerian degeneration in the areas that are connected by these WM structures,[102,103] such as the hippocampus, and as such lead to higher MD. Alternatively the accumulation of intraneuronal tau in the hippocampus [121] may lead to axonal damage and lower microstructural integrity of the hippocampus, and as a consequence to loss of function.

Ideally a surrogate marker for dementia identifies those at higher risk during the pre-clinical period, because identification of this disease at an earlier stage (that is, before the irreversible damage has been done), might lead to early intervention with for instance vascular risk factors as a target. In turn, individuals identified as having a lower risk of developing dementia, can possibly be comforted. DTI of the hippocampus might identify the persons at risk in an earlier stage than possible with conventional MRI and may be a promising a surrogate marker of disease progression for use in therapeutic trials. However to answer the question whether dementia can really be prevented by treating or targeting these vascular risk factors we need randomized clinical trials with large sample size and sufficient long follow-up.[122]

In conclusion, results of our study show that hippocampal diffusion parameters at baseline are associated with an increased risk of developing dementia five years later, even when macroscopical loss of hippocampal volume is not apparent. These results might suggest that diffusion changes in the hippocampus precede volumetric changes as seen in dementia. DTI may offer an earlier insight in the development of dementia, and therefore provide an earlier window for possible preventive strategies.



5.

Baseline white matter microstructural integrity is not related to cognitive decline after 5 years

Ingeborg W.M. van Uden, Helena .M. van der Holst, Pauline Schaapsmeerders, Anil M. Tuladhar, Anouk .G.W. van Norden, Karlijn F. de Laat, David G. Norris, Jurgen A.H.R Claassen, Ewoud J. van Dijk, Edo Richard, Roy P.C. Kessels and Frank-Erik de Leeuw

Biochimica et Biophysica acta Clinical. 2015;4:108-14

Abstract

Objectives: Traditional markers of cerebral small vessel disease (cSVD) are related to cognition and cognitive decline, but this relation is weak. Therefore other factors may determine the transition from intact cognitive performance to cognitive decline, such as the damage of the cerebral white matter at the microstructural level. Little is known about the association between microstructural integrity of the white matter and changes in cognition. In this study we investigated the relation between baseline microstructural integrity and change in cognitive function.

Methods: 503 participants of the RUN DMC study with cSVD without dementia, 398 of whom (79.1%) underwent repeated cognitive testing at follow-up, with a mean follow-up time of 5.4 years (± SD 0.2), and amongst others FLAIR MRI and diffusion tensor imaging (DTI). At baseline mean diffusivity (MD) and mean fractional anisotropy (FA) was measured in both white matter hyperintensities (WMH) and normal appearing white matter (NAWM). A linear regression analysis was performed assessing the association between baseline diffusion parameters and decline in cognitive domains.

Results: An inverse association was found between baseline MD in the NAWM and decline in cognitive index, (β=0.17; p=0.035), adjusted for age, sex, education, presence of depressive symptoms at baseline, normalized TBV, lacunes and WMH volume. However, no significant associations were found between diffusion parameters and decline in any cognitive domain after Bonferroni correction.

Conclusions: In contrast to cross-sectional studies, in older adults with cSVD microstructural integrity of the white matter as assessed with DTI, is not related to decline in global cognitive function or any other sub domain.

Introduction

Cerebral small vessel disease (cSVD) is very common in older adults[6] and is related to cognitive decline and dementia.[41] However, not everyone with cSVD visible on conventional structural MRI eventually develops cognitive decline or dementia. Therefore other factors may determine the transition from intact cognitive performance to cognitive decline, such as the damage of the cerebral white matter at the microstructural level. The interconnected neural networks, crucial for cognitive performance, are hypothesized to be disconnected by this damage in the white matter microstructure, also known as the "disconnection syndrome".[35]

As identical appearing white matter hyperintensities (WMH) on FLAIR MRI scanning are histopathologically heterogeneous,[123] possibly only WMH with the highest loss of structural integrity are related to cognitive decline. Furthermore the degree of structural integrity of the surrounding Normal Appearing White Matter (NAWM) might be important in cognitive decline. As conventional MRI is not sensitive to detect subtle damage of the white matter (WM), diffusion tensor imaging (DTI), using the diffusion properties of water molecules might be of use to provide an early marker for this cognitive decline.[25,80] A low Fractional Anisotropy (FA) and high Mean Diffusivity (MD) are believed to represent low microstructural integrity.[56]

A low microstructural integrity of the WM has been associated with lower cognitive performance in both population based studies [124-126] and older adults with cSVD, albeit at the cross-sectional level. [95,127-129] Some of these studies even found that DTI parameters correlated better with cognitive performance than traditional markers of cSVD in patients with cognitive impairment,[128] suggesting an important role of low WM microstructural integrity in cognitive impairment. At a cross-sectional level we showed that cognitive performance was associated with white matter microstructural integrity independent of traditional markers of cSVD, both in the WMH and NAWM [95] and in specific WM tracts.[129] However, we additionally showed that DTI of the NAWM and WMH had only limited additional value to the traditional cSVD parameters in explaining the variance in cognitive function.[43] Two smaller prospective DTI studies did not find an association between microstructural integrity and cognitive functioning at follow-up.[72,76] A larger longitudinal study using diffusion weighted imaging (DWI) in individuals with SVD showed that DWI parameters within the NAWM were related to cognitive decline after 3 years follow-up.[104] We, however, recently showed no relation between microstructural integrity of the WM and incident dementia after five years.[44] Taken together, results found in prospective studies are weak and conflicting.

We therefore investigated whether baseline microstructural integrity as assessed by DTI, both within the WMH and the NAWM, independently of traditional cSVD characteristics predicts decline in several cognitive domains after 5 years. Furthermore we investigated if this relation was different in those with low, moderate and high WMH severity at baseline.

Material and methods

Study population

The Radboud University Nijmegen Diffusion Tensor and Magnetic resonance Cohort (RUN DMC) study prospectively investigates risk factors and clinical consequences of brain changes as assessed by MRI among 503, 50 to 85 year old non-demented older adults with cerebral SVD. The selection procedure of the participants and the study rationale and protocol were described in detail previously.[60] In short, on the basis of established research criteria, cSVD was a radiological diagnosis, defined as the presence of lacunes and/or WMH on neuro-imaging.[61] Symptoms of cSVD include acute symptoms, such as TIAs or lacunar syndromes, or sub acute manifestations such as cognitive, motor disturbances and/or depressive symptoms.[61] The baseline data collection was performed in 2006. Main exclusion criteria were dementia, (psychiatric) disease interfering with cognitive testing or follow-up, WMH or cSVD mimics and MRI contra-indications or known claustrophobia.[60]

Follow-up was completed in 2012 (mean follow-up time 5.2 years (SD 0.7). Of the 503 baseline participants, 2 were lost to follow-up (but not deceased according to the Dutch Municipal Personal Records database) and 49 had died. From all remaining 442 participants follow up was available (face-to-face follow-up was performed in 398 participants, 54 consented to the collection of clinical endpoints via their general practitioner (Figure 1).

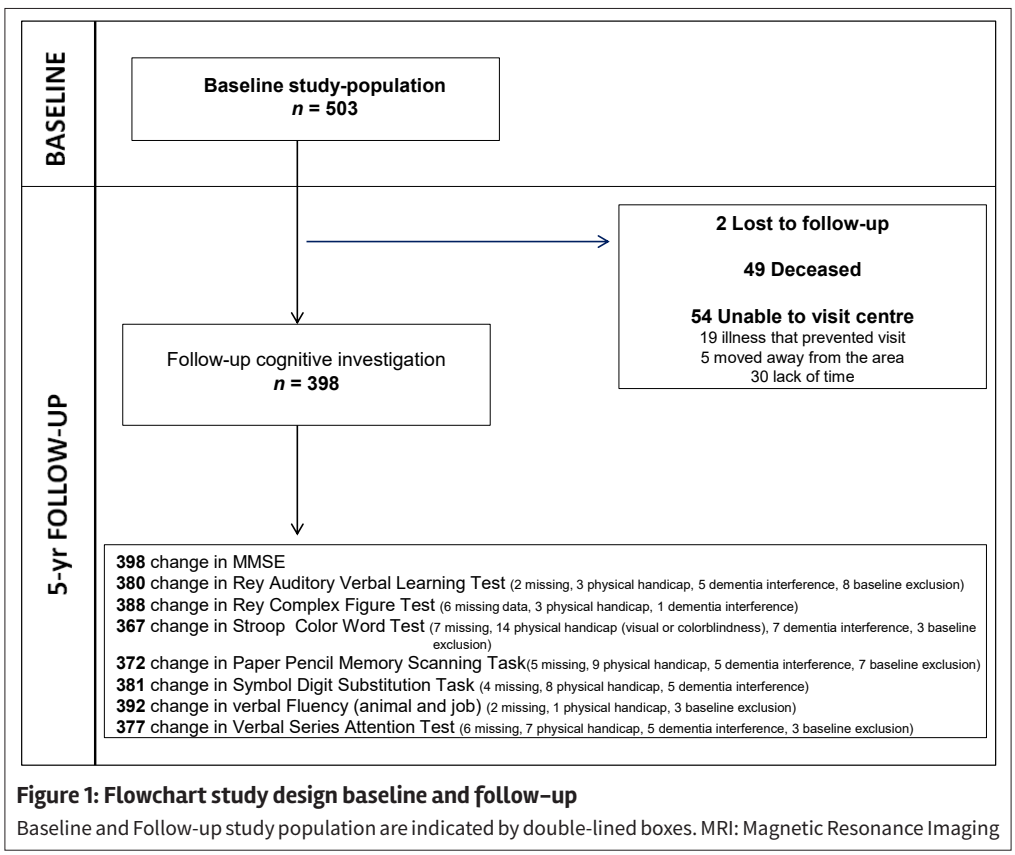


Figure 1: Flowchart study design baseline and follow–up

Baseline and Follow-up study population are indicated by double-lined boxes. MRI: Magnetic Resonance Imaging

Cognitive function

Participants underwent the same neuropsychological test battery both at baseline and during follow-up examination, covering the main cognitive domains. These tests have been previously applied in large-scale epidemiological studies.[53,130] The test battery included the Mini–Mental State Examination (MMSE),[82] verbal fluency (animals and profession naming),[131] Rey Auditory Verbal Learning Test (RALVT; 3-trial version),[132,133] Symbol Digit Substitution Task (SDST),[134] Stroop Colour Word Test (short form),[135] Paper-Pencil Memory Scanning Task,[136] Rey Complex Figure Task (RCFT)[137] and Verbal Series Attention test (VSAT).[138] The same versions of the tests were used for baseline and follow-up assessment.

Speed-Accuracy Trade-Off (SAT) scores were calculated where appropriate [accuracy(%)/reaction time], to adjust for a number of faults. Performance across tests was made comparable by transforming the raw test scores into z-scores (individual test score minus mean test score, divided by the standard deviation). Z-scores for both baseline and follow-up were calculated using the mean and SD of the baseline tests.[139] Higher z-scores always indicate a better performance.

Change in cognitive functioning for separate cognitive domains was calculated within-subject, by subtracting the baseline domain compound score from the follow-up domain compound score.

Subsequently, compound scores for global cognitive function (Cognitive Index), memory (verbal and visospatial memory) and executive function (psychomotorspeed, fluency, inhibition and attention) were calculated. The Cognitive Index was constructed to obtain a more robust outcome measure for global cognition. This was calculated as the mean of the z-scores of the SAT score of the 1-letter subtask of the Paper-Pencil Memory Scanning Task, the mean of the SAT score of the reading task of the Stroop test, the mean of the SDST, and the mean of the added score on the three learning trials of the RAVLT and the mean of the delayed recall of this test.[53]

Verbal memory is a compound score of the mean of z-scores of the total correct words on the three learning trials of the RALVT and the delayed recall of this test. Visuospatial memory is calculated from the mean of the z-scores of the immediate recall and delayed recall trial of the RCFT. Psychomotor speed was calculated as the mean of the z-scores of the SAT score of the 1-letter subtask of the Paper-Pencil Memory Scanning Task, the mean of the SAT score of the reading task of the Stroop test and the mean of the SDST. Verbal fluency was calculated from the mean of the z-scores of both fluency conditions. Inhibition was measured using the following formula: dividing the Stroop part III SAT score by the mean of the SAT scores of parts I and II. Afterwards a z-score for inhibition was calculated. Attention was computed as the z-score of the SAT score of the total time of the VSAT. If one test of a particular domain was missing, the domain score was computed using the remaining tests of that domain (this occurred in less than 6.3% in the sub domains). For 98% of all participants a score for Cognitive Index was available, of whom 90% completed all five subtests without recording of any test problems.

MRI resonance imaging protocol

MRI scans of all participants were acquired on a single 1.5-Tesla MRI scanner (Magnetom Sonata, Siemens Medical Solutions, Erlangen, Germany). The protocol included the following whole brain scans: a T1-weighted 3D magnetization-prepared rapid gradient-echo (MPRAGE) imaging (TR/TE/TI 2250/3.68/850ms;flip angle15°; voxel size 1.0 x 1.0 x 1.0mm); Fluid-attenuated inversion recovery (FLAIR) pulse sequences TR/TE/TI 9000/84/2200ms; voxel size 1.0 x 1.2 x 5.0mm, with an interslice gap of 1 mm); a transversal T2*weighted gradient echo sequence (TR/ TE 800/26ms; voxel size 1.3 x 1.0 x 6.0mm, with an interslice gap of 1 mm) and a Diffusion Tensor Imaging (DTI) sequence (TR/TE 10100/93ms; voxel size 2.5x2.5x2.5mm; 4 unweighted scans, 30 diffusion weighted scans with b-value = 900 $s \cdot mm^{-2}$).[60]

MRI analysis

WMH were manually segmented on FLAIR images and the total WMH volume was calculated by summing the segmented areas multiplied by slice thickness. The ratings of lacunes and microbleeds were revised according to the recently published STRIVE-criteria, by trained raters blinded to all clinical data.[12] Excellent intra and inter-rater reliabilities were found with weighted kappa of 0.87 and 0.95 respectively for the presence of lacunes and 0.85 and 0.86 for the presence of microbleeds, calculated in 10 percent of the scans. Inter-rater reliability (assessed using the intra-class correlation coefficient) for total WMH volume was 0.99.

To obtain the grey matter (GM), WM and cerebrospinal fluid (CSF) volume, segmentation of the T1 MPRAGE images was revised using a recent version of Statistical Parametric Mapping 12 unified segmentation routines (SPM12; Wellcome Department of Cognitive Neurology, University College London, UK (http://www.fil.ion.ucl.ac.uk/spm/software/spm12/). All images were visually checked for co-registration errors and for motion and/or segmentation artefacts. The intracranial volume (ICV) was calculated by summing the volumes of GM, WM and CSF, by multiplying the probabilistic tissue segmentations by the voxel volume. Total brain volume (TBV) was taken as the sum of total GM and WM. All volumes were normalized to total ICV.

DTI–analysis

Diffusion data were pre-processed and analyzed according to an extensively earlier described procedure. [60] The diffusion weighted images of each participant were realigned on the unweighted image using mutual information based co-registration routines from SPM5. The diffusion tensor and its eigenvalues were computed using linear regression, using an SPM5 add-on (http://sourdeforge.net/projects/spmtools). The spurious negative values were set to zero, after which the tensor derivates MD and FA were calculated[64] The mean unweighted image was used to compute the co-registration parameters to the anatomical T1 image, (SPM5 mutual information co-registration), which were then applied to all diffusion weighed images and derivates. All images were visually checked for motion artefacts and

co-registration errors. The mean MD and FA were then calculated in the WMH, NAWM and total WM.

Other parameters

Education was classified using 7 categories (1 being less than primary school, 7 reflecting academic degree) and then dichotomized in a group having only or less than primary school and a group having more than primary education.[65] Depressive symptoms were assessed with the 20-item Centre of Epidemiologic Studies Depression Scale (CES-D); they were considered present in participants with CES-D ≥ 16 and/or participants who currently used anti-depressive medication, taken for depression.[48,60]

Statistical analysis

Baseline characteristics are presented as mean and standard deviation (SD) for the participants who had a face-to-face follow-up and those without. For the WMH median and inter quartile range (IQR) is shown. Group-differences between participants and non-participants are calculated with age and sex-adjusted ANOVA or logistic regression for categorical variables. The associations between baseline microstructural integrity of the NAWM and WMH and the decline in different cognitive domains were assessed by means of linear regression analysis. The variance inflation factor (VIF) was calculated for all regression models to investigate if multicollinearity was present. The VIF scores were low for all multiple regression models (scores below 3, where scores above 5 is considered high multicollinearity). Data were presented as standardized betas. To correct for multiple testing, Bonferroni correction was used, therefore α was set to 0.007. The analyses were adjusted for the possible confounders age, sex, education level, presence of depressive symptoms, TBV, lacunes and for WMH volume where appropriate. A secondary analysis using stepwise backward selection was performed to confirm these results. First, age, sex and education were forced into the model and a backward stepwise selection procedure was used on the full regression model to remove the variables from the model one at a time, until these had p values smaller than 0.10. To investigate if the microstructural integrity of the WM played a different role in the decline in cognition in those who have limited cSVD on FLAIR MRI vs. those who have a higher degree of cSVD, we repeated this analysis in strata (tertiles) of WMH volume. A post-hoc analysis was performed, using age and sex-adjusted ANOVA, to investigate whether the microstructural integrity within the WMH and NAWM of the 10% least decliners and the 10% worst decliners in cognitive index differed.

Results

Baseline demographics and neuro-imaging characteristics of the 398 participants in the in-person follow-up examination and the 105 subjects who did not participate are shown in Table 1. Average mean follow-up duration was 5.4 years (SD 0.2; range 4.5 to 6.2). Participants who did not return for in person-follow-up were significantly older at baseline, had a higher

Table 1: Baseline characteristics study–population

	Follow-up-complete	No Follow-up examination	p-value for difference^
Demographics	**(n=398)**	**(n=105)**	
Age at baseline (SD)	64.5 (8.5)	70.0 (8.4)	p<0.001
Sex, male (n, %)	227 (57.0)	57 (54.3)	p=0.554[i]
Education, only primary (n,%)	33 (8.3)	16 (15.2)	p=0.396[i]
MMSE (SD)	28.3 (1.6)	27.6 (1.8)	**p=0.042**
Cognitive Index (SD)*	0.10 (0.76)	- 0.44 (0.70)	**p<0.001**
Depressive symptoms (n,%)**	266 (67.5)	65 (62.5)	p=0.378[i]
MRI characteristics	**(n=397)**	**(n=105)**	
Intracranial volume, ml (SD)	1459.0 (134.7)	1445.9 (147.3)	p=0.707
White matter volume, ml (SD)	468.0 (39.6)	450.9 (57.1)	p =0.285
WMH volume, ml (IQR)†	6.0 (3.2-15.1)	14.4 (6.0-27.2)	**p=0.004**
NAWM volume, ml (SD)	455.6 (43.4)	430.5 (62.6)	p=0.055
Lacunes, presence (n, %)	90 (22.7)	44 (41.9)	**p=0.008**[i]
Microbleeds, presence (n, %)***	58 (14.6)	23 (21.9)	P=0.502[i]
Territorial infarcts, presence (n, %)	40 (10.1)	16 (15.2)	P=0.422[i]
Grey matter volume, ml (SD)	621.5 (49.9)	595.3 (48.7)	**p=0.022**
Total brain volume, ml (SD)	1089.4 (70.3)	1046.2 (77.3)	p=0.012
Hippocampal volume, ml (SD)****	6.83 (0.94)	6.68 (0.97)	p=0.879
Global DTI characteristics [e]	**(n=395)**	**(n=104)**	
White matter, mean FA, (SD)	0.33 (0.02)	0.32 (0.02)	**p=0.029**
WMH, mean FA, (SD)	0.34 (0.03)	0.33 (0.03)	p=0.424
NAWM, mean FA, (SD)	0.33 (0.02)	0.32 (0.02)	p=0.026
White matter, mean MD,(SD)	0.88 (0.04)	0.91 (0.04)	**p=0.012**
WMH, mean MD, (SD)	0.99 (0.06)	1.02 (0.07)	p=0.172
NAWM, mean MD, (SD)	0.88 (0.04)	0.91 (0.04)	**p=0.016**

Data shown represent numbers of subjects (%), mean (SD) or median † (inter quartile range), ^age and sex adjusted where appropriate (ANOVA or logistic regression[i]). MMSE: Mini-mental State Examination, ml: millilitres, SD: standard deviation, WMH: White Matter Hyperintensities, NAWM: Normal Appearing White Matter, FA: Fractional Anisotropy, MD: Mean Diffusivity (10^{-3} mm2/s). Brain volumes represent normalized brain volumes to the total ICV. *one was excluded because of missing cognitive data ** five were excluded because of missing values of depressive symptoms, ***three were excluded because of missing values of microbleeds.**** three were excluded because of missing values of hippocampal volume. [e]3 were additionally excluded for the DTI analysis because of baseline DTI-scan artefacts.

Bold values indicate significance at $p < 0.05$. They performed worse on the raw test scores of almost all cognitive domains at baseline compared with participants who participated (Supplementary Tables A and B).

WMH volume, more lacunes, lower GM volume and a lower microstructural integrity of the WM compared with those with follow-up examination, adjusted for age and sex.

They performed worse on the raw test scores of almost all cognitive domains at baseline compared with participants who participated (supplementary Table A and B).
Figure 2 shows compound z-scores of the cognitive domains at baseline and follow-up. Decline in cognitive performance is observed in all domains except visuospatial memory and concept shifting.
A correlation matrix with the predictors in the dataset is presented as supplementary Table C. Low microstructural integrity (measured by MD) in the NAWM was related to decline in cognitive index (β=0.17;p=0.035), adjusted for age, sex, education, presence of depressive symptoms at baseline, normalized TBV, lacunes and WMH volume, however this was no longer significant after Bonferroni correction. No significant relation was found between white matter microstructural integrity and decline in any of the other cognitive sub-domains, adjusted for the above mentioned confounders after Bonferroni correction (Table 2). There was no significant relation found between diffusion parameters in the total white matter and any of the cognitive domains. Backward stepwise selection of all models confirmed these results (data not shown).

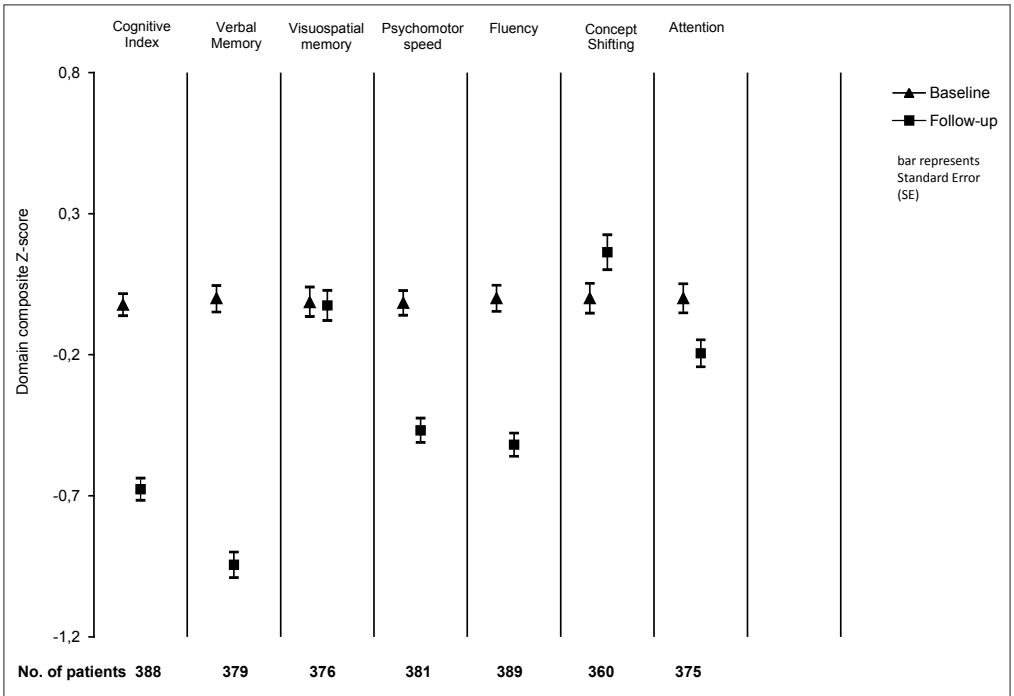


Figure 2: Composite z–scores at Baseline and Follow–up
Bar represents Standard Error. Apart from the domains visuospatial memory and concept shifting, participants score on average worse on follow-up test than at baseline. z-scores of the follow-up are calculated with the mean and standard deviation from the baseline

Table 2 : The relation between DTI parameters in both the white matter hyper intensities and the normal appearing white matter and decline in global cognitive performance

	White Matter Hyperintensities		Normal Appearing White Matter	
	Mean Diffusivity	**Fractional Anisotropy**	**Mean Diffusivity**	**Fractional Anisotropy**
Global cognitive function				
MMSE	0.04; p=0.556	-0.07; p=0.180	0.14; p=0.083	-0.06 ; p=0.318
Cognitive Index	0.10; p=0.122	0.01; p=0.838	0.17; p=0.035	-0.07; p=0.230
Memory				
Verbal memory	0.07; p=0.305	0.01; p=0.903	0.21; p=0.008	-0.08; p=0.190
Visuospatial memory	0.00; p=0.984	-0.03; p=0.555	0.03; p=0.729	-0.11; p=0.068
Executive function and attention				
Psychomotor speed	0.04; p=0.532	0.02; p=0.717	0.01; p=0.889	-0.02; p=0.717
Fluency	0.14; p=0.027	-0.07; p=0.188	0.19; p=0.015	-0.15; p=0.016
Inhibition (concept shifting)	0.01; p=0.916	0.02; p=0.787	-0.03; p=0.718	-0.12; p=0.054
Attention	0.09; p=0.163	-0.18; p=0.001	0.03; p=0.708	-0.06; p=0.317

Numbers represent standardized β's and are adjusted for age, sex, education, depressive symptoms, normalized total brain volume, lacunes and in the NAWM also for log normalized white matter hyper intensities. Composite Z-score of Follow-up is standardized to the baseline; (FU-test – mean Baseline)/(SD Baseline). Significance after Bonferroni correction p<0.007

After stratification in tertiles of baseline WMH severity, we did not find a relation between white matter microstructural integrity and cognitive decline, in those with mild, moderate and WMH load, adjusted for the above mentioned confounders.(data not shown)
A post-hoc analysis investigating the microstructural integrity within the WMH and NAWM in the 10% with the least decline in cognitive index and the 10% highest decliners, showed no significant difference in the mean MD or FA in both the WMH and NAWM, adjusted for age and sex.

Discussion

In older adults with cSVD, microstructural integrity in the white matter was not related with decline in global cognitive performance in all separate cognitive domains after adjustment for possible confounders, after 5 years of follow-up. This finding was independent of WMH severity. This finding is in line with our previous findings, in which we found only limited additional value to conventional cSVD parameters in explaining the variance in cognitive function,[43] and the lack of relation between diffusion parameters and incident dementia after 5 years.[44] Probably other factors, apart from WM microstructural integrity play a role in cognitive decline over time.
Several methodological issues must be addressed. First and foremost, 79.1% of the baseline study population was available at follow-up for cognitive testing. The dropout might lead to attrition bias, although the response rate is considered high after 5 years. Drop-outs at follow-up were significantly older at baseline, performed less on cognitive testing at baseline and had a higher WMH volume, more lacunes and a lower microstructural integrity at baseline. Since these variables were independently associated with cognitive performance in other studies, [51,53,129] exclusion of drop-outs might resulted in an underestimation of the effect of WM integrity on several cognitive domains. The above mentioned issue is a well known paradox of follow-up studies: To prove a causal relation over time, long follow-up is required, however, the longer the follow-up period, the higher the risk of selective drop-out, which itself reduces the magnitude of the effect.
Second, the same cognitive tests have been administered at baseline and follow-up examination. Therefore it is possible that ***learning effects*** may have occurred. Especially cognitive tests with a memory-component are prone for this learning effect.[140] We think this would have had little effect in our study, because of the relatively long interval between the two moments of testing, and because in almost all cognitive domains, participants declined (Figure 2), which would be the opposite when learning effects would have great impact. A strength of our study design is that we collected our data in a single centre, which allowed us to administer baseline and follow-up assessments according to identical procedures, using the same test-instructions and even interview-rooms, reducing measurement errors (non-systematic errors of the test score because of coincident fluctuations in concentration, motivation or mood, or differences in the test-procedure), as much as possible.[140] Finally, we were not informed on the genetic APOE status, CSF biomarkers or PET scan at baseline

of our participants, which prevented us from further excluding possible neurodegenerative processes.

Three prospective studies described the relation between diffusion parameters and cognitive decline preciously. First, a large prospective study in older adults with cSVD[104] reported a relation between baseline diffusion parameters and decline in executive function, memory and speed after a follow-up period of 3 years, after adjustment for age, sex, education, TBV, WMH and lacunes. However, they did not take depressive symptoms into account as a possible confounding factor. This is especially relevant since the same authors reported that depressive symptoms predicted cognitive decline and dementia in their cohort.[118] In our study, the adjustment for depressive symptoms weakened the associations between baseline microstructural integrity and cognitive decline. Additional analyses confirmed no mediation effect of depressive symptoms in this relation. This was not expected because the associations between depressive symptoms and worse cognitive performance in older participants has previously extensively been described. [141]. Second, a small prospective study (n=35) with one-year follow-up in participants with cSVD found a relation between diffusion parameters and executive function at a cross-sectional level, however they could not relate diffusion parameters to cognitive decline,[72] possibly by the relatively short follow-up period and small numbers. The third study (n=84) in middle-aged community dwelling individuals found no correlations between baseline DTI parameters and changes in working memory, but showed that decline in working memory was correlated to decline in DTI parameters after 2 years.[76] This study however did not adjust for possible confounders. Taking the previous findings into account, in the future it could be interesting to investigate if the ***change*** in microstructural integrity over time instead of the baseline microstructural status, causes cognitive decline.

Finally we have to consider we missed the (very weak) association between baseline DTI parameters and cognitive decline, because of a type A error. Possibly due to limited power and selective drop-out at follow-up, which was in our study, despite the high response, 20.9%. As a result, our sample may consist of participants with a relatively good health and cognitive performance at baseline who may have less chance to deteriorate in cognitive functioning over time. However in our study this seems to be not the case, for post-hoc analyses comparing the microstructural integrity in of the 10% least decliners versus the 10% who declined most at follow-up showed no difference in any of the diffusion parameters, both within the WMH or NAWM. Moreover, participants at follow-up with low test-scores because of cognitive problems or dementia, have had difficulties performing complex neuropsychological tests which may have resulted in missing data (such as task 3 of the Stroop or the Rey Complex Figure). This may have resulted in an underestimation of the decline in the domains assessed by these tests, which might have weakened the strengths of the associations. Finally, the cognitive change profile in our study shows predominantly a change in verbal memory, which does not reflect the core profile of cSVD where deficits in executive function, such as speed and fluency, are most prominent. Probably other factors

than white matter microstructural integrity play a role in cognitive decline. Such a factor could be the microstructural integrity of other areas in the brain known to be related to cognitive performance, such as the hippocampus, as we previously showed.[45]

In summary, in older adults with cSVD, microstructural integrity of the WM was not associated with decline in cognitive performance after a 5-year follow-up. These results are not in line with cross-sectional reports, and therefore unexpected. The lack of association might be due to a type A error, due to selective drop-out at follow-up, however this was not supported by a post-hoc analysis. Other factors than microstructural integrity of the white matter, might underlie cognitive decline in older adults with cSVD.

Supplementary Table A: Raw test–scores of participants with both baseline and follow–up examination

Cognitive tests	Baseline (2006-2007) All	Baseline (2006-2007) Completed both B and FU test	Follow--up (2011-2012) Completed both B and FU test
Mini Mental State examination (range 0-30)			
	28.1 (1.6)	28.3 (1.6)	27.8 (2.8)
Rey Auditory Verbal Learning Test (no. of words recalled)			
immediate recall trial 1	5.1 (1.8)	5.3 (1.7)	3.6 (1.6)
immediate recall trial 2	7.3 (2.3)	7.6 (2.2)	5.5 (2.1)
immediate recall trial 3	8.6 (2.6)	9.0 (2.4)	6.7 (2.4)
delayed recall	6.0 (3.1)	6.4 (3.0)	3.9 (2.7)
Stroop Color Word Test (time in sec.-faults)			
part 1 (word reading)	25.8 (6.3) - 0.2	25.1 (6.1) - 0.2	26.3 (6.0) - 0.3
part 2 (colour naming)	33.2 (7.7) - 0.2	32.2 (7.4) - 0.2	33.4 (8.6) - 0.4
part 3 (interference)	63.8 (22.1) - 1.0	59.9 (19.4) - 0.9	60.9 (24.1) - 1.1
Paper Pencil Memory Scanning Task (time in sec.-faults)			
1 character	45.2 (13.7) - 0.7	43.2 (12.3) - 0.6	51.9 (18.9) - 0.6
2 characters	62.4 (19.8) - 3.1	60.3 (17.8) - 2.8	72.7 (27.4) - 2.8
3 characters	77.6 (26.8) - 2.6	74.6 (23.8) - 2.5	90.2 (35.8) - 2.1
Symbol Digit Substitution Task (no. in 60 sec.)			
	27.0 (9.7)	28.5 (9.4)	23.2 (8.5)
Verbal Fluency (no. in 60 sec.)			
animals	22.0 (6.5)	22.6 (6.5)	19.2 (5.5)
professions	16.5 (5.3)	17.10 (5.3)	14.3 (5.0)
Verbal Series Attention Test (time in sec. - faults)			
total time	96.0 (38.2) - 2.4	90.1 (34.6) - 2.1	95.9 (35.5) - 2.3
Rey Complex Figure Test (range 0-36)			
copy trial	33.4 (3.4)	33.8 (3.0)	33.2 (4.3)
immediate recall trial	18.2 (6.8)	18.9 (6.6)	18.7 (6.8)
delayed recall trial	18.1 (6.6)	18.7 (6.5)	18.6 (7.0)

Numbers represent means (SD) . Drop-out rate for change-score per test, because of baseline and/or Follow-up missing data. MSSE: 0%, RAVLT: 4.5%, Stroop 7.8%, Paper Pencil Test 6.5%, SDST 4.3%, Verbal Fluency 1.5%, VSAT 5.3%, RCFT copy trial 2.5%, delayed recall trial: 8.8%.

Supplementary Table B: Cognitive domain scores at baseline of participants and non–participants at follow–up

	BASELINE (2006-2007)				
	Follow-up-completed	**No Follow-up examination**	**p-value for difference***	**Deceased participants**	**Refusers**
Cognitive domains					
Global cognitive function					
MMSE	28.3 (SD 1.6)	27.6 (SD 1.8)	**p=0.026**	27.2 (SD 1.9)	27.9 (SD 1.7)
Cognitive Index	0.10 (0.76)	- 0.44 (0.70)	**p<0.001**	-0.71 (SD 0.72)	-0.23 (SD 0.61)
Memory					
Verbal memory	0.11 (SD 0.92)	-0.42 (SD 0.94)	**p<0.001**	-0.76 (SD 0.94)	-0.18 (SD 0.85)
Visuospatial memory	0.07 (SD 0.97)	-0.34 (SD 1.01)	p=0.051	-0.39 (SD 0.99)	-0.29 (SD 1.05)
Executive function and attention					
Psychomotor speed	0.10 (SD 0.85)	-0.46 (SD 0.78)	**p<0.001**	-0.68 (SD 0.76)	-0.27 (SD 0.78)
Fluency	0.10 (SD 0.92)	-0.40 (SD 0.81)	**p=0.001**	-0.48 (SD 0.87)	-0.30 (SD 0.76)
Inhibition (concept shifting)	0.07 (SD 0.94)	-0.27 (SD 1.17)	p=0.544	-0.39 (SD 1.01)	-0.21 (SD 1.30)
Attention	0.11 (SD 1.01)	-0.43 (SD 0.84)	**p=0.001**	-0.37 (SD 0.85)	-0.47 (SD 0.84)

Data shown represent composite Z-scores of different neuropsychological tests to calculate cognitive domains, * age and sex adjusted difference between participants with and without Follow-up participation (ANCOVA). MMSE: Mini-mental State Examination, ml: millilitres, SD: standard deviation. Composite Z-score of Follow-up is standardized to the baseline; (FU-test – mean Baseline) /(SD Baseline).

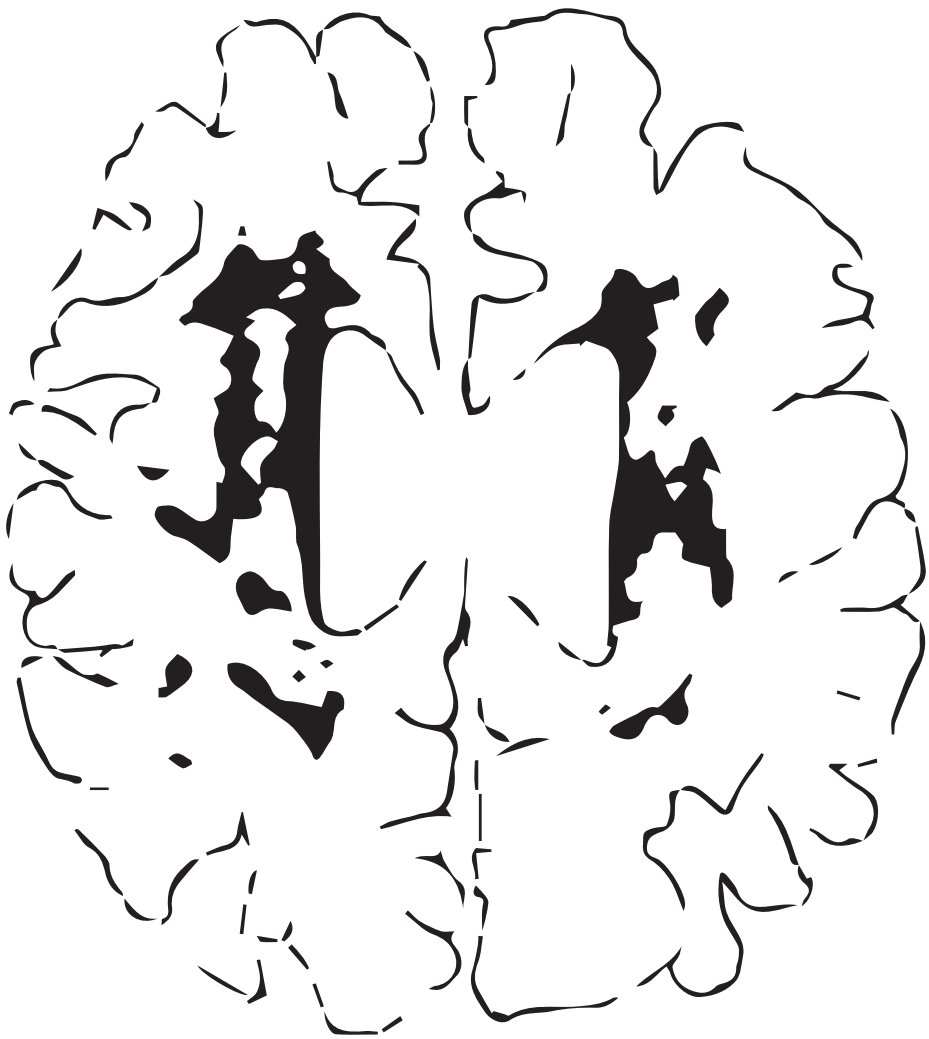
Supplementary Table C: Correlation coefficients for demographical and imaging predictors

	Age	Education	Depressive symptoms	TBV, mL	Lacunes	WMH Volume ▲	NAWM mean MD	WMH mean MD	WM mean MD	NAWM mean FA	WMH mean FA
Age	…										
Education	-0.20**	…									
Depressive Symptoms	0.01	- 0.21**	…								
TBV, mL	-0.62**	0.13*	0.02	…							
Lacunes, (p)	0.18**	- 0.08	0.04	-0.27**	…						
WMH Volume	0.47**	- 0.18*	0.05	-0.39**	0.40**	…					
NAWM mean MD	0.26**	-0.04	-0.01	-0.63**	0.30**	0.57**	…				
WMH mean MD	0.41**	-0.11*	0.02	-0.58**	0.28**	0.57**	0.73**	…			
WM mean MD	0.27**	-0.18**	0.00	-0.63**	0.30*	0.61**	1.00**	0.86**	…		
NAWM mean FA	-0.26**	0.14*	-0.09	0.20**	-0.22**	-0.53**	-0.73**	-0.68**	-0.74**	…	
WMH mean FA	-0.29**	0.14*	-0.12*	0.26**	-0.23**	-0.40**	-0.42**	-0.55**	-0.43**	0.58**	…
WM mean FA	-0.26**	0.14*	-0.09	0.20**	-0.22**	-0.53**	-0.73**	-0.68**	-0.74**	1.00**	0.59**

*correlation is significant at 2-tailed p<0.05; **p<0.001. For binary characteristics a Spearman Rho is used, whereas for the continuous variables a Pearson Correlation coefficient is shown. Education: only primary education, Depressive symptoms: presence of depressive symptoms, TBV: total brain volume, Lacunes: presence of lacunes, WMH: White matter hyperintensities, ▲ log transformed, NAWM: normal appearing white matter, MD: mean diffusivity, FA: Fractional Anisotropy, WM: white matter. Note the inclusion of all the DTI measures (FA and MD within WMH and NAWM) in the matrix, though these measures were not included together in a regression model.

Part III:

Depressive symptoms in cerebral SVD and its relation with cognition



6.

Depressive symptoms and amygdala volume in elderly with cerebral small vessel disease

Ingeborg W.M. van Uden, Anouk G.W. van Norden, Karlijn F. de Laat, Lucas J.B. van Oudheusden, Rob A.R. Gons, Indira Tendolkar, Marcel P. Zwiers and Frank-Erik de Leeuw

Journal of Aging Research 2011;(1-7):647869

Abstract

Introduction: Late onset depressive symptoms (LODS) frequently occur in elderly with cerebral small vessel disease (cSVD). cSVD cannot fully explain LODS, therefore a contributing factor could be amygdala volume. We investigated the relation between amygdala volume and LODS, independent of cSVD in 503 participants with symptomatic cerebral SVD.

Methods: Patients underwent FLAIR and T1 scanning. Depressive symptoms were assessed with structured questionnaires, amygdala and WML were manually segmented. The relation between amygdala volume and LODS and early onset depressive symptoms (EODS) was investigated and adjusted for age, sex, intracranial volume(ICV) and cSVD.

Results: Participants with LODS had a significantly lower left amygdala volume than those without (1.6 mL (SD 0.3) vs. 1.8 mL (SD 0.5); p = 0.02), independent of cSVD. Each decrease of total amygdala volume (by mL) was related to a increased risk of LODS (OR = 1.77; 95%CI 1.02-3.08; p = 0.04).

Conclusion: Lower left amygdala volume is associated with LODS, independent of cSVD. This may suggest differential mechanisms, in which individuals with a small amygdala might be vulnerable to develop LODS.

Introduction

A first depressive episode at older age is defined as late onset depressive symptoms (LODS). LODS are usually defined by their occurrence after the age of 60 years, while early onset depressive symptoms (EODS) appear before. The prevalence of LODS and EODS ranges from 10% to 32% in the elderly population.[67,142-144] Patients with EODS show a higher rate of family history of depression than patients with LODS, therefore genetic factors appear to be important. While psychological and genetic factors presumably play an important role in EODS,[38] there are probably other non-genetic factors that play a role in LODS such as structural changes in the brain.[37,38,145,146]

Population-based neuroimaging studies suggest a possible role for frequently occurring white matter lesions (WMLs) in the aetiology of LODS.[36,147-149] Their dominant view is that WMLs disrupt white matter tracts connecting cortical and subcortical structures (e.g., fronto-striatal circuits), which result in LODS.[150,151]

As WML are part of the cerebral small vessel disease (cSVD) spectrum, also including lacunar infarcts, it could be that the association between WML and LODS is driven by lacunar infarcts as they reflect more severe structural damage than WML. In addition, cSVD might not fully explain the presence of depressive symptoms in elderly as there are patients with LODS without cSVD.

A functional or structural change in the amygdala may explain the residual depressive symptoms as the amygdala is involved in mood regulation, and structural MRI studies showed a lower amygdala volume in patients with both LODS and EODS than in healthy controls.[152-155] However, these studies did not adjust for the possible confounding role of cSVD. Conversely, none of the population-based studies investigating cSVD and LODS, took amygdala volume into account. We therefore aimed to investigate the association between amygdala volume and the presence of depressive symptoms in older adults with cSVD, with adjustment for degree of this cSVD.

Patients and Methods

Study population

The Radboud University Nijmegen Diffusion tensor and Magnetic resonance imaging Cohort (RUN DMC) study is a prospective cohort study that investigates the risk factors and cognitive, motor, and mood consequences of functional and structural brain changes among elderly with cerebral SVD.

Cerebral SVD is characterized on neuroimaging by either WML or lacunar infarcts. Symptoms include acute symptoms, such as transient ischemic attacks (TIAs) or lacunar syndromes, or sub acute manifestations, such as cognitive, motor (gait) and/or mood disturbances.[34] As the onset of cerebral SVD is often insidious, clinically heterogeneous and typically with mild symptoms, it has been suggested that the selection of subjects with cerebral SVD in clinical studies should be based on the more consistent brain imaging features.[61]

Accordingly, in 2006, consecutive patients who visited the department of neurology, between October 2002 and November 2006, were selected for participation. Inclusion criteria were (a) age between 50 and 85 years; (b) cerebral SVD on neuroimaging (WML and/or lacunar infarcts). Subsequently the above-mentioned acute or sub acute clinical symptoms of SVD were assessed by standardized structured assessments. Patients who were eligible because of a lacunar syndrome were included only >6 months after the event to avoid acute effects on the outcomes.

Exclusion criteria were (a) dementia and (b) Parkinson(ism) according to the international diagnostic criteria;[85,156,157] (c) life expectancy of less than six months; (d) intracranial space occupying lesion; (e) (psychiatric) disease interfering with cognitive testing or follow-up; (f) recent or current use of acetylcholine-esterase inhibitors, neuroleptic agents, L-dopa, or dopa-a(nta)gonists; (g) WML mimics (e.g., multiple sclerosis and irradiation induced gliosis); (h) prominent visual or hearing impairment; (i) language barrier; (j) MRI contraindications or known claustrophobia.

Patients were selected in a three-step approach. After reviewing medical records 1004 individuals were invited by letter, 727 were eligible after contact by phone, and 525 agreed to participate. In 22 subjects exclusion criteria were found during their visit to our research centre (14 with unexpected claustrophobia, one died before MRI scanning, one was diagnosed with multiple sclerosis, in one there was a language barrier, one subject fulfilled the criteria for Parkinson's disease, and four met the dementia criteria), yielding a response of 71.3% (503/705). From these 503 individuals one group had symptoms of TIA or lacunar syndrome (*n* = 219), and the remaining (*n* = 284) had cognitive disturbances, motor disturbances, depressive symptoms, or a combination thereof. All participants signed an informed consent form. The Medical Review Ethics Committee region Arnhem-Nijmegen approved the study.

MRI acquisition

Imaging was performed on a 1,5 Tesla MRI scanner (Magnetom, Sonata; Siemens Medical Solutions, Erlangen, Germany). The protocol included 3D MPRAGE imaging (TR/TE/TI 2250/3.68/850 ms; flip angle15°; voxel size 1.0 × 1.0 × 1.0mm) and fluid attenuated inversion recovery (FLAIR) sequences (TR/TE/TI 9000/84/2200 ms; voxel size 1.0×1.2×5.0 mm, with an interslice gap of 1.0 mm). All scans were performed on the same scanner.

Amygdala volumetry

One investigator, blinded to the clinical and other imaging data (IvU), performed manual segmentation of the amygdala, using the interactive software program "ITK-SNAP"[88] (http://www.itksnap.org/). Briefly, this program allowed simultaneous viewing of volumes in coronal sagittal and transversal view, thereby permitting a neat handling of anatomical borders while segmenting the regions of interest. Left and right amygdalae were manually segmented in the coronal plane, from posterior to anterior. Next, segmentations were reviewed in the sagittal plane, because then boundaries were better visualized.[158-160] Segmentation was

performed according to previously published protocols,[160,161] and the correct segmentation of anatomical boundaries was [161,162] In short, the first slice of the amygdala, the posterior border, was identified superior to the hippocampus at the point where the white matter first starts to appear superior to the alveus and laterally to the hippocampal head. The anterior border of the amygdala was defined at the level where the amygdala no longer has an ovoid shape. The medial border is marked by the medial margin of the temporal lobe, which borders cerebrospinal fluid (CSF). The lateral/inferior border is the surrounding white matter and the inferior horn of the lateral ventricle. The amygdala and hippocampus were carefully separated on the sagittal view, moving from the medial to the lateral side of the brain.
Segmentations were done in a standardized way by rating the left amygdala first for half of the participants and the right amygdala first for the other half. Volume was calculated for the left and right amygdala separately by summing all segmented areas, multiplied by slice thickness. Intrarater on a random sample yielded an intra-class correlation coefficient for both left and right amygdala of 0.8.

WML volumetry and lacunar infarcts

White matter signal hyperintensities on FLAIR scans, which were not, or only faintly hypointense on T1-weighted images, were considered WML, except for gliosis surrounding infarcts. WMLs were manually segmented on transversal FLAIR images, by 2 trained raters, (IvU, LvO), blinded for all clinical data and amygdala volumes. Total WML volume was calculated in the same fashion as for both amygdalae. Inter-rater variability for total WML volume was determined on a random sample of ten percent and yielded an intra-class correlation coefficient of 0.99.
Lacunar infarcts were defined as areas with a diameter >2mm and <15mm with low signal intensity on FLAIR and T1, ruling out enlarged perivascular spaces and infraputaminal pseudolacunes.[163] Evaluation of infarcts was performed by one person, with a good intra-rater variability with a weighted kappa of 0.80. In ten percent of the scans inter-rater variability was calculated with a weighted kappa of 0.88.

Brain volumetry

Gray (GM), white matter (WM) tissue and CSF probability maps were computed using SPM5 routines (Wellcome Department of Cognitive Neurology, University College London, UK). Total GM, WM and CSF volumes were calculated by summing all voxel volumes that had a $p > 0.5$ for belonging to the tissue class. Intra-cranial volume (ICV) was taken as the sum of total GM, WM and CSF.

Assessment of depressive symptoms

Depressive symptoms were assessed with the Centre of Epidemiologic Studies Depression Scale (CES-D).[164] Depressive symptoms were considered present in patients with a CES-D score ≥ 16 and/or current use of anti-depressive medication, taken for depression, irrespective of

their actual CES-D score, because depressive symptoms were considered to be the indication for the medication prescription.
In addition, all patients were asked about their history of depressive episodes. If depressive episodes had occurred, the patients were asked for the age of onset and whether the episodes had prompted them to seek medical advice. A history of depression was considered present if depressive episodes in the past had required attention of a general practitioner, psychologist, or psychiatrist.[67]
According to the literature we used the age of 60 years as cut-off point to distinguish between LODS and EODS.[67] Patients at the age or older than 60 years with a CES-D ≥ 16 and/or current use of anti-depressive medication, taken for depression, without a history of depressive episodes before or at the age of 60, were classified as having LODS. Individuals with a CESD < 16, without a history of depressive symptoms and without the current use of anti-depressive medication, formed the reference group. All others fulfilled the criteria for EODS (first depressive episode <60 years).

Statistical analysis

We compared the amygdala and WML volumes (overall, left, right) between the EODS and LODS group with the reference using ANCOVA. Adjustments for age, sex, ICV, WML (or amygdala volume with WML being dependent variable) and presence of lacunar infarcts were made.
The risk of LODS and EODS per millilitre increases in amygdala volume, WML volume, and presence (yes/no) of lacunar infarcts was calculated (expressed as the odds ratio (OR) with a 95% confidence interval) by means of age, sex, ICV, adjusted logistic regression analysis with additional adjustment for the appropriate structural MRI measures (WML volume, lacunar infarcts or amygdala volume). All data were analyzed using SPSS statistical software, version 16.0. P-values < 0.05 were considered statistical significant.

Results

Of the 503 patients one was excluded because of an automatic segmentation problem that could not be solved manually. Two patients did not complete the CES-D questionnaire and of two patients the history of depression was not known. There were 101 individuals with LODS (20.3%) and 108 with EODS (21.7%); the reference group comprised 289 persons (58.0%).
Demographic and neuroimaging characteristics of 498 patients are shown in Table 1. Mean age of the population was 65.6 years (SD 8.8), and 56.4% were male. Mean age of the LODS group was 71.4 years and of the EODS group 59.1 years. Mean age of onset of EODS was 45.1 years (SD 10.9).
Total amygdala volume was 3.4mL (SD 0.5), and mean volume of left amygdala was 1.8mL (SD 0.3) and differed significantly ($P < 0.001$), from the right amygdala 1.6 mL (SD 0.3). Amygdala volume decreased significantly with age ($\beta = -0.34$; $P < 0.001$), and women had smaller amygdala (3.2 mL; SD 0.4) than men (3.5 mL; SD 0.5; $P < 0.001$).

Table 1: Baseline Characteristics of patients with Late Onset Depressive Symptoms (LODS), Early Onset Depressive Symptoms (EODS) and patients without depressive symptoms

Depressive symptoms	Late onset	Early onset	Reference	Overall
	(n=101) (20.3%)	(n=108) (21.7%)	(n=289) (58.0%)	(n=498) (100%)
Demographics				
Age, yrs, (SD)	71.4 (6.0)	59.1 (6.2)	65.9 (9.0)	65.6 (8.8)
Sex (male/female)	47/54	60/48	174/115	281/217
MSSE, (SD)	27.4 (1.7)	28.4 (1.5)	28.3 (1.6)	28.1 (1.6)
Education (> primary school (%))	77.2%	95.4%	93.4%	90.6%
Mean CES-D, (SD)	21.0 (8.0)	16.9 (11.0)	7.7 (5.0)	12.4 (9.3)
Use of anti-depressive medication, no.	30 (29.7%)	30 (27.8%)	0 (0%)	60 (12.0%)
imaging characteristics				
WM volume, mL (SD)	438.4 (57.1)	484.7 (62.9)	465.3 (68.8)	464.1 (66.9)
GM volume, mL (SD)	597.4 (66.7)	649.8 (60.5)	632.3 (65.8)	629.0 (67.1)
ICV, mL (SD)	1660.8 (156.1)	1659.9 (146.5)	1687.9 (159.4)	1676.3 (156.3)
Median WML volume, mL	16.8	4.5	8.0	8.0
Number of lacunar infarcts	40 (39.6%)	30 (27.8%)	101 (34.9%)	171 (34.3%)

Number represent mean (SD) or number. MMSE: Mini Mental State Examination; CES-D: Centre for Epidemiological Studies Depression Scale; WM: white matter; GM: gray matter; ICV: intra cranial volume; WML: white matter lesions

Table 2 shows that patients with LODS had a higher WML volume (21.8mL; SD 20.2) than the reference group (14.7mL; SD 18.0), although not significant (*P* = 0.06). Independent of cSVD, patients with LODS had a significant lower left amygdala volume (1.6mL; SD 0.3; *P* = 0.017) than the reference group (1.8 mL; 0.28); this difference was not found for the right amygdala (1.5mL; SD 0.3, *P* = 0.432).

Patients with EODS did not differ from the reference group with respect to WML volume, amygdala volume and the proportion of lacunar infarcts. We found no significant differences between left, right and total amygdala volume, WML volume and presence of lacunar infarcts between patients with LODS and EODS.

Table 3 shows the risk of LODS and EODS and per mL decrease in total, left, and right amygdala volume, WML volume (mL), and presence of lacunar infarcts. Each decrease of both total and left amygdala volume (mL) showed a significant increased risk of the presence of LODS (OR = 1.77; 95%CI 1.02–3.08; *P* = 0.04, in total amygdala volume and OR 2.92; 95%CI 1.22–7.01; *P* = 0.02 in left amygdala volume), independent of cSVD. In addition, there was a nearly significant (*P* = 0.08) increased risk for LODS per increase of WML volume. This was not found for EODS and decrease of amygdala volume (OR = 0.95; 95CI 0.56–1.61; *P* = 0.86) per increase of WML volume, or presence of lacunar infarcts.

Table 2: Adjusted mean Amygdala volume, White Matter Lesions (WML) volume and Lacunar Infarcts in patients with Late Onset Depressive Symptoms (LODS), Early Onset Depressive Symptoms (EODS) and patients without depressive symptoms

	Patients with LODS (n=101)	**p-value †**	**Patients with EODS (n=108)**	**p-value †**	**Reference group (n=289)**
Total amygdala volume(mL) *	3.2 (0.5)	0.05	3.5 (0.5)	0.82	3.4 (0.5)
Left amygdala (mL)*	1.6 (0.3)	**0.02**	1.8 (0.3)	0.71	1.8 (0.28)
Right amygdala (mL)*	1.5 (0.3)	0.43	1.7 (0.3)	0.90	1.6 (0.28)
WML volume (mL)**	21.8 (20.2)	0.06	9.5 (13.8)	0.94	14.7 (18.0)
Lacunar infarcts (%)***	32.9%	0.42	35.8%	0.47	32.0%

Number represent mean (SD) or number. Data is shown of the comparison of LODS/EODS versus the reference group * adjusted for age, sex, ICV , WML volume and presence of lacunar infarcts , ** adjusted for age, sex, ICV, presence of lacunar infarcts and amygdala volume, *** adjusted for age, sex, ICV, WML and amygdala volume. † compared with the reference group.

Table 3: The risk of LODS and EODS per decrease in amygdala volume, white matter lesion (WML) volume and lacunar infarcts.

	Patients with LODS (n=101)		Patients with EODS (n=108)	
	OR	**95% CI**	**OR**	**95% CI**
Total amygdala volume*	1.77	1.02 - 3.08 †	0.95	0.56 – 1.61
Left amygdala volume*	2.92	1.22 – 7.01 †	0.98	0.41 – 2.30
Right amygdala volume*	1.53	0.59 – 4.03	0.85	0.34 – 2.14
WML volume**	0.99	0.97 – 1.00	1.01	0.99 – 1.03
Lacunar infarcts ***	1.32	0.78 – 2.23	0.80	0.46 – 1.39

Number represent odds ratio (OR), 95% confidence interval (CI); * adjusted for age, sex, WML, ICV volume and presence of lacunar infarcts; ** adjusted for age, sex, total amygdala volume, ICV and presence of lacunar infarcts; *** adjusted for age, sex, ICV, WML and amygdala volume; † significance $p < 0.05$

Discussion

In this study of 498 elderly patients with cerebral SVD, left amygdala volume was related to LODS, independent of WML volume and the presence of lacunar infarcts; this was not found for EODS and amygdala volume (left, right nor total).

Before conclusions can be drawn, there are some methodological issues that need to be addressed. The proportion of patients with depressive symptoms in our study is relatively high (42%) for LODS and EODS together. In other studies the proportion of patients with depressive symptoms varies between 9.6%–32%.[67,142-144,165] A possible explanation for our relatively high proportion of patients with depressive symptoms could be the fact that we purposely included patients on the basis of presence of cSVD; consequently the median degree of WML volume in our study is higher than in population-based studies.[6] As these lesions are related to LODS it seems reasonable to expect a concomitant increased presence of depressive symptoms. Our finding of the (borderline significant) association between WML volume and LODS is in line with findings from these population-based studies.[67,142,166] Another explanation is that we classified patients as suffering from depressive symptoms once they had had a depressive episode in their medical history or when they used anti-depressive drugs at baseline examination (while previous studies usually did not assess detailed information on the use of medication).[167] The third explanation could be that we included patients with a history of lacunar stroke and transient ischemic attacks. It is known that in this population the prevalence of depressive symptoms is higher compared to the general population.[168]

To elucidate the etiological mechanisms of LODS its current widely used definition suffers from a conceptual problem, due to the fact that age during the first depressive episode determines the classification. It could very well be that recovery after EODS occurs while depressive symptoms develop again after sixty years of age. Despite the fact that these

patients still fulfil the definition of EODS because of their history, they may have developed their LODS on the basis of another underlying pathology including cSVD. This could have led to overrepresentation of WML among the EODS group.

There is a conceptual problem with LODS and EODS. By definition the EODS sample included many subjects with major depression. All had consulted a doctor or were at one time treated with antidepressants. By contrast, the LODS sample was defined primarily by the CES-D score (and/or current antidepressant use). Some may have had major depression and some minor depression. These potential differences may affect results both ways.

Another limitation is the cross-sectional nature of our study, which prevents us from proving causality. The RUN DMC study has a longitudinal design, and follow-up is already planned to evaluate the effect of brain changes on depressive symptoms.[60]

Strengths of our study include its design of a homogeneous population that covers the whole spectrum of cerebral SVD, its size and high response rate of over 70%, and the use of a single expert who segmented the amygdala, blinded to clinical information. Particularly the definition of the anatomic boundaries of the amygdala is a notorious problem in amygdala segmentation. Our use of one single, experienced rater minimized the effect of differential segmentation between several raters thereby limiting the effect misclassification. Our results are in line with those from meta-analyses on amygdala volume in nonclinical samples that found a mean volume of both left and right amygdala in 39 studies of 1.7 mL, with a range from 1.0 to 3.9mL.[161]

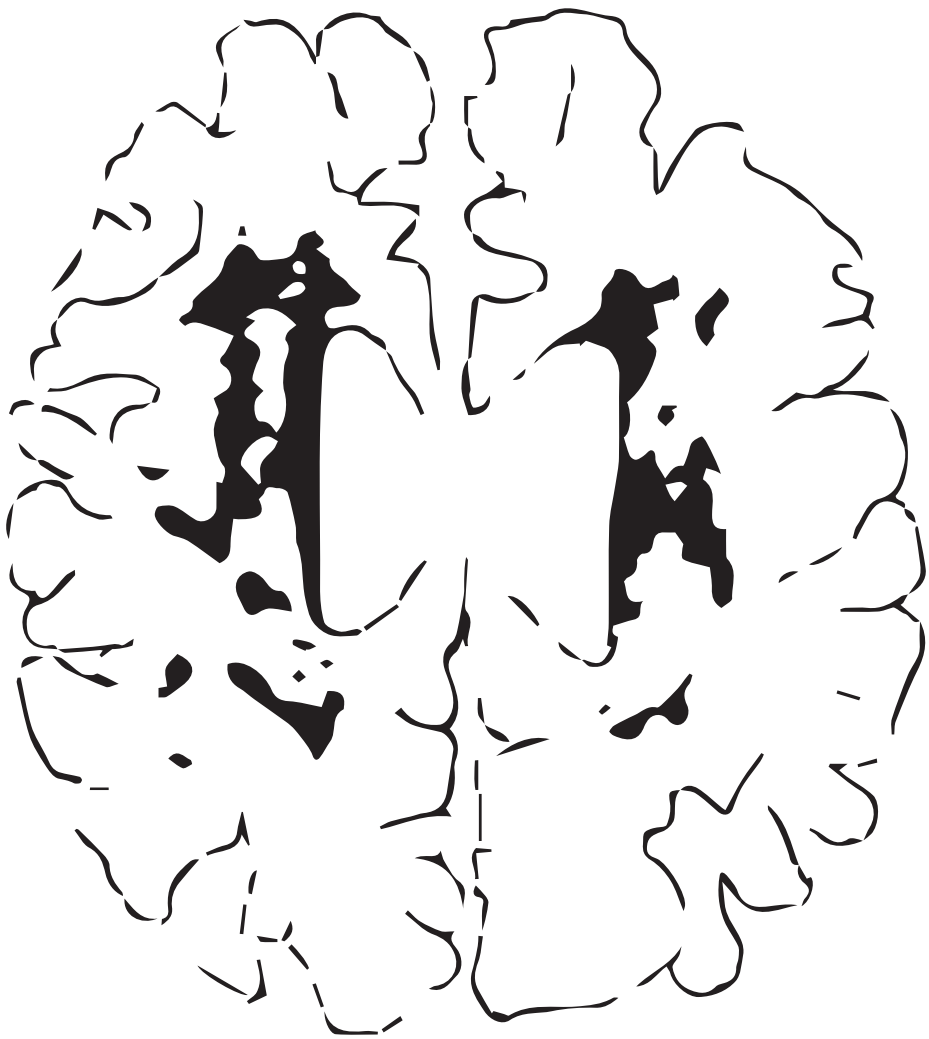
Although the amygdala is critical to the interpretation of emotion[153,154] and implicated in mood disorders, volumetric studies of the amygdala in patients with mood disorders have provided inconsistent results.[155] Studies of chronic or recurrent depressive patients have found identical,[169,170] smaller[171-173] but also larger[174] amygdala volumes compared to controls. Post-mortem studies showed a smaller amygdala in depressed patients compared to controls, probably due to fewer glial cells.[152]

Our data showed a significant relationship with LODS and left amygdala volume; we did not find this relation with the volume of the right amygdala. This is concordant with previous neuroimaging and post-mortem studies that have reported left lateralized atrophy in the prefrontal cortex and amygdala in mood disorders,[152,173,175,176] however previous results are inconclusive as others also report on an association between a decrease in right amygdala volume and mood.[172] In addition some functional imaging studies have shown lateralization, with activation of the left amygdala, in relation to emotion and emotional information processing.[177,178]

A possible explanation for a smaller amygdala in patients with LODS could be the coexistent cSVD, abnormalities in blood flow, metabolism, and neurotransmitter receptors[179,180] which may, either directly or indirectly, lead to amygdala atrophy. Intact connectivity of the fronto-striatal circuits is important in mood regulation.[151,181] According to the “vascular depression” hypothesis[151,166] cSVD, that tends to have a high prevalence in the fronto-striatal regions, disrupts fibre tracts within these circuits, probably leading to depressive symptoms.[36,166]

There are two other studies that investigated the role of amygdala morphometry in LODS patients. One study showed that, despite insignificant amygdala volumetric findings, variations of amygdala shape can be detected and localized.[182] They investigated so in 11 healthy elderly individuals and 14 depressed elderly individuals. A population-based cohort study found a relation between a history of depression, particularly early onset, and an increased risk of for Alzheimer's disease; however this risk was not mediated by smaller hippocampal or amygdala volumes at baseline[165]. In contrast to our results they did not find a relation between a history of depression or depressive symptoms at baseline and smaller amygdala volume. As discussed earlier, this could be because of the difference in degree of cSVD between this population-based cohort and our study sample in which we included only subjects with some degree of cSVD. As amygdala volume is related to the degree of cSVD,[183] our study sample will probably have smaller amygdalae. In addition there was a difference in WML segmentation, most studies used semi-quantitative methods, and in contrast we manually segmented the WML volume. This in combination with the higher proportion of depressive symptoms in our study sample (42% versus 27%) can explain the relation we found between amygdala volume and LODS in contrast to the findings of this population-based cohort. Finally, the relation between LODS and amygdala volume in our cohort was mainly driven by the left amygdala, while this population-based cohort summed both amygdalae in their analysis.

In conclusion, amygdala volume is associated with LODS, independent of cSVD and not with EODS. Future research should consist of prospective studies in order to assess whether baseline presence of cSVD increases the risk of amygdala atrophy at follow-up and whether this coincides with LODS. Innovative MRI techniques including diffusion tensor imaging (DTI) could offer promising tools in order to identify white matter tracts between the amygdala and other parts of the brain and the effects of cSVD in those tracts with respect to the incidence of LODS.



7.

White matter integrity and depressive symptoms in cerebral small vessel disease

Ingeborg W.M. van Uden*, Anil M. Tuladhar*, Karlijn F. de Laat, Anouk G.W. van Norden, David G. Norris, Ewoud J. van Dijk, Indira Tendolkar and Frank-Erik de Leeuw

American journal of geriatric psychiatry 2015;23(5):525-35

**both authors contributed equally*

Abstract

Objectives: Depressive symptoms are common in elderly with cerebral small vessel disease (cSVD). As not every individual with cSVD experiences depressive symptoms, other factors might play a role. We therefore investigated the white matter (WM) integrity of the white matter tracts in elderly in elderly with depressive symptoms, independent of global cognitive function, by applying the tract-based spatial statistics (TBSS).

Design: Prospective cohort study with cross-sectional baseline data.

Setting: Radboud University Nijmegen Medical Centre, The Netherlands.

Participants: 438 individuals between 50-85 years, with cSVD without dementia.

Measurements: Diffusion tensor imaging parameters and depressive symptoms, assessed with the Centre of Epidemiologic Studies Depression Scale.

Results: Compared to non-depressed participants (n=287), those with depressive symptoms (n=151) had lower fractional anisotropy in the genu and body of the corpus callosum, bilateral inferior fronto-occipital fasciculus, uncinate fasciculus and corona radiata. These differences disappeared after adjustment for white matter hyperintensities (WMH) and lacunar infarcts. Mean-, axial- and radial diffusivity were higher in these areas in participants with depressive symptoms. After additional adjustment for WMH and lacunar infarcts, the changes observed in radial diffusivity also disappeared. Adding global cognition, as confounding variable altered the diffusion parameters only slightly.

Conclusions: This study indicates that elderly with depressive symptoms show a lower WM integrity, independent of global cognitive function, and that the presence of cSVD is mostly responsible, affecting the fronto-subcortical regions and hereby disrupting the neural circuitry involved in mood regulation.

Introduction

Cerebral small vessel disease (cSVD), including amongst others white matter hyperintensities (WMHs) and lacunar infarcts, is commonly seen in the elderly.[6] Cognitive and motor impairment are known consequences of cSVD. The relation between cSVD and depressive symptoms is however less known.[36,67,184] Depressive symptoms are also common in the elderly, with a prevalence ranging from 1% to 35%.[67,185] cSVD might cause disruption of the fronto-striatal white matter tracts of the neural circuit mediating emotion perception and mood regulation, and thereby increases the risk of developing depressive symptoms in an older individual.[39,186] However, as not every individual with cSVD experiences depressive symptoms, other factors apart from cSVD-related lesions seen on conventional MRI might be related to depressive symptoms at older age. One of these factors could be damage to the structural integrity of the normal-appearing white matter (NAWM) surrounding the WMH, which cannot be assessed by conventional MRI.

This microstructural organization of the WM, including the NAWM can now be assessed by diffusion tensor imaging (DTI), an MRI technique, that provides valuable information on the microstructural organization of the WM, including the NAWM by measuring the molecular motion (diffusion) of water in biological tissue.[80] Several common DTI-derived indices can be measured indexing global characteristics of WM integrity, such as fractional anisotropy, mean, radial and axial diffusivity and mode of anisotropy.

To date, relatively few DTI studies have examined the microstructural integrity of WM in patients with depressive symptoms later in life.[187-190] These studies reported compromised integrity of the WM in regions involved in emotional processing. However, these studies did not have large sample size (n<48), only used the fractional anisotropy as a global measure, and did not adjust for the possible confounders such as WM lesions and cognitive performance.[187,191,192] Tract Based Spatial Statistics (TBSS) can be used to investigate the structural integrity, by projecting the DTI indices on a virtual skeleton that is located at the centre of the white matter tracts. So far, few studies have performed TBSS in patients with depressive symptoms.[193,194] Although they found a lower fractional anisotropy in bilateral frontal, right temporal and midbrain in depressed participants, these studies also have limitations, such as relative small sample sizes and not adjusting for possible confounders such as cognition.

Our aim was to investigate the regional differences in microstructural integrity of the white matter tracts in a large sample of elderly people with cSVD and with or without depressive symptoms. We investigated the full spectrum of DTI-derived indices using TBSS. We hypothesized that participants with depressive symptoms have a compromised microstructural integrity (low fractional anisotropy and high mean diffusivity) of the WM compared with participants without depressive symptoms, located in critical regions for emotional processing. Additionally we hypothesized that these compromised regions are partially explained by the presence of cSVD and independent of global cognition.

Materials and Methods

Study sample

This study sample is embedded within the Radboud University Nijmegen Diffusion tensor and MRI Cohort (RUN DMC) study that prospectively investigates risk factors and cognitive, motor and mood consequences of brain changes, as assessed by MRI in elderly with cSVD.[60] On the basis of established research criteria cSVD was defined as the presence of lacunar infarcts and/or WMH on neuroimaging.[61] The presence of any of these manifestations of cerebral SVD qualified for the cut-off for presence of cSVD. The selection procedure of the participants and the study rationale and protocol were described previously in detail .[60] In short, in 2006, consecutive patients referred to the Department of Neurology between October 2002 and November 2006, were selected for participation. Inclusion criteria were: (a) age between 50 and 85 years; (b) cerebral SVD on neuroimaging (WMH and/or lacunar infarcts). Exclusion criteria were: (a) dementia[195] (b) Parkinson(-ism)[156](c) life expectancy of less than six months; (d) intracranial space occupying lesion; (e) current (psychiatric) disease interfering with cognitive testing or follow-up, serious mental illness in their history, or a current depression (f) recent/current use of acetylcholine-esterase inhibitors, neuroleptic agents, L-dopa or dopa-a(nta)gonists; (g) WMH or cSVD mimics (e.g. multiple sclerosis and irradiation induced gliosis); (h) prominent visual or hearing impairment; (i) language barrier; (j) MRI contraindications or known claustrophobia. This study comprises of 503 elderly without dementia or parkinsonism, aged between 50 and 85 years with cerebral cSVD. For the present study, 65 participants were additionally excluded because of the presence of territorial infarcts (n = 55), inadequate quality of the MRI image (n=4) and incomplete data on neuropsychological tests (n=6). This study was approved by the Medical Review Ethics Committee region Arnhem-Nijmegen and all participants gave written informed consent prior to inclusion.

Assessment of depressive symptoms

Depressive symptoms were assessed with the Centre of Epidemiologic Studies Depression Scale (CES-D).[196] Depressive symptoms were considered present in patients with a CES-D score ≥ 16 and/or current use of anti-depressive medication, taken for depression, irrespective of their actual CES-D score, because depressive symptoms were considered to be the indication for the medication prescription. Those who mentioned psychiatric diagnoses during medical history taking and/or when this became apparent when retrieving the information form medical records, were excluded from participation, apart from those with a diagnosis of depression in their history. A total of 53 participants used antidepressants: 30 participants had a CES-D score ≥ 16, while other participants had a CES-D score < 16. All participants were taking antidepressant for depression (and not for other reasons such as anxiety, insomnia or neuropathic pain). All other participants in the RUN DMC study sample were considered as the comparison non-depressed group.

MRI acquisition

MRI scans of all participants were acquired on a single 1.5-Tesla MRI (Magnetom Sonata, Siemens Medical Solutions, Erlangen, Germany). The protocol included, among other sequences, 3-D T1 MPRAGE (TR/TE/TI 2250/3.68/850ms; flip angle15°; voxel size 1.0 x 1.0 x 1.0mm), a FLAIR pulse sequence (TR/TE/TI 9000/84/2200ms; voxel size 1.0 x 1.2 x 5.0mm, interslice gap 1mm) and a DTI sequence (TR/TE 10100/93ms voxel size 2.5 x 2.5 x 2.5 mm, 4 unweighted scans, 30 diffusion weighted scans with b-value of 900 s/mm^2).[60]

Conventional MRI analysis

WMHs were manually segmented on FLAIR images by two trained raters and the total WMH volume was calculated by summing the segmented areas multiplied by slice thickness.[60] WMHs were defined as hyperintense lesions on FLAIR MRI without corresponding cerebrospinal fluid like hypo-intense lesions on the T1 weighted image. Gliosis surrounding lacunar and territorial infarcts is not considered to be WMHs.[163] Inter-rater variability (assessed by intra-class correlation coefficient) for total WMH volume was 0.99. Lacunar infarcts are defined as hypo-intense areas greater than 2 mm and less than or equal to 15mm on FLAIR and T1, ruling out enlarged perivascular spaces (≤ 2 mm, except around the anterior commissure, where perivascular spaces can be large) and infraputaminal pseudolacunes. [163] The intra- and inter-rater reliability for the lacunar infarcts yielded a weighted kappa of 0.80 and 0.88.[60] To obtain the grey matter (GM), WM and cerebrospinal spinal fluid (CSF) volume, the T1 MPRAGE images were segmented using Statistical Parametric Mapping 5 unified segmentation routines. Total GM, WM and CSF volumes were subsequently calculated by summing all voxel volumes that had a $p > 0.5$ for belonging to that tissue class. The sum of GM and WM volume was considered as total brain volume (TBV). The intracranial volume (ICV) was a summation of all tissue classes. To normalize for the head size, TBV was expressed as percentage of total ICV.

DTI analysis

The in-house developed algorithm named 'PATCH' (www.ru.nl/neuroimaging/diffusion)[91] was employed to the raw diffusion data to detect and correct head and cardiac motion artefacts using an iteratively re-weighted-least-squares algorithm. Corrections of eddy current and motion artefacts from affine misalignment were performed simultaneously, which was based on minimization of the residual diffusion tensor error. Next, fractional anisotropy, mean diffusivity, axial and radial diffusivity, and mode of anisotropy images were calculated using DTIFit within the FSL toolbox, which were fed into the TBSS pipeline.[93] The thinning procedure was conducted on the mean fractional anisotropy image to create a common skeleton using a threshold value 0.3 for the fractional anisotropy, which represents the core-structure of the white matter tract. The threshold value 0.3 was chosen to include major white matter tracts and to reduce inter-subject variability. These skeleton projection vectors were then applied to the mean, radial and axial diffusivity, and mode of anisotropy.

Fractional anisotropy represents a normalized ratio of diffusion directionality and can be used as a measurement of structural integrity of the WM. Mean diffusivity, which measures the averaged diffusion in all directions, can be used as a marker for fibre density. A low fractional anisotropy and/or a high mean diffusivity is typically inferred to the loss of microstructural integrity of the WM, due to various underlying pathophysiological mechanisms. Axial diffusivity reflects the diffusivity parallel to the white matter tracts, while radial diffusivity measures the diffusivity perpendicular to these tracts. Changes in axial diffusivity may implicate axonal damage, whereas changes in radial diffusivity indicates loss of myelin integrity.[25] Mode of anisotropy denotes the shape of the diffusion tensor ranging from planar to linear anisotropy,[197] and can reflect selective degeneration of fibres and/or reorganization of these fibres. The investigation of these diffusivities and mode of anisotropy[197] may yield important information about these underlying mechanisms. Studies showed that DTI can be used to assess the cSVD-induced changes in the WM and that areas with WMH could have quite different DTI characteristics . To this end, a comprehensive analysis of the full spectrum of DTI-derived indices is necessary.

Other measurements

Age, sex, level of education,[65] normalized TBV and global cognitive function were considered as possible confounders. Global cognitive function was evaluated by the Mini-Mental State Exam (MMSE) [82] and the cognitive index . The cognitive index is a compound score that was calculated as the mean of the z-scores of the 1-letter subtask of the Paper-Pencil Memory Scanning Task, the mean of the reading subtask of the Stroop test, the mean of the Symbol-Digit Substitution Task and the mean of the added score on the three learning trials of the RAVLT and the delayed recall of this last test.[53] Global cognitive function was considered as a possible confounder. For assessment of vascular risk factors, structured questionnaires were used together with measurements of blood pressure taken on separate occasions. The risk factors included presence of hypertension (mean blood pressure ≥ 140/90 mmHg and/or use of anti-hypertensive medications), diabetes (treatment with anti-diabetic medications), hypercholesterolemia (treatment with lipid-lowering drugs) and smoking status.

Statistical analyses

Demographic and neuro-imaging characteristics in Tables 1 and 2 were tested through univariate analysis, by an independent t-test, a Chi-square test or a Mann-Whitney U test. When the Levene's test for equality of variances was significant, the significance of the difference between the groups was computed using Welch's ***t***-test (equal variances not assumed). To compare the DTI parameters (fractional anisotropy, mean, radial and axial diffusivity, and mode of anisotropy) between those with or without depressive symptoms, we performed two-sample t-test using a permutation-based statistical interference as a part of FSL toolbox ('randomise'), with a standard number of permutation tests set to 5000. Significant clusters were identified using the threshold-free cluster enhancement with a p-value less than 0.05,

family-wise error corrected to control for multiple comparisons. A stepwise analysis was performed. First, age, sex, educational level and normalized TBV were added as nuisance covariates when performing the group comparisons, to keep comparability with other studies. Second, the same analyses were done with additional adjustments for WMH volume and number of lacunar infarcts. Finally, we executed the analysis additionally adjusted for MMSE and cognitive index, to adjust for the possible confounding effect global cognition may have on the relation between microstructural integrity and depressive symptoms.

Results

Demographic, clinical and neuroimaging characteristics are shown in Table 1. Mean age did not significantly differ between participants with (65.6 years SD 8.6) or without depressive symptoms (64.8 years SD 8.9). There were no significant differences for total WMH volume, lacunar infarcts, and normalized TBV between the groups. In both groups, WMHs were predominately periventricular located - especially in the frontal regions - as shown in Figure 1.

A lower overall fractional anisotropy and a higher overall mean and radial diffusivity within the white matter skeleton was seen in participants with depressive symptoms compared to those without (see Table 2). Axial diffusivity did not differ significantly between the groups.

The regions of the significant differences between the groups for each DTI parameters are shown in Figure 2. Participants with depressive symptoms exhibited lower fractional anisotropy in the genu and the body of the corpus callosum, bilateral inferior fronto-occipital fasciculus, uncinate fasciculus and corona radiata, than those without. Figure 3 displays the location of these white matter tracts. These differences disappeared after adjustment for WMHs and lacunar infarcts. The mean, axial and radial diffusivity were significantly higher in participants with depressive symptoms in the genu and body of the corpus callosum, bilateral inferior fronto-occipital fasciculus, uncinate fasciculus, and corona radiate, than in participants without depressive symptoms. This was also seen for fractional anisotropy (Figure 2). In addition to these regions, the spatial patterns of these diffusivities changes also include bilateral anterior cingulum bundle, internal and external capsule, and right inferior longitudinal fasciculus and parietal lobe. The mean diffusivity remained significantly higher in the genu and body of corpus callosum, right anterior cingulum bundle, bilateral superior corona radiata after adjustment for WMH's and lacunar infarcts, as was the axial diffusivity in the right superior corona radiata and right superior longitudinal fasciculus. There were no regions with a higher mean, axial and radial diffusivity in patients without depressive symptoms. The changes observed for radial diffusivity disappeared after additional adjustment for WMH and lacunar infarcts. We observed a trend for lower mode of anisotropy in the body of corpus callosum, right superior corona radiata and right inferior fronto-occipital fasciculus not surpassing the P-value < 0.05 family-wise error-corrected (data not shown). Among those with depressive symptoms, there was no relationship between

Table 1: Baseline characteristics of the participants with and without depressive symptoms

	All participants (n=438)	participants without depressive symptoms (n = 287)	participants with depressive symptoms (n=151)	Significance
Demographic and clinical characteristics				
Age (SD)	65.1 (8.8)	64.8 (8.9)	65.6 (8.6)	p = 0.377 (a)
Men, no. (%)	239 (54,6)	169 (58.9)	70 (46.4)	p = 0.012 (b)
CES-D, (SD)	11.1 (9.4)	5.9 (4.5)	21.0 (8.3)	p < 0.001 (a)
Anti depressive medication, no. (%)	53	0	53	N.A.
Only primary education, no. (%)	41 (9.4)	19 (6.6)	22 (14.6)	p = 0.007 (b)
MMSE, (SD)	28.2 (1.6)	28.4 (1.5)	27.8 (1.7)	p < 0.001 (c)
Cognitive index, (SD)*	0.00 (0.45)	0.04 (0.43)	- 0.08 (0.47)	p = 0.006 (a)
Vascular risk factors				
Hypertension no. (%)	314 (71.7)	202 (70.4)	112 (74.2)	p = 0.403 (b)
Diabetes no. (%)	60 (13.7)	35 (12.2)	25 (16.6)	p = 0.207 (b)
Hypercholesterolemia no. (%)	192 (43.8)	118 (41.1)	74 (49.0)	p = 0.114 (b)
Smoking status no. (%) ∞	305 (69.7)	205 (71.4)	100 (66.2)	p = 0.351 (b)

Data represent number of participants, mean (standard deviation). MMSE: Mini Mental State Examination; CES-D: Centre of Epidemiologic Studies on Depression. Smoking status defined as current and former smoking. * 6 were additionally excluded because of incomplete neuropsychological data. Univariate analysis a) Independent T-test; df = 436. b) Chi-square test; df = 1, ∞ df = 2. c) Mann-Whitney U test. N.A. = not applicable

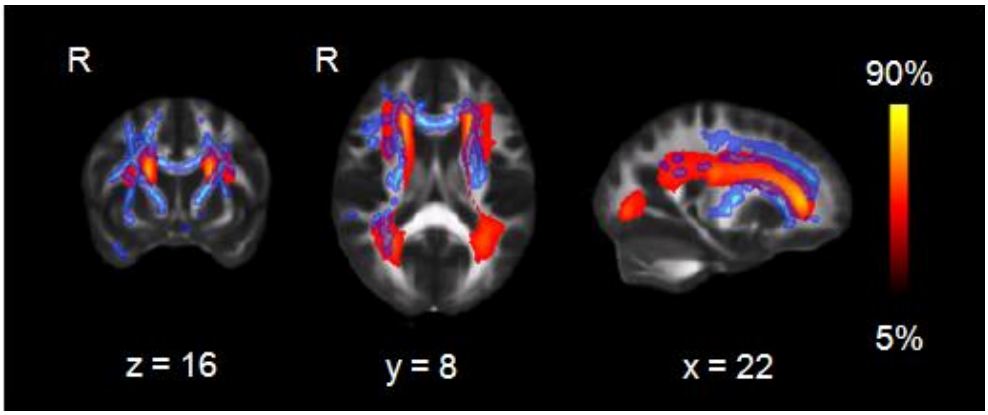


Figure 1: The probability distribution of the white matter hyperintensities

WMHs are in red and colour-coded in percent (colour bar), thresholded from 5 to 90%. These images are projected onto the spatially normalized (Montreal Neurological Institute stereotactic space) and averaged (n=438) structural map. The statistical map (mean diffusivity) of the group comparisons adjusted for age, sex, education level, is presented in blue.

Table 2: Neuro imaging characteristics of the participants with depressive symptoms and without depressive symptoms

	participants without depressive symptoms (n = 287)	participants with depressive symptoms (n=151)	significance
Neuro imaging			
WMH volume, mL† (IQR)	6.0 (3.2-15.3)	7.7 (3.3-20.7)	p = 0.127 (a)
Lacunar infarcts , presence, no. (%)	82 (28.6)	52 (34.4)	p = 0.205 (b)
Normalized TBV, mL (SD)	1105.7 (87.0)	1097.2 (85.6)	p = 0.332 (c)
Mean FA skeleton (SD)	0.423 (0.0292)	0.417 (0.0297)	**p = 0.037** (c)
Mean MD skeleton (SD)	8.14×10^{-4} (4.57×10^{-5})	8.23×10^{-4} (4.19×10^{-5})	**p = 0.038** (c)
Mean AD skeleton (SD)	1.20×10^{-3} (4.16×10^{-5})	1.20×10^{-3} (3.54×10^{-5})	p = 0.051 (c)
Mean RD skeleton (SD)	6.16×10^{-4} (5.14×10^{-5})	6.26×10^{-4} (4.86×10^{-5})	**p = 0.049** (c)

Date represent mean (Standard deviation). TBV: Total Brain Volume. WMH: White Matter Hyperintensities. † represents median, IQR: inter quartile range; TBV is normalized to the intracranial volume. A) Independent T-test; df = 436. b) Chi-square test; df = 1; c) Independent t-test.

severity of depressive symptoms (measured by CES-D score) and the DTI measures (fractional anisotropy and mean diffusivity).

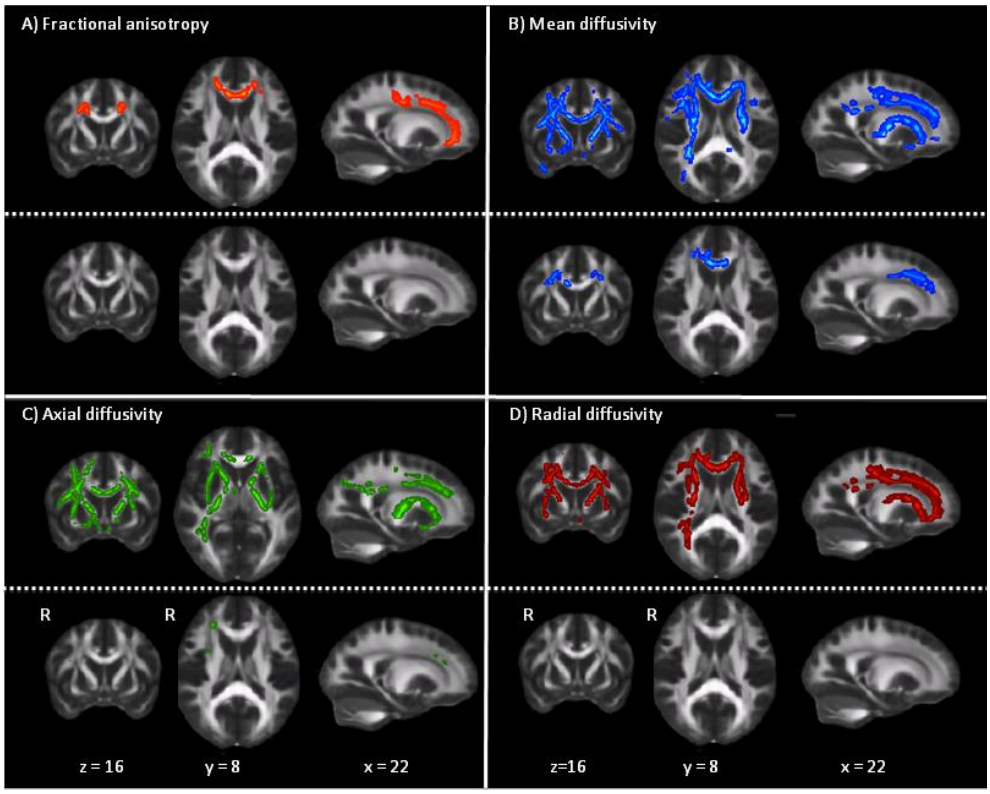


Figure 2: Spatially distributed differences in DTI parameters between participants with depressive symptoms and without depressive symptoms

Voxel-wise analysis of the DTI parameters, A) fractional anisotropy, B) mean diffusivity, C) axial diffusivity and D) radial diffusivity. Fractional anisotropy is reduced in frontal regions in participants with depressive symptoms, while the mean, axial and radial diffusivity is increased in mainly the frontal regions ($p<0.05$, family-wise error corrected for multiple comparisons). The upper panels within each box display the statistical maps of the group comparisons adjusted for age, sex, educational level and normalized total brain volume. The lower panels within each box displays the statistical maps additionally adjusted for WMH volume and lacunar infarcts.

As antidepressant medication in itself can have effects on the structural integrity of the white matter, we repeated the analyses while controlling for current use of antidepressant medication. These analyses did not alter our findings.

Adjustment for MMSE did not markedly alter our findings. After additional adjustment for cognitive index, the difference in fractional anisotropy between those with or without depressive symptoms disappeared. Higher mean, axial and radial diffusivity for patients with depressive symptoms compared with those without remained present in the similar regions as in the unadjusted analysis.

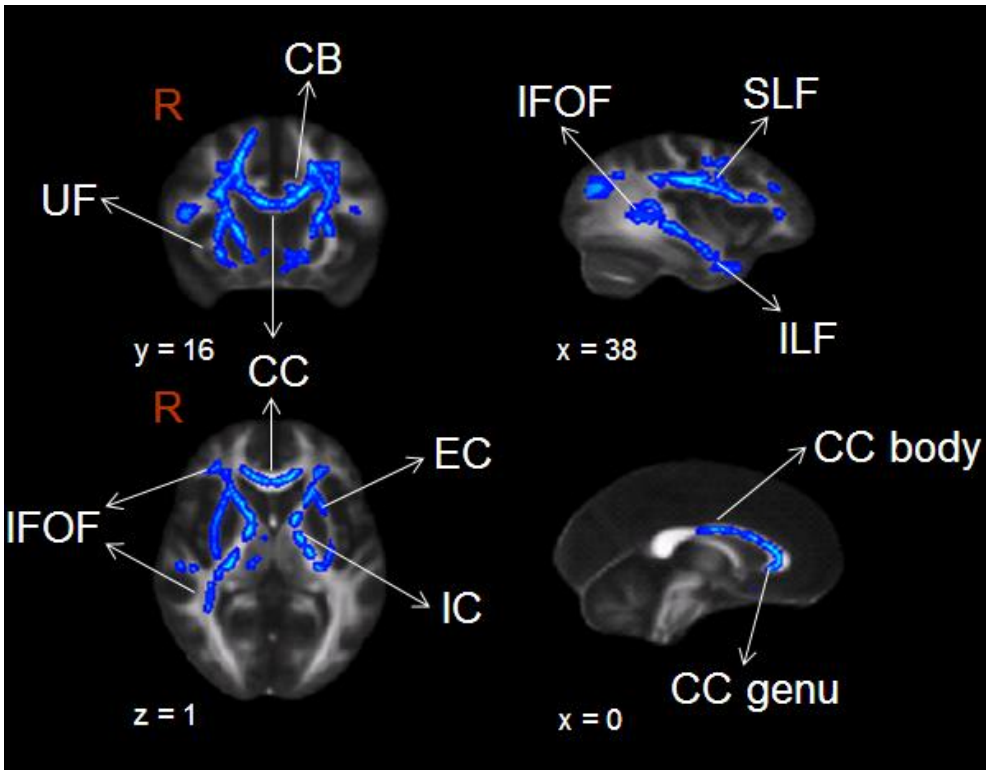


Figure 3: Map of the location of specific white matter tracts, with decreased white matter structural integrity, in participants with depressive symptoms

This map shows the mean diffusivity parameters of the group comparisons adjusted for age, sex, educational level and normalized total brain volume. CB: cingulum bundle, UF: uncinate fasciculus, CC: corpus callosum (body and genu), IFOF: inferior frontal occipital fasciculus, ILF: inferior longitudinal fasciculus, SLF: superior longitudinal fasciculus, IC: internal capsule, EC: external capsule.

Discussion

This study clearly shows regional differences in the microstructural integrity of the WM using TBSS in a large sample of elderly people with or without depressive symptoms. We found that participants with depressive symptoms had a lower microstructural integrity in several WM regions, predominantly located in the prefrontal white matter tracts. These differences were independent of global cognition and might, at least in part, be explained by the presence of cSVD. To the best of our knowledge, this is the first study conducting TBSS in a large group of participants, while controlling for multiple possible confounders, including global cognitive function.

Several methodological issues need to be addressed. First, a limitation is the cross-sectional nature of our study, which prevents us from making causal inferences. Second, participants with ≥ 16 points on CES-D and/or current use of anti-depressive medication were considered

to be participants with depressive symptoms. Using these criteria, not all participants with depressive symptoms will be diagnosed as major depressive disorder.[198] Third, the CES-D is commonly used as a screening tool and could be considered a screen for general affective distress, including depression and anxiety. However, one has to keep in mind that it is not a specific screening tool for depressive symptoms. In order to exclude other psychiatric disorders that might have proven screen-positive with the CES-D, we excluded participants at baseline with serious mental illness, including an anxiety disorder by medical history taking and structured screening of medical records and subsequent adjudication of the psychiatric events to be excluded. Fourth, we applied TBSS, which is criticized for problems with the repetitive registration process required and for issues related to constraining analyses to only large and highly organized fiber bundles. Nevertheless, this observer-independent analysis method mitigates several methodological constraints of voxel-based analysis, such as misalignment due to registration procedure and smoothing effects. Finally, the proportion of participants with depressive symptoms in our study is relatively high (34.5%). The high rate of depressive symptoms is not so much a methodological limitation, but rather a consequence of the fact that we selected participants on the basis of cSVD, which is characterized (among other) by depressive symptoms.

On the other hand, major strengths of our study include its design of a homogeneous population that covers the whole spectrum of cerebral SVD, its large sample size, the single centre design, the use of a single scanner and high response rate over 70%. Furthermore, we manually segmented the WMH and the analyses were adjusted for various possible confounders. We intentionally did not adjust for vascular risk factors, such as hypertension or diabetes, as they were considered a part of the causal chain between cSVD and depressive symptoms.

In this study, participants with depressive symptoms showed compromised microstructural integrity in several white matter tracts, predominantly located in the prefrontal regions. Especially frontal-subcortical circuits are thought to play an important role in the pathogenesis of mood, but also cognitive and motor symptoms.[191] The vascular depression hypothesis [166] entails that WMHs, caused by cerebrovascular disease, disrupt white matter tracts within the fronto-striatal-subcortical circuit and/or interrupt the connections with limbic structures.[39,199] Disruption of these circuits, which are involved in mood-regulation,[200] may lead to a "disconnection syndrome" [35] and consequently to depressive symptoms. Brain areas that connect the prefrontal cortex with subcortical areas, such as the amygdala and the ventral striatum, are crucially involved in the (patho)physiology of mood. In a recent DTI study,[201] we were able to show that subtle WM changes in the connectivity of the amygdala and medial prefrontal cortex might be key factors in the pathophysiology of major depressive disorder, which may account for functional changes. Indeed, a recent meta-analysis[202] of patients with major depressive disorder showed changes in the white matter fascicles connecting the prefrontal cortex within cortical and subcortical areas (amygdala and hippocampus).

We found lower microstructural integrity in the inferior longitudinal fasciculus. This is an associative bundle connecting the occipital and temporal lobes. These long fibres connect the visual areas to the amygdala and hippocampus, which are important components of the limbic system. The corpus callosum is the largest fibre bundle of the human brain and is involved in several perceptual, cognitive and motor functions. The genu (anterior portion) connects the prefrontal and orbitofrontal regions, and the body (central portion) connects the precentral frontal regions and the parietal lobes.[203] Lower microstructural integrity was also evident in the genu and body of the corpus callosum in participants with depressive symptoms, which is consistent with previous findings.[204] We found loss of microstructural integrity in the anterior cingulum bundle in patients with depressive symptoms, unlike a recent TBSS study, in which patients with depression showed a lower fractional anisotropy in the middle cingulate cortex.[205] This region is activated during anger and fear and is reported as abnormal in functional and structural imaging studies.[147] Most other areas in which we found lower microstructural integrity are consistent with other findings, such as the loss of structural integrity in the uncinate fasciculus [206] and the superior longitudinal fasciculus.[207] Both have frequently been demonstrated to be involved in mood regulation. Taken together, these findings suggest that the changes in white matter tracts might underlie the occurrence of depressive symptoms in participants with cSVD by disrupting the critical white matter tracts involved in emotional circuitry. These findings have implications in the understanding of neurobiological circuit of depression in elderly participants with cSVD. Furthermore the disruption of the frontal-subcortical circuits has been associated with the presence of motivational symptoms. This also could play a role in the underlying pathophysiology of depressive symptoms in elderly with cSVD.

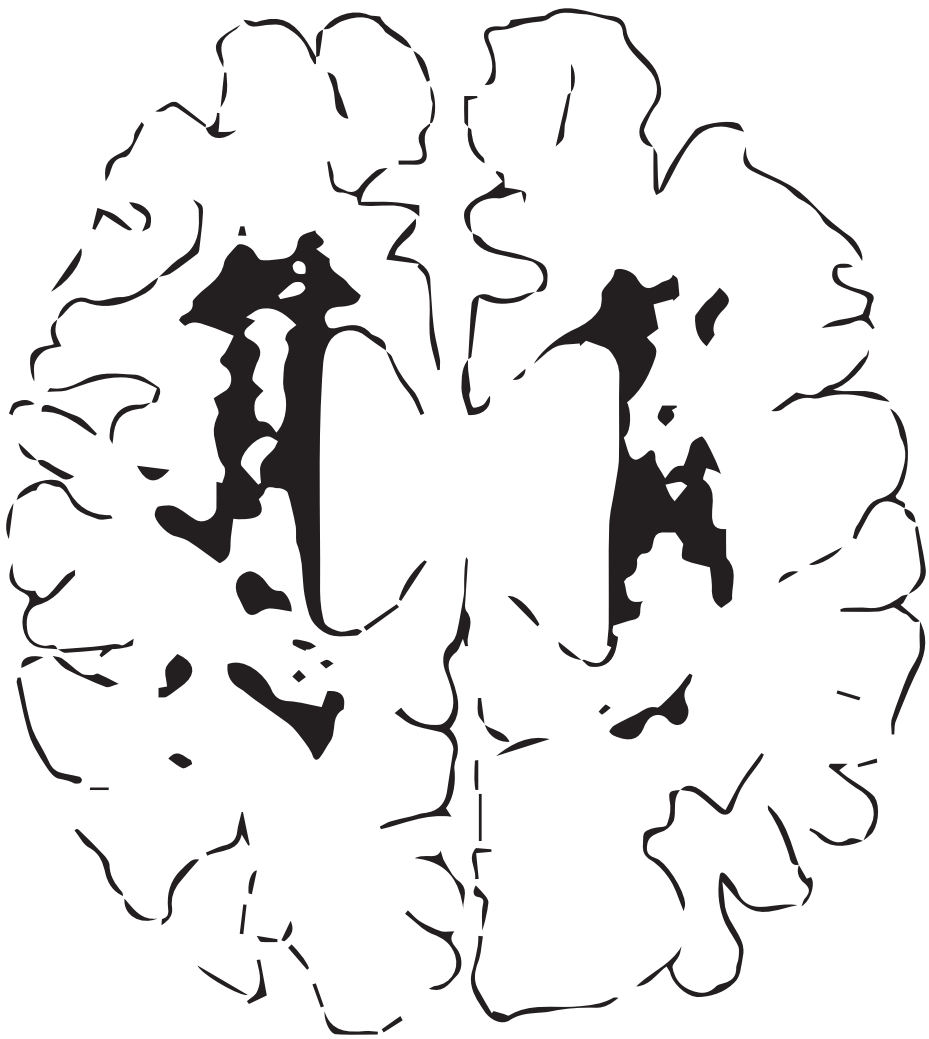
The occurrence of depressive symptoms in elderly might be a psychological reaction to (perceived) lower cognitive function.[208] On the other hand, loss of the structural integrity of the WM in specific regions may increase susceptibility to depression and thereby, loss of motivation and can be expressed clinically as cognitive impairment. These cognitive changes add to the severity of symptoms and disability that older depressed patients experience.[209] To exclude the possibility of the effect of global cognition on the depressive symptoms, additional analyses were performed while adjusting for global cognition. These analyses did not markedly alter the conclusion, though increased the variance explained by global cognitive functioning.

During the last decade, studies have shown higher volume of WMHs in elderly depressed patients compared to age-matched controls.[210] In our study, the significant differences of the DTI parameters between the groups diminished after additional adjustment for the WMHs and lacunar infarcts. This suggests a major role of cSVD in the loss of microstructural WM integrity in the participants with depressive symptoms. However, after adjustment for WMHs and lacunar infarcts, these participants exhibit significantly higher mean and axial diffusivity, suggesting the occurrence of mechanisms other than cSVD. This is consistent with the concept that different pathological mechanisms often coexist in one participant.[211] It is

interesting to note that the few white matter tracks that remained significant, such as genu of the corpus callosum and anterior cingulum bundle, have all been previously related to mood dysregulation, while other regions, which were no longer significant after adjustment for cSVD, such as internal/external capsule, have not been related to mood dysregulation.

Several pathophysiological mechanisms might have occurred governing the diffusion changes (lower fractional and higher mean, axial and radial diffusivity) in participants with depressive symptoms. First, the microstructural abnormalities of the WM could be caused by ischemic damage due to cSVD, hereby increasing the blood-brain permeability, subsequently leading to reduced myelination, axonal degeneration and/or gliosis.[212] Second, indirect effects of SVD can lead to decreased microstructural integrity by means of antero- and retrograde degeneration of neuronal fibres surrounding the WMHs, e.g. Wallerian degeneration.[213] Third, other factors might play a role in the aetiology of depression, independent of ischemic disease. The microstructural changes could also partially reflect the disorganization of the white matter tracts, alterations of the intracellular compartment and glial changes.[214] This is further corroborated by the findings of the frontal WM infrastructure alterations in participants with major depression in post-mortem studies.[215] These changes can lead to water molecule diffusion increases in every direction, which might govern the increase in mean, radial and axial diffusivity, and to a lesser extent, in the decrease of fractional anisotropy, as seen in our study. As our study is cross-sectional, the differences in structural integrity between those with or without depressive symptoms can also be a consequence of depressive symptoms rather than a cause.

In conclusion, our study provides evidence for microstructural damage in participants with depressive symptoms, independent of global cognition. This damage is predominantly located at the prefrontal white matter fibres, disrupting the fronto-subcortical circuits, which are involved in emotional processing. The loss of microstructural integrity in our participants with depressive symptoms might, at least in part, be related to the presence of cSVD. However, other mechanisms might also play a role in the aetiology of depression, independent of cerebrovascular disease. The analysis of the full spectrum of DTI-derived parameters is relevant and provides additional insights into the pathophysiological mechanisms that underline the occurrence of the depressive symptoms in elderly with cSVD. Both direct and indirect effects of cSVD may results in axonal loss (higher AD) and demyelization (higher RD). It also helps us to refine the imaging endo-phenotype of patients with depressive symptoms for future studies. Future studies are, however, needed to investigate the influence of cSVD on not only the structural connectivity, but also on functional connectivity and the effect of treatment. This information is important in understanding the pathology of depressive symptoms in elderly with cSVD and might be of added value to find a more specific medical treatment for elderly with depressive symptoms.



8.

Late onset depressive symptoms increase the risk of dementia in cerebral small vessel disease

Ingeborg W.M. van Uden, Helena M. van der Holst, Esther M.C. van Leijsen, Anil M. Tuladhar, Anouk G.W. van Norden, Karlijn F. de Laat, Jurgen A.H.R Claassen, Ewoud J. van Dijk, Roy P.C. Kessels, Edo Richard, Indira Tendolkar and Frank-Erik de Leeuw

Neurology. 2016;87(11):1102-9.

Abstract

Objective: We prospectively investigated the role of depressive symptoms (DS) on all-cause dementia in a population with cerebral small vessel disease (cSVD), considering onset-age of DS and cognitive performance.

Methods: The RUN DMC study is a prospective cohort study among 503 older adults with cSVD on MRI without dementia at baseline (2006), with a follow-up of 5-years (2012). Kaplan-Meier curves stratified for DS and dementia risk were compared using log-rank test. We calculated hazard ratios using Cox-regression analyses.

Results: Follow-up was available for 496 participants (mean baseline age 65.6 years (SD 8.8); mean follow-up time 5.2 years). All-cause dementia developed in 41 participants. The 5.5-year dementia risk was higher in those with DS (HR 2.7; 95%CI 1.4-5.2), independent of confounders. This was driven by those with late onset depressive symptoms (LODS). The five-year cumulative risk difference for dementia was higher in depressed participants with high baseline cognitive performance (no DS 0.0% vs. DS 6.9% log-rank p<0.001), compared with those who had low cognitive performance at baseline.

Conclusions: Late-onset DS increase the dementia risk, independent of cSVD. Especially in those with relatively high cognitive performance, DS indicate a higher risk. In contrast to current practice, clinicians should monitor those with DS who also show relatively good cognitive test scores.

Introduction

Depressive symptoms (DS) occur in approximately 30% of patients with dementia[216] and have been coined a risk factor for,[217] an early symptom of,[218] or a reaction to perceived cognitive decline. Both cross-sectional and longitudinal population-based studies have investigated this, showing conflicting results.[219-223]

Late-onset DS (LODS), initially occurring in older age, should be distinguished from early-onset DS (EODS), for a different aetiology is presumed.[37,38] Particularly LODS, rather than EODS, were found to be related to incident dementia.[224] It is unclear whether LODS increase dementia risk alone or whether this is mediated by the same underlying cerebral small vessel disease (cSVD).[38 36,41,225] cSVD refers to neuroradiological findings, including white matter hyperintensities (WMH), lacunes of presumed vascular origin (lacunes) and microbleeds.[12]

One recent study in older adults with cSVD has already shown that DS were associated with an increased risk of cognitive decline after 3 years, independent of WMH.[118] However, important adjustments for other aspects of the cSVD spectrum as well as grey matter (GM and hippocampal volume were not made.

Therefore, we prospectively investigated the role of baseline DS on the risk of all-cause dementia in adults with cSVD, also considering cSVD characteristics and other well-known confounders. Second, we investigated the risk of dementia, stratified by age of onset of DS, as LODS and EODS might have different relations with incident dementia. Finally, we investigated the relation between DS and incident dementia in those with high and low cognitive performance at baseline.

Methods

Study population

The Radboud University Nijmegen Diffusion Tensor and Magnetic resonance Cohort (RUN DMC) study, a prospective cohort study investigating risk factors and clinical consequences of brain changes during aging as assessed with MRI among 503 older adults without dementia aged 50 to 85 year with cerebral SVD. The recruitment, study rationale, and protocol were described previously in detail.[60] cSVD was defined on established research criteria (presence of any WMH and/or lacunes of presumed vascular origin),[61] as cSVD onset is often insidious and clinically heterogeneous with acute, (TIAs or lacunar syndromes), or sub acute (cognitive, motor and/or mood disturbances) symptoms. Baseline data collection was performed in 2006. Main exclusion criteria were dementia, WMH or cSVD mimics, current (psychiatric) disease interfering with cognitive testing (such as current major depression) or follow-up, history of serious mental illness, and MRI contra-indications (e.g. pacemaker, vascular clips and known claustrophobia).[60]

Follow-up occurred in 2011-2012. Of 503 baseline participants, 2 were lost to follow-up (not deceased; supplementary Figure S1); five additional participants were excluded because of missing values on the CES-D, yielding a final sample of 496. This study was approved by the

Medical Review Ethics Committee region Arnhem-Nijmegen. All participants gave written informed consent prior to inclusion.

Assessment of depressive symptoms

DS were assessed with the 20-item Centre of Epidemiologic Studies Depression Scale (CES-D).[196] DS were considered present when participants had a CES-D score ≥16 and/or currently used antidepressants, taken for depression. Sixty-one participants used antidepressive medication.[48]

Furthermore, participants were interviewed about their history of depressive episodes, onset-age and whether they asked for medical advice. If depressive episodes had required attention of a general practitioner, psychologist, or psychiatrist, a history of depression was considered present.[67] To distinguish between LODS and EODS we used the age of 60 as the cut-off point.[67] Those with a CES-D score ≥ 16 and/or current use of antidepressants, age 60 years or older, without a history of depressive episodes before the age of 60, were classified as having LODS. Those with a CESD score < 16, without a history of DS and without current use of antidepressants, formed the reference group. The others (first depressive episode < 60 years), fulfilled the criteria for EODS.[47] (Supplementary Table S1)

Dementia case finding

At baseline all participants were free of dementia. Dementia case-finding, and assessment of time-to-dementia were described in detail previously.[44,45] In short, dementia case finding was performed for all 501 participants: those who participated in the in-person follow-up and among those who could not (died or refused) (Supplementary Figure S1). Participants who screened positive at in-person follow-up (MMSE score ≤26 or a decline ≥3 points from baseline) were subsequently examined for dementia at the Radboud Alzheimer Centre. For those who refused this examination, the panel, consisting of members of the same Radboud Alzheimer Centre (to ensure a standardized approach), came to a consensus diagnosis. This panel reviewed all available neuropsychological[45,60] and imaging information next to interference in daily functioning, confirmed by family or caregivers.

For those who were not available for an in-person assessment, the same panel reviewed the medical records. Furthermore we contacted patients' general practitioners and medical specialists for information on cognitive status. The dementia diagnosis was based on the Diagnostic and Statistical manual of mental disorders (IV) criteria.[62] Incident dementia was diagnosed in 43 cases. Data on baseline DS were not available for 2 patients who developed dementia; therefore, 41 cases of incident dementia are included in the analysis (Supplementary Figure S1).

MRI resonance imaging protocol and analyses

MRI scans were performed on a single 1.5-Tesla MRI, on average 19 days after assessment at our research centre. The protocol included, among other sequences, a T1-weighted

3-dimensional magnetization-prepared rapid acquisition gradient-echo (MPRAGE) imaging (voxel size 1.0×1.0×1.0mm); a Fluid-attenuated inversion recovery (FLAIR); voxel size 1.0×1.2×5.0mm, with an interslice gap of 1 mm); and a transversal T2* weighted gradient echo sequence (voxel size 1.3×1.0×6.0mm, with an interslice gap of 1 mm).[60]

WMH were manually segmented on FLAIR images. Total WMH volume was calculated by summing the segmented areas multiplied by slice thickness. Lacunes and microbleeds were assessed by raters blinded to all clinical data, according to the Standards for Reporting Vascular changes on neuro-imaging (STRIVE) criteria,[12] with good intra- and inter-rater variability.[45] To obtain GM (cortex, basal ganglia and thalamus), white matter (WM) and cerebrospinal spinal fluid (CSF) volume, the T1 MPRAGE images were segmented using Statistical Parametric Mapping 12 unified segmentation routines (SPM12; Wellcome Department of Cognitive Neurology, University College London, UK http://www.fil.ion.ucl.ac.uk/spm/software/spm12/).[45] To obtain GM, WM and CSF volumes all voxel volumes that had a $p > 0.5$ for belonging to that tissue class were summed. Intracranial volume (ICV) was a summation of total GM, total WM and CSF volume. Total brain volume (TBV) was a summation of GM and WM volume. We visually checked all images for co-registration errors and for motion and/or segmentation artefacts. Hippocampal volumes (HV) were manually segmented on the MPRAGE image using the interactive software program ITK-SNAP, as described previously.[87,88] (http://www.itksnap.org). To adjust for head size, all volumes were normalized to the ICV.

Other measurements

Education was classified using 7 categories (1 represented less than primary school, 7 an academic degree).[65] We then dichotomized in primary or less (levels 1-2), or more than primary education (levels 3-7). Speed-accuracy trade-off (SAT) scores were calculated where appropriate [accuracy(%)/reaction time], to adjust for a number of faults. The Cognitive Index was constructed to obtain a more robust outcome measure for global cognition. In short, this was calculated as the mean of the z-scores of the SAT score of the Paper and Pencil Memory Scanning Task, the mean of the SAT score of the reading task of the Stroop test, the mean of the Symbol Digit Substitution Task, and the mean of the added score on the three learning trials of the Rey Auditory Verbal Learning Task, and the mean of the Delayed Recall of this test.[46] Functional independence was assessed using the Barthel Index (range, 0–20).[60] All other measurements were done at initial assessment.

Statistical analyses

WMH volume was log-transformed because of the skewness of the data. Cumulative risk of incident dementia was estimated with Kaplan-Meier analysis stratified by presence of baseline DS. Person-years at risk were calculated for each participant from date of the baseline assessment, until onset of dementia, death, or date of the follow-up assessment. Those who did not reach the endpoint or died were censored. Subsequently, we used Incidence curves to compare between subgroups using log-rank test. We used Cox regression

analyses to calculate hazard ratios (HRs) with 95% confidence intervals (CIs) for presence of baseline DS (total, EODS, and LODS), adjusted for age, sex, and education (model 1), additional for baseline Cognitive Index and TBV (model 2), additional for WMH volume, presence of lacunes and microbleeds (model 3), or additional for GM and hippocampal volume (model 4). Verification of proportionality of hazards was performed by examining Schoenfeld residuals; there were no indications that this assumption was violated. Finally, to identify the role of DS on the dementia risk in "high cognitive performers" and "low cognitive performers" at baseline, (defined by compound Z-score Cognitive Index below the median at baseline; Supplementary Table S2) we performed Kaplan-Meier analyses and compared subgroups with log-rank test.

Results

Baseline demographic and neuro-imaging characteristics (n=496) are shown in Tables 1 and Supplementary Table 3, respectively. At baseline, 33.7% of participants had DS. Dementia developed in 41 of the 496 participants during a mean follow-up of 5.2 years (SD0.7). Mean age at baseline was 65.6 years (SD 8.8), and 56.9% were men. Mean MMSE score at baseline was 28.1 (SD 1.6). The 5.5-year cumulative risk of dementia was higher in participants with DS at baseline compared with those without (DS 18.5% vs. no DS 7.4%, log-rank p = 0.003) (Figure 1).

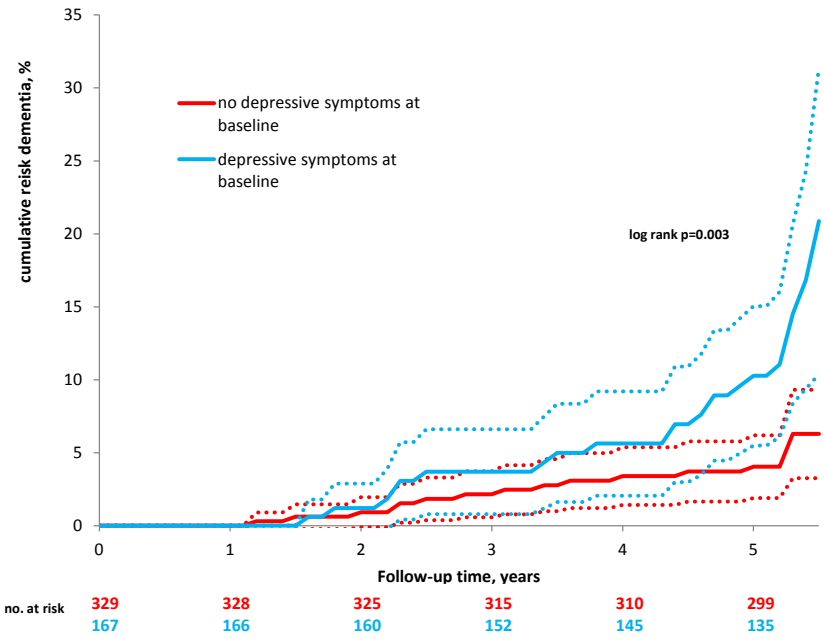


Figure 1: Cumulative risk for dementia, stratified by presence of depressive symptoms at baseline

Cumulative risk for dementia stratified by presence of baseline depressive symptoms, unadjusted percentages. Log-rank test pair wise over strata.

Table 1: Baseline characteristics of participants with and without depressive symptoms at baseline assessment

	Total, n=496	Depressive symptoms at baseline, n=167	No depressive symptoms at baseline, n=329	p-value for difference °
Demographics				
Age at baseline (SD)	65.6 (8.8)	65.9 (8.7)	65.4 (8.8)	p=0.607 °
Men, n (%)	282 (56.9)	81 (48.5)	201 (61.1)	p=0.007 ∞
Only primary education, n (%)	48 (9.7)	26 (15.6)	22 (6.7)	p=0.002∞
CES-D at baseline, (SD)	11.0 (9.4)	21.1 (8.5)	5.9 (4.4)	p=0.001 α
Anti depressive medication use at baseline, n. (%)	61 (12.3)	61 (36.5)	0 (0.0)	NA
History of depression, n (%)	127 (25.6)	85 (50.9)	42 (12.8)	p<0.001 ∞
MMSE baseline (SD)	28.1 (1.6)	27.7 (1.7)	28.3 (1.6)	p<0.001 α
Compound Z-score Cognitive Index (SD) [a]	-0.0 (0.8)	-0.3 (0.7)	0.1 (0.8)	p<0.001 °
Low cognitive performance, n(%) [a]	247 (49.8)	107 (64.1)	140 (42.6)	p<0.001∞
Dementia at follow-up, n (%)	41 (8.3)	22 (13.2)	19 (5.8)	p=0.005∞
Barthel Index (SD)	19.7 (0.8)	19.5 (1.1)	19.8 (0.6)	p=0.011 °

Of the 503 participants at baseline, 2 were Lost to follow-up. 5 were additionally excluded because of missing values on the status of depression at baseline, [a]one was excluded because of missing cognitive tests at baseline. The Cognitive Index is a compound Z-score of the paper pencil test, Stroop test, SDST, and immediate and delayed recall of the 15 RALVT. MMSE: Mini-mental State Examination. Low cognitive performance is defined by a compound Z-score of the cognitive index below the median at baseline. SD: standard deviation, FU: Follow-up, Data shown are unadjusted values, and represent numbers (%), mean (SD) [o]comparison of those with and without depressive symptoms at baseline.[x](Means are compared with independent ° T-test, ∞ Chi-square test or α Mann-Whitney-U)

Baseline DS significantly predicted incident dementia after 5 years of follow-up, adjusted for age, sex, and education (HR 2.7, 95% CI 1.4-5.2). Additional adjustment for baseline Cognitive Index and TBV; cSVD characteristics (WMH volume, lacunes and microbleeds), or GM structures (hippocampal and GM volume), did not significantly alter these findings (data not shown). The unadjusted cumulative risk of incident dementia in those with EODS and LODS separately compared to those who never experienced DS is presented in Figure 2. LODS predicted incident dementia (HR 2.5, 95% CI 1.3-4.8); adjustments for the above-mentioned confounders did not change this relation substantially (data not shown). For EODS this relation with incident dementia was not found (Table 2).

Finally, Figure 3 shows the cumulative risk of dementia in strata of baseline cognitive performance for participants with and without DS. In depressed participants with high baseline cognitive performance there was a significant 5-year cumulative risk difference for dementia (no DS 0.0% vs. DS 6.9%, log-rank p<0.001), whereas no such difference was found between participants with and without DS with low cognitive performance (no DS 9.7% vs. DS 12.2%, log-rank p=0.514). Adjustment for demographic and imaging confounders in Cox regression analyses in both good and poor cognitive performance did not change this relation. Low dementia risk in participants without DS with good cognitive performance, led to wide confidence intervals (HR 40.2, 95% CI 4.5-361.8).

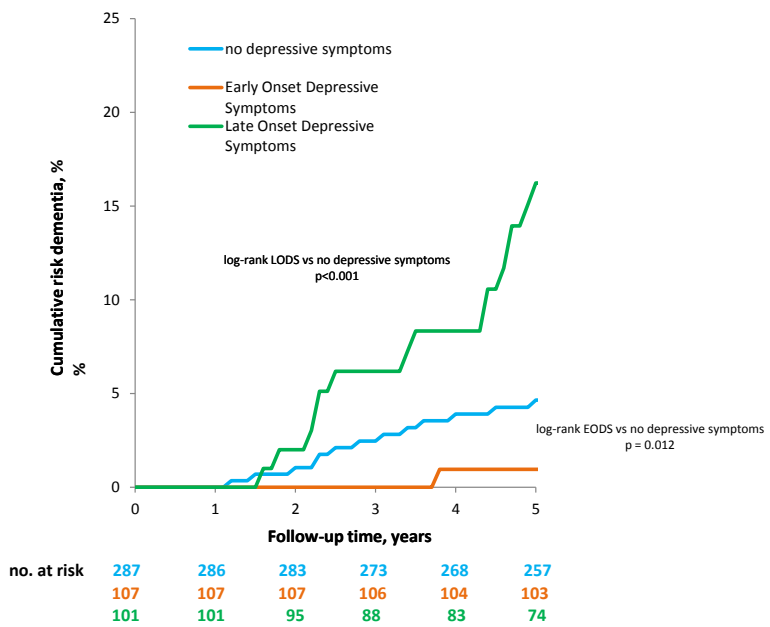


Figure 2: Cumulative risk for dementia, stratified by onset of depressive symptoms

Cumulative risk for dementia stratified by onset of depressive symptoms, unadjusted percentages. Log-rank test pair wise over strata.

Table 2: Cox Proportional Hazards for Dementia by baseline depression status, Early Onset Depressive Symptoms and Late Onset Depressive Symptoms

	Progression to dementia, n, (%)	Hazard Ratio + 95% CI Adjusted age, sex, education
Depressive symptoms		
no depressive symptoms. (n =329)	19 (5.8%)	**1**
depressive symptoms. (n= 167)	22 (13.2%)	**2.7 (1.4-5.2)**
Early onset (<60 yr)		
no depressive symptoms. (n =287)	19 (6.6%)	1
Early Onset Depressive Symptoms (n=107)	1 (0.9%)	0.2 (0.02-1.7)
Late onset (=/> 60 yr)		
no depressive symptoms (n =287)	19 (6.6 %)	**1**
Late Onset Depressive Symptoms (n=101)	21 (20.6%)	**2.5 (1.3-4.8)**

Of the 503 participants at baseline, 2 were Lost to follow-up. 5 were additionally excluded because of missing values on the status of depression at baseline resulting in a total of 496 participants. Late onset depressive symptoms: Patients at the age or older than 60 years with a CES-D ≥ 16 and/or current use of anti-depressive medication, taken for depression, without a history of depressive episodes before or at the age of 60. Early onset depressive symptoms: a CES-D ≥ 16 and/or current use of anti-depressive medication, taken for depression, and/or a history of depressive episodes before or at the age of 60. Individuals with a CESD < 16, without a history of depressive symptoms and without the current use of anti-depressive medication formed the reference group. Verification of proportionality of hazards was performed by examining Schoenfeld residuals.

The interaction between DS and cognitive performance on dementia risk showed a significant effect (HR 25.3, 95% CI 2.6-238.7;p=0.005). We did not find a significant interaction between sex or low education and DS (data not shown).

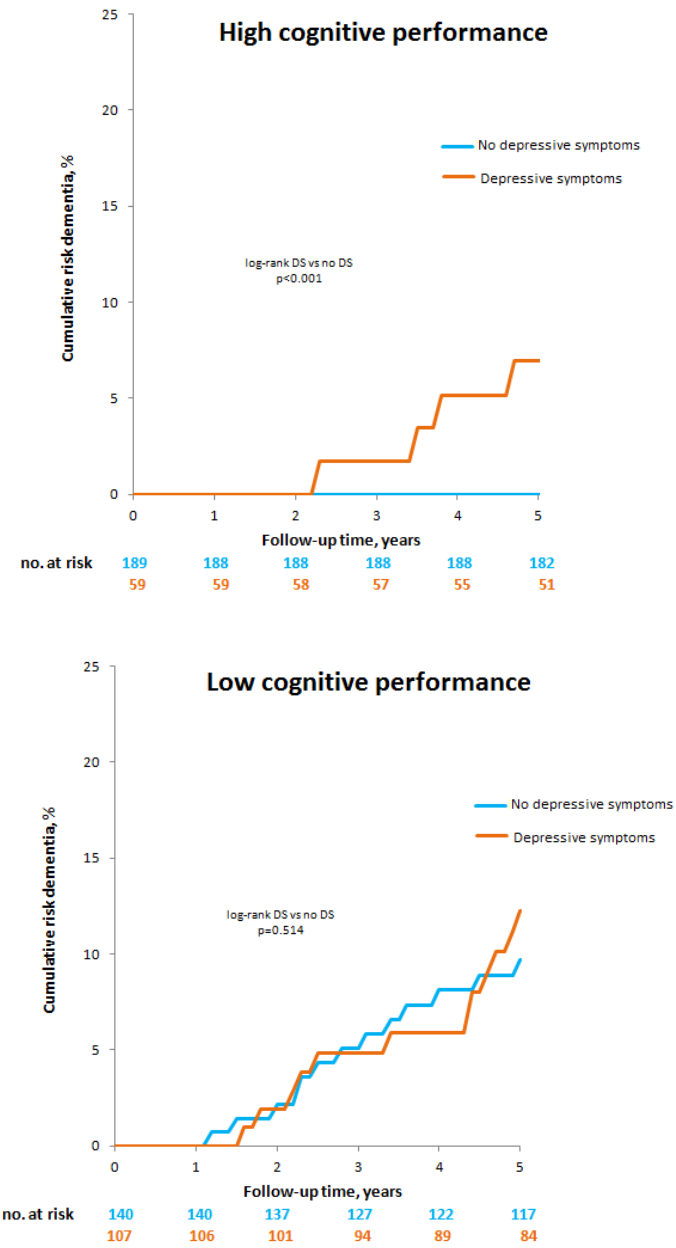


Figure 3: Cumulative risk for dementia, stratified by presence of depressive symptoms at baseline, in those with high and low cognitive performance at baseline.

Cumulative risk for dementia stratified by presence of baseline depressive symptoms, in those with high and low cognitive performance at baseline, unadjusted percentages. Log-rank test pair wise over strata. High cognitive performance defined as the upper half cognitive index at baseline, low cognitive performance defined as the lower half cognitive index at baseline. DS = depressive symptoms

Discussion

Our results show that DS increase the dementia risk after 5 years in older adults with cSVD, a relation which is driven by those who develop a first depressive episode at later age. This relation is independent of SVD characteristics and GM and hippocampal volume, suggesting additional factors besides cSVD play a role in our cohort in development of dementia. We showed that DS are related to an increased risk of dementia in those without objective cognitive problems. The magnitude of this relation, however, cannot be estimated because of the small number of incident cases in this subgroup. Below we will discuss the significance of these findings in more detail.

Strengths of our study include its longitudinal and single-centre design allowing us to collect both baseline and follow-up data according to identical procedures, reducing the risk of procedural bias. Subsequently, the large cohort that covers the whole spectrum of cerebral SVD and the high follow-up rate of 99.6% are major strengths. We manually segmented WMH and hippocampal volumes without prior knowledge of clinical data. Finally, the relation between DS and incident dementia was investigated with adjustment for demographics, baseline global cognition, cSVD and GM structures, reducing confounds.

Several methodological issues need to be addressed. First, we investigated "overall dementia" as an outcome measure and did not investigate the risk of Alzheimer disease (AD) and Vascular Dementia (VaD) separately. This distinction is difficult to make in elderly persons since at a later age vascular diseases and neurodegeneration often co-occur.[30] Our dementia diagnosis was a clinical diagnosis, supported by MRI most closely performed to the moment of diagnosis. It is possible that we have missed few cases of incident dementia in participants who were not available for in-person follow-up, because of incomplete medical records or uninformed general practitioners. In those cases, misclassification could have occurred in relatively early stages of dementia. Second, although widely used, the cut-off point of 26 in the MMSE, might not be sensitive enough to detect all incident dementia cases, especially for those in early stage of the disease, those with VaD, or in participants with dementia who have higher education levels. Therefore, misclassification, which might have led to an underestimation of the effect, might have occurred. Third, the relation between DS and incident all-cause dementia was adjusted for demographic and imaging characteristics and for baseline cognitive performance, reducing confounders. However, the small number of dementia cases in some of the subgroups resulted in wide confidence intervals, preventing a reliable estimate of the magnitude of the effect. Therefore, these results should be interpreted with caution. Furthermore, our analyses were not adjusted for other relevant confounders such as lifestyle risk factors and functional health, which might have led to residual confounding. Finally, we used the CES-D and/or current use of anti-depressive medication to assess DS. Although used in large population-based studies,[219,226] it is also considered a screen for general affective distress, and therefore not a specific screening tool for DS. To account for this, we excluded participants at baseline with serious mental illness.

We think that our study has an excellent external validity regarding vulnerability for cognitive and affective symptoms in patients between 50 and 85 years of age presenting with cSVD on neuroimaging in a neuropsychiatric clinic. All of our participants were functionally independent at baseline, with a mean MMSE score of 28.1 (SD 1.6), reflecting estimates in the general population. The incidence rate for dementia in our SVD cohort is higher than in a large population-based study.[86] This is not surprising as cSVD is associated with incident dementia.[41] When comparing our incidence rate of dementia with another cSVD cohort,[118] our incidence rate was actually lower. This could be explained by the approximately 10 years younger mean age of our population and also the less severe WMH at baseline; both factors are known to be related to the incidence of dementia.[86,100]

Our findings that DS are associated with all-cause dementia are accounted for by those participants with LODS. At the same time, we showed that EODS were not related to incident dementia in older adults with cSVD. This is an important finding because (longer existing) EODS would be expected to increase dementia risk, for they have shown to be associated with neurodegeneration through neurotoxic mechanisms, including hippocampus atrophy.[227,228] This could suggest that LODS present not a causal factor for incident dementia, but rather are an early symptom of the spectrum, ultimately leading to dementia, for they present relatively short before dementia onset.

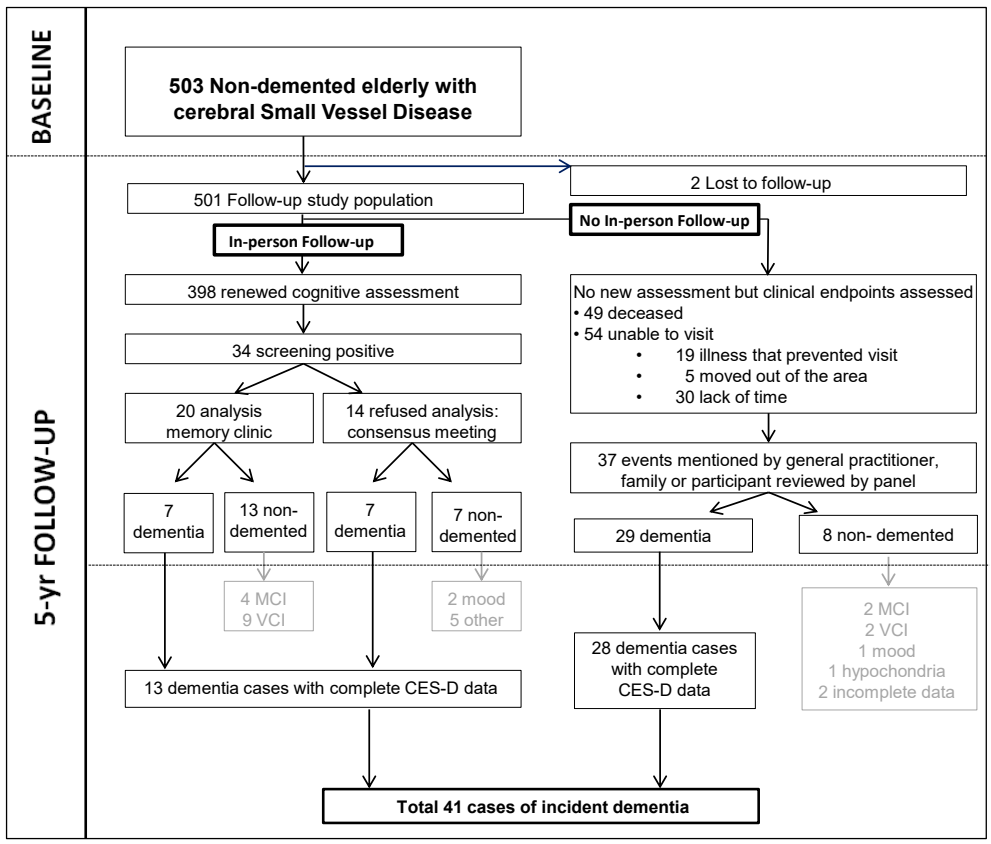
There are several hypothesized explanations for (late-onset) DS as an early symptom of dementia.[229] Disruption of the subcortical-frontal circuit, for example, by cSVD or neurodegenerative processes could result in both LODS and cognitive difficulties, such as dysexecutive problems and slower speed of information processing.[48,230-232] This structural defect might also explain resistance to treatment, which is common in late-life depression. Of note, in our study we found that the association between DS and incident dementia remained present after adjustment for cSVD characteristics, suggesting that additional factors besides cSVD play a role that may not be necessarily depicted at the macro-anatomic level. This was in line with other longitudinal studies on depression and dementia.[118,233]

We showed in an cSVD population that participants with DS have a higher risk of incident dementia compared to those without DS while performing relatively well cognitively. This finding could not be merely explained by the level of education, as post hoc analyses showed that all of those with incident dementia in the group that performed relatively well cognitively had more than primary education (data not shown). While it seems somewhat counterintuitive at first glance, it may very well be that only in those with relatively high cognitive performance, DS are indicative for incident dementia. Because cognitive performance at baseline is a very strong risk factor for incident dementia, it is possible that in the group with relatively low cognitive performance, presence of DS adds no significant value to the overall dementia risk. This is another argument suggesting that both (cognitive and affective) symptoms are part of the same underlying pathophysiology, whereby DS may present an early symptom rather than a risk factor for dementia.

The information presented above could imply that presence of DS might be a sensitive tool to investigate future dementia-risk, possibly more sensitive than cognitive evaluation, in those with a relatively higher cognitive status. In contrast to current clinical practice, these patients in particular should be adequately monitored for occurrence of cognitive decline, while those without DS have a very low 5-year risk of developing dementia. This is important to acknowledge, beginning with the general practitioner, which must recognize these first DS at older age as an initial sign of dementia. After this, the diagnostic path calls for a multidisciplinary approach in which there must be a good cooperation between psychiatrists, clinical neuropsychologists and neurologists.

Our results show that LODS increase the risk of all-cause dementia in elderly persons with cSVD, but independent thereof, suggesting that other factors also have a role. It is possible that DS in older age are an early symptom rather than an independent risk factor for incident dementia. General practitioners and medical specialists should be alert on presence of DS, and also monitor also those with relatively good cognitive test scores while presenting with DS.

Supplementary Figures



Supplementary Figure S1: Flowchart diagnostic work–up dementia

MCI: Mild cognitive impairment, VCI: Vascular cognitive impairment, CES-D: Centre of Epidemiologic Studies Depression Scale

Supplementary Tables

Supplementary Table S1: Baseline characteristics stratified by onset of depressive symptoms

	Not ever depressive symptoms n=287	Early Onset Depressive Symptoms, n=107	Late Onset Depressive Symptoms, n=102
Demographics			
Age at baseline (SD)	65.8 (8.8)	59.2 (6.2)	71.6 (6.2)
Men, n (%)	175 (61.0)	60 (56.1)	47 (46.1)
Only primary education, n (%)	20 (7.0)	5 (4.7)	23 (22.5)
CES-D at baseline, (SD)	5.7 (4.4)	16.1 (11.0)	20.1 (8.2)
Anti depressive medication use at baseline, n. (%)	0 (0.0)	30 (28.0)	31 (30.4)
History of depression, n (%)	0 (0.0)	88 (82.2)	39 (38.2)
MMSE baseline (SD)	28.3 (1.6)	28.4 (1.5)	27.4 (1.7)
Compound Z-score Cognitive Index (SD) [a]	0.08 (0.76)	0.21 (0.72)	-0.53 (0.65)
Low cognitive performance, n(%) [a]	125 (43.6)	40 (37.4)	82 (80.4)
Dementia at follow-up, n (%)	19 (6.6)	1 (0.9)	21 (20.6)
Barthel Index (SD)	19.8 (0.7)	19.7 (1.1)	19.4 (0.8)

Of the 503 participants at baseline, 2 were Lost to follow-up. 5 were additionally excluded because of missing values on the status of depression at baseline, [a]one was excluded because of missing cognitive tests at baseline. The Cognitive Index is a compound Z-score of the paper pencil test, Stroop test, SDST, and immediate and delayed recall of the 15 RALVT. MMSE: Mini-mental State Examination. Low cognitive performance is defined by dichotomization at the median of the compound Z-score cognitive index at baseline. SD: standard deviation, FU: Follow-up, Data represent numbers (%), mean (SD) [c]comparison of those with Early and Late Onset Depressive Symptoms.[x](age and gender adjusted difference by binary logistic regression or ANOVA where appropriate)

Supplementary Table S2: distribution of cognitive scores for high and low cognitive performers

	Overall	Low cognitive performance	High cognitive performance
MMSE (SD)	**28.1 (1.6)**	**27.4 (1.7)**	**28.8 (1.2)**
Stroop words, time (sec)	25.8 (6.3)	29.3 (6.7)	22.4 (3.5)
Stroop faults, no	0.2 (0.5)	0.2 (0.6)	0.2 (0.4)
Paper Pencil, time (sec)	45.2 (13.7)	53.3 (13.7)	37.5 (7.9)
Paper Pencil faults, no	0.7 (1.4)	1.1 (1.8)	0.4 (0.8)
SDST correct in 1 min, no	27 (9.8)	20.0 (6.5)	33.9 (7.1)
RAVLT, 3 trials correct, no	21.1 (5.9)	17.3 (4.4)	24.6 (4.9)
Delayed recall, correct, no	6.0 (3.1)	4.1 (2.2)	7.7 (2.7)
Compound Z-score Cognitive Index (SD) [a]	-0.02 (0.8)	-0.66 (0.4)	0.62 (0.4)

MMSE: Mini-mental State Examination, SD: standard deviation, no: number, sec: seconds, SDST: symbol digit substitution task; RALVT: Rey Auditory Verbal Learning Task. The Cognitive Index is a compound Z-score of the paper pencil test, Stroop test, SDST, and immediate and delayed recall of the 15 RALVT.

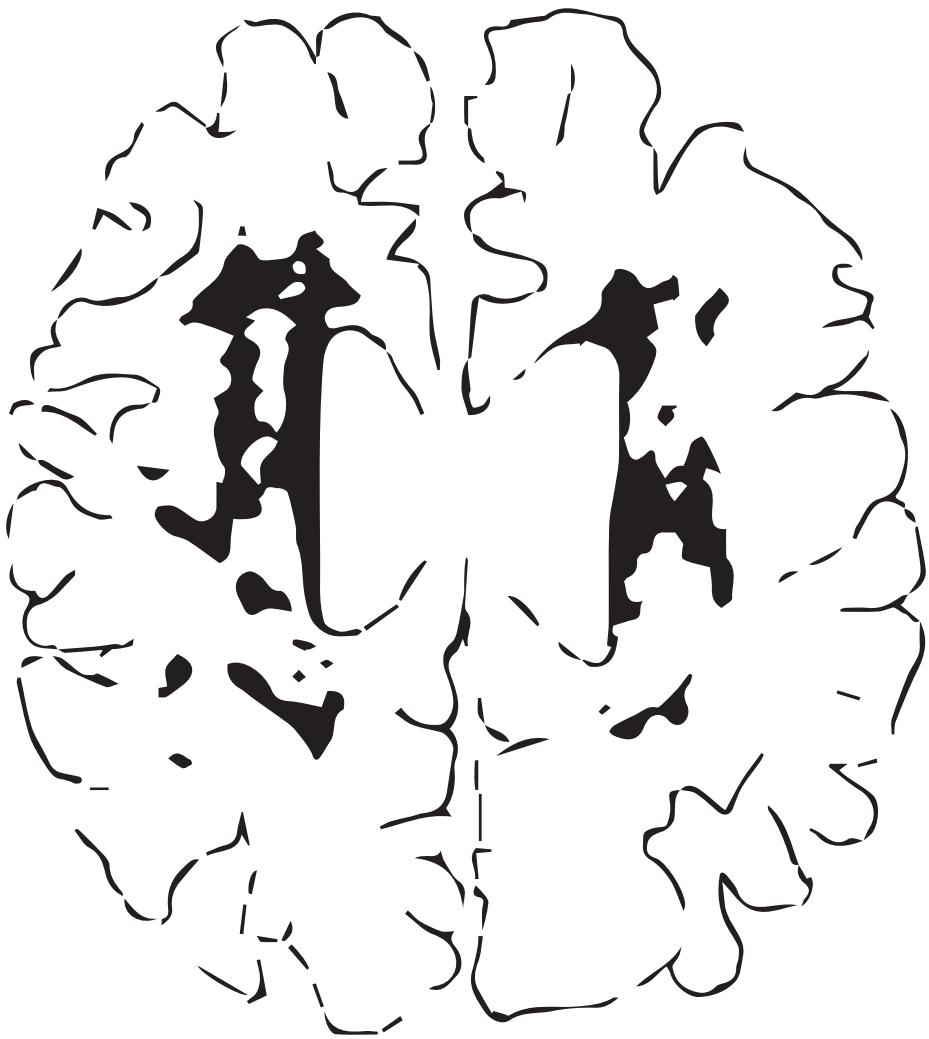
Supplementary Table S3: Baseline neuro–imaging characteristics of participants with and without baseline depressive symptoms

	Total, n=495	Depressive symptoms at baseline, n=167	No depressive symptoms at baseline, n=329	p-value for difference ◦
Baseline neuro-imaging characteristics				
White matter volume, mL (SD)	464.5 (44.3)	467.7 (37.1)	462.9 (47.6)	p=0.255º
WMH volume, mL (IQR)†	7.2 (3.5-18.0)	9.1 (3.5-22.2)	6.5 (3.4-17.1)	p=0.085 α
NAWM volume, mL (SD)	450.4 (49.0)	451.7 (42.4)	449.7 (52.2)	p=0.661º
Lacunes, presence, n (%)	132 (26.7)	49 (29.3)	83 (25.3)	p=0.327∞
Microbleeds, presence, n (%)*	80 (16.2)	26 (15.6)	54 (16.5)	p=0.787∞
Territorial infarcts, presence, n (%)	55 (11.1)	13 (7.8)	42 (12.8)	p=0.095∞
Grey matter volume, mL (SD)	616.0 (50.9)	612.3 (53.3)	617.8 (49.5)	p=0.253º
Hippocampal volume, mL (SD)**	6.8 (0.95)	6.8 (0.98)	6.8 (0.93)	p=0.594º

Of the 503 participants at baseline, 2 were Lost to follow-up. 5 were additionally excluded because of missing values on the status of depression at baseline, one was additionally excluded because of baseline T1 artefacts ml: millilitres, WMH: White Matter Hyperintensities, NAWM: Normal Appearing White Matter, Brain volumes represent normalized brain volumes to the total ICV. Data shown are unadjusted values, and represent numbers (%), mean (SD) or median † (inter quartile range). *two were excluded because of missing values of microbleeds. ** three were excluded because of missing values of hippocampal volume. ◦comparison of those with and without depressive symptoms at baseline.ˣ(Means are compared with independent º T-test, ∞ Chi-square test or α Mann-Whitney-U)

Part IV:

The clinical consequences of white matter microstructural damage



9.

White matter microstructural damage on diffusion tensor imaging in cerebral small vessel disease: The clinical consequences

Marco Pasi, Ingeborg W.M. van Uden, Anil M. Tuladhar,
Frank-Erik de Leeuw and Leonardo Pantoni

Stroke. 2016 Jun;47(6):1679-84

Introduction

Cerebral small vessel disease (cSVD) is a major health problem for its contribution to about 45% of dementias, and about a fifth of all strokes worldwide, representing one of the most important causes of disabilities.[234] The term cerebral small vessel disease refers to a group of pathological processes with various aetiologies that affect the small arteries, arterioles, venules, and capillaries of the brain. The most common forms are age- and hypertension-related SVD and cerebral amyloïd angiopathy (CAA).[13] Vessel wall changes may lead to both ischemic and hemorrhagic consequences: 1) a state of chronic hypoperfusion or vascular dysfunction responsible for incomplete infarction[235,236] and 2) acute focal necrosis (lacunar infarct), or 3) vessel rupture manifesting as hemorrhagic cSVD. The clinical consequences of cSVD are various and mainly consist of cognitive, mood, and motor dysfunctions leading to functional disability in the late stages of the disease.

MRI has become crucial in the diagnosis of cSVD enabling the evaluation of the disease progression both in the clinical and research settings. However, correlations between clinical features of cSVD and conventional MRI measures have been partially discordant. Some authors suggested that the cumulative effect of cSVD lesions, rather than the individual lesions themselves determines the clinical impact,[237] whereas others suggested that the presence and severity of alterations non-visible on conventional MRI might also be an explanation.[127]

In the last decade, diffusion tensor imaging (DTI) has been increasingly used for the evaluation of cSVD patients because it is very sensitive to tissue damage and can show abnormalities in both areas of white matter hyperintensities (WMH) and in normal appearing WM (NAWM). However, despite the high sensitivity in detecting cerebral damage, DTI has a low specificity on detecting the underlying cause. In fact, we can only infer that DTI changes reflect a loss of WM integrity due to damage to structures that restrict molecular movement along the primary axis of the axon such as axonal cell membranes, myelin sheaths, and neurofilaments. DTI is suited to study cortical disconnection because it provides indices of microstructural integrity within interconnected neural networks. Most DTI studies evaluated WM microstructural damage in aging, Alzheimer disease (AD) and mild cognitive impairment (MCI) patients, but recently, studies in cSVD patients have documented a significant association between WM microstructural damage and clinical features, providing new insight in the biological basis of this condition.[48,129,238]

The aim of this review is to analyze the evidence of the role of WM microstructural damage beyond the standard structural MRI imaging sequences, evaluated with DTI, in the clinical consequences of cerebral SVD. Although some definitions have been proposed,[239] it is explicitly not our intention to clinically define a 'cSVD' patient, as cSVD is often accompanied by other processes, such as aging and neurodegeneration leading to a broad spectrum of clinical manifestations. Our review is based on those studies that enrolled patients with a predominant cSVD pathology and does not include those that enrolled non-SVD participants (e.g., healthy aging, AD, MCI) even though they evaluated the association between cSVD markers and WM microstructural damage.

The review first part briefly examines the methodological aspects of DTI and the role of DTI in understanding cSVD pathophysiology analyzing also the relationship between risk factors and WM microstructural damage. In the second part, we review clinical studies that reported on the association between DTI and different clinical manifestations of cSVD, focusing on cognition, mood disorders, and motor dysfunctions. The last part of the review outlines possible future applications of DTI, in particular its role as a sensitive marker to evaluate SVD progression in clinical trials.

Article search strategy

Articles were identified through Pubmed searches using these terms: DTI, diffusion, WM microstructural damage, WM integrity, structural network, brain connectivity AND each of the following: SVD, subcortical small vessel, Binswanger, vascular cognitive impairment, CAA, CADASIL, vascular dementia, motor symptoms, gait, falls, balance, parkinsonism, depression, depressive symptoms, mood, smoking, hypertension, blood pressure, diabetes, blood glucose, body mass index, hypercholesterolemia, physical activity, leukoaraiosis, lacunes, from any date to November 15, 2015.

Methodological aspects

DTI is a quantitative MR technique that measures the movement of water within the tissue microstructure applying a magnetic diffusion gradient in more directions (at least six) to acquire a diffusion tensor.[57] From the tensor, two commonly derived quantitative measures that provide information about the ***in vivo*** WM microstructure are fractional anisotropy (FA) and mean diffusivity (MD). FA is a measure of anisotropic water diffusion, which reflects the degree of directionality of cellular structures within the WM tracts and ranges from zero (diffusivity equal in all directions) to one (entirely unidirectional). MD is the average rate of diffusion in the non-collinear directions and an increasing value represents an increase in water diffusion. A lower FA and corresponding higher MD are generally believed to reflect lower microstructural connectivity. Other tensor indices have been proposed as markers of neuronal damage, such as axial diffusivity and radial diffusivity. Once FA and MD maps are generated, post-processing procedures start with mainly three approaches: region of interest (Figure 1, panel A), tract-based spatial statistics (TBSS) (Figure 1, panel B), and voxel-based analysis (Figure 1, panel C). Each of these techniques has pro and cons. Technical aspects related to brain network analysis are briefly described in the figure (Figure 1, panel D).

DTI and cerebral SVD

Among DTI studies in patients with sporadic cSVD, mainly defined as presence of moderate/severe WM hyperintensities and/or lacunar infarcts not related to a monogenic disease, the predominant findings are that FA is decreased and MD is increased both in NAWM and WMH suggesting decline in the composition and integrity of the WM.[240] Similar results have been reported also in CADASIL patients (Supplementary Table S1).

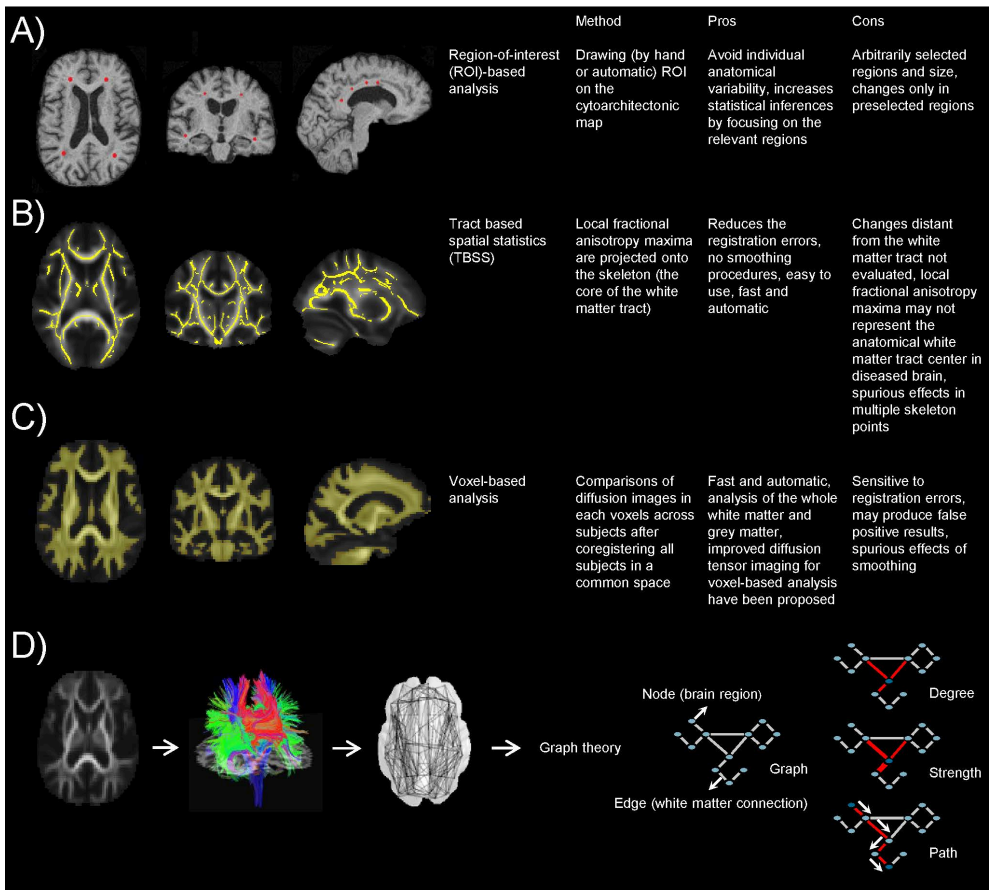


Figure 1: Diffusion tensor imaging (DTI) post processing techniques with pros and cons, construction of structural network based on diffusion tensor tractography and network measures.

A) Region-of-interest (ROI-) based analysis, B) Tract-based spatial statistics (TBSS), C) Voxel-based analysis. D) Construction of structural network based on DTI followed by tractography. Each individual network is represented as a graph, a mathematical model of a network with nodes (brain regions) linked by edges (white matter connections), which can then be explored using graph theory. There is a wide variety of network measures, only some of which are depicted in the figure. Basic network measures, including degree and strength of a node, are depicted in the figure. Degree of a node represents the number of connections linked to a node in a network. Strength of a node is defined as the sum of the weights of all edges connected to a node. Characteristic path length is defined as the shortest path or the minimum number of connections between two given nodes. Efficiency is inversely related to path length, which is easier to use in a disconnected graph.

A substantial loss of anisotropy in the regions of WMH compared with NAWM has been reported[56] while NAWM showed lower FA values close to WMH compared to more distant areas.[241] For this reason, the term WM penumbra has been proposed to describe the area just surrounding the WMH which is still composed of NAWM based on conventional MRI, but with already lower structural integrity as compared with more remote areas of WM.[241]

A positive correlation has been reported between the total WMH load and diffuse WM injury in NAWM, suggesting WM damage to be more widespread rather than region-specific. Normal WM, WMH penumbra, and WMH all show a similar decline in WM integrity over time.[241,242] Accordingly, NAWM regions that ultimately converted into WMH had already significant lower FA and higher MD at baseline in both growing WMH (defined as WMH expanding from already present WMH at baseline) and de novo WMH (defined as a new WMH not adhering to an already present WMH at baseline) compared to persistent NAWM.[24] These results highlight that WMH develop gradually, and that WMH are only the tip of the iceberg of WM pathology.

DTI tractography can be used to spatially characterize WM diffusion abnormalities along the pathway of a specific tract. Using a reconstructed WM tract containing a lacunar infarct, Reijmer and collaborators showed that WM microstructural damage attenuates with increasing distance from the primary lesion.[243] This finding was replicated also in CAA patients.[244] Duering and colleagues applied serial cortical thickness measurements and tractography in CADASIL patients and showed focal cortical thinning in cortical regions with high probability of connectivity with the incident infarct. This result provided evidence for cortical neurodegeneration after subcortical ischemia as one mechanism for brain atrophy in cerebrovascular disease.[245] The same group has replicated this finding in a non-CADASIL cohort.[79]

Recently, new advances in network analysis have been used to study the whole brain connectivity employing graph theory. This can be applied after structural networks have been reconstructed from diffusion tensor tractography (Figure 1, panel D). cSVD patients have been reported to have networks less densely connected, and reductions in both global and local efficiency, compared to controls, especially in interhemispheric and prefrontal tracts.[246] A similar approach showed network disturbances, most pronounced in the occipital, parietal, and posterior temporal lobes, in CAA patients.[237]

Risk factors for microstructural damage within the WM in cSVD

To date, the Radboud University Nijmegen Diffusion Tensor and Magnetic resonance Cohort (RUN DMC) is the only study investigating cross-sectionally the role of vascular risk factors on microstructural changes in cSVD[247,248] (Supplementary Table S2). Increased blood pressure (average of 3 measurements of systolic and diastolic blood pressures) and hypertension (defined as blood pressure >140/80 mmHg and/or use of blood pressure–lowering agents) resulted associated with loss of WM integrity in both the NAWM and WMH.[247] In particular, hypertension was associated with lower FA in the splenium of the corpus callosum and higher MD in both the anterior body and the splenium of the corpus callosum. These associations disappeared after adjustment for other cSVD markers, such as WMH volume, lacunes, and gray matter atrophy, evoking the possible role of mediator of cSVD between hypertension and low microstructural integrity.[248]

In one cSVD cohort, both history and duration of smoking were associated with a low WM microstructural integrity, and diffusion values were comparable between those who had quit

smoking more than 20 years and those who had never smoked. This may suggest a beneficial role of quitting smoking on WM structural integrity.[249]

One cross-sectional study, using TBSS, investigated the relationship between physical activity and WM microstructural integrity showing that poor physical activity was associated with lower microstructural integrity in almost all voxels of the TBSS skeleton.[250]

No study has so far investigated the association between diabetes mellitus and microstructural damage specifically in a cSVD population. A recent review, however, reported on five cross-sectional studies examining the relation between DTI parameters and diabetes mellitus and all found lower microstructural integrity in patients with type 2 diabetes compared to controls, adjusted for different confounders.[251]

Taken together, these studies may suggest that vascular risk factors could damage WM integrity in elderly patients with cSVD and that their control might be associated with better DTI parameters. This may not apply to normal aging because recently in a population-based cohort cardiovascular risk factors were not associated with longitudinal changes in white matter microstructure.[252]

Clinical expressions of cSVD and WM microstructural damage

Cognition

cSVD patients are prone to develop cognitive impairment and their neuropsychological profile is generally characterized by a predominant impairment of executive functions, attention and psychomotor speed. One of the most accepted mechanisms of cognitive impairment in cSVD is based on the disconnection theory by which it is hypothesized that impairment in attention, processing speed, and executive function is related to the disruption of fronto-subcortical circuits. Indeed, it has been demonstrated in both sporadic cSVD and CADASIL patients that the forceps minor and the thalamic radiation are strategic WM tracts for processing speed.[253,254]

O'Sullivan and colleagues demonstrated that in cSVD patients DTI indices, especially in NAWM, correlated more strongly with cognitive function than T2-lesion volume, after controlling for conventional MRI parameters.[240] Similarly, diffusion changes predict faster decline in psychomotor speed, executive functions, and working memory regardless of conventional MRI findings.[104] Other groups have confirmed the strong association between WM microstructural damage and cognitive impairment in sporadic cSVD patients, especially in terms of executive functions, attention and psychomotor speed (Supplementary Table S3). In CADASIL patients, executive performances were reported to be correlated with MD in the frontal WM and through the major antero-posterior fasciculus of the cingulum bundle.[255]

A further contribution to the understanding of the relationship between WM microstructural damage and cognition comes from the RUN DMC study in which more than 500 independently living, non-demented patients with cerebral SVD, aged between 50 and 85 years, were enrolled. In this large cohort, the microstructural integrity of both WMH and NAWM was related to global cognitive function, memory and executive function.[95] Moreover, TBSS post-processing

analyses were performed and corpus callosum especially in the genu and splenium showed the highest significant relation with global cognitive index. Analyses for each cognitive domain showed the strongest relationship between 1) cingulum bundle microstructural integrity and verbal memory performance, and 2) frontal WM and psychomotor speed.[129] However, in the same cohort, the main predictors for the development of incident dementia at 5 years were WM and hippocampal volumes[44] while baseline WM integrity was not associated with decline in cognitive performances.[46]

In the Vascular Mild Cognitive Impairment Tuscany study, WM microstructural damage was more strongly reflected in Montreal Cognitive Assessment (MoCA) than Mini Mental Status Examination performances,[256] possibly for the presence in MoCA of items reflecting executive functions and psychomotor speed.

Interesting insights in the development of cognitive impairment related to cSVD come from the evaluation of network connectivity. In both cSVD and CAA cohorts, the importance of network disruption as a mediating mechanism between cSVD MRI burden and cognitive dysfunction, especially in executive functions, has been demonstrated.[237,246,257] Moreover, it has been shown that structural network efficiency is a predictor of conversion to dementia.[257]

Depressive symptoms

Previous cross-sectional studies showed a positive association between conventional cSVD characteristics and depressive symptoms in older age, both at a cross-sectional level[36] and prospectively.[258] DTI studies performed in patients with late life depression consistently showed lower microstructural integrity in the fronto-striatal and limbic networks.[259]

To date, four studies investigated the role of the WM microstructure in cSVD in relation to depressive symptoms (Supplementary Table S4).[48,260-262] The first study found that microstructural WM damage, measured by median FA, at least partially mediated the association between SVD and depression.[260] The second study, using TBSS, showed that low WM microstructural integrity in the genu and body of the corpus callosum, bilateral inferior fronto-occipital fasciculus, uncinate fasciculus, and corona radiata was associated with depressive symptoms. These associations almost fully disappeared after adjustment for WMH and lacunes, suggesting that the visible cSVD drives the association.[48] The third study reported an association between WM microstructural damage and depressive symptoms in MCI patients with cSVD independently of disability or cognitive or motor impairment.[261] The last study evaluated the relationship between FA and both apathy and depression, finding that only apathy was related to damage of cortical-subcortical networks.[262]

The majority of these studies suggest that the association between WM microstructural damage and depressive symptoms might be mediated by the underlying cSVD and to a lower extent by other factors such as disability.

Motor problems

Only a small number of studies have investigated the relation between WM integrity and motor impairment (gait, parkinsonism, falls and balance) in cSVD using DTI (Supplementary Table S5).[90,96,237,238,263-267] Loss of WM integrity, most pronounced in the corpus callosum, especially the genu,[90,263] was associated with lower gait velocity at a cross-sectional level.[90,96,237,263] This association with gait was seen for both NAWM and WMH. Network efficiency results related with gait velocity in CAA patients, suggesting a role of network disruption in this relation.[237] Other studies investigated the cross-sectional associations between microstructural integrity and a clinical scale measuring extrapyramidal motor deficits,[264] extrapyramidal movement disorders, such as freezing of gait,[265] and mild parkinsonian signs.[238] Three studies found an association between extrapyramidal motor symptoms and low microstructural integrity in both supratentorial (frontal lobes),[238,266,267] and infratentorial (pedunculopontine nucleus) regions. A prospective study showed a low baseline microstructural integrity of several bi-frontal WM tracts involved in movement control in participants with incident vascular parkinsonism in comparison to those without also after adjustment for cSVD characteristics.[266] These studies uniformly support the notion that, in cSVD, disturbances of frontal WM microstructure, especially the genu of the corpus callosum, are associated with motor deficits, and related to incident vascular parkinsonism.

Future directions and conclusions

The evaluation of WM microstructural damage has gained attention over the last 15 years in the study of cSVD because it provides in vivo an understanding of the pathogenesis of important clinical and neuroimaging consequences of cSVD. The majority of the studies that have used DTI demonstrated a very good correlation between WM microstructural damage and several clinical measures linked to cSVD such as cognition, mood disorders and motor performances. In the studies where a multimodal approach was used, DTI indices were generally strongly associated with clinical outcome measures also after correction for multiple conventional neuroimaging markers of cSVD. Furthermore, longitudinal studies showed that changes in DTI parameters could be detected over a period of 1 or 2 years.[72,76] In CADASIL patients for example, Molko and collaborators found important changes in DTI parameters over a period of 20 months, while no changes were detected in the control group.[268] These findings suggest that DTI might be considered a sensitive biomarker to monitor the progression of WM damage in patients with cSVD. This may be particularly relevant because DTI indices were shown to be predictors of clinical progression in both sporadic cSVD and CADASIL.[72,75,76,268] Therefore, the measurement of diffusion will possibly become one important surrogate marker in future preventive trials in cSVD.

There are at least three possible ways in which DTI can be of aid in a better understanding of the aetiology and clinical consequences of cSVD: 1) It may provide new insights in the understanding of the mechanisms of the main clinical consequences of cSVD, particularly by evaluating the structural integrity of the cerebral WM architecture; 2) it may furnish a

reliable surrogate marker, especially in clinical trials, of cSVD progression over time in order to appreciate the effects of beneficial therapeutic interventions; 3) it may help to better appreciate the real cSVD burden and its progression.

Supplementary Table 1. White matter microstructural damage in CADASIL.

Study	Sample size and age (years ± SD)	DTI Method	Adjustments	Significant associations with cognition	Other results
O'Sullivan, 2005	CADASIL pts: 18 46.3±11.3	Voxel based analyses, WM MD and FA	None	- MD, FA in the left cingulum bundle and left precuneus: Trail making Test B and Symbol digit	
Chabriat, 1999	CADASIL pts: 16 59.3±7.6 Controls pts: 10 54.6±7.2	ROI, NAWM and WMH, MD	Age	- WMH MD: MMSE	- NAWM MD and FA in SVD differ significantly from controls - WMH MD differed between patients mild vs severe impairment
Holtmannspotter, 2005	**Longitudinal study:** CADASIL pts: 62 44±9	Whole brain WM MD (average value, peak height and location)	Age, gender, systolic blood pressure, homocysteïne level, T2-lesion volume	- Whole brain WM MD: SIDAM scale	- MD at baseline significant predictor for changes of the Rankin score, of the SIDAM score and newly occurring strokes - 1 year follow up: average values and peak locations of MD significantly increased
Viswanathan, 2010	CADASIL pts: 147 51.8±11.2	Whole brain DWI ADC histogram	Age, gender, education level, WMH, brain volume, normalized lacunes volume, microbleeds	- Whole brain mean ADC histogram: Mattis Dementia Rating scale (also MMSE, results not shown)	- Brain atrophy plays the most important role in disability and cognitive impairment
Jouvent, 2007	CADASIL pts: 42 69.1±7.8	Whole brain DWI ADC histogram	Age, hypercholesterolemia, gender, WMH, lacunes, microbleeds	- Not evaluated	- Brain volume was significantly associated with whole brain mean ADC

Molko, 2002	**Longitudinal study** CADASIL pts: 22 54±11 Controls pts: 12 51±11 14 CADASIL pts and 5 controls repeated MRI at 21±6 months (average time) 7 CADASIL underwent a third MRI	Whole brain DTI, trace of the diffusion tensor [Trace (D)].	None	- Diffusion parameters: baseline MMSE - In the subgroup of 14 patients, these correlations remained significant at both the 1st and 2st scan times.	- Whole brain mean value of Trace (D) was higher in patients than in controls. - An increase in the mean Trace (D) value and a decrease in the peak height of Trace (D) histograms were observed overtime in patients, while no change was found in the control group

DTI: diffusion tensor imaging, Pts: patients, WMH: white matter hyperintensities, NAWM: normal appearing white matter, WM: white matter, SIDAM : structured Interview for the diagnosis of Dementia of the Alzheimer Type, multi-infarct dementia and other type of dementia. MMSE: mini-mental state examination test, DWI: diffusion weighted imaging, MD: mean diffusivity, FA: Fractional Anisotropy, ROI: Region of Interest

Supplementary Table 2. White matter microstructural damage in cerebral small vessel disease and vascular risk factors.

Study	Sample size and age (years ± SD)	SVD definition	Risk factors	DTI method	Adjustments	Significant association with vascular risk factor	Other
Gons, 2010	SVD pts: 499 65.6±8.8	Presence of WMH and/ or lacunes	Blood pressure (>140/90 and/ or use of anti-hypertensive medication)	ROI: Mean FA and mean MD in NAWM and WMH, and lobar ROI's	Age, sex, DM hypercholesterol-aemia, smoking and BMI	- Mean FA and MD in the WMH: both systolic and diastolic blood pressure - Mean FA in the NAWM: both systolic and diastolic blood pressure - Mean MD in the NAWM: systolic blood pressure - Hypertensive subjects had lower mean FA and higher mean MD in WMH and NAWM than normotensives	- Treated uncontrolled had lower mean FA and higher mean MD values than the treated controlled subjects
Gons, 2012	SVD pts: 499 65.6±8.8	Presence of WMH and/ or lacunes	Blood pressure (>140/90 and/ or use of anti-hypertensive medication)	ROI mean FA and mean MD in the genu, anterior body, posterior body and splenium of the CC	Age, sex and cardiovascular risk factors Additionally GM volume, number of lacunes and WMH	- Low FA in the splenium and high MD in the anterior body and splenium of the CC and presence of hypertension - Treated uncontrolled subjects had both lower FA and higher MD in both the anterior body and splenium	- FA and MD in the genu, anterior body and splenium were associated with global cognitive and executive functions
Gons, 2011	SVD pts: 499 65.6±8.8	Presence of WMH and/ or lacunes	Cigarette smoking (pack-years, and status never/ former/ current)	ROI Mean FA and mean MD in NAWM and WMH	Age, sex, alcohol intake, education and cardiovascular risk factors, including DM hypercholesterol-aemia, HT and BMI	- MD in WMH and NAWM and smoking status Lower MD and higher FA in the NAWM and years of smoking cessation - Smoking cessation >20 years had identical MD and FA values compared with never smokers	- MD and FA in the NAWM and global cognition (by cognitive index)

Gons, 2013	SVD pts: 440 Age range 50-85	Presence of WMH and/ or lacunes	Physical activity, MET	TBSS MD, AD, RD and FA And ROI: mean FA and MD in the NAWM and WMH	Age, sex, education, normalized TBV, and cardiovascular risk factors.	- MD in the WMH and NAWM and physical activity - In almost all voxels of the skeleton MD, AD and RD related to physical activity, whereas no association with FA	- Association remained present after adjustment for confounders

SVD: small vessel disease, HT: hypertension, HC: healthy controls; WMH: white matter hyper intensities, MRI: Magnetic Resonance Imaging, DTI: Diffusion Tensor Imaging, MRS: Magnetic Resonance Spectroscopy, NAA: N-acetylaspartate, tCho: total cholines, tCr: total creatines, mI: myo-inositol, MD: mean diffusivity, FA: Fractional Anisotropy, ROI: Region of Interest, DM: diabetes mellitus, BMI: Body Mass Index, CC: corpus callosum, MET: metabolic equivalent, TBV: total brain volume

Supplementary Table 3. White matter microstructural damage and cognition in cerebral small vessel disease.

Study	Sample size and age (years ± SD)	SVD definition	Cognitive Tests	DTI Method	Adjustments	Significant associations with cognition	Other results
Lawrence, 2013	SVD pts: 121 70.0±9.7 Controls: 57 70.4±9.2	Clinical lacunar stroke + lacunar infarct on MRI and WMH (Fazekas ≥ 2)	Verbal fluency, modified Wisconsin card sort test, Grooved Pegboard Task, digit symbol substitution, BMIPB Speed of Information Processing	Whole brain NAWM: FA, MD, AD, RD (mean, median, inter quartile range, SD, skew, kurtosis, peak height and location)	Age, sex , premorbid IQ, WMH, lacunes, brain volume	- Peak height MD: psychomotor speed - Peak height RD: executive functions	- Mean FA < in SVD than controls - Mean MD > in SVD than controls - RD more altered than AD in SVD
O'Sullivan, 2004	SVD pts: 36 69.5±8.8 Controls: 24 71.6±7.5	Diffuse/ confluent WMH + clinical lacunar stroke	MMSE, WAIS-R digit span, digit symbol, full scale IQ, WCST, Reitan trail making, verbal fluency, WAIS-R digit symbol and digit span backwards, logical memory, paired associate learning subtests, Benton facial recognition	ROIs in WMH and NAWM: FA, MD	Age, sex, WMH volume, parenchymal volume	- NAWM MD: IQ and WCST	- NAWM MD and FA in SVD differ significantly from controls
List, 2003	SVD pts: 20 72.3±4.3 Controls: 20 70.6 ±3.6	WMH (Fazekas ≥ 2)	TMT A+B, verbal and semantic fluency, AVLT, ROCF, digit span and block tapping, working memory performance	Whole brain WM, ROIs: mean FA	Age	- Mean whole brain WM FA: executive functions, working and verbal memory	- In SVD, FA of all brain areas, except cerebellum < than controls
Nitkunan, 2008	**Longitudinal study:** Baseline: SVD pts: 35 68.8±9.3 1 year Follow-up SVD pts: 27	Lacunar stroke (at least 3 months before the enrolment)+ Fazekas≥ 2	National adult reading test, WAIS: vocabulary and matrix reasoning subtests, MMSE, Wechsler Memory scale III (verbal memory, digit span), verbal fluency, TMT B, Trails Motor Speed subtest from the Delis-Kaplan Executive Function System	Whole brain WM: FA and MD (peak height, median, mode)	WMH volume, brain volume, lacunar infarcts age, sex, and premorbid IQ	- Peak height FA: executive functions (composite score).	- 1-year follow-up: increase in median MD, MD at peak height, and reduction in peak height FA

Xu, 2010	SVD pts: 42 Age: 69.1±7.8	Moderate and severe WMH + lacunar infarct	MMSE, TMT, Stroop colour word test, category verbal fluency test, RAVL test, ROCF, Boston naming test	Whole brain WMH or NAWM: FA, MD	Age, sex, education	- Whole brain WMH and NAWM DTI indices: attention, executive and memory functions	- Cognitive impairment pts had > MD and <FA in both WMH and NAWM than pts without cognitive impairment
Zhou, 2011	SVD pts: - 18 with cognitive impairment no dementia -18 no cognitive impairment	WMH + ≥ 1 lacunar infarct	TMT A, B, Stroop colour word test, category verbal fluency test, AVLT, ROCF, Boston naming test (30 words)	VBA: MD and FA average value, histogram peak height and location	None	- FA peak location, average MD, and mean MD peak location of WM: general intellect (composite z-scores)	- SVD pts with cognitive impairment had < FA in bilateral frontal lobes, occipital lobes, temporal lobes, and insula than no cognitive impairment SVD pts
Tuladhar, 2015	SVD pts: 444 65.3±8.9	Presence of WMH and/or lacunes	MMSE, 1-letter subtask of the paper–pencil memory scanning task, Stroop test, symbol–digit, RAVL, ROCF, verbal fluency, verbal series attention test	TBSS, ROIs (corpus callosum): FA, MD	Age, sex, education, depressive symptoms, normalized TBV, WMH and number of lacunes	- WM MD, FA: psychomotor speed, concept shifting. - WM FA: cognitive index, verbal memory - cingulum bundle and corpus callosum FA: verbal memory - integrity of genu and splenium of the corpus callosum: cognitive index, executive domains, psychomotor speed and concept shifting - integrity of body of corpus callosum: memory	- Association of MD and cognition in corpus callosum resulted driven by changes in RD, and not AD

Van Norden, 2012	SVD pts: 499 65.6±8.8	Presence of WMH and/or lacunes	Same as reference [17]	WMH and NAWM FA, MD	Age, sex, education, depressive symptoms, TBV, lacunes and WMH	- WMH MD, FA: attention, concept shifting - WMH MD: global function, psychomotor speed. -NAWM MD, FA: concept shifting, psychomotor speed, attention, verbal memory - NAWM MD: visuospatial memory, fluency	- Pts with severe WMH had MD and FA changes more related to worst performances in many cognitive domains than those with mild or moderate WMH
Van der Holst, 2013	SVD pts: 440 65.2± 8.9	Presence of WMH and/or lacunes	Same as reference [17]	TBSS, ROIs (cingulum): FA MD	Age, sex, educational level, depressive symptoms TBV, hippocampal volume, WMH, lacunes	- FA and MD in the cingulum, corpus callosum: immediate memory, delayed recall, delayed recognition and overall verbal memory performance - mid/posterior (ROI approach): cingulum FA and memory	- MD, FA of all ROIs differed significantly between pts with good and poor hippocampal integrity
Pasi, 2015	SVD pts: 76 75.8±6.8	WMH (Fazekas ≥ 2) and MCI	MoCA test, MMSE	Whole brain WM: median FA, MD	Age, education level, sex, lacunar infarcts, WMH, global cortical atrophy, medial temporal lobe atrophy	FA, MD: MoCA test	- No correlation was found between MD, FA and MMSE

O'Sullivan 2001	SVD pts: 30 69.7±8.9 Controls: 17 71.8±7.9	WMH + history of a clinical lacunar event	MMSE, Wisconsin Card Sorting test	ROI: NAWM MD, FA (anterior and posterior PV, centrum semiovale)	None	- Anterior PV FA and MMSE - Centrum semi-ovale: executive functions (Wisconsin card sorting test)	- FA in PV NAWM < in SVD
Kim SH, 2011	SVD pts: 61 73.3±6.9 - MCI: 27 - Demented: 34	WMH: PV cap or band ≥ 10 mm + deep lesion ≥ 25 mm and MCI or dementia	ROCF, Seoul verbal learning test, Boston naming Test, digit span test, word fluency tests, and Stroop colour reading test	VBM: FA, MD	Age, education	- MD values in temporal area: delayed recall - FA in the splenium of the corpus callosum and FA and MD in posterior PV areas: praxia - FA in the left medial temporal area: language - FA, MD in PVWM and deep WM: digit span - FA and MD in the left PVWM: phonemic fluency - FA in the posterior PVWM and MD in right medial temporal, corpus callosum, anterior cingulate gyrus and lingual gyrus: attention	- Disruption of the posterior WM integrity is related to poor performance on cognitive tests in the task for frontal functioning.
Li, 2012	SVD pts: 20 65.1±7.0 Controls: 20 65.8±8.0	WMH + history of a clinical lacunar event	MMSE, TMT B, verbal fluency (phonemic and meaning), digit span backwards, digit symbol	ROI: mean MD, FA (anterior, posterior horn)	None	- Anterior PV WM FA: executive functions - Anterior PV WM MD: executive functions, fluency, memory, MMSE - Posterior PV WM: MD and executive functions	- MD > and FA < in PV WM in SVD

Lin, 2015	SVD pts: 50 VCIND pts: 22 72±6.8 NC pts: 28 70.9±8.2	Moderate WMH + lacunar infarcts	MoCA test	ROI: FA, MD	None	- FA and MD values of all projections, commissural and associational fibres: MoCA score	- MD > VMCI than NC - FA < VMCI than NC
Jokinen, 2013	SVD pts: 340 73.9±5.1	Mild to severe WMH	MMSE, VADAS, Stroop test and TMT A and B-A	DWI: WMH mean ADC	Age, sex, education, WMH, lacunes, global brain atrophy	- WMH mean ADC: TMT A	- WMH mean ADC related to a faster rate of decline in the TMT A scores
Della Nave, 2007	SVD pts: 36 77±4.5	Mild to severe WMH	MMSE, Stroop test, Symbol digit, Maze Task and Verbal Fluency Test	VBM FA histograms and whole brain MD	None	- Whole brain MD: Stroop test, Maze task, digit and verbal fluency	
Van Uden, 2015	**Longitudinal study** SVD pts:500 65.6±8.8	Presence of WMH and/or lacunes	Same as reference 17	NAWM, WMH FA,MD	Age, gender, education, MMSE, WMH, lacunes, MB, GM volumes, hippocampal volumes	- DTI parameters did not predict dementia (42 pts after 5 years)	- WM and hippocampal volume predicted the risk of dementia at 5 years
Van Uden 2015	**Longitudinal study** SVD pts: 398	Presence of WMH and/or lacunes	Same as reference 17	NAWM, WMH FA,MD	Age, gender, education, depressive symptoms, WMH, lacunes, TBV	- NAWM MD with decline in cognitive index - no significant association after Bonferroni correction	

Tuladhar, 2015	SVD pts:436 65.2±8.8	Presence of WMH and/or lacunes	Same as reference 17	Weighted structural connectivity network from DTI	Age, gender, education, depressive symptoms, WMH, lacunes, MB, total brain volume, MD	-Higher global efficiency with higher scores on cognitive index and psychomotor speed - Lower global network efficiency with increased risk of incident all-causes dementia	- WMH, MB, TBV, MD, indirectly related to cognitive index and psychomotor speed via global efficiency - Nodes with the strongest associations with cognitive index and psychomotor speed were in frontal, parietal, occipital lobes

DTI: diffusion tensor imaging, Pts: patients, MD: mean diffusivity, FA: fractional anisotropy, RD: radial diffusivity, AD: axial diffusivity, TBSS: Tract-based spatial statistics, VBM: Voxel based morphometry, VBA: Voxel based analysis, WMH: white matter hyperintensities, NAWM: normal appearing white matter, ARWMG: age-related with matter changes, SVD: small vessel disease, MCI: mild cognitive impairment, PV: periventricular, VCIND: vascular cognitive impairment, NC: normal cognition, MoCA: Montreal Cognitive Assessment, MMSE Mini-Mental State Examination, IQ: Intelligence quotient, VADAS: Vascular Dementia Assessment Scale–Cognitive Subscale, NABT: Normal appearing brain tissue, BMIPB: Birt Memory & Information Processing Battery, WAISR: Wechsler adult intelligence scale–revised, WCST: Wisconsin card sorting test, TMT: Trail making test, AVLT: Auditory Verbal Learning Test, ROCF: Rey-Osterrieth complex figure test, RAVL: Rey auditory verbal learning test, TBV: Total Brain volume, GM: Gray matter, MB: microbleeds, DWI: diffusion weighted imaging, ROI: region of interest

Supplementary Table 4. Microstructural damage in cerebral small vessel disease and depressive symptoms

Author	Sample size and age (years ± SD)	SVD definition	Depression-scale	DTI method	Adjustments	Significant association with depressive symptoms	Other
Brookes, 2014	SVD pts: 101 71±9.5 Controls: 203 67.1±9.4	Clinical lacunar stroke syndrome with radiologic (MRI) confirmation	GDS	TBSS median FA	Global cognitive deficit, functional disability	- Median FA and depression: lower white matter integrity associated with depressive symptoms	- Association between median FA and disability and with global cognition - No association between median FA and quality of life
Van Uden, 2014	SVD pts: 438 65.1±8.8	Presence of WMH and/or lacunes	CES-D	TBSS FA, MD, RD, AD	Age, sex, education and TBV Additionally: WMH and lacunes, cognition, anti-depressant use	- Low mean FA, high MD and RD in pts with depressive symptoms compared to those without In pts. with depressive symptoms compared to those without: - FA, AD and RD: genu and body of the CC, bilateral IFOF, UF and corona radiata - For AD and RD(additionally): CB, internal and external capsule, ILF and parietal lobe - For MD: genu and body of the CC, CB, corona radiata	- The associations with FA, AD and RD disappeared after adjustment for WMH and lacunes - Adjustment for use of anti-depressive medication or cognition did not alter the results

Pasi, 2015	SVD pts: 76 75± 6.8	Evidence on MRI of moderate to severe degrees of WMH according to the modified version of the Fazekas scale	GDS	Whole brain Mean FA and mean MD	Age, WMH, global cognitive, functional and motor performance, anti-depressant use	- Median FA and MD and depressive symptoms	- This association was not mediated by disability, cognitive, and motor impairment
Hollocks, 2015	SVD pts: 118 69.9±9.8 Controls: 398 61.9±13.5	Clinical lacunar stroke syndrome with radiologic (MRI) confirmation	GDS	VBA FA MD	Age, IQ, global cognitive function and apathy	- Median FA with apathy but not depression	- Association between median FA and quality of life - Association between Median FA and MD and global cognitive function

Pts: patients, SD: standard deviation, DTI: diffusion tensor imaging, SVD: small vessel disease, GDS: geriatric depression scale, TBSS: tract-based spatial statistics, FA: fractional anisotropy, CES-D: centre for epidemiologic studies depression Scale, WMH: white matter hyperintensities, MD: mean diffusivity, RD: radial diffusivity, AD: axial diffusivity, WM: white matter, CC: corpus callosum, IFOF: inferiorfronto-occipital fasciculus, UF: uncinate fasciculus, ILF: inferior longitudinal fasciculus, CB: cingulum bundle; VBM: voxel based morphometry, VBA: voxel based analysis, IQ: intelligence quotient.

Supplementary Table 5. White matter microstructural damage and motor impairment in cerebral small vessel disease

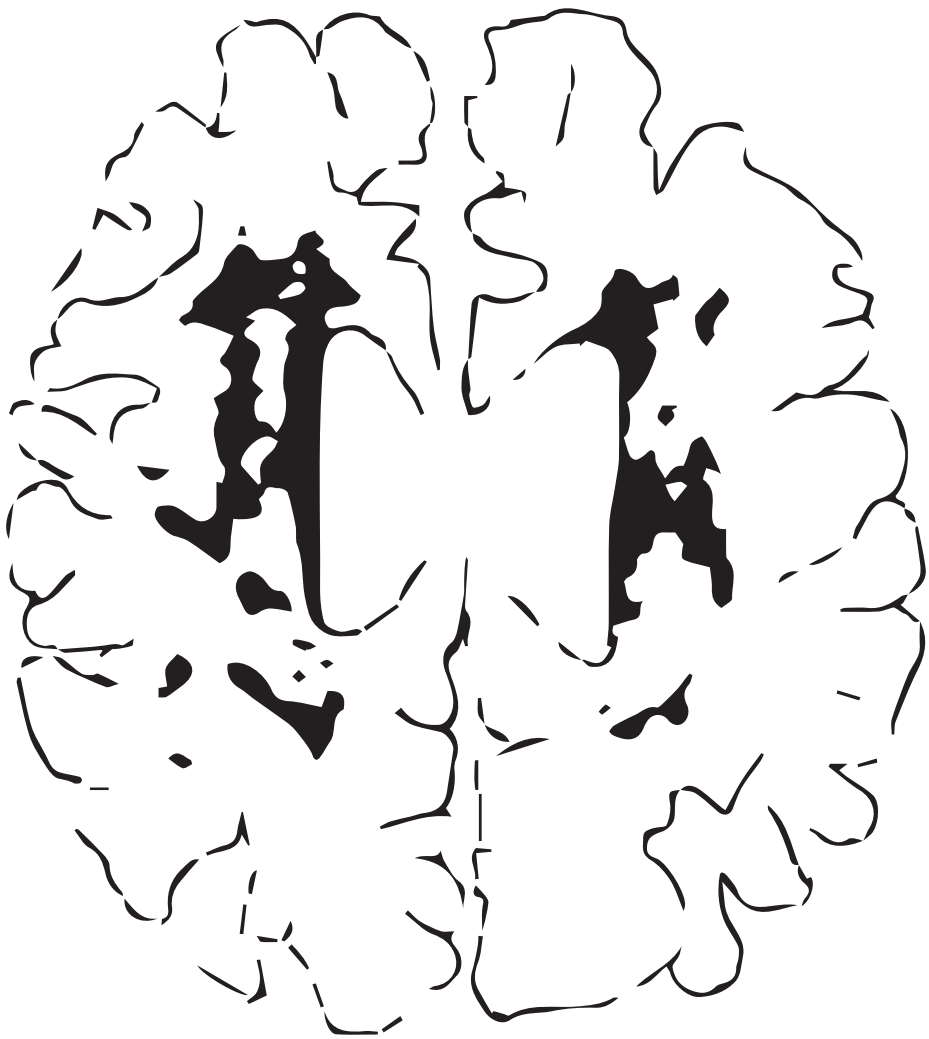
Author	Sample size and age (years ± SD)	SVD definition	Motor score	DTI method	Adjustments	Significant association with motor symptoms	Other
Della Nave, 2007	SVD pts: 36 77±4.5	ARWMC changes of any degree	Gait velocity, Single-leg stance test SPPB	VBM FA histograms and whole brain MD	None	- Wide clusters of high MD and smaller clusters of FA in both the corpus callosum and pericallosal WM with 1) gait velocity and 2) SPPB	- No results with single-leg stance test were shown
de Laat, 2011	SVD pts: 484 65.6±8.9	Presence of WMH and/or lacunes	Gait velocity, Stride length, Stride width, Tinetti, TUG	ROI based Mean FA and MD in WMH, NAWM	Age, sex, height and TBV additionally: WMH, lacunes	- Higher MD in the WMH: gait speed, cadence, step width, stride width, Tinetti and TUG. Lower FA in the WMH and Tinetti	- Both FA and MD in the NAWM were associated with several gait parameters, of which only the MD in the NAWM remained present after adjustment for WMH
de Laat, 2011	SVD pts: 429 65.2±8.9	Presence of WMH and/or lacunes	Gait velocity, Stride length, Stride width, Cadence	TBSS -VBM FA, MD, AD and RD 3 ROI's in the CC Mean FA, MD AD and RD	Age, sex and height additionally: WMH, lacunes, TBV	- FA positively associated with gait velocity, stride length and negatively to stride width. MD was negatively associated with the same parameters. - Voxels with the highest relation between FA and MD and gait were located in the total CC, and for MD also in the internal capsule	- After additional adjustment for WMH and lacunes most associations disappeared. - DTI parameters in the genu of the CC showed strongest associations with gait
Kim, 2011	MCI pts: 27 73.6±6.7 Dementia pts:34 73.0±7.5	A cap or band ≥10 mm as well as a deep WMH ≥25 mm, as modifed from Fazekas ischemia criteria	Pyramidal and Extra-pyramidal scale for motor deficits	VBM FA and MD	Age	- Low FA with total PEPS score and gait in the brainstem, CC, cerebellum, Corona radiata. - High MD with PEPS in the brainstem, bilateral PVWM and corona radiate. - High MD with extrapyramidal scores in the PVWM and forceps major	- Low FA and high corticospinal score in the internal capsule, corona radiate, CC and posterior PVWM - High MD and corticospinal score in the internal capsule and corona radiate - Corticobulbar symptoms and low diffusion parameters in brainstem

Youn, 2012	FOG pts: 14 81±5.5 Controls: 26 79±5.4	ARWMC 2 or more	Freezing of gait	ROI FA and ADC	FOG and control group did not differ in age, sex, vascular risk factors and ARWMC scale	- Low FA and FOG in the bilateral pedunculopontine nucleus (PPN), superior premotor cortex, right orbitofrontal area and left supplement motor area	- No significant association with ADC values - Compared to controls the FOG group fibre tracking showed lower fibre bundle volume in the PPN ROI
Reijmer, 2015	CAA pts: 31 68.9±9.9 Controls: 29 71.3± 7.1	Probable or definite CAA	Gait velocity (TUG)	DWI; structural brain network by network density, global efficiency	Age, sex, education level, WMH, MB TBV or median FA	- Low global network efficiency was associated with worse gait velocity. This was not independent of median FA	
De Laat, 2012	SVD pts: 483 65.6±8.9	Presence of WMH and/or lacunes	Mild parkinsonian signs by UPDRS	ROI based Mean FA and MD in WMH, NAWM	Age, sex and TVB WMH and lacunes	- Low FA in the WMH and MPS, independent of WMH volume. - Low FA or high MD in the NAWM increased the presence of MPS 2 fold, which disappeared after adjustment for WMH and lacunes. - Low FA and high MD and MPS in the periventricular ROIs, independent of WMH and lacunes	- In the subcortical ROIs mainly presenting NAWM no association with MPS
Van der Holst, 2015	**Longitudinal study** SVD pts: 436 65.6±8.8 at baseline	Presence of WMH and/or lacunes	Parkinsonism (clinical diagnosis)	TBSS Mean FA, mean MD	Age, sex, baseline UPDRS score and TBV, WMH, WM, lacunes and MB	- low FA and high MD of bi-frontal WM tracts (CC, CI, SLF, forceps minor, IFOF, cingulum, superior Corona radiata, thalamic radiation) with incident Parkinsonism after 5 years. The association with FA remains present after adjustment for SVD.	- There was no association with high MD and incident parkinsonism after 5 years after adjustment for SVD.

Pts: patients, SD: standard deviation, DTI: diffusion tensor imaging, SVD: small vessel disease, ARWMC: age-related white matter changes, SPPB: short physical performance battery, VBM: voxel-based morphometry, FA: fractional anisotropy, MD: mean diffusivity, WMH: white matter hyperintensities, NAWM: normal appearing white matter, TUG: timed up and go-test, ROI: region of Interest, TBV: total brain volume, TBSS: tract-based spatial statistics, AD: Axial Diffusivity, RD: Radial Diffusivity, CC: Corpus Callosum, MCI: Mild Cognitive Impairment, PEPS: Pyramidal and Extra-pyramidal scale, MRI: Magnetic Resonance Imaging, PVWM: peri-ventricular white matter, FOG: freezing of gait, ADC: apparent diffusion coefficient, PPN: pedunculopontine nucleus, MPS: mild parkinsonian signs, CAA: cerebral amyloïd angiopathy, DWI: diffusion weighted imaging, MB: microbleeds, UPDRS: unified Parkinson's disease rating scale, WM: white matter, CI: internal capsule, SLF: superior longitudinal fasciculus, IFOF: inferior fronto-occipital fasciculus.

Part V:

The temporal dynamics of cerebral small vessel disease



10.

The Rise and Fall of cerebral small vessel disease

Ingeborg W.M. van Uden*, Esther M.C. van Leijsen*, Mohsen Ghafoorian, Mayra I. Bergkamp, Valerie Lohner, Eline C.M. Kooijmans, Helena M. van der Holst, Anil M. Tuladhar, David G. Norris, Ewoud J. van Dijk, Loes C.A. Rutten-Jacobs, Bram Platel Catharina J.M. Klijn and Frank-Erik de Leeuw

Submitted

**Both authors contributed equally*

Abstract

Cerebral small vessel disease (cSVD) progression is increasingly recognized as the most important vascular contributor to the development of dementia. However, our understanding of ***who*** will show cSVD progression and ***when*** this will occur is limited due to the lack of studies with more than one follow-up assessment. In this study we aimed to investigate the temporal dynamics of cSVD progression by three consecutive imaging assessments in participants with cSVD.

We assessed changes in cSVD markers at three time-points over nine years in 276 participants of the RUN DMC cohort. We assessed white matter hyperintensities (WMH) volume by semi-automatic segmentation, and rated lacunes and microbleeds manually. We categorized baseline WMH severity as mild, moderate or severe according to the Fazekas scale. We performed mixed-effects regression analysis including a quadratic term for increasing age during follow-up.

Mean WMH progression over nine years was 4.7 mL (0.54 mL/yr; IQR 0.95–5.5 mL), 20.3% of the participants had incident lacunes (2.3%/yr) and 18.9% incident microbleeds (2.2%/yr). WMH volume declined in 9.4% of the participants, and lacunes vanished in 3.6% and microbleeds in 5.7% of the participants. WMH progression accelerated over time: including a quadratic term for increasing age during follow-up significantly improved the model ($p<0.001$). cSVD progression was predominantly seen in participants with moderate to severe WMH at baseline, whereas those with mild WMH barely progressed (OR 35.5; 95% CI 15.8-80.0; $p<0.001$ for WMH progression; OR 5.7; 95% CI 2.8-11.2; $p<0.001$ for incident lacunes and OR 2.9; 95% CI 1.4-5.9; $p=0.003$ for incident microbleeds).

Our findings demonstrate that cSVD progression is a non-linear, dynamic and highly variable process, sometimes interrupted by vanishing cSVD. cSVD progression was predominantly seen in participants with severe WMH, suggesting that therapeutic strategies to prevent cSVD progression should particularly be deployed in patients with moderate to severe WMH.

Introduction

Markers of cerebral small vessel disease (cSVD) are present on neuroimaging in virtually every individual over 60 years of age, although in highly variable degree. They include white matter hyperintensities (WMH), lacunes of presumed vascular origin, cerebral microbleeds and (sub)cortical brain atrophy [12]. cSVD, and its progression, has been recognized as the most important vascular contributor to the development of dementia [29,41]. Therefore understanding of not only ***who*** will show progression of cSVD but also ***when*** this will occur, will result in better understanding of both aetiology and consequences of cSVD, and may in time lead to personalized treatment approaches.

Current knowledge regarding temporal dynamics of cSVD is limited due to lack of studies with more than one follow-up assessment. Consequently, these studies could only report the average, presumably linear change in cSVD severity between two time-points. Average WMH progression ranged between 0.2 and 2.5 mL/yr, depending on the study population [14,15,22,269]. Yearly incidence of lacunes varied between 0.7 and six percent [17,18], and that of microbleeds between three and nine percent [19,20]. Previous studies have suggested that cSVD progression may in fact be a non-linear process accelerating over time [270,271], and that, besides age, baseline lesion load may predict cSVD progression [22,270-272]. Actual proof for these assumptions is lacking.

Recently, decrease of WMH volume [270-274] as well as decrease in number of lacunes [275,276] and microbleeds [277,278] have been reported, further challenging the assumption of linear progression of cSVD markers. Neither the time-course, nor the magnitude of this "vanishing cSVD" has been investigated.

There is increasing awareness that cSVD not only exerts its clinical effects in a direct way by disconnecting white matter tracts, but also by affecting remote brain structure and function [79,279]. The temporal relation between changes in cSVD and the subsequent atrophy of remote brain structures remains unknown because of the same methodological limitation of having only one follow-up moment.

In this study we investigated the temporal dynamics ("the rise and fall") of cSVD by three consecutive assessments over a period of nine years in participants with cSVD, distinguishing progression from regression. In addition, we investigated the effect of cSVD progression on remote brain regions including grey and white matter.

Methods

Study population

This study is part of the Radboud University Nijmegen Diffusion tensor and Magnetic resonance imaging Cohort (RUN DMC) study that prospectively investigates risk factors and clinical consequences of cSVD. The detailed study protocol has been published previously [60]. Of 503 baseline participants, 281 underwent repeated MRI assessment at three time points (baseline in 2006, first follow-up in 2011 and second follow-up in 2015). Five participants were excluded because of insufficient scan quality, yielding a final sample of 276 participants

for the present study (Supplementary Fig. 1). The Medical Review Ethics Committee region Arnhem-Nijmegen approved the study and all participants gave written informed consent.

MRI protocol

Images were acquired at three time-points on 1·5-Tesla MRI (2006: Siemens, Magnetom Sonata; 2011 and 2015: Siemens, Magnetom Avanto) and included the following whole brain scans: T1-weighted 3D MPRAGE, FLAIR and T2*-weighted gradient echo sequence. Full acquisition details have been described previously [60]. We made slight adjustments in the FLAIR sequence between baseline and first follow-up (baseline: voxel size 1·2×1·0×5·0 mm; interslice gap 1·0 mm; first and second follow-up: voxel size 1·2×1·0×2·5 mm; interslice gap 0·5 mm). Follow-up FLAIR images were resliced using linear interpolation to match slice thickness of baseline images to prevent differences in partial volume effects. The same head coil was used at all three time points.

Cerebral small vessel disease and brain volumetry

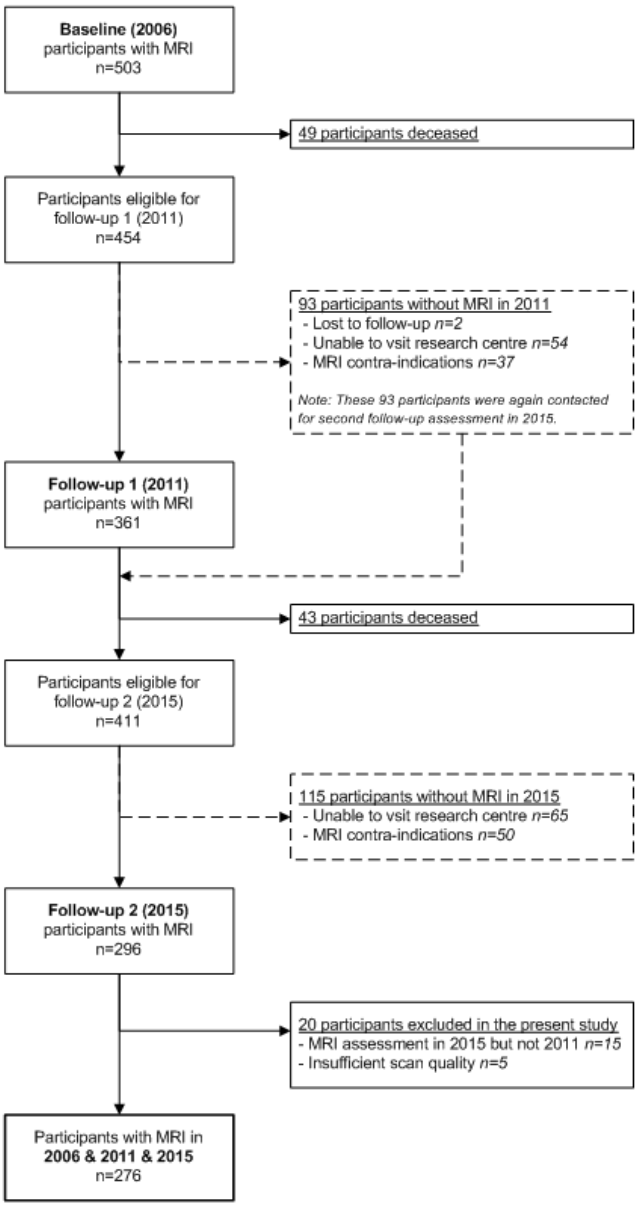
cSVD was rated according to the STRIVE criteria [12]. WMH volumes were calculated by a semi-automatic WMH segmentation method at all time points [280]. Segmentations were visually checked for segmentation errors by one trained rater, blinded for clinical data. Because of its easy use and clinical applicability, we used the modified Fazekas scale to categorize WMH severity at baseline (mild: Fazekas 0-1; moderate: Fazekas 2; and severe: Fazekas 3) [281]. Lacunes and microbleeds were rated manually on FLAIR/T1-weighted scans and T2*-weighted MRI scans by two trained raters blinded for clinical data. Inter and intra-rater reliability were excellent [45].

Grey matter (GM), white matter (WM) and CSF probability maps were computed using SPM12 (http://www.fil.ion.ucl.ac.uk/spm/) unified segmentation routines on T1 images corrected for WMH. All images were visually checked for co-registration and segmentation artefacts. GM, WM and CSF volumes (GMV, WMV and CSFV) were computed by summing all voxels belonging to that tissue class multiplied by voxel volume in mL. Intracranial volume (ICV) was determined by summing GMV, WMV and CSFV and total brain volume (TBV) by summing GMV and WMV.

Longitudinal brain changes

We calculated brain volumes (TBV, GMV, WMV) and WMH volume (WMHV)) at all time-points in native space. To account for inter-scan-effects we corrected for differences in ICV between baseline and follow-up. We normalized all volumes to baseline ICV to account for head size [89]. We determined longitudinal volume change by calculating differences in volumes between the three time-points. To correct for WM atrophy we also calculated WMH as percentage of WM. Change in the number of lacunes and microbleeds was expressed as difference between 2015, 2011 and 2006, and included both increase and decrease. Incidence was expressed as number of participants with new lacunes or microbleeds.

Supplementary Figures



Supplementary Figure 1: Flowchart

Design of the RUN DMC study. Imaging assessments were performed at three time-points over the course of nine years at baseline in 2006, at first follow-up in 2011 and at second follow-up in 2015. Note that the 93 participants who were unable to undergo first follow-up assessment in 2011 were again contacted for second follow-up assessment in 2015 (dotted lines). In total 281 participants underwent imaging assessments at all three time-points, of whom 276 participants were included in the present study.

Vascular risk factors

We assessed presence of hypertension, smoking, alcohol use, diabetes and hypercholesterolemia, by standardized questionnaires, as described previously [60].

Statistical analysis

We calculated differences in baseline characteristics between participants and those without follow-up using univariate analyses. We created WMH probability maps and distribution maps of lacunes for the three time-points separately as well as for change. WMH decline was defined as more than 0.25 mL volume decline, as previously this was shown to be smallest change that could be confirmed visually [274]. Changes of WMH volume with increasing age were plotted at individual level using R package ggplot2 (version 2.1.0) [282]. R package lme4 was used to perform mixed-effects regression analysis with maximum likelihood estimation to analyse WMH change as function of baseline age and time (version 1.1-12) [283]. We used a random intercept and random slope model, which permits the estimation of an average slope across the whole cohort while allowing for inter-individual variability. By smoothed curves using loess smoothing we explored average WMH change with increasing age. To evaluate a possible quadratic relationship, indicating non-linear progression of cSVD, we compared model fit between the full model and the full model with a quadratic term for increasing age during follow-up included, using χ^2 test and change in Akaike information criterion (AIC).
Multicollinearity between different cSVD markers was investigated using regression analysis. To determine remote effects of cSVD progression, we analyzed the relation between WMH progression in the first follow-up interval, and GM, WM and total brain atrophy in the second, adjusted for age, sex, baseline volume, and atrophy during the first follow-up period, by means of linear regression analysis.
We created WMH probability maps stratified by baseline age (<60 years, 60-70 years and >70 years) and by baseline WMH severity (mild, moderate and severe). We repeated mixed-effects regression analysis stratified by baseline WMH severity to explore change in WMH within these groups separately. We calculated odds of cSVD progression according to baseline Fazekas 0-1 versus Fazekas 2-3 by logistic regression analysis, adjusted for age and sex.
Statistical analyses were performed using SPSS Statistics version 20 and R Programming Language version 3.2.1.

Results

Baseline characteristics are presented in Table 1. Mean age at baseline was 62.5 ± 7.7 years and 59·1% was male. Mean follow-up duration was 5.4 ± 0.2 years until first and 8.7 ± 0.2 years until second follow-up. Those lost to follow-up were significantly older, had more severe baseline cSVD characteristics, and more often had hypertension, diabetes and hypercholesterolemia compared to participants (Supplementary Table 1).

Table 1: Demographic and imaging characteristics

	Baseline (2006) n=276	Follow-up 1 (2011) n=276	Follow-up 2 (2015) n=276	Change 2006-2011	Change 2011-2015	Overall change 2006-2015
Demographics						
Age (years), mean (SD)	62.5 (7.7)	67.8 (7.7)	71.2 (7.8)	5.3 (0.2)	3.4 (0.2)	8.7 (0.2)
Time until follow-up, yrs	-	5.4 (0.2)	8.7 (0.2)	-	-	-
Sex, male, no (%)	163 (59.1)	-	-	-	-	-
Education >primary school, no (%)	259 (93.8)	-	-	-	-	-
MMSE score, mean (SD)	28.6 (1.3)	28.4 (1.8)	28.2 (2.0)	-0.23 (1.7)	-0.22 (1.6)	-0.45 (2.0)
SVD characteristics						
White matter hyperintensities, ml, median (IQR)	2.3 (0.8-6.1)	2.8 (1.2-7.7)	4.7 (2.0-11.5)	0.52 (0.03-1.6)	1.5 (0.51-3.8)	2.1 (0.95-5.5)
White matter hyperintensities, ml, mean (SD)	5.8 (9.5)	7.4 (11.5)	10.5 (14.4)	1.6 (3.6)	3.1 (4.3)	4.7 (6.6)
% WMH of WM, mean (SD)	1.3 (2.3)	1.7 (2.9)	2.5 (3.8)	0.42 (1.0)	0.79 (1.2)	1.2 (1.9)
Participants with any lacunes, n (%)	55 (19.9)	70 (25.4)	77 (27.9)	15 (5.4)	7 (2.5)	22 (8.0)
Total number of lacunes	117	165	203	48	38	86
Participants with any microbleeds, n (%)º	36 (13.6)	49 (18.5)	66 (24.9)	13 (4.9)	17 (6.4)	30 (11.4)
Total number of microbleedsº	140	186	219	46	32	79
Participants with any territorial infarcts, n (%)	23 (8.3)	27 (9.8)	29 (10.5)	4 (1.4)	2 (0.7)	6 (2.2)
Brain volumes						
White matter volume, ml (SD)	465.6 (38.9)	455.2 (43.8)	444.0 (45.6)	-10.5 (13.7)	-11.2 (11.9)	-21.6 (17.2)
Grey matter volume, ml (SD)	620.7 (48.9)	610.5 (49.8)	598.4 (51.2)	-10.2 (16.8)	-12.1 (13.5)	-22.2 (20.4)

Data shown are unadjusted values and represent numbers (%), mean (SD) or median (IQR). Change represents the number of participants without lacunes or microbleeds at baseline who developed lacunes or microbleeds during follow-up.ºFor ratings of microbleeds 12 participants were additional excluded based on missing T2*or scan artefacts at any time point.

Temporal dynamics of cSVD

Probability maps of presence and increase of WMH are shown in Fig. 1A, Fig. 1B and Supplementary Movie 1. Mean WMH progression was 0.54 mL/yr (median 0.24; IQR 0.11-0.64 mL/yr). Progression of WMH increased with higher baseline age (Fig. 2, Supplementary Movie 2). In mixed-effects regression analysis including a random intercept and random slope, each year increase of age at baseline resulted in an increase in WMH as percentage of WM of 0.10% (95% CI: 0.07-13.7%). Including a quadratic term for increasing age during follow-up significantly improved the model (AIC base model 2995.2 versus AIC extended model 2932.9, χ^2 p<0.001).

Fifty-six participants (20.3%) developed new lacunes over nine years (2.3%/yr; Table 2). The distribution of lacunes is shown in Fig. 1C and Supplementary Movie 3. Incidence of lacunes was higher for the second follow-up period (3.5%/yr) than for the first follow-up period (2.7%/yr).

Fifty participants (18.9%) developed new microbleeds over nine years (2.2%/yr) (Table 3). Incidence of microbleeds in the second follow-up period (4.2%/yr) was higher than in the first follow-up period (1.7%/yr).

Multicollinearity between cSVD markers is shown in Supplementary Table 2.

Vanishing SVD

Besides progression we also noticed cSVD regression. We observed decline in WMH volume in 26 participants (9.4%; median decline -0.5 mL; IQR: -0.9 to -0.3 mL) during the first follow-up period and in 5 participants (1.8%; median -0.5 mL; IQR: -0.9 to -0.4 mL) during the second follow-up period. In one participant WMH volume declined over the course of nine years (0.4%; -0.4 mL). In 10 participants (3.6%) 14 lacunes could not be found at follow-up imaging (Table 2). In 15 participants (5.7%) 37 microbleeds vanished after nine years of follow-up (Table 3). Examples of vanishing lacunes and microbleeds are shown in Fig. 3.

Remote effects of SVD

Mean WM atrophy was 21.6 mL over nine years (0.54%/yr) and mean GM atrophy was 22.2 mL (0.41%/yr). WM and GM atrophy were higher for the second follow-up period (WM 0.73%/yr; GM 0.58%/yr) compared to the first period (WM 0.43%/yr; GM 0.30%/yr). WMH progression in the first follow-up period was associated with total brain atrophy in the second follow-up period (β=0.124; p=0.040) as well as with atrophy of WM (β=0.149; p=0.013) but not with atrophy of remote GM (β=0.045; p=0.461).

Heterogeneity in temporal dynamics of SVD

Mean WMH progression over nine years was 2.4 mL for participants with mild WMH at baseline, 12.0 mL for those with moderate WMH and 15.2 mL for those with severe WMH (Fig.4, Fig.5 and Supplementary Movie 4). From participants with mild WMH at baseline, six percent showed WMH progression beyond measurement error, compared with 75% of participants

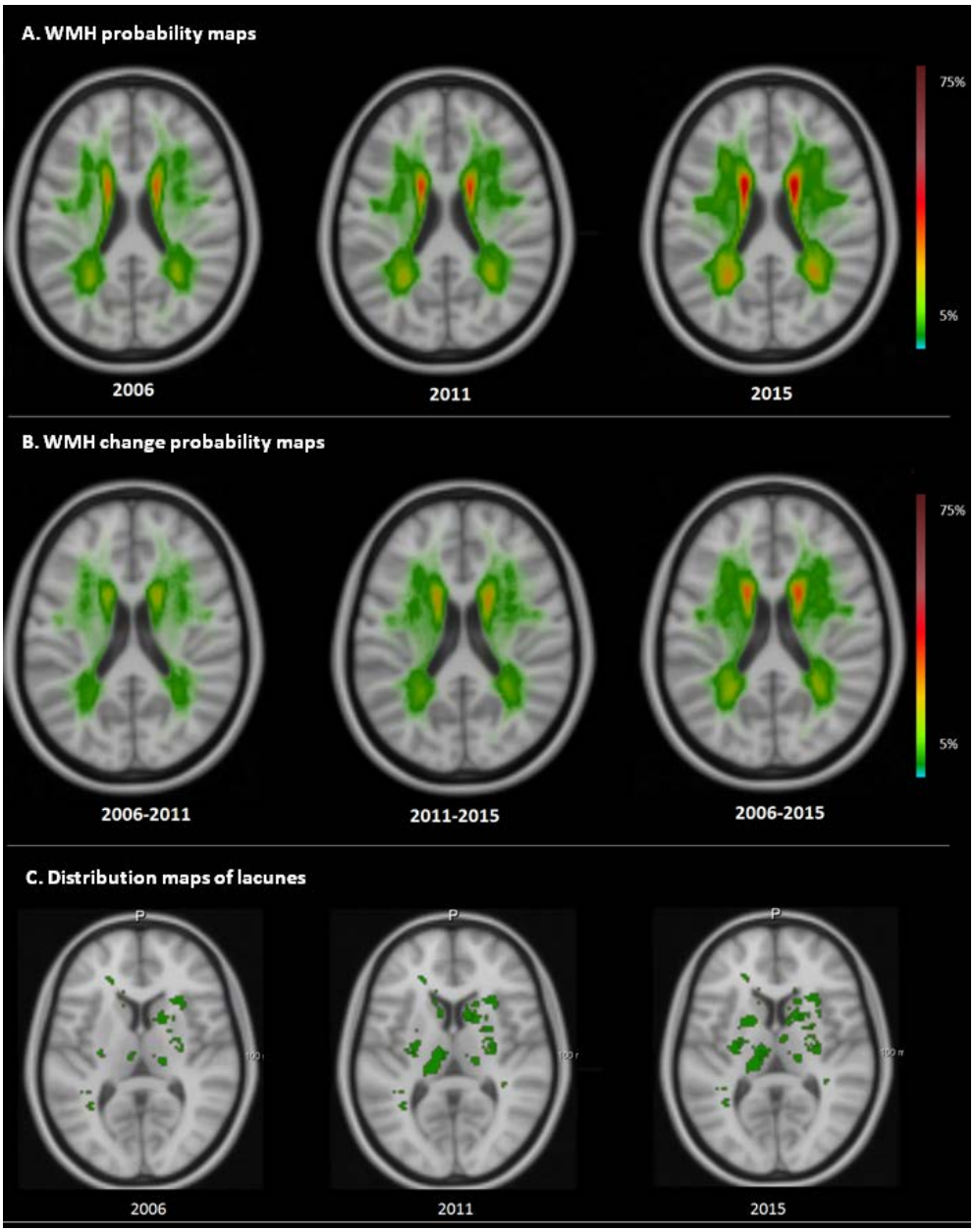


Figure 1: Probability maps of WMH and lacunes

Probabilities of presence of WMH at three time-points (A) and probabilities to increase over these three time-points (B), colour-coded in percentage from 5 to 75%. Probability maps through the whole brain can be seen in Supplementary Movie 1. Panel C shows the distribution of lacunes at three time-points. Whole brain distribution maps can be seen in Supplementary Movie 2.

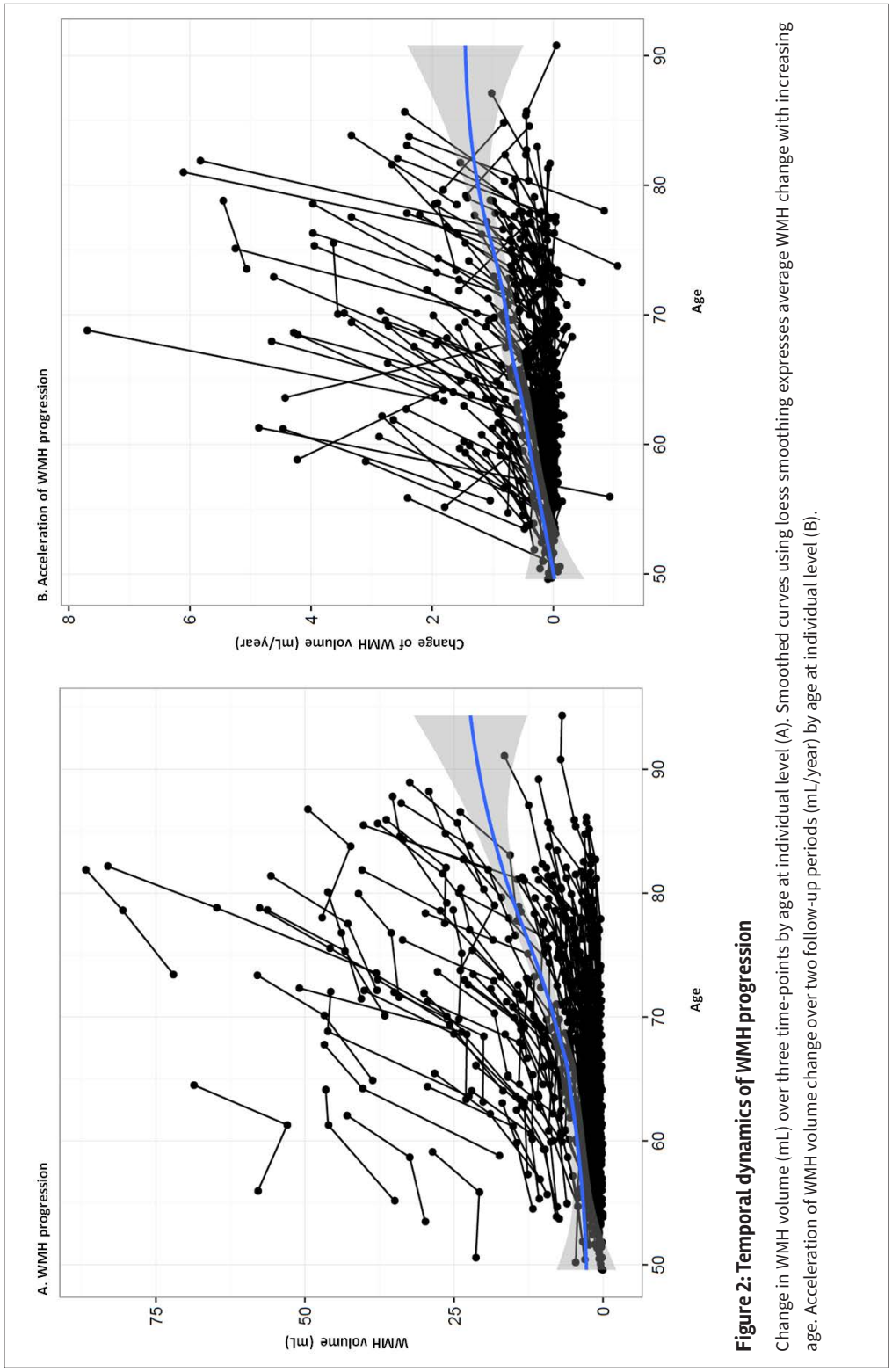


Figure 2: Temporal dynamics of WMH progression

Change in WMH volume (mL) over three time-points by age at individual level (A). Smoothed curves using loess smoothing expresses average WMH change with increasing age. Acceleration of WMH volume change over two follow-up periods (mL/year) by age at individual level (B).

Table 2: Lacunes per brain location

	Baseline (2006) n=276	Follow-up 1 (2011) n=276	Follow-up 2 (2015) n=276	Number of participants with incident lacunes	Number of participants with vanishing lacunes
Subcortical					
Frontal, no (%)	22 (8.0)	27 (9.8)	31 (11.2)	18 (6.5)	3 (1.1)
Parietal, no (%)	9 (3.3)	10 (3.6)	10 (3.6)	3 (1.1)	2 (0.7)
Occipital, no (%)	1 (0.4)	1 (0.4)	1 (0.4)	0 (0.0)	1 (0.4)
Temporal, no (%)	9 (3.3)	9 (3.3)	11 (4.0)	3 (1.1)	0 (0.0)
Any subcortical, no (%)	31 (11.2)	34 (12.3)	40 (14.5)	21 (7.6)	6 (2.2)
Deep					
Basal Ganglia[i], no (%)	25 (9.1)	39 (14.1)	46 (16.7)	35 (12.7)	5 (1.8)
Thalamus, no (%)	4 (1.4)	7 (2.5)	10 (3.6)	6 (2.2)	0 (0.0)
Internal Capsule, no (%)	4 (1.4)	5 (1.8)	7 (2.5)	3 (1.1)	0 (0.0)
Any deep, no (%)	29 (10.5)	44 (15.9)	52 (18.8)	41 (14.9)	5 (1.8)
Infratentorial [d]					
Any infratentorial, no (%)	13 (4.7)	20 (7.2)	21 (7.6)	10 (3.6)	0 (0.0)
Any lacunes, no (%)	**55 (19.9)**	**70 (25.4)**	**77 (27.9)**	**56 (20.3)**	**10 (3.6)**

Data represent number of participants (%) with lacunes or change in lacunes per brain location. [i] Basal ganglia include Globus Pallidus, Putamen and Caudate Nucleus. [d]Infratentorial includes pons, mesencephalon, medulla oblongata and cerebellum.

Table 3: Microbleeds per brain location

	Baseline (2006) n=264	Follow-up 1 (2011) n=264	Follow-up 2 (2015) n=264	Number of participants with incident microbleeds	Number participants with vanishing microbleeds
Subcortical					
Frontal, no (%)	18 (6.8)	21 (7.9)	27 (10.2)	20 (7.6)	6 (2.3)
Parietal, no (%)	8 (3.0)	10 (3.8)	19 (7.2)	17 (6.5)	2 (0.8)
Occipital, no (%)	8 (3.0)	9 (3.4)	9 (3.4)	4 (1.5)	4 (1.5)
Temporal, no (%)	11 (4.2)	12 (4.5)	15 (5.7)	8 (3.1)	5 (2.0)
Any subcortical, no (%)	30 (11.3)	35 (13.2)	49 (18.5)	36 (13.6)	14 (5.3)
Deep					
Basal Ganglia[i], no (%)	9 (3.4)	15 (5.7)	17 (6.4)	11 (4.2)	1 (0.4)
Thalamus, no (%)	4 (1.5)	4 (1.5)	6 (2.3)	3 (1.2)	2 (0.8)
Internal Capsule, no (%)	4 (1.5)	4 (1.5)	3 (1.1)	1 (0.4)	2 (0.8)
Any deep, no (%)	14 (5.3)	23 (8.7)	30 (11.3)	21 (7.9)	3 (1.1)
Infratentorial [d]					
Any infratentorial, no (%)	5 (1.9)	11 (4.2)	17 (6.4)	12 (4.6)	1 (0.4)
Any microbleeds, no (%)	**36 (13.6)**	**49 (18.5)**	**66 (24.9)**	**50 (18.9)**	**15 (5.7)**

Data represent number of participants (%) with microbleeds or change in microbleeds per brain location. [i] Basal ganglia include Globus Pallidus, Putamen and Caudate Nucleus. [d]Infratentorial includes pons, mesencephalon, medulla oblongata and cerebellum.

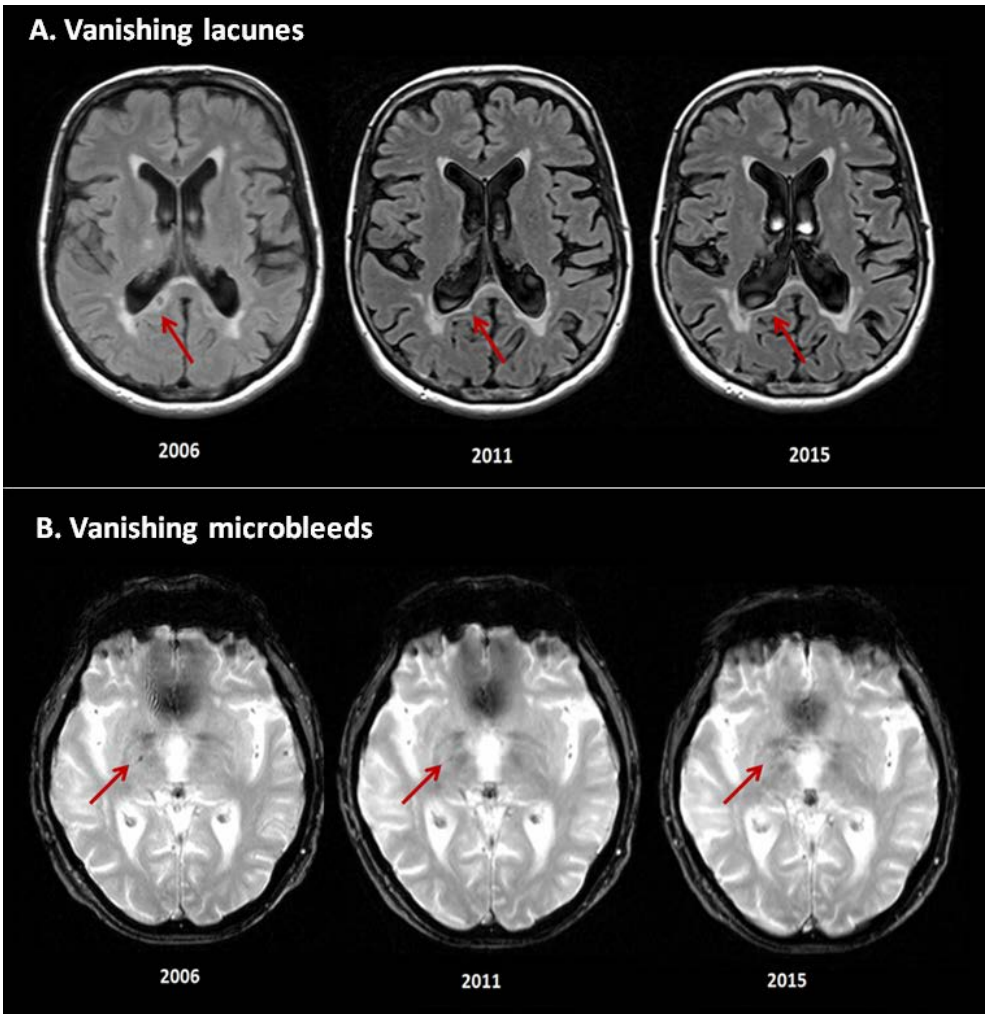


Figure 3: Vanishing lacunes and microbleeds

Examples of a vanishing lacune (A), who appear to be assimilated by the ventricle. Vanishing microbleeds (B) appear to have faded away over time.

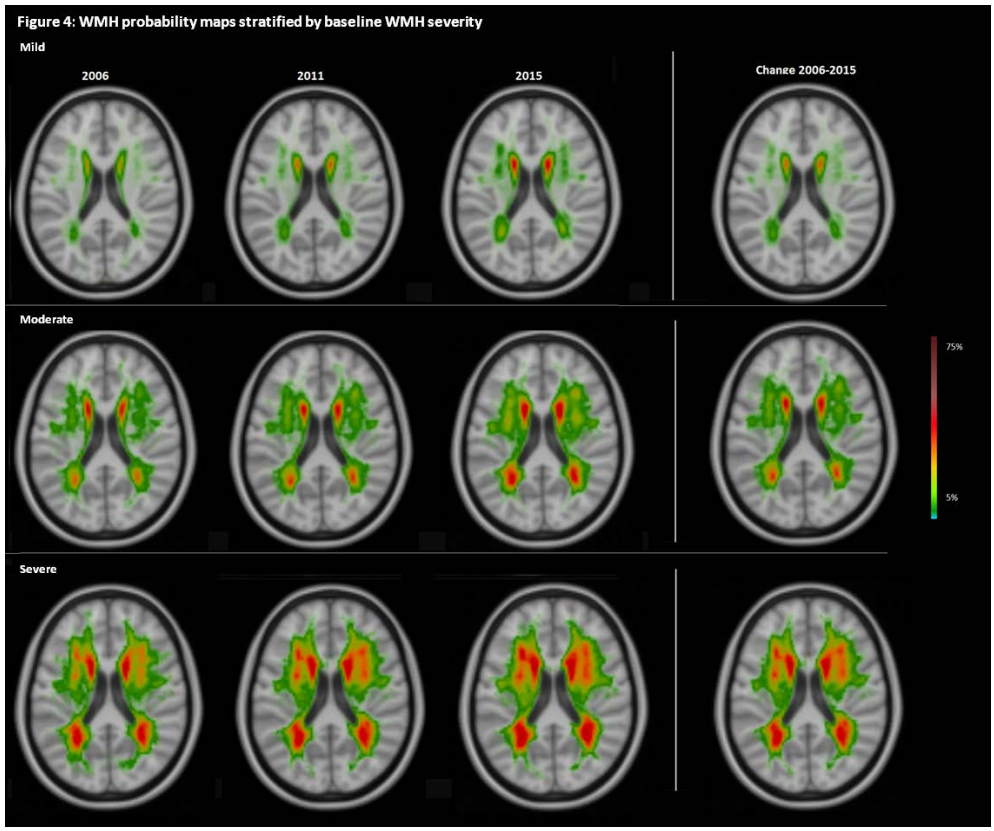


Figure 4: WMH probability maps stratified by baseline WMH severity

Probabilities of presence of WMH stratified by baseline WMH severity, colour-coded in percentage from 5 to 75%. Baseline WMH severity is determined as mild (Fazekas 0-1; n=211), moderate (Fazekas 2; n=33) and severe (Fazekas 3; n=20). The overall 9-year change is shown in the right column. Probability maps through the whole brain can be seen in Supplementary Movie 4.

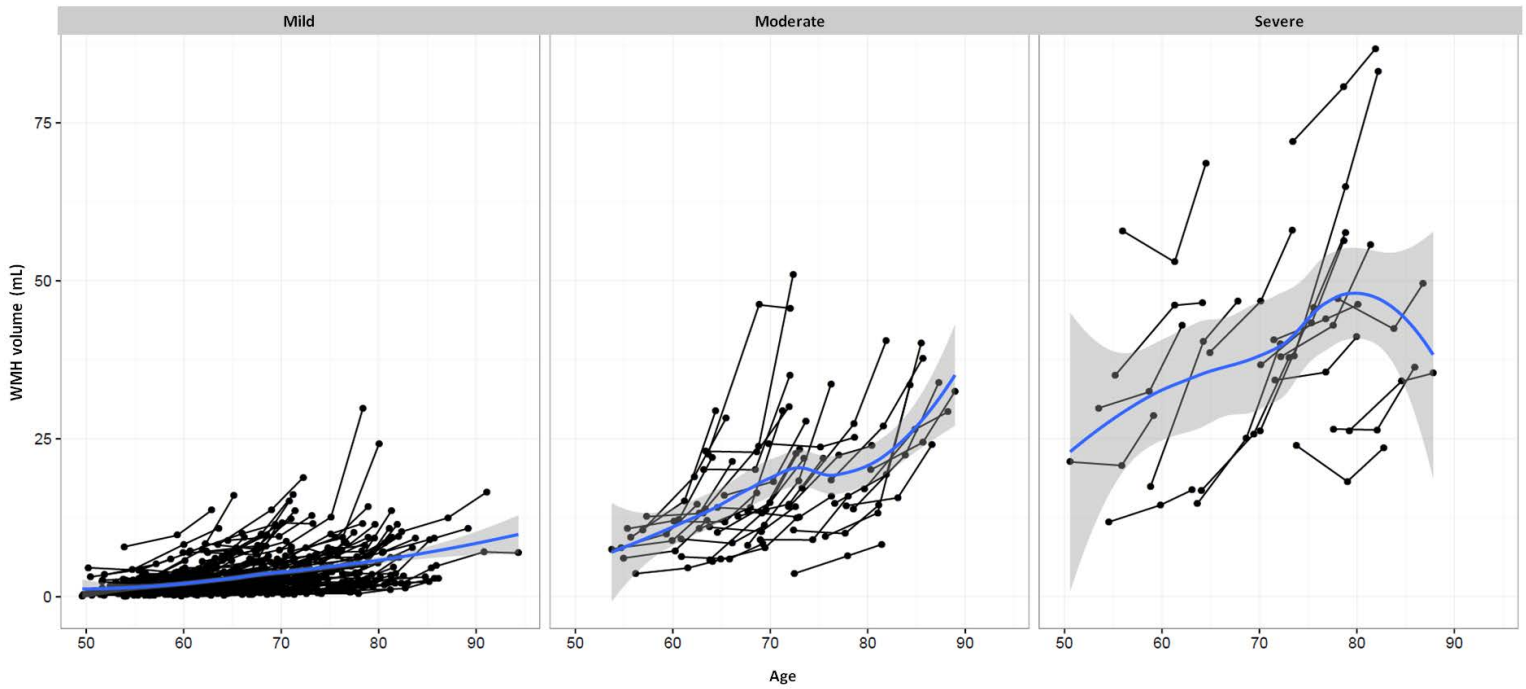


Figure 5: WMH progression stratified by baseline WMH severity

Change in WMH volume (mL) over three time-points by age at individual level stratified by baseline cSVD severity. Smoothed curves using loess smoothing expresses average WMH change with increasing age.

with moderate or severe WMH. Participants with moderate to severe WMH at baseline had 36 times higher risk of WMH progression compared to participants with mild WMH (OR 35.5; 95% CI 15.8-80.0; p<0.001). Participants with moderate to severe WMH also more often developed incident lacunes (OR 5.7; 95% CI 2.8-11.2; p<0.001) and microbleeds (OR 2.9; 95% CI 1.4-5.9; p=0.003) compared to participants with mild WMH at baseline.

Discussion

In this study we showed the temporal dynamics of cSVD, revealing both cSVD progression and regression, using three imaging assessments over a period of nine years. We demonstrated that progression of all cSVD markers occurred in a non-linear fashion, accelerating over time consistent with a quadratic course. Progression of WMH was associated with atrophy of adjacent white matter, but not with atrophy of remote grey matter. In addition, we showed that participants with moderate or severe WMH had a high likelihood of progression of their cSVD, whereas participants with only mild cSVD rarely showed progression, not even over a period of nine years.

This is the first study to demonstrate that cSVD progression is not linear but accelerates with increasing age. While the average progression in our study is comparable with other studies [14,15,17-20,22,269], the use of three imaging assessments allowed us to show that cSVD progression accelerated over time, providing evidence for a non-linear process [270,271]. Moreover, our results suggest that a quadratic course of SVD progression over time is plausible, since including a quadratic term for increasing age during follow-up significantly improved the model. Although we would need more than three time-points to further study exponential functions, our study indicates non-linear temporal dynamics of cSVD progression. Our findings do not support the hypothesized ceiling effect in which WMH progression reaches a certain threshold at high age and high lesion volume,[284] as we also saw WMH progression in those at high age and with high cSVD lesion load.

Imaging assessments at three time-points also enabled us to identify vanishing of cSVD markers followed by progression, in a cohort that on average showed progression. This observation provides further evidence that cSVD does not gradually evolve but is a dynamic process, with progression interrupted by regression in some. Thus far only few other studies have reported a decline in WMH volume [270,271,273,274], possibly because WMH decline within a certain time window was compensated by WMH progression thereafter (or vice versa). Two imaging assessments do not allow disentangling of episodes with regression from those with progression.

The observed decline in WMH may have several explanations. First, WMH decline in the first follow-up period could be explained in part by partial volume effects caused by slight adjustments in FLAIR sequences between baseline and first follow-up. However, we think this explanation is unlikely because WMH volumes calculated from even and odd slices were identical and because we also found WMH decline between the second and third MRI assessment, performed with identical imaging protocols. Second, recently developed WMH

might represent areas of tissue oedema. Reduction in tissue oedema at a later stage could then lead to reduced WMH volume [273]. Third, improved control of vascular risk factors or factors influencing the blood-brain-barrier might play a role by reducing WMH volume [273,274]. Disappearance of lacunes could be due to partial volume effects, due to "collapsing" lacunes or to incorporation of the lacune into the ventricle (Fig. 3) [275,276]. Vanishing microbleeds may be explained by partial volume effects as well as by clearance of hemosiderin-containing macrophages [278]. Our findings are in line with the latter hypothesis. In most cases microbleeds seemed to "fade away" between 2006 and 2011 and were no longer visible in 2015.

The relation between WMH progression in the first follow-up period and occurrence of WM atrophy and TBV atrophy during the second follow up, suggests that cSVD affects adjacent brain structures. In contrast to other studies [79,279], we did not find an association with overall remote GM atrophy. WM atrophy might be the result of disconnected white matter tracts due to cSVD, leading to axonal loss by anterograde or retrograde degeneration, and subsequently the loss of brain volume [29,103]. The clinical observation that patients with similar cSVD burden show heterogeneity in clinical symptoms might be explained by disconnection of WM tracts.

All cSVD markers at baseline were important predictors for cSVD progression, in a non-linear way and independent of age. The differential progression of cSVD suggests heterogeneity in aetiology of mild versus severe cSVD. Small WMH volumes, representing punctuate WMH or small periventricular caps, probably are of mixed origin, consisting of enlarged perivascular spaces and subependymal gliosis [22]. On the contrary, confluent WMH represent a continuum of ischemic tissue damage, ranging from mild fibre loss to complete infarction and may have a more malignant course in terms of cognitive deterioration. These different aetiologies call upon a different diagnostic and therapeutic approach.

Strengths of this study include the large cohort of elderly participants with cSVD and the long duration of follow-up of almost nine years. Furthermore, imaging assessments at three points in time allowed us to characterize change in cSVD over time, including vanishing cSVD. cSVD was rated according to standardized procedures [12], minimizing risk of misclassification. Moreover, semi-automatic quantification of WMH volumes reduced risk of information bias [41]. Finally, to reduce chance of misclassification, brain volumes were determined with the newest segmentation routines of SPM12 and corrected for segmentation errors using WMH masks. Finally, our study has high external validity for older patients with cSVD in a general neurology clinic.

A limitation of our study is change of MRI scanner between baseline and first follow-up leading to a distortion of the T1-images. However, by taking into account the third MRI assessment we are able to capture most of this possible bias. A slight adjustment in FLAIR sequence between baseline and first follow-up may have caused an overestimation of incident lacunes. However, we were able to limit the possible negative effects by reslicing follow-up to baseline FLAIR images before rating lacunes. Finally, selection bias may have led to an underestimation of progression of cSVD, since those who dropped-out at follow-up

were older, had more vascular risk factors and more severe cSVD at baseline than participants who completed follow-up examination.

Our study demonstrates that cSVD progression is a non-linear, dynamic and highly variable process, predominantly seen in patients with moderate or severe WMH at baseline. And, equally important, those with no or mild WMH rarely show progression over a nine year course. Since cSVD progression has been linked to cognitive decline and development of dementia, our study now allows for the early identification of those at high risk for cSVD progression. It is likely, although not directly investigated in this study, that these patients are also at the highest risk to develop dementia, suggesting that therapeutic strategies to prevent cSVD progression are particularly needed for patients with moderate to severe WMH. Further understanding of temporal dynamics of remote effects of cSVD can be obtained by performing serial imaging at much shorter time intervals.

Supplementary Table 1: Baseline characteristics of participants compared with those lost to follow-up

	Participants n=276	Lost to follow up n=227	significance
Demographics			
Age (years), mean (SD)	62.5 (7.7)	69.5 (8.5)	**p<0.001**
Sex, male, no (%)	163 (59.1)	121 (53.3)	p=0.207
Education >primary school, no (%)	259 (93.8)	32 (14.1)	p=0.004
MMSE score, mean (SD)	28.6 (1.3)	27.6 (1.8)	**p<0.001**
SVD Characteristics			
White matter hyperintensities, ml, median (IQR)	2.3 (0.8-6.1)	7.7 (2.6-16.2)	**p<0.001**
White matter hyperintensities, ml, Mean (SD)	5.8 (9.5)	11.9 (13.6)	**p<0.001**
% WMH of WM, mean (SD)	1.3 (2.3)	2.9 (4.4)	**p<0.001**
Participants with any lacunes, n (%)	55 (19.9)	77 (33.9)	**p<0.001**
Total number of lacunes	117	135	**p=0.038**
Participants with any microbleeds, n (%) º	36 (13.1)	47 (20.9)	p=0.022
Total number of microbleedsº	140	159	p=0.496
Territorial Infarcts, n (%)	23 (8.3)	34 (15.0)	**p=0.023**
Modified Fazekas score			
Mild WMH (0-1), no (%)	218 (79.0)	114 (50.2)	**p<0.001**
Moderate WMH (2), no (%)	38 (13.8)	70 (30.8)	**p<0.001**
Severe WMH (3), no (%)	20 (7.2)	43 (18.9)	**p<0.001**
Brain volumes			
White matter volume, ml (SD)	465.6 (38.9)	441.5 (50.2)	**p<0.001**
Grey matter volume, ml (SD)	620.7 (48.9)	588.7 (51.6)	**p<0.001**
Vascular risk factors			
Smoking, ever, no (%)	196 (71.0)	157 (69.2)	p=0.696
Alcohol, glasses/week, mean (SD)	8.3 (9.0)	7.5 (9.7)	p=0.367
Glucose lowering drugs, no (%)	23 (8.3)	43 (18.9)	**p=0.001**
Hypertension, no (%)	190 (68.8)	179 (78.9)	**p=0.015**
BMI, mean (SD)	27.1 (4.1)	27.2 (4.2)	p=0.778
Lipid-lowering drugs, no (%)	118 (42.8)	119 (52.4)	**p=0.032**

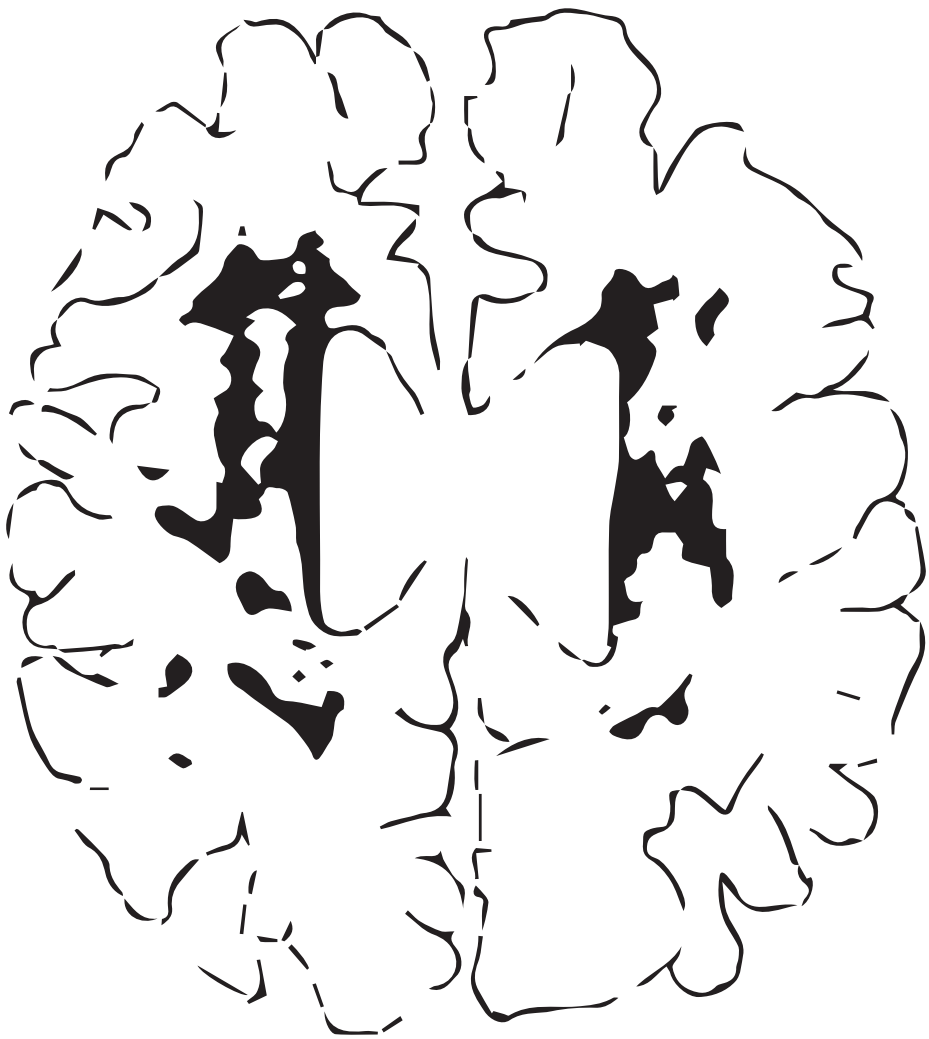
Data are represented as numbers (%), mean (SD) or median (IQR). Comparisons between participants and those lost to follow-up were performed by t-test, Chi-square or Mann-Whitney-U test .ºFor ratings of microbleeds 4 participants were additional excluded based on missing T2*or scan artefacts at baseline.

Supplementary Table 2: Correlation matrix for baseline SVD characteristics and SVD progression

	Age	Sex	Baseline WMH volume	Baseline lacunes	Baseline microbleeds	Baseline WM volume	Baseline GM volume	Change WMH volume	Incident lacunes	Incident microbleeds	Change WM volume	Change GM volume
Age	…	…	…	…	…	…	…	…	…	…	…	…
Sex	-0.010	…	…	…	…	…	…	…	…	…	…	…
Baseline WMH volume	**0.315*** **	0.088	…	…	…	…	…	…	…	…	…	…
Baseline lacunes	**0.189** **	-0.102	**0.310*** **	…	…	…	…	…	…	…	…	…
Baseline microbleeds	**0.127***	0.006	**0.222*** **	**0.237*** **	…	…	…	…	…	…	…	…
Baseline WM volume	**-0.460*** **	0.001	**-0.244*** **	**-0.205** **	**-0.132***	…	…	…	…	…	…	…
Baseline GM volume	**-0.531*** **	**0.354*** **	**-0.258*** **	**-0.265*** **	**-0.161** **	**0.283*** **	…	…	…	…	…	…
Change WMH volume	**0.299*** **	0.101	**0.577*** **	**0.229*** **	0.110	**-0.182** **	**-0.281*** **	…	…	…	…	…
Incident lacunes	**0.137***	-0.054	**0.299*** **	**0.447*** **	**0.129***	**-0.165** **	**-0.151***	**0.207** **	…	…	…	…
Incident microbleeds	**0.226*** **	0.049	**0.256*** **	**0.153***	0.101	-0.104	**-0.147***	**0.161** **	**0.173** **	…	…	…
Change WM volume	**-0.379*** **	**0.164** **	**-0.186** **	**-0.166** **	**-0.131***	**0.202** **	**0.266*** **	**-0.295*** **	-0.085	**-0.172** **	…	…
Change GM volume	**-0.131***	0.038	-0.036	-0.109	0.026	0.046	-0.093	-0.051	-0.112	-0.115	**0.189** **	…

Correlations between baseline cSVD characteristics and progression of cSVD markers were determined by Spearman Rho for binary characteristics and by Pearson Correlation coefficients for continuous variables. Correlations were significant at 2-tailed *p<0.05; **p<0.01; ***p<0.001.

Part VI:
Summary and general discussion



11.
Summary

Cerebral small vessel disease (cSVD) is frequently seen on FLAIR MRI of older adults. White matter hyperintensities (WMH), lacunes of presumed vascular origin (lacunes), microbleeds and (sub)cortical atrophy are the well known 'traditional cSVD makers'. cSVD is very common in persons aged above 60 years, it's incidence increases non-linearly with age, and its progression is thought to be related to baseline cSVD load and vascular risk factors. The clinical spectrum and the prognosis of cSVD is highly heterogeneous and ranges from very mild symptoms to full blown stages of dementia and even death. With the aid of conventional MRI it has proven to be very difficult to predict which patients with cSVD are at highest risk to develop these severe clinical symptoms, and who will not. Therefore other factors apart from the 'traditional cSVD markers' might determine the transition to incident dementia in some, while leaving others unaffected. Such 'other factors' could be the microstructural integrity of the white matter, (cSVD extending beyond lesions visible on conventional MRI) and brain volumes of structures known to be related to behavioural symptoms.
Studies in this thesis are based on data from the RUN DMC study; a prospective cohort study among 503 independently living, non-demented elderly with cerebral cSVD between 50 and 85 years old, that investigates causes and consequences of cSVD with baseline assessment in 2006-2007 with a 5- and 9-year follow-up period. We describe the associations between baseline imaging characteristics (both macro- and microstructural) and the attendant behavioural consequences 5 years later. The focus of this thesis is on cognitive decline and depressive symptoms. Finally the 9 year temporal dynamics of traditional cSVD characteristics is described.

Macro– versus microstructural MRI in cognition and incident dementia

In **Part I (chapter 2)** we report on the possible (additional) clinical value of DTI over conventional MRI sequences with respect to cognitive performance at the cross sectional level. All MRI parameters together accounted for 1–6% of the variance in cognitive function on top of 22–36% already explained by age, gender, and level of education. Of the MRI parameters, the WM microstructural integrity did not substantially contribute to the explained variance of cognitive function (1%). The MD of the WM however had the strongest association with cognitive performance. Therefore we concluded that the cross-sectional relation between WM microstructural integrity and cognitive performance is rather weak, and has limited additional value to the macrostructural MRI parameters. **Part II** prospectively describes the relation between baseline brain structures, both assessed with MRI (FLAIR, T1 and DTI) and the development of incident dementia or cognitive decline after 5 years. In **chapter 3** we showed a 5.5-year cumulative risk of all-cause dementia of 11.1% (95% CI 7.7–14.6) in our study. Furthermore we showed that both low white matter volume (HR 0.65 (95% CI 0.44-0.96)) and low hippocampal volume (HR 0.68 (95% CI 0.47-0.99)) were related to the development of dementia 5 years later, independent of cSVD. Interestingly there was no additional diagnostic value of any of the cSVD characteristics (including global DTI parameters) in predicting the occurrence of dementia. We showed in **chapter 4** that diffusion

parameters in the hippocampus, independent of hippocampal volume, brain volume and cSVD markers, are related to an increased dementia risk (HR 1.44 (95% CI 1.10-1.88)). Sub analyses revealed that low hippocampal microstructural integrity more than doubled the dementia risk in the group of healthy older adults without evidence of hippocampal atrophy (HR 2.66 (95% CI 1.30-5.44)). Therefore DTI of the hippocampus might identify persons at risk for dementia at an earlier stage than using volumetric measures of the hippocampus alone, and possibly serves as a surrogate marker of disease progression. In **chapter 5** we report on baseline global DTI parameters and decline in cognition after 5 years. We found that baseline DTI parameters in the white matter were not related with decline in global cognitive performance or in other cognitive domains after adjustment for demographics and imaging markers (both volumetric and cSVD). This was in line with the baseline finding in which we showed only limited added value of global DTI measures over other MRI measures **(chapter 2)** and the lack of relation between global DTI parameters and incident dementia **(chapter 3)**.

Depressive symptoms in cerebral small vessel disease

In **Part III** we report on depressive symptoms, and the relation between depressive symptoms and cognition in older adults with cSVD. The association between amygdala volume and depressive symptoms is described in **chapter 6** on a cross-sectional level. We showed that lower volume of the left amygdala was associated with depressive symptoms occurring for the first time later in life (LODS), independent of cSVD markers. Each decrease of total amygdala volume (by mL) was related to an increased risk of LODS (OR = 1.77; 95% CI 1.02-3.08; p=0.04). In **chapter 7** we investigated the white matter integrity of older adults with and without depressive symptoms, using Tract-Based-Spatial-Statistics (TBSS), on a cross-sectional level. We showed that older adults with depressive symptoms had a lower microstructural integrity of the white matter compared to those without depressive symptoms, predominantly located in the prefrontal white matter tracts, which are thought to play an important role in the pathogenesis of mood. This was independent of global cognitive performance. After adjustment for cSVD markers, the magnitude of this association grossly disappeared suggesting that cSVD plays a role. In **chapter 8** we prospectively report on the role of depressive symptoms on incident all-cause dementia after 5 years follow-up, taking both the age of onset and baseline cognitive performance into account. We showed a higher dementia risk in those with depressive symptoms at baseline (HR 2.7 (95%CI 1.4-5.2)), which was driven by LODS, and was independent of cSVD markers. In those with a high cognitive performance at baseline depressive symptoms are indicative for incident dementia, whereas there was no such risk difference between those with and without depressive symptoms in participants with a lower cognitive performance at baseline (no depressive symptoms 0.0% vs. depressive symptoms 6.9%; log-rank p<0.001).

In **part IV (chapter 9)** we reviewed the clinical consequences; (cognitive, motor, mood) and the risk factors of microstructural damage of the white matter, as assessed with diffusion tensor imaging.

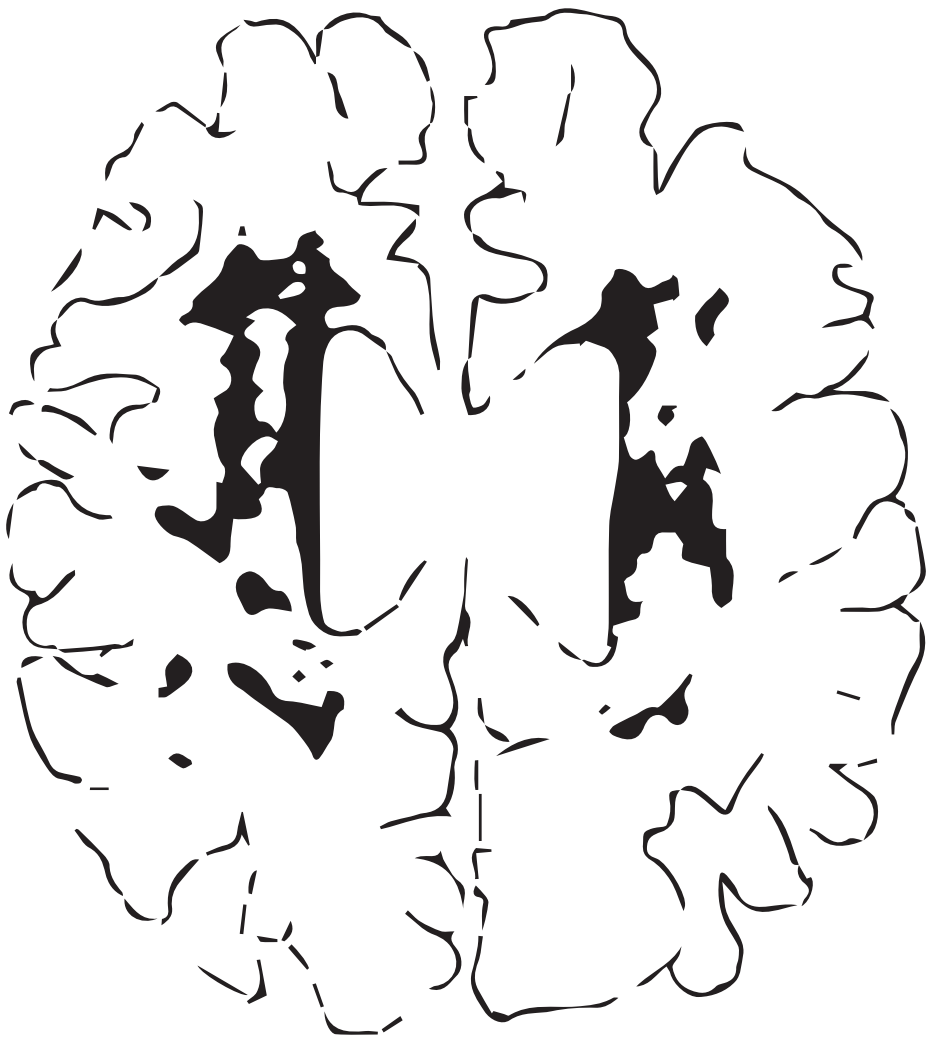
Temporal dynamics in cerebral small vessel disease

Finally in **part V** we described the 9-year change of cSVD markers, using two follow-up moments (2011 and 2015). In **chapter 10** we elaborate on the dynamics of cSVD, taking both new developed as well as apparent decline of the traditional cSVD markers into account. Mean WMH progression over nine years was 4.7 mL (0.54 mL/yr; IQR 0.95–5.5 mL), 20.3% of patients had incident lacunes (2.3%/yr) and 18.9% incident microbleeds (2.2%/yr). WMH volume declined in 9.4% of the participants, and lacunes vanished in 3.6% and microbleeds in 5.7% of the participants. These numbers were calculated using 2 follow-up moments, allowing us to report on the non-linear progression of cSVD. Furthermore we showed that those with very little cSVD at baseline showed little to no progression in contrast to those with already moderate to severe baseline cSVD load, who show most progression (OR 35.5; 95% CI 15.8-80.0; p<0.001 for WMH progression; OR 5.7; 95% CI 2.8-11.2; p<0.001 for incident lacunes and OR 2.9; 95% CI 1.4-5.9; p=0.003 for incident microbleeds).

Conclusion

The studies described in this thesis identified imaging markers which can help to gain more insight into the pathophysiological processes that underlie the behavioural consequences of cerebral small vessel disease. At the same time, studies underline the limited additional value of diffusion tensor imaging on top of the macrostructural imaging markers in an elderly population with already long-standing cSVD. Furthermore in those developing depressive symptoms for the first time at older age, while performing cognitively well during objective testing, depressive symptoms might be the first indication of incident dementia.

Future research should address whether an ***increase*** in cSVD (incident lacunes or microbleeds, decline in microstructural integrity, or increase of WMH volume), or brain atrophy (change in hippocampal, amygdalar, WM and GM volumes), precede newly developed behavioural consequences, and possibly has a better clinical correlate than the baseline imaging markers.



12.
General discussion and future perspectives

The overall aim of the studies described in this thesis was to gain better insight in the role of macro and microstructural aspects of markers of cerebral small vessel disease (cSVD) and the attendant behavioural consequences. Furthermore the relation between depressive symptoms and the risk of incident dementia was studied. Finally we described the frequency, severity and the temporal dynamics (both progression and reduction) of the macrostructural cSVD markers over a 9-year period in our cohort.

These studies are based on data from the RUN DMC study; a prospective cohort study among 503 independently living, non-demented elderly with cerebral cSVD between 50 and 85 years old, that investigates causes and consequences of cSVD with baseline assessment in 2006-2007. In 2011-2012 and in 2015 both the extensive cognitive and motor assessments and also the cerebral MRI were repeated, resulting respectively in a 5- and 9-year follow-up period.

In the previous chapters the individual studies have been addressed. In this chapter the overall methodological considerations will be discussed. Subsequently, the main findings of the studies will be put in perspective and potential clinical implications will be discussed. Finally suggestions for future research are given.

Methodological considerations

The studies in this thesis are based on cross-sectional[43,47,48] and longitudinal data (5.4 years follow-up[44-46,49] and 8.7 years follow-up[285]). The use of cross-sectional data limits causal inference. However our longitudinal design allowed us to investigate causal relations between imaging characteristics and incident dementia and decline in cognition, as well as the change in imaging characteristics. Because of the observational nature of our study, we have to interpret these results with caution. Further methodological considerations (precision, internal validity and external validity) will be discussed on the basis of principles described by Rothman and Greenland.[286]

Precision: minimizing 'random error'

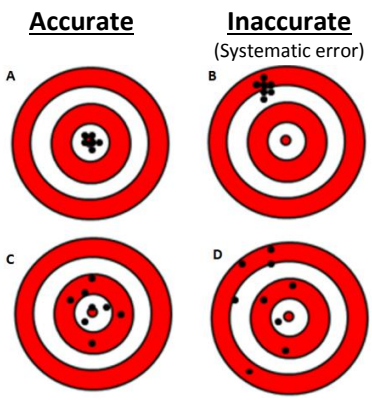
Precision (or reliability) reflects the amount of random error (or non-differential misclassification) in a measurement[286] and with that the reproducibility of that measurement. Random error may result in imprecise (or unreliable) estimates, resulting in broad confidence intervals (**PANEL 1**).

Especially in a longitudinal study random error tends to cause 'regression towards the mean', which makes it more difficult to detect a relation between determinant and outcome. A possible solution to reduce random error, and increase precision, is to increase the number of measurements.

In addition, in our study precision was enhanced by having a large sample size, n=503 at baseline to begin with.

Precision in measurement of the outcome "cognitive performance" was enhanced by performing multiple neuropsychological tests. Tests within one compound score assessed the same cognitive domain, resulting in multiple measurements of the same variable.[139] By doing so, the intra-individual variability was reduced, giving a better estimate of the true

PANEL 1: Precision and Validity



Adapted from Ahlbom & Norell, 1990, *Introduction to modern epidemiology*

A: Precise and Accurate	**OR**	Reliable and Valid
B: Precise but Inaccurate	**OR**	Reliable but not Valid
C: Accurate but Imprecise	**OR**	Valid but not Reliable
D: Imprecise and Inaccurate	**OR**	not Valid and not Reliable

value per domain. Cognitive scores were adjusted for a number of faults by using SAT scores, giving a better estimate of the test-score.

The precision in assessing depressive symptoms was increased by the use of a valid questionnaire,[196] used in large population based studies.[67]

Although commonly used as a screening tool with high sensitivity and specificity for depressive symptoms,[287] the CES-D is not specific for depressive symptoms, and could be considered a screening for general affective distress. Additional usage of the Geriatric Depression Scale (GDS) could have added to precision in measuring depressive symptoms in our study of older adults.

Internal validity: limiting systematic error or bias

Validity (or accuracy) involves minimizing systematic error in a measurement. Systematic error may result in invalid, (or inaccurate) results, not reflecting the 'true value' (**PANEL 1**). Three types of systematic error often are identified: 1. ***selection*** bias, 2. ***information*** bias (or differential misclassification) and 3. ***confounding***.

1 Selection bias

In the case of selection bias the study sample is not representative of the population that is to be analyzed.[286] This occurs when the relation between the determinant (imaging markers)

and outcome (behavioural consequences) is not the same for participants as for the non-participants, potentially eligible for the study. It is impossible to adjust for selection bias in the analyses, therefore the only thing one can do is to obtain a maximal response.
The response rate at baseline was ***71.3%***, which is high but incomplete**.** Non-participants were older, and had more severe WMH (as rated on the scan prior to inclusion) than participants. For both are related to cognitive performance and depressive symptoms, it is possible that those with the highest degree of cSVD and cognitive disturbances were not included in our study, resulting in an underestimation of the effect of cSVD on behavioural symptoms. To obtain the highest possible response at follow-up, participants were annually contacted by sending them both a Christmas card and a news letter in which we informed them on the study progression and the presumed start of the next follow-up. This resulted in an extremely high response of ***99.6%*** for our primary outcome measure 'incident dementia' in 2011, resulting in virtually no selection bias with respect to this outcome.
The response for in-person testing of the cognitive domains (2011) was ***79.1%*** and in 2015 ***54.9%*** underwent MRI scanning for the third time in a row. Those without follow-up assessment, again, were older and had a worse baseline cSVD load. In addition they performed lower on cognitive testing, had lower brain volumes and more vascular risk factors at baseline. Since these variables were independently associated with cognitive performance and SVD progression,[51,53,129] this probably resulted in an underestimation of the effect of imaging characteristics on cognitive decline, and an even further underestimation of cSVD progression after 9 years. This issue is a well-known paradox of follow-up studies: to prove a causal relation over time, long follow-up is required, however, the longer the follow-up period the higher the risk of selective drop-out, leading to selection bias.

2. Information bias

Information bias is also named (differential) misclassification. It results from incorrect information on the determinant, influenced by the outcome variable, or vice versa. For example, rating of the MRI-scans (determinant) can be influenced by prior knowledge of a dementia diagnosis (outcome) in that person. This error might lead to an over or underestimation of the results, affecting validity.
In our study both at baseline and follow-up, the assessments of imaging markers and the outcome variables (depressive symptoms, cognitive performance) were performed blinded for, and independent of each other. Moreover, assessment of vascular risk factors and demographics was performed independently and blinded from the MRI scan. Thus it is very unlikely that misclassification has been differential, and with that the assessment of both these determinants and outcome variables suffer from non-random error. Remaining issues on differential misclassification are discussed below.

Possible systematic error in MRI measures

All baseline scans were performed on one single MRI-scanner, without adjustment of scan-protocol or software. Moreover, brain volumes (WMH, amygdalar and hippocampal volume) were manually segmented by one trained rater (IvU), with high inter- and intra-rater correlations on random subsamples of 10%, reducing systematic error.

The change in MRI-scanner between baseline (SONATA) and the first follow-up (AVANTO) might have led to differences (geometric distortion of theT1 images) between baseline and follow-up images. To minimize these effects, follow-up brain volumes were corrected to the baseline intracranial volume before normalization. Furthermore, comparing brain volumes from the second follow-up with the first (both performed on the same MRI scanner), the scanner change did not seem to be a major problem. To further prevent systematic error the 'traditional' cSVD markers were manually revised according to the STRIVE criteria, (including the baseline cSVD markers), each time by the same person, which is the gold standard. WMH were assessed volumetric which is superior in a longitudinal design in order to show progression, for visual rating scales provide a ceiling effect.[284,288] At follow-up WMH volumes were segmented using a (semi)automated WMH detection method, lowering misclassification.

Brain volumes (WM, GM and CSF and ICV) were segmented using the newest segmentation version SPM 12 (instead of SPM 5) which is currently the most up-to date version. Because the use of this newest version, the amount of non-brain tissue being misclassified as GM or CSF is reduced.[289]

Possible systematic error in cognitive testing and incident dementia

Possibly some patients with incident dementia might have been missed. The cut-off point of 26 points or lower on the MMSE (used as a screening tool for dementia) might not have been sensitive enough for cases of dementia in early stage of the disease, VaD (most likely to express executive dysfunction that is not adequately captured by the MMSE), or in participants with higher education levels, leading to an underestimation of the dementia diagnosis in the relatively early stages. However the additional high IADL scores and the confirmation of little or no interference in daily functioning in screen-negatives, is an indication that if any misclassification is present, it is presumably limited.

Furthermore, incident all-cause dementia, was a clinical diagnosis, based on the DSM-IV,[62] preferably confirmed by a visit to the Radboud Alzheimer center. When a visit to the Radboud Alzheimer centre was not possible, all available information (neuropsychological testing, medical records, baseline/follow-up imaging or information from caregivers) was reviewed by a panel. The use of the MRI as a supportive feature (diagnosis was based on cognitive testing and interference in daily functioning) might have led to differential misclassification (incorporation bias) when we dealt with difficult cases with fully normal or pathological MRI-scans. Finally we were not informed on the genetic APOE status, CSF biomarkers or PET scan

at baseline, which could have further improved our dementia diagnosis and we obviously did not have pathological confirmation.

3. Confounding

Confounding occurs when a third variable is accountable for the found relation between the determinant and the outcome variable.[286] This is of particular importance in longitudinal research aiming to identify causal realations.[290] Confounding variables are associated with both the determinant and outcome variable, but are not part of the 'causal chain' (intermediate factor) leading to the outcome. In our study we adjusted for these possible confounders in our multivariate regression models.

Because the large number of subjects in our study in most of the analyses, we were able to adjust for multiple confounders. If this was not possible at once (in some of the longitudinal analyses), we investigated these relations using different models with different groups of confounders per model.

Age, gender, level of education, depressive symptoms, brain volumes, but also some of the other cSVD characteristics were considered possible confounders.

We intentionally did not adjust for vascular risk factors[291] in our analyses, as they were thought to be an 'intermediate factor' (e.g. causing WMH and lacunes and microbleeds). Including these in the models, might cause an existing relation between MRI markers of cSVD and behavioral consequences to disappear, due to over-adjustment. In addition, since WMH and lacunes are presumed to result from largely the same underlying pathology, treating them as confounders in an analysis might have led to an underestimation of the effect of the determinant, for the above-mentioned reason. Finally, as WM microstructural integrity is now considered to be a part of the SVD-spectrum, the WMH (and other traditional cSVD markers) that are thought to cause this microstructural damage, probably are part of the causal chain, and therefore adjustments for these markers might have led to over-adjustment.

External Validity –– Generalization

An important aspect of study design is whether the results obtained in the study group represent the wider population in real world situations, and thus can be extended to make predictions about the entire population.[286]

Our study was performed in an university hospital-based cohort of older adults with cerebral cSVD on neuro imaging. We think that our results can be generalized to patients aged between 50-85 years old with cSVD, (which is likely to be related to arteriolosclerosis and vascular risk factors), visiting a general hospital. This is based on the inclusion of all consecutive patients that were referred to the outpatient clinic of the neurology department who underwent neuro-imaging. The majority was referred by their general practitioner and seen as a first opinion. Moreover, patients were selected based on neuro-imaging characteristics of cSVD,[61] and cSVD is a common disorder with a prevalence over 90% in older adults over 60 years of age.

Main findings

Imaging markers that precede cognitive decline after 5 years

In our cohort, 'traditional' cSVD markers were not associated with incident dementia after adjustment for confounders, as was shown previously.[41] WM and hippocampal volume did predict the dementia risk after 5 years.[44] WM (subcortical) atrophy is now also seen as a part of the cSVD spectrum. The WM represents structural connectivity between different brain structures, including the neocortex and hippocampus, which are crucial in cognitive function. Therefore it is not surprising that a lower WM volume is a predictor for incident dementia. Possibly this WM atrophy occurs through remote (or better: adjacent) effects of cSVD. For example when lacunes occur, WM volume does not only diminish through local necrosis, but probably also by anterograde or Wallerian degeneration.[103] Possibly this relatively severe brain damage (WM atrophy) has such severe clinical consequences (incident dementia), that the traditional cSVD markers do not substantially contribute to the (severity of the) clinical dementia syndrome in the presence of brain atrophy.

Hippocampal atrophy is a well-known characteristic in dementia, and is thought to be a major cause of the profound deficit in episodic memory consolidation in dementia.[33] We further investigated this hippocampus at a microstructural level, and found that a lower hippocampal microstructural integrity, especially hippocampi which are volumetrically intact, predict incident dementia.[45] This might provide an early insight in the development of dementia.

We were the first study to investigate WM microstructural integrity and the risk of incident dementia, for at a cross sectional level WM microstructural integrity was suggested to play a role in dementia.[81] We found no additional value of WM microstructural integrity on top of the macrostructural imaging characteristics on the risk of dementia. Possibly this loss of microstructural integrity of the WM is not severe enough to result in a clinical dementia syndrome[44] (or cognitive decline)[46] in the presence of WM and hippocampal atrophy, which probably both have a stronger relation with incident dementia.

Taken together, in older adults with cSVD, WM volume, hippocampal volume and hippocampal microstructural integrity are independently related to the 5 year cumulative risk of dementia in older adults with cSVD. We did not find this for 'traditional' cSVD markers and WM microstructural integrity. These findings might provide a better insight in the development of dementia. Another important conclusion is the limited clinical added value of diffusion tensor imaging in quantifying the risk of dementia.

Depressive symptoms in cSVD and its relation to incident dementia

Traditional cSVD characteristics were known to be related with depressive symptoms,[36] but little was known about the role of the WM microstructural integrity in relation to depressive symptoms. We showed that older adults with depressive symptoms had a lower WM integrity compared to those without,[48] especially in the fronto-subcortical region, independent of global cognitive functioning. After adjustment for traditional cSVD markers these results

almost disappeared, suggesting a role of this 'visible' cSVD. These findings suggest that microstructural WM damage might underlie the development of depressive symptoms in cSVD patients at older age, by disruption of the circuits that are involved in mood regulation. Furthermore we showed in our longitudinal analyses that depressive symptoms independently increased the dementia risk after 5 years,[49] which was driven by those who develop depressive symptoms for the first time later in life. Our results suggest that depressive symptoms are a first symptom of cognitive decline later in life, rather than a risk factor, as we did not find the association with early onset depressive symptoms and incident dementia. Especially in those with good objective cognitive performance the depressive symptoms indicate a higher risk for the development of dementia 5 years later. This is an interesting finding, for in current practice in those who present with both subjective cognitive problems and depressive symptoms (but perform well during objective cognitive testing), the depressive symptoms are thought to be the cause of the perceived cognitive problems. Our findings suggest that those with a high performance on cognitive testing while presenting with depressive symptoms for the first time later in life, should be monitored more closely for the development of dementia in the following years.

In those with low cognitive performance, depressive symptoms did not differentiate between those who are and are not at risk for dementia. Probably because low cognitive performance is a strong risk factor for incident dementia, depressive symptoms have no significant contribution to the overall dementia risk in this group.

Small vessel disease; a dynamic process

Thus far, little is known about cSVD progression, especially not over a long time period. We showed that, over a 9 year period, cSVD is a dynamic process which is, in general, progressive. Having a closer look into this process, it encompasses not only progression but also reduction, which is thus far not or only partly understood.[285] WMH regression, as we found in some, could possibly be present due to reduction of tissue oedema, as could be explained by those individuals with WMH progression due through acute infarction.[292] Few suggested a hypothesis for this reduction of lacunes and microbleeds, however evidence is still lacking. [293] [278] Because of the availability of 2 follow-up moments in the RUN DMC study over almost a decade, we were able to provide evidence for the non-linear progression of WMH volume, incident lacunes and incident microbleeds which was not fully explained by increasing age. Those with low (macrostructural) cSVD load at baseline rarely showed progression even after 9 years, whereas those with moderate or severe cSVD had highest risk to progress. We could not find a 'ceiling effect' for this cSVD progression (the progression reaches a certain threshold) as was suggested previously.[41]

Clinical relevance and implications

Because patients with cSVD have an increased risk of not only behavioural symptoms, but also a tripled risk on stroke and even death,[41,294,295 294,296] it is important to identify those at risk as early as possible, preferably before the irreversible damage has been done.

Those who present with (or even without) cognitive problems, and have a relatively low WM or hippocampal volume on brain MRI, have a higher risk to develop dementia in the years to come compared to those with intact WM and hippocampal volumes. This implies a pre-clinical period, where intervention might be possible before the development of dementia. Persons with low WM and hippocampal volume should possibly be monitored more closely, and medical care should be optimized. Probably our study does not change the current MRI protocols used in clinical practice, for demographic characteristics (foremost age and cognitive performance) remain the main predictors for conversion to dementia, and in the clinical practice hippocampal atrophy measurements are already used in the diagnostic process. In those with relatively intact hippocampal volumes, hippocampal DTI parameters may be of added value in dementia risk stratification. In clinical practice however it is questionable if DTI scanning is of usage, as diffusion tensor imaging takes a lot of time, and this technique is not available in most clinical practices yet.[68] In conclusion, these findings provide an early insight into the pathophysiology of cognitive decline in older adults with cSVD, however DTI does not, or only limited, add to conventional MRI in explaining clinical expressions of cSVD.

Because depressive symptoms increase the cumulative risk of dementia 5 years later, in clinical practice it is important to monitor those with depressive symptoms that first occur at older age, for these might precede dementia. Especially in the patients performing relatively well in objective cognitive testing, depressive symptoms could be an early symptom of dementia. Therefore these patients should be monitored in contrast to current clinical practice. On the other hand, those who present with both good objective test scores and without depressive symptoms have very little chance to develop dementia, and can be comforted.

Finally cSVD progression, is predominantly seen in participants with severe WMH at baseline, whereas participants with mild WMH, though highly prevalent, rarely showed progression, not even over a period of nine years. This knowledge results in better understanding of both the aetiology as the consequences of cSVD, for it is likely that those with most severe WMH progression are at highest risk to develop behavioural consequences. Personalized treatment strategies to prevent cSVD progression should be optimized for all those with mild, moderate ***and*** severe WMH. Those with mild cSVD are at a low risk to progress and therefore can probably be reassured together with optimal vascular care. Preventive strategies are especially needed for those with moderate and severe WMH at all ages, for we did not find a ceiling effect and progression was independent of age.

Preventing cSVD progression therefore is useful in all cSVD patients, for reducing cSVD progression might have a beneficial effect on the number of people suffering from the

behavioural consequences of cSVD, and might eventually be a therapeutic strategy to reduce cognitive decline.

Possible treatment targets

As we showed in our study, the association between cSVD and cognitive decline is weak. Possibly the additional concept of brain 'reserve' plays a role, in which individuals with more reserve capacity (including both 1. brain reserve such as microstructural integrity or volume because of genetic factors or good nutrition and 2. cognitive reserve representing better coping mechanisms), presumably better tolerate cSVD and with that express less clinical symptoms with the same cSVD load than those with less 'reserve'.[297] As genetic factors cannot be modified, continuing a healthy lifestyle at older age, preventing malnutrition and stimulating older adults to remain cognitively active might help in better toleration of present cSVD. Finally as depressive symptoms increase the dementia risk, it could be suggested that treatment of depression at older age could influence the risk for cognitive decline.[298]

Until now no randomized clinical trials have been performed to study the different preventive and therapeutic strategies in those with cSVD on the risk of behavioral consequences.[299] Possibly specific clinics such as in Florence (VAS-COG clinic), are a first step into a better understanding of cSVD patients with behavioural consequences, using more specified screening, diagnostic methods and treatment strategies. [300]

Future research

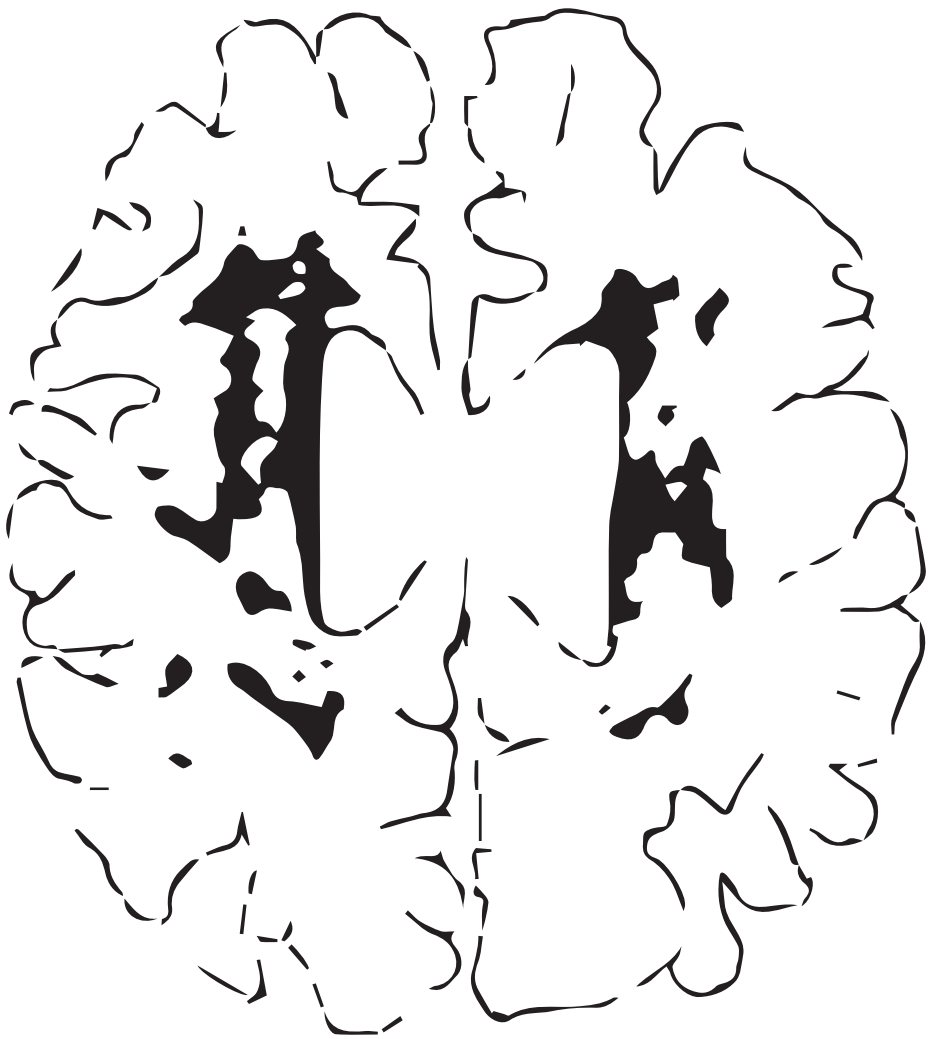
One of the remaining questions is whether an ***increase*** in traditional cSVD markers (incident lacunes or microbleeds, decline in microstructural integrity, or increase of WMH volume), or brain atrophy (change in hippocampal, amygdalar, WM and GM volumes), precede newly developed behavioural consequences. Because of the availability of 2 follow-up moments in the RUN DMC study, temporal dynamics of cSVD can be assessed. Moreover, it is interesting to investigate if microstructural integrity changes precede macrostructural changes, and whether these changes in this microstructural integrity better correlate with incident behavioural consequences than baseline imaging characteristics, or macrostructural changes over time.[301] Finally the follow-up of the RUN DMC study should investigate the 'remote effects' of cSVD, taking 'change in cSVD' and subsequent brain atrophy (WM, GM and hippocampal volume) into account.

An important clinical challenge is the identification of intervention targets, in order to provide a possible therapy. Therefore it is important to identify which (vascular) risk factors are most important in causing cSVD progression and incident dementia. With the availability of the 2 follow-up moments in the RUN DMC study, we are in a unique position to investigate these riskfactors.[302] Eventually randomized clinical trials have to be performed to identify if treatment or prevention of these vascular risk factors not only diminish cSVD progression, but also have a clinical effect by reducing incident dementia.

In future studies novel imaging markers of cSVD can be used as surrogate markers for behavioural symptoms, possibly explaining another part of the variability in the relation between cSVD and cognition or mood. Such imaging markers are cortical superficial siderosis (cSS), micro-infarcts. Novel quantitative imaging techniques including MR Perfusion imaging with arterial spin labelling (ASL), functional MRI (fMRI) and Positron Emission Tomography (PET) might give new insights in the mechanisms causing cSVD.[29] Network analyses to study the whole brain connectivity, for example the aid of the graph theory, are interesting new techniques in investigating the change of behavioural consequences in relation to (change in) network disruption (both connectivity and functional).[303]

In order to gain more insight into the aetiology of cSVD, studies with repetitive imaging, using diffusion weighted imaging, should be conducted to further investigate the contribution of acute ischemia to cSVD progression as was suggested by a small pilot study.[292] This study has already emerged from the RUN DMC study, and started in 2016. 50 Participants of the RUN DMC study will be monthly scanned to gain more insight into the contribution of acute ischemic pathology in the development of cSVD.

It could be interesting to investigate if the total burden of the individual cSVD markers are possibly more representative for the severity of the brain damage, rather than every individual marker separately.[291,304] The sum of these markers might correlate better with incident dementia, cognitive decline or mood disturbances than the (macrostructural and microstructural) cSVD markers separately.



13.
Nederlandse samenvatting

Cerebrale micro-angiopathie wordt in het Engels ook wel 'cerebral small vessel disease' (cSVD) genoemd, en beschrijft het pathologische proces van schade aan de kleine bloedvaten van de hersenen. Witte stof afwijkingen (WSA), lacunes (kleine herseninfarcten), microbloedingen en (sub)corticale atrofie zijn de bekende of 'traditionele' cSVD markers. cSVD wordt zeer regelmatig gezien op MRI-scans van mensen ouder dan 60 jaar. De incidentie neemt niet-lineair toe met de leeftijd, en de progressie van cSVD lijkt gerelateerd te zijn aan de hoeveelheid cSVD op baseline en de aanwezigheid van vasculaire risicofactoren. Het klinische spectrum en de prognose van cSVD zijn enorm heterogeen. Het klinisch spectrum varieert van zeer milde cognitieve- of stemmingsklachten tot het eindstadium van dementie of zelfs overlijden. Met behulp van conventionele MRI technieken is het moeilijk gebleken om te voorspellen welke patiënten met cSVD het hoogste risico hebben op het ontwikkelen van deze ernstige klinische symptomen, en voor wie dat niet geldt. We denken daarom dat ook andere factoren (náást de "traditionele" cSVD karakteristieken), misschien wel meer bepalend zijn voor wie uiteindelijk dementie ontwikkelen, en wie niet. Zulke 'andere factoren' kunnen onder andere de microstructurele integriteit van de witte stof zijn (dat wil zeggen: cSVD welke je niet kunt zien op conventionele MRI) en hersenvolumina van structuren waarvan we weten dat deze geassocieerd zijn met gedrag (zoals bijvoorbeeld de hippocampus en amygdala).
De studies in dit proefschrift zijn gebaseerd op data van de RUN DMC studie. Dit is een prospectieve cohort studie onder 503 niet-demente, zelfvoorzienende ouderen (tussen de 50 en 85 jaar oud) met enige vorm van cSVD op cerebrale beeldvorming. In deze studie onderzoeken we de oorzaken en gevolgen van cSVD, waarbij het eerste onderzoek (baseline) heeft plaatsgevonden in 2006-2007 met tweemaal een vervolg (follow-up), 5 jaar en 9 jaar later. We beschrijven de associaties tussen de MRI- karakteristieken (zowel macro- als microstructureel) op baseline en de gevolgen voor het gedrag 5 jaar later. De focus van dit proefschrift ligt op cognitieve achteruitgang en depressieve symptomen. Ten slotte beschrijven we het dynamische verloop (zowel de progressie als de regressie) van de traditionele cSVD karakteristieken over een periode van 9 jaar.

Cognitie en incidente dementie: macro– versus microstructurele MRI

In **Deel I (hoofdstuk 2)** beschrijven we op cross-sectioneel niveau de mogelijke (additionele) klinische waarde van diffusion tensor imaging (DTI) boven op de bekende (conventionele) MRI sequenties, met betrekking tot het cognitief functioneren. We laten zien dat álle MRI parameters gezamenlijk 1 tot 6% van de variantie in cognitief functioneren verklaren, bovenop de 22-36% die al werd verklaard door leeftijd, geslacht en opleidingsniveau. Van deze MRI parameters (die de additionele 1 tot 6% verklaarden), droegen de DTI parameters (markers voor de structurele integriteit van de witte stof) niet substantieel bij aan de verklaarde variantie van cognitief functioneren (slechts 1%). Van deze DTI parameters, had de MD (mean diffusivity) de sterkste associatie met cognitief functioneren. We concluderen dat de cross-sectionele relatie tussen de microstructurele integriteit van de witte stof en cognitief

functioneren zwak is, en dat DTI een zeer beperkte toegevoegde klinische waarde heeft naast de conventionele beeldvorming.

In **Deel 2** beschrijven we prospectief de relatie tussen de hersenstructuren op baseline, onderzocht met MRI (FLAIR, T1 en DTI) en het ontstaan van dementie en cognitieve achteruitgang na 5 jaar. In **hoofdstuk 3** laten we een 5.5-jaar cumulatief risico van 11.1% (95%CI 7.7-14.6) zien op het ontwikkelen van all-cause dementie. Daarnaast tonen we aan dat zowel een laag witte stof volume (HR 0.65 (95% CI 0.44-0.96)) als een laag hippocampus volume (HR 0.68 (95% CI 0.47-0.99)), zijn gerelateerd aan het ontwikkelen van dementie, onafhankelijk van cSVD. Opvallend was dat we in deze studie in het geheel geen toegevoegde diagnostische waarde vonden van de cSVD karakteristieken (ook niet van de DTI parameters) op het voorspellen van het dementierisico na 5 jaar. In **hoofdstuk 4** laten we zien dat diffusieparameters in de hippocampus, onafhankelijk van hippocampus volume, hersenvolume en cSVD markers, zijn gerelateerd aan een verhoogd risico op dementie (HR 1.44 (95% CI 1.10-1.88)). Sub analyse in de deelnemersgroep zonder duidelijke hippocampusatrofie, liet zien dat een lage hippocampale microstructurele integriteit het risico op dementie meer dan verdubbelde (HR 2.66 (95% CI 1.30-5.44)). Mogelijk geeft een toegevoegde DTIscan van de hippocampus ons de mogelijkheid om personen, die risico lopen op het ontwikkelen van dementie, eerder te identificeren dan wanneer alléén volumetrische metingen van de hippocampus worden gebruikt. Tevens zou DTI van de hippocampus gebruikt kunnen worden als een surrogaat marker van ziekteprogressie. In **hoofdstuk 5** beschrijven we prospectief globale DTI parameters op baseline en cognitieve achteruitgang gemeten over verschillende domeinen ,5 jaar later. Ook hier tonen we aan dat DTI parameters van de witte stof niet gerelateerd zijn aan achteruitgang in globaal cognitief functioneren, of in andere cognitieve domeinen, na correctie voor demografische karakteristieken en MRI parameters. Deze bevinding is in overeenstemming met de resultaten op cross-sectioneel niveau waar we slechts zeer beperkte toegevoegde waarde van DTI parameters vonden bovenop de andere MRI maten in relatie met cognitief functioneren **(hoofdstuk 2)** en het gebrek aan relatie tussen globale DTI parameters en incidente dementie na 5 jaar **(hoofdstuk 3)**.

Depressieve symptomen en cerebral small vessel disease

In **Deel 3** bespreken we depressieve symptomen in relatie tot hersenstructuren en de relatie tussen depressieve symptomen en cognitief functioneren, bij ouderen met cSVD. De associatie tussen amygdala volume en depressieve symptomen (op cross-sectioneel niveau) is het onderwerp van **hoofdstuk 6**. Hierin laten we zien dat een lager volume van de linker amygdala is geassocieerd met depressieve symptomen die voor het eerst voorkomen op latere leeftijd (LODS), onafhankelijk van cSVD markers. Elke afname van amygdala volume (per mL) is gerelateerd aan een hoger risico op LODS (OR = 1.77; 95% CI 1.02-3.08; p=0.04).

In **hoofdstuk 7** rapporteren we over de integriteit van de witte stof van oudere volwassenen met en zonder depressieve symptomen, op cross-sectioneel niveau. Hierbij gebruikten we Tract-Based Spacial-Statistics (TBSS). We laten zien dat ouderen met depressieve

symptomen een lagere microstructurele integriteit van de witte stof hebben in vergelijking met degene zonder depressieve symptomen. Deze verschillen bevinden zich met name in de prefrontale wittestof banen, welke een belangrijke rol lijken te spelen in de (patho)genese van gedrag en stemming. Dit verschil is onafhankelijk van cognitief functioneren. Onze data suggereren dat cSVD een rol speelt in het verschil in wittestof integriteit tussen personen met en zonder depressieve symptomen, want na correctie voor cSVD markers verdwijnt deze associatie vrijwel volledig.
In **hoofdstuk 8** onderzoeken we prospectief de relatie tussen het hebben van depressieve symptomen en het ontstaan van 'all-cause' dementie na 5 jaar. Hierbij nemen we zowel de leeftijd waarop de depressieve symptomen zijn ontstaan en het cognitief functioneren op baseline in beschouwing. We laten zien dat diegenen met depressieve symptomen op baseline een hoger risico hebben op het ontwikkelen van dementie (HR 2.7 (95%CI 1.4-5.2)) dan de ouderen zonder depressieve symptomen bij de eerste meting. Deze relatie wordt bepaald door diegenen met depressieve symptomen voor het eerst in hun leven op latere leeftijd (LODS), en blijken onafhankelijk van cSVD markers. Juist bij de deelnemers die op baseline goed scoorden op de cognitieve testen, geven depressieve symptomen op latere leeftijd een hoger risico op het ontwikkelen van dementie (geen depressieve symptomen 0.0% vs depressieve symptomen 6.9%; log-rank p<0.001). Een dergelijk verschil vonden we niet tussen de deelnemers met en zonder depressieve symptomen in de groep die lager presteerde bij de cognitieve testen op baseline.

In **Deel IV (hoofdstuk 9)** reviewen we de klinische consequenties; (cognitief, motoor en stemming) en de risicofactoren van microstructurele schade van de witte stof (gemeten middels diffusion tensor imaging (DTI)).

Het beloop van cerebral small vessel disease in de tijd

Tenslotte beschrijven we in **Deel V** het verschil in cSVD markers gemeten over een periode van 9 jaar, waarbij we naast de baseline meting (2006) twee follow-up momenten gebruiken (2011 en 2015). In **hoofdstuk 10** weiden we uit over het dynamische proces van cSVD, waarbij we zowel de nieuw ontwikkelde cSVD als de ogenschijnlijke afname van de traditionele cSVD markers in beschouwing nemen. De gemiddelde WSA progressie 9 jaar later was 4.7 mL (0.54mL/jaar; IQR 0.95-5.5mL), 20.3% van de deelnemers had incidente lacunes (2.3%/jaar) en 18.9% incidente microbloedingen (2.2%/jaar). WSA volume nam af bij 9.4% van de deelnemers, en lacunes verdwenen bij 3.6% en microbloedingen bij 5.7%. Deze getallen zijn berekend over 2 follow-up momenten, hetgeen ons de mogelijkheid geeft om de niet-lineaire progressie van WSA te beschrijven. Bovendien rapporteerden we dat diegenen die op baseline zeer weinig cSVD hadden, weinig tot geen progressie lieten zien, in tegenstelling tot diegenen die al matige tot ernstige cSVD op baseline toonden (OR 35.5; 95% CI 15.8-80.0; p<0.001 voor WSA progressie; OR 5.7; 95%CI 2.8-11.2; p<0.001 voor incidente lacunes en OR 2.9; 95%CI 1.4-5.9; p=0.003 voor incidente microbloedingen).

Conclusie

De studies in dit proefschrift identificeerden MRI-markers die ons kunnen helpen om meer inzicht te krijgen in de pathofysiologische processen die ten grondslag liggen aan de cognitieve c.q. stemmingsveranderingen als gevolg van cerebral small vessel disease. Tegelijkertijd laten we in dit proefschrift de zeer beperkte toegevoegde waarde op dit gebied zien van diffusion tensor imaging bovenop de bekende macrostructurele MRI-markers in een oudere populatie met al langer bestaande cSVD. Depressieve symptomen die zich voor het eerst openbaren op hogere leeftijd, juist bij personen die tegelijkertijd goed scoren op cognitieve testen, wijzen op een verhoogd risico op het ontwikkelen van dementie. Ten slotte is cerebral small vessel disease een niet-lineair, dynamisch proces, dat voornamelijk progressie vertoont wanneer de jaren verstrijken, maar bij enkele personen ook afname.

In de toekomst zouden we moeten onderzoeken of een ***toename*** van cSVD (incidente lacunes of microbloedingen, toename van WSA volume, of afname in microstructurele integriteit van de witte stof) of atrofie van de hersenen (afname in hippocampus-, amygdala-, witte stof- en grijze stof volumina), voorafgaan aan nieuw ontwikkelde cognitieve of stemmingsproblemen. Mogelijk dat deze ***verandering*** in MRI-markers een beter correlaat met de klinische symptomen laat zien dan de baseline MRI-markers.

Part VII:
Appendices

List of abbreviations

3D: Three Dimensional
AD: Alzheimer's Disease
AD: Axial Diffusivity
AIC: Akaike Information Criterion
AVLT: Auditory Verbal Learning Test
ANCOVA: Analysis of Covariance
ANOVA: Analysis of Variance
APOE: Apo lipoprotein E
ARWMC: Age-related with matter changes
BMIPB: Birt Memory & Information Processing Battery
BMI: Body Mass Index
CAA: Cerebral Amyloïd Angiopathy
CADASIL: Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leucoencofalopathy
CARASIL: Cerebral Autosomal Recessive Arteriopathy with Subcortical Infarcts and Leucoencofalopathy
CB: Cingulum bundle
CC: Corpus callosum
CES-D: Centre of Epidemiologic Studies Depression Scale
95% CI: Confidence Interval
CI: Cognitive Index
CSF: Cerebrospinal Fluid
cSS: cortical Superficial Siderosis
cSVD: cerebral Small Vessel Disease
CT: Compute Tomography
df: degrees of freedom
DM: Diabetes Mellitus
DS: Depressive Symptoms
DSM-IV: Diagnostic and Statistical Manual of Mental Disorders - IV
DTI: Diffusion Tensor Imaging
DWI: Diffusion Weighted Imaging
EC: External capsule
EODS: Early Onset Depressive Symptoms
EPI: Echo planar Imaging
EPVS: Enlarged Perivascular Spaces
FA: Fractional Anisotropy
FLAIR: Fluid Attenuated Inversion Recovery
FSL: FMRIB's Software Library

FTD: Fronto-Temporal Dementia
FU: Follow-up
GDS: Geriatric Depression Scale
GM: Grey matter
HC: Healthy controls
HT: Hypertension
HV: Hippocampus volume / Hippocampal volume
HR: Hazard ratio
IC: Internal capsule
ICV: Intracranial Volume
IFOF: Inferior Frontal Occipital Fasciculus
ILF: Inferior Longitudinal Fasciculus
IQ: Intelligence Quotient
IQR: Inter Quartile Range
Lacunes: Lacunes of presumed vascular origin
LODS: Late Onset Depressive Symptoms
MB: Microbleeds
MCI: Mild Cognitive Impairment
MD: Mean Diffusivity
MET: Metabolic Equivalent
mI: myo-inositol
MINI: Mini International Neuropsychiatric Interview
mL: milliliter
MMSE: Mini–Mental State Examination
MNI space: Montreal Neurological Institute stereotactic space
MO: Mode of Anisotropy
MoCA: Montreal Cognitive Assessment
MPS: Mild parkinsonian signs
MPRAGE: Magnetization Prepared RApid Gradient Echo
MRI: Magnetic Resonance Imaging
MRS: Magnetic Resonance Spectroscopy
MS: Multiple sclerosis
NAA: N-acetylaspartate
NABT: Normal Appearing Brain Tissue
NAWM: Normal Appearing White Matter
NC: Normal cognition
NIA-AA: National Institute of Aging/Alzheimer's Association
NINDS-AIREN: National Institute of Neurological Disorders and Stroke - Association Internationale pour la Recherche et l'Enseignement en Neurosciences
n: number

OR: Odds ratio
PEPS: Pyramidal and Extra-Pyramidal Scale
PET: Positron Emission Tomography
PPN: Pedunculopontine nucleus
PTs: patients
PV: Periventricular
PVR: Perivascular spaces
RAVLT: Rey Auditory Verbal Learning Test
RCFT: Rey Complex Figure Task
RD: Radial Diffusivity
ROI: Region of Interest
ROCF: Rey-Osterrieth complex figure test
RUN DMC: Radboud University Nijmegen Diffusion Tensor and Magnetic resonance Cohort
SAT: Speed-Accuracy Trade-Off
SD: Standard deviation
SDST: Symbol Digit Substitution Task
SIDAM : structured Interview for the diagnosis of Dementia of the Alzheimer Type, multi-infarct dementia and other type of dementia
SLF: Superior Longitudinal Fasciculus
SPM5: Statistical Parametric Mapping 5
SPPB: Short Physical Performance Battery
STRIVE: Standards for research into small vessel disease
SVD: small vessel disease
TBSS: Tract-Based Spatial Statistics
TBV: Total Brain Volume
tCho: total Cholines,
tCr: total Creatines
TE: Echo time
TI: Inversion time
TIA: Transient Ischemic Attack
TMT: Trail making test
TR: Time repetition
UF: Uncinate Fasciculus
UPDRS: Unified Parkinson's Disease Rating Scale
UK: United Kingdom
VaD: Vascular Dementia
VADAS: Vascular Dementia Assessment Scale–Cognitive Subscale
VBA: Voxel Based Analysis
VBM: Voxel based Morphometry
VCI: Vascular Cognitive Impairment

VIF: Variance Inflation Factor
vs.: versus
VSAT: Verbal Series Attention test
WAISR: Wechsler adult intelligence scale–revised
WCST: Wisconsin card sorting test
WM: White matter
WMH: White matter hyperintensities
WML: White matter lesions

References

1. Poirier J, Derouesne C. [The concept of cerebral lacunae from 1838 to the present]. ***Rev Neurol (Paris)*** 1985;(1): 3-17.
2. Durand-Fardel M. Traité clinique et pratique des maladies des vieillards Baillière. 1854.
3. Binswanger O. Die Abgrenzung der allgemeinen progressiven paralyse ***Berl Klin Wochenschr*** 1894;(31): 1103-5.
4. Roman GC. On the history of lacunes, etat crible, and the white matter lesions of vascular dementia. ***Cerebrovasc Dis*** 2002: 1-6.
5. Hachinski VC, Potter P, Merskey H. Leuko-araiosis. ***Arch Neurol*** 1987;(1): 21-3.
6. de Leeuw FE, de Groot JC, Achten E, et al. 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;(1): 9-14.
7. van Dijk EJ, Prins ND, Vermeer SE, Koudstaal PJ, Breteler MM. Frequency of white matter lesions and silent lacunar infarcts. ***J Neural Transm Suppl*** 2002;(62): 25-39.
8. Cordonnier C, Al-Shahi Salman R, Wardlaw J. Spontaneous brain microbleeds: systematic review, subgroup analyses and standards for study design and reporting. ***Brain*** 2007;(Pt 8): 1988-2003.
9. Doubal FN, MacLullich AM, Ferguson KJ, Dennis MS, Wardlaw JM. Enlarged perivascular spaces on MRI are a feature of cerebral small vessel disease. ***Stroke*** 2010;(3): 450-4.
10. Charidimou A, Gang Q, Werring DJ. Sporadic cerebral amyloid angiopathy revisited: recent insights into pathophysiology and clinical spectrum. ***J Neurol Neurosurg Psychiatry*** 2012;(2): 124-37.
11. van Veluw SJ, Zwanenburg JJ, Rozemuller AJ, Luijten PR, Spliet WG, Biessels GJ. The spectrum of MR detectable cortical microinfarcts: a classification study with 7-tesla postmortem MRI and histopathology. ***J Cereb Blood Flow Metab*** 2015;(4): 676-83.
12. Wardlaw JM, Smith EE, Biessels GJ, et al. Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. ***Lancet Neurol*** 2013;(8): 822-38.
13. Pantoni L. Cerebral small vessel disease: from pathogenesis and clinical characteristics to therapeutic challenges. ***Lancet Neurol*** 2010;(7): 689-701.
14. Gouw AA, van der Flier WM, van Straaten EC, et al. Reliability and sensitivity of visual scales versus volumetry for evaluating white matter hyperintensity progression. ***Cerebrovasc Dis*** 2008;(3): 247-53.
15. Maillard P, Crivello F, Dufouil C, Tzourio-Mazoyer N, Tzourio C, Mazoyer B. Longitudinal follow-up of individual white matter hyperintensities in a large cohort of elderly. ***Neuroradiology*** 2009;(4): 209-20.
16. Schmidt R, Ropele S, Enzinger C, et al. White matter lesion progression, brain atrophy, and cognitive decline: the Austrian stroke prevention study. ***Ann Neurol*** 2005;(4): 610-6.
17. Akoudad S, Ikram MA, Koudstaal PJ, et al. Cerebral microbleeds are associated with the progression of ischemic vascular lesions. ***Cerebrovasc Dis*** 2014;(5): 382-8.
18. Gouw AA, van der Flier WM, Pantoni L, et al. On the etiology of incident brain lacunes: longitudinal observations from the LADIS study. ***Stroke*** 2008;(11): 3083-5.

19. Ding J, Sigurdsson S, Garcia M, et al. Risk Factors Associated With Incident Cerebral Microbleeds According to Location in Older People: The Age, Gene/Environment Susceptibility (AGES)-Reykjavik Study. ***JAMA Neurol*** 2015;(6): 682-8.
20. Klarenbeek P, van Oostenbrugge RJ, Rouhl RP, Knottnerus IL, Staals J. Higher ambulatory blood pressure relates to new cerebral microbleeds: 2-year follow-up study in lacunar stroke patients. ***Stroke*** 2013;(4): 978-83.
21. van Dijk EJ, Breteler MM, Schmidt R, et al. The association between blood pressure, hypertension, and cerebral white matter lesions: cardiovascular determinants of dementia study. ***Hypertension*** 2004;(5): 625-30.
22. Schmidt R, Enzinger C, Ropele S, Schmidt H, Fazekas F, Austrian Stroke Prevention S. Progression of cerebral white matter lesions: 6-year results of the Austrian Stroke Prevention Study. ***Lancet*** 2003;(9374): 2046-8.
23. Fazekas F, Kleinert R, Offenbacher H, et al. Pathologic correlates of incidental MRI white matter signal hyperintensities. ***Neurology*** 1993;(9): 1683-9.
24. de Groot M, Verhaaren BF, de Boer R, et al. Changes in normal-appearing white matter precede development of white matter lesions. ***Stroke*** 2013;(4): 1037-42.
25. Pierpaoli C, Jezzard P, Basser PJ, Barnett A, Di Chiro G. Diffusion tensor MR imaging of the human brain. ***Radiology*** 1996;(3): 637-48.
26. Ikram MA, Vrooman HA, Vernooij MW, et al. Brain tissue volumes in the general elderly population. The Rotterdam Scan Study. ***Neurobiol Aging*** 2008;(6): 882-90.
27. Barber R, Scheltens P, Gholkar A, et al. White matter lesions on magnetic resonance imaging in dementia with Lewy bodies, Alzheimer's disease, vascular dementia, and normal aging. ***J Neurol Neurosurg Psychiatry*** 1999;(1): 66-72.
28. Kapasi A, Schneider JA. Vascular contributions to cognitive impairment, clinical Alzheimer's disease, and dementia in older persons. ***Biochim Biophys Acta*** 2016;(5): 878-86.
29. Banerjee G, Wilson D, Jager HR, Werring DJ. Novel imaging techniques in cerebral small vessel diseases and vascular cognitive impairment. ***Biochim Biophys Acta*** 2016;(5): 926-38.
30. Viswanathan A, Rocca WA, Tzourio C. Vascular risk factors and dementia: how to move forward? ***Neurology*** 2009;(4): 368-74.
31. de Leeuw FE, Barkhof F, Scheltens P. White matter lesions and hippocampal atrophy in Alzheimer's disease. ***Neurology*** 2004;(2): 310-2.
32. 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;(9): 2248-52.
33. Squire LR, Stark CE, Clark RE. The medial temporal lobe. ***Annu Rev Neurosci*** 2004: 279-306.
34. Roman GC, Erkinjuntti T, Wallin A, Pantoni L, Chui HC. Subcortical ischaemic vascular dementia. ***Lancet Neurol*** 2002;(7): 426-36.
35. Geschwind N. Disconnexion syndromes in animals and man. I. ***Brain*** 1965;(2): 237-94.
36. Herrmann LL, Le Masurier M, Ebmeier KP. White matter hyperintensities in late life depression: a systematic review. ***J Neurol Neurosurg Psychiatry*** 2008;(6): 619-24.

37. Krishnan KR, McDonald WM, Doraiswamy PM, et al. Neuroanatomical substrates of depression in the elderly. ***Eur Arch Psychiatry Clin Neurosci*** 1993;(1): 41-6.
38. Mendlewicz J, Baron M. Morbidity risks in subtypes of unipolar depressive illness: differences between early and late onset forms. ***Br J Psychiatry*** 1981: 463-6.
39. Seminowicz DA, Mayberg HS, McIntosh AR, et al. Limbic-frontal circuitry in major depression: a path modeling metanalysis. ***Neuroimage*** 2004;(1): 409-18.
40. van Sloten TT, Sigurdsson S, van Buchem MA, et al. Cerebral Small Vessel Disease and Association With Higher Incidence of Depressive Symptoms in a General Elderly Population: The AGES-Reykjavik Study. ***Am J Psychiatry*** 2015;(6): 570-8.
41. Prins ND, Scheltens P. White matter hyperintensities, cognitive impairment and dementia: an update. ***Nat Rev Neurol*** 2015;(3): 157-65.
42. Schmidt R, Scheltens P, Erkinjuntti T, et al. White matter lesion progression: a surrogate endpoint for trials in cerebral small-vessel disease. ***Neurology*** 2004;(1): 139-44.
43. van Norden AG, van Uden IW, de Laat KF, van Dijk EJ, de Leeuw FE. Cognitive function in small vessel disease: the additional value of diffusion tensor imaging to conventional magnetic resonance imaging: the RUN DMC study. ***J Alzheimers Dis*** 2012;(3): 667-76.
44. van Uden IW, van der Holst HM, Tuladhar AM, et al. White Matter and Hippocampal Volume Predict the Risk of Dementia in Patients with Cerebral Small Vessel Disease: The RUN DMC Study. ***J Alzheimers Dis*** 2016;(3): 863-73.
45. van Uden IW, Tuladhar AM, van der Holst HM, et al. Diffusion tensor imaging of the hippocampus predicts the risk of dementia; the RUN DMC study. ***Hum Brain Mapp*** 2016;(1): 327-37.
46. van Uden IW, van der Holst HM, Schaapsmeerders P, et al. Baseline white matter microstructural integrity is not related to cognitive decline after 5 years: The RUN DMC study. ***BBA Clin*** 2015: 108-14.
47. van Uden IW, van Norden AG, de Laat KF, et al. Depressive Symptoms and Amygdala Volume in Elderly with Cerebral Small Vessel Disease: The RUN DMC Study. ***J Aging Res*** 2011: 647869.
48. van Uden IW, Tuladhar AM, de Laat KF, et al. White matter integrity and depressive symptoms in cerebral small vessel disease: The RUN DMC study. ***Am J Geriatr Psychiatry*** 2015;(5): 525-35.
49. van Uden IW, van der Holst HM, van Leijsen EM, et al. Late-onset depressive symptoms increase the risk of dementia in small vessel disease. ***Neurology*** 2016;(11): 1102-9.
50. Pasi M, van Uden IW, Tuladhar AM, de Leeuw FE, Pantoni L. White Matter Microstructural Damage on Diffusion Tensor Imaging in Cerebral Small Vessel Disease: Clinical Consequences. ***Stroke*** 2016;(6): 1679-84.
51. Vermeer SE, Prins ND, den Heijer T, Hofman A, Koudstaal PJ, Breteler MM. Silent brain infarcts and the risk of dementia and cognitive decline. ***N Engl J Med*** 2003;(13): 1215-22.
52. van Norden AG, van den Berg HA, de Laat KF, Gons RA, van Dijk EJ, de Leeuw FE. Frontal and temporal microbleeds are related to cognitive function: the Radboud University Nijmegen Diffusion Tensor and Magnetic Resonance Cohort (RUN DMC) Study. ***Stroke*** 2011;(12): 3382-6.
53. de Groot JC, de Leeuw FE, Oudkerk M, et al. Cerebral white matter lesions and cognitive function: the Rotterdam Scan Study. ***Ann Neurol*** 2000;(2): 145-51.

54. Vernooij MW, Ikram MA, Tanghe HL, et al. Incidental findings on brain MRI in the general population. ***N Engl J Med*** 2007;(18): 1821-8.
55. Awad IA, Johnson PC, Spetzler RF, Hodak JA. Incidental subcortical lesions identified on magnetic resonance imaging in the elderly. II. Postmortem pathological correlations. ***Stroke*** 1986;(6): 1090-7.
56. Jones DK, Lythgoe D, Horsfield MA, Simmons A, Williams SC, Markus HS. Characterization of white matter damage in ischemic leukoaraiosis with diffusion tensor MRI. ***Stroke*** 1999;(2): 393-7.
57. Basser PJ, Mattiello J, LeBihan D. MR diffusion tensor spectroscopy and imaging. ***Biophys J*** 1994;(1): 259-67.
58. Basser PJ, Pierpaoli C. Microstructural and physiological features of tissues elucidated by quantitative-diffusion-tensor MRI. ***J Magn Reson B*** 1996;(3): 209-19.
59. Le Bihan D, Mangin JF, Poupon C, et al. Diffusion tensor imaging: concepts and applications. ***J Magn Reson Imaging*** 2001;(4): 534-46.
60. van Norden AG, de Laat KF, Gons RA, et al. Causes and consequences of cerebral small vessel disease. The RUN DMC study: a prospective cohort study. Study rationale and protocol. ***BMC Neurol*** 2011: 29.
61. Erkinjuntti T. Subcortical vascular dementia. ***Cerebrovasc Dis*** 2002: 58-60.
62. Association AP. Diagnostic and statistical manual of mental disorders, 4th ed. Washington DC; 2000.
63. Ashburner J, Friston KJ. Unified segmentation. ***Neuroimage*** 2005;(3): 839-51.
64. Basser PJ, Jones DK. Diffusion-tensor MRI: theory, experimental design and data analysis - a technical review. ***NMR Biomed*** 2002;(7-8): 456-67.
65. Hochstenbach J, Mulder T, van Limbeek J, Donders R, Schoonderwaldt H. Cognitive decline following stroke: a comprehensive study of cognitive decline following stroke. ***J Clin Exp Neuropsychol*** 1998;(4): 503-17.
66. Rosendorff C, Black HR, Cannon CP, et al. Treatment of hypertension in the prevention and management of ischemic heart disease: a scientific statement from the American Heart Association Council for High Blood Pressure Research and the Councils on Clinical Cardiology and Epidemiology and Prevention. ***Circulation*** 2007;(21): 2761-88.
67. de Groot JC, de Leeuw FE, Oudkerk M, Hofman A, Jolles J, Breteler MM. Cerebral white matter lesions and depressive symptoms in elderly adults. ***Arch Gen Psychiatry*** 2000;(11): 1071-6.
68. Patel B, Markus HS. Magnetic resonance imaging in cerebral small vessel disease and its use as a surrogate disease marker. ***Int J Stroke*** 2011;(1): 47-59.
69. Yamauchi H, Fukuyama H, Ogawa M, Ouchi Y, Kimura J. Callosal atrophy in patients with lacunar infarction and extensive leukoaraiosis. An indicator of cognitive impairment. ***Stroke*** 1994;(9): 1788-93.
70. Mungas D, Jagust WJ, Reed BR, et al. MRI predictors of cognition in subcortical ischemic vascular disease and Alzheimer's disease. ***Neurology*** 2001;(12): 2229-35.
71. Schiavone F, Charlton RA, Barrick TR, Morris RG, Markus HS. Imaging age-related cognitive decline: A comparison of diffusion tensor and magnetization transfer MRI. ***J Magn Reson Imaging*** 2009;(1): 23-30.

72. Nitkunan A, Barrick TR, Charlton RA, Clark CA, Markus HS. Multimodal MRI in cerebral small vessel disease: its relationship with cognition and sensitivity to change over time. ***Stroke*** 2008;(7): 1999-2005.
73. Viswanathan A, Godin O, Jouvent E, et al. Impact of MRI markers in subcortical vascular dementia: a multi-modal analysis in CADASIL. ***Neurobiol Aging*** 2010;(9): 1629-36.
74. Taylor WD, Bae JN, MacFall JR, et al. Widespread effects of hyperintense lesions on cerebral white matter structure. ***AJR Am J Roentgenol*** 2007;(6): 1695-704.
75. Holtmannspotter M, Peters N, Opherk C, et al. Diffusion magnetic resonance histograms as a surrogate marker and predictor of disease progression in CADASIL: a two-year follow-up study. ***Stroke*** 2005;(12): 2559-65.
76. Charlton RA, Schiavone F, Barrick TR, Morris RG, Markus HS. Diffusion tensor imaging detects age related white matter change over a 2 year follow-up which is associated with working memory decline. ***J Neurol Neurosurg Psychiatry*** 2010;(1): 13-9.
77. Miwa K, Tanaka M, Okazaki S, et al. Multiple or mixed cerebral microbleeds and dementia in patients with vascular risk factors. ***Neurology*** 2014;(7): 646-53.
78. Achterberg HC, van der Lijn F, den Heijer T, et al. Hippocampal shape is predictive for the development of dementia in a normal, elderly population. ***Hum Brain Mapp*** 2014;(5): 2359-71.
79. Duering M, Righart R, Csanadi E, et al. Incident subcortical infarcts induce focal thinning in connected cortical regions. ***Neurology*** 2012;(20): 2025-8.
80. Basser PJ, Mattiello J, LeBihan D. Estimation of the effective self-diffusion tensor from the NMR spin echo. ***J Magn Reson B*** 1994;(3): 247-54.
81. Stebbins GT, Murphy CM. Diffusion tensor imaging in Alzheimer's disease and mild cognitive impairment. ***Behav Neurol*** 2009;(1): 39-49.
82. 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;(3): 189-98.
83. Sheehan DV, Lecrubier Y, Sheehan KH, et al. 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: 22-33;quiz 4-57.
84. McKhann GM, Knopman DS, Chertkow H, et al. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. ***Alzheimers Dement*** 2011;(3): 263-9.
85. Roman GC, Tatemichi TK, Erkinjuntti T, et al. Vascular dementia: diagnostic criteria for research studies. Report of the NINDS-AIREN International Workshop. ***Neurology*** 1993;(2): 250-60.
86. Ott A, Breteler MM, van Harskamp F, Stijnen T, Hofman A. Incidence and risk of dementia. The Rotterdam Study. ***Am J Epidemiol*** 1998;(6): 574-80.
87. van Norden AG, Fick WF, de Laat KF, et al. Subjective cognitive failures and hippocampal volume in elderly with white matter lesions. ***Neurology*** 2008;(15): 1152-9.
88. Yushkevich PA, Piven J, Hazlett HC, et al. User-guided 3D active contour segmentation of anatomical structures: significantly improved efficiency and reliability. ***Neuroimage*** 2006;(3): 1116-28.

89. Colliot O, Chetelat G, Chupin M, et al. Discrimination between Alzheimer disease, mild cognitive impairment, and normal aging by using automated segmentation of the hippocampus. ***Radiology*** 2008;(1): 194-201.
90. de Laat KF, Tuladhar AM, van Norden AG, Norris DG, Zwiers MP, de Leeuw FE. Loss of white matter integrity is associated with gait disorders in cerebral small vessel disease. ***Brain*** 2011;(Pt 1): 73-83.
91. Zwiers MP. Patching cardiac and head motion artefacts in diffusion tensor imaging. ***Proc Intl Soc Magn Reson Med*** 2009: 1423.
92. Andersson JL, Skare S. A model-based method for retrospective correction of geometric distortions in diffusion-weighted EPI. ***Neuroimage*** 2002;(1): 177-99.
93. Smith SM, Jenkinson M, Johansen-Berg H, et al. Tract-based spatial statistics: voxelwise analysis of multi-subject diffusion data. ***Neuroimage*** 2006;(4): 1487-505.
94. Vernooij MW, Ikram MA, Vrooman HA, et al. White matter microstructural integrity and cognitive function in a general elderly population. ***Arch Gen Psychiatry*** 2009;(5): 545-53.
95. van Norden AG, de Laat KF, van Dijk EJ, et al. Diffusion tensor imaging and cognition in cerebral small vessel disease: the RUN DMC study. ***Biochim Biophys Acta*** 2012;(3): 401-7.
96. Della Nave R, Foresti S, Pratesi A, et al. Whole-brain histogram and voxel-based analyses of diffusion tensor imaging in patients with leukoaraiosis: correlation with motor and cognitive impairment. ***AJNR Am J Neuroradiol*** 2007;(7): 1313-9.
97. Dufouil C, Godin O, Chalmers J, et al. Severe cerebral white matter hyperintensities predict severe cognitive decline in patients with cerebrovascular disease history. ***Stroke*** 2009;(6): 2219-21.
98. van Norden AG, van Dijk EJ, de Laat KF, Scheltens P, Olderikkert MG, de Leeuw FE. Dementia: Alzheimer pathology and vascular factors: from mutually exclusive to interaction. ***Biochim Biophys Acta*** 2012;(3): 340-9.
99. Breteler MM. Vascular risk factors for Alzheimer's disease: an epidemiologic perspective. ***Neurobiol Aging*** 2000;(2): 153-60.
100. Prins ND, van Dijk EJ, den Heijer T, et al. Cerebral white matter lesions and the risk of dementia. ***Arch Neurol*** 2004;(10): 1531-4.
101. Au R, Massaro JM, Wolf PA, et al. Association of white matter hyperintensity volume with decreased cognitive functioning: the Framingham Heart Study. ***Arch Neurol*** 2006;(2): 246-50.
102. Seo SW, Ahn J, Yoon U, et al. Cortical thinning in vascular mild cognitive impairment and vascular dementia of subcortical type. ***J Neuroimaging*** 2010;(1): 37-45.
103. Dziedzic T, Metz I, Dallenga T, et al. Wallerian degeneration: a major component of early axonal pathology in multiple sclerosis. ***Brain Pathol*** 2010;(5): 976-85.
104. Jokinen H, Schmidt R, Ropele S, et al. Diffusion changes predict cognitive and functional outcome: The LADIS study. ***Ann Neurol*** 2013;(5): 576-83.
105. Wiesmann M, Kiliaan AJ, Claassen JA. Vascular aspects of cognitive impairment and dementia. ***J Cereb Blood Flow Metab*** 2013;(11): 1696-706.
106. van Norden AG, de Laat KF, Fick I, et al. Diffusion tensor imaging of the hippocampus and verbal memory performance: the RUN DMC study. ***Hum Brain Mapp*** 2012;(3): 542-51.

107. Fellgiebel A, Dellani PR, Greverus D, Scheurich A, Stoeter P, Muller MJ. Predicting conversion to dementia in mild cognitive impairment by volumetric and diffusivity measurements of the hippocampus. ***Psychiatry Res*** 2006;(3): 283-7.
108. Geuze E, Vermetten E, Bremner JD. MR-based in vivo hippocampal volumetrics: 2. Findings in neuropsychiatric disorders. ***Mol Psychiatry*** 2005;(2): 160-84.
109. Manjon JV, Coupe P, Concha L, Buades A, Collins DL, Robles M. Diffusion weighted image denoising using overcomplete local PCA. ***PLoS One*** 2013;(9): e73021.
110. Zwiers MP. Patching cardiac and head motion artefacts in diffusion-weighted images. ***Neuroimage*** 2010;(2): 565-75.
111. Zola-Morgan S, Squire LR, Amaral DG. Human amnesia and the medial temporal region: enduring memory impairment following a bilateral lesion limited to field CA1 of the hippocampus. ***J Neurosci*** 1986;(10): 2950-67.
112. Alvarez P, Zola-Morgan S, Squire LR. Damage limited to the hippocampal region produces long-lasting memory impairment in monkeys. ***J Neurosci*** 1995;(5 Pt 2): 3796-807.
113. den Heijer T, der Lijn F, Vernooij MW, et al. Structural and diffusion MRI measures of the hippocampus and memory performance. ***Neuroimage*** 2012;(4): 1782-9.
114. Cherubini A, Peran P, Spoletini I, et al. Combined volumetry and DTI in subcortical structures of mild cognitive impairment and Alzheimer's disease patients. ***J Alzheimers Dis*** 2010;(4): 1273-82.
115. Fellgiebel A, Wille P, Muller MJ, et al. Ultrastructural hippocampal and white matter alterations in mild cognitive impairment: a diffusion tensor imaging study. ***Dement Geriatr Cogn Disord*** 2004;(1): 101-8.
116. Carlesimo GA, Cherubini A, Caltagirone C, Spalletta G. Hippocampal mean diffusivity and memory in healthy elderly individuals: a cross-sectional study. ***Neurology*** 2010;(3): 194-200.
117. Launer LJ. Demonstrating the case that AD is a vascular disease: epidemiologic evidence. ***Ageing Res Rev*** 2002;(1): 61-77.
118. Verdelho A, Madureira S, Moleiro C, et al. Depressive symptoms predict cognitive decline and dementia in older people independently of cerebral white matter changes: the LADIS study. ***J Neurol Neurosurg Psychiatry*** 2013;(11): 1250-4.
119. Fratiglioni L, De Ronchi D, Aguero-Torres H. Worldwide prevalence and incidence of dementia. ***Drugs Aging*** 1999;(5): 365-75.
120. Clerx L, Visser PJ, Verhey F, Aalten P. New MRI markers for Alzheimer's disease: a meta-analysis of diffusion tensor imaging and a comparison with medial temporal lobe measurements. ***J Alzheimers Dis*** 2012;(2): 405-29.
121. H B, E B. Diagnostic criteria for neuropathologic assessment of Alzheimer's disease. Neurobiol Aging; 1997.
122. Richard E, Moll van Charante EP, van Gool WA. Vascular risk factors as treatment target to prevent cognitive decline. ***J Alzheimers Dis*** 2012;(3): 733-40.
123. Matsusue E, Sugihara S, Fujii S, Ohama E, Kinoshita T, Ogawa T. White matter changes in elderly people: MR-pathologic correlations. ***Magn Reson Med Sci*** 2006;(2): 99-104.

124. Charlton RA, Barrick TR, McIntyre DJ, et al. White matter damage on diffusion tensor imaging correlates with age-related cognitive decline. ***Neurology*** 2006;(2): 217-22.
125. Kerchner GA, Racine CA, Hale S, et al. Cognitive processing speed in older adults: relationship with white matter integrity. ***PLoS One*** 2012;(11): e50425.
126. Shenkin SD, Bastin ME, Macgillivray TJ, et al. Cognitive correlates of cerebral white matter lesions and water diffusion tensor parameters in community-dwelling older people. ***Cerebrovasc Dis*** 2005;(5): 310-8.
127. O'Sullivan M, Morris RG, Huckstep B, Jones DK, Williams SC, Markus HS. Diffusion tensor MRI correlates with executive dysfunction in patients with ischaemic leukoaraiosis. ***J Neurol Neurosurg Psychiatry*** 2004;(3): 441-7.
128. Xu Q, Zhou Y, Li YS, et al. Diffusion tensor imaging changes correlate with cognition better than conventional MRI findings in patients with subcortical ischemic vascular disease. ***Dement Geriatr Cogn Disord*** 2010;(4): 317-26.
129. Tuladhar AM, van Norden AG, de Laat KF, et al. White matter integrity in small vessel disease is related to cognition. ***Neuroimage Clin*** 2015: 518-24.
130. Moller JT, Cluitmans P, Rasmussen LS, et al. Long-term postoperative cognitive dysfunction in the elderly ISPOCD1 study. ISPOCD investigators. International Study of Post-Operative Cognitive Dysfunction. ***Lancet*** 1998;(9106): 857-61.
131. Van der Elst W, Van Boxtel MP, Van Breukelen GJ, Jolles J. Normative data for the Animal, Profession and Letter M Naming verbal fluency tests for Dutch speaking participants and the effects of age, education, and sex. ***J Int Neuropsychol Soc*** 2006;(1): 80-9.
132. Brand N, Jolles J. Learning and retrieval rate of words presented auditorily and visually. ***J Gen Psychol*** 1985;(2): 201-10.
133. Van der Elst W, van Boxtel MP, van Breukelen GJ, Jolles J. Rey's verbal learning test: normative data for 1855 healthy participants aged 24-81 years and the influence of age, sex, education, and mode of presentation. ***J Int Neuropsychol Soc*** 2005;(3): 290-302.
134. van der Elst W, van Boxtel MP, van Breukelen GJ, Jolles J. The Letter Digit Substitution Test: normative data for 1,858 healthy participants aged 24-81 from the Maastricht Aging Study (MAAS): influence of age, education, and sex. ***J Clin Exp Neuropsychol*** 2006;(6): 998-1009.
135. Houx PJ, Jolles J, Vreeling FW. Stroop interference: aging effects assessed with the Stroop Color-Word Test. ***Exp Aging Res*** 1993;(3): 209-24.
136. Sternberg S. Memory-scanning: mental processes revealed by reaction-time experiments. ***Am Sci*** 1969;(4): 421-57.
137. Caffarra P, Vezzadini G, Dieci F, Zonato F, Venneri A. Rey-Osterrieth complex figure: normative values in an Italian population sample. ***Neurol Sci*** 2002;(6): 443-7.
138. RK Marhurin NC. Verbal series attention test: Clinical utility in the assessment of dementia. ***The Clinical Neuropsychologist*** 1996;(1): 43-53.
139. Prins ND, van Dijk EJ, den Heijer T, et al. Cerebral small-vessel disease and decline in information processing speed, executive function and memory. ***Brain*** 2005;(Pt 9): 2034-41.

140. M. Hendriks RK, M. Gorissen B. Schmand. Neuropsychologische diagnostiek; de klinische praktijk. Amsterdam: Boom; 2006.
141. Sheline YI, Barch DM, Garcia K, et al. Cognitive function in late life depression: relationships to depression severity, cerebrovascular risk factors and processing speed. ***Biol Psychiatry*** 2006;(1): 58-65.
142. Godin O, Dufouil C, Maillard P, et al. White matter lesions as a predictor of depression in the elderly: the 3C-Dijon study. ***Biol Psychiatry*** 2008;(7): 663-9.
143. Baune BT, Suslow T, Arolt V, Berger K. The relationship between psychological dimensions of depressive symptoms and cognitive functioning in the elderly - the MEMO-Study. ***J Psychiatr Res*** 2007;(3-4): 247-54.
144. Castro-Costa E, Dewey M, Stewart R, et al. Prevalence of depressive symptoms and syndromes in later life in ten European countries: the SHARE study. ***Br J Psychiatry*** 2007: 393-401.
145. Baldwin RC. Poor prognosis of depression in elderly people: causes and actions. ***Ann Med*** 2000;(4): 252-6.
146. Lesser IM, Miller BL, Boone KB, et al. Brain injury and cognitive function in late-onset psychotic depression. ***J Neuropsychiatry Clin Neurosci*** 1991;(1): 33-40.
147. Ebmeier KP, Donaghey C, Steele JD. Recent developments and current controversies in depression. ***Lancet*** 2006;(9505): 153-67.
148. Paranthaman R, Burns AS, Cruickshank JK, Jackson A, Scott ML, Baldwin RC. Age at onset and vascular pathology in late-life depression. ***Am J Geriatr Psychiatry*** 2012;(6): 524-32.
149. Paranthaman R, Greenstein AS, Burns AS, et al. Vascular function in older adults with depressive disorder. ***Biol Psychiatry*** 2010;(2): 133-9.
150. Brown FW, Lewine RJ, Hudgins PA, Risch SC. White matter hyperintensity signals in psychiatric and nonpsychiatric subjects. ***Am J Psychiatry*** 1992;(5): 620-5.
151. Krishnan KR, Hays JC, Blazer DG. MRI-defined vascular depression. ***Am J Psychiatry*** 1997;(4): 497-501.
152. Bowley MP, Drevets WC, Ongur D, Price JL. Low glial numbers in the amygdala in major depressive disorder. ***Biol Psychiatry*** 2002;(5): 404-12.
153. Young AW, Aggleton JP, Hellawell DJ, Johnson M, Broks P, Hanley JR. Face processing impairments after amygdalotomy. ***Brain*** 1995: 15-24.
154. Scott SK, Young AW, Calder AJ, Hellawell DJ, Aggleton JP, Johnson M. Impaired auditory recognition of fear and anger following bilateral amygdala lesions. ***Nature*** 1997;(6613): 254-7.
155. Campbell S, MacQueen G. An update on regional brain volume differences associated with mood disorders. ***Curr Opin Psychiatry*** 2006;(1): 25-33.
156. Gelb DJ, Oliver E, Gilman S. Diagnostic criteria for Parkinson disease. ***Arch Neurol*** 1999;(1): 33-9.
157. 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;(7): 939-44.
158. Analysis CfM. General Brain Segmentation; 2004.

159. van de Pol LA, Hensel A, van der Flier WM, et al. Hippocampal atrophy on MRI in frontotemporal lobar degeneration and Alzheimer's disease. ***J Neurol Neurosurg Psychiatry*** 2006;(4): 439-42.
160. Geuze E, Vermetten E, Bremner JD. MR-based in vivo hippocampal volumetrics: 1. Review of methodologies currently employed. ***Mol Psychiatry*** 2005;(2): 147-59.
161. Brierley B, Shaw P, David AS. The human amygdala: a systematic review and meta-analysis of volumetric magnetic resonance imaging. ***Brain Res Brain Res Rev*** 2002;(1): 84-105.
162. Duvernoy H. The Human Hippocampus, Functional Anatomy, Vascularization and Serial Sections with MRI. New York, USA: Springer; 1997.
163. Herve D, Mangin JF, Molko N, Bousser MG, Chabriat H. Shape and volume of lacunar infarcts: a 3D MRI study in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. ***Stroke*** 2005;(11): 2384-8.
164. Radloff LS. The use of the Center for Epidemiologic Studies Depression Scale in adolescents and young adults. ***J Youth Adolesc*** 1991;(2): 149-66.
165. Geerlings MI, den Heijer T, Koudstaal PJ, Hofman A, Breteler MM. History of depression, depressive symptoms, and medial temporal lobe atrophy and the risk of Alzheimer disease. ***Neurology*** 2008;(15): 1258-64.
166. Alexopoulos GS, Meyers BS, Young RC, Campbell S, Silbersweig D, Charlson M. 'Vascular depression' hypothesis. ***Arch Gen Psychiatry*** 1997;(10): 915-22.
167. Ashtari M, Greenwald BS, Kramer-Ginsberg E, et al. Hippocampal/amygdala volumes in geriatric depression. ***Psychol Med*** 1999;(3): 629-38.
168. Lenzi GL, Altieri M, Maestrini I. Post-stroke depression. ***Rev Neurol (Paris)*** 2008;(10): 837-40.
169. Frodl T, Meisenzahl EM, Zetzsche T, et al. Larger amygdala volumes in first depressive episode as compared to recurrent major depression and healthy control subjects. ***Biol Psychiatry*** 2003;(4): 338-44.
170. Mervaala E, Fohr J, Kononen M, et al. Quantitative MRI of the hippocampus and amygdala in severe depression. ***Psychol Med*** 2000;(1): 117-25.
171. Hastings RS, Parsey RV, Oquendo MA, Arango V, Mann JJ. Volumetric analysis of the prefrontal cortex, amygdala, and hippocampus in major depression. ***Neuropsychopharmacology*** 2004;(5): 952-9.
172. Sheline YI, Gado MH, Price JL. Amygdala core nuclei volumes are decreased in recurrent major depression. ***Neuroreport*** 1998;(9): 2023-8.
173. Siegle GJ, Konecky RO, Thase ME, Carter CS. Relationships between amygdala volume and activity during emotional information processing tasks in depressed and never-depressed individuals: an fMRI investigation. ***Ann N Y Acad Sci*** 2003: 481-4.
174. Lange C, Irle E. Enlarged amygdala volume and reduced hippocampal volume in young women with major depression. ***Psychol Med*** 2004;(6): 1059-64.
175. Rajkowska G, Miguel-Hidalgo JJ, Wei J, et al. Morphometric evidence for neuronal and glial prefrontal cell pathology in major depression. ***Biol Psychiatry*** 1999;(9): 1085-98.
176. Hamidi M, Drevets WC, Price JL. Glial reduction in amygdala in major depressive disorder is due to oligodendrocytes. ***Biol Psychiatry*** 2004;(6): 563-9.

177. Wager TD, Phan KL, Liberzon I, Taylor SF. Valence, gender, and lateralization of functional brain anatomy in emotion: a meta-analysis of findings from neuroimaging. ***Neuroimage*** 2003;(3): 513-31.
178. Phelps EA, O'Connor KJ, Gatenby JC, Gore JC, Grillon C, Davis M. Activation of the left amygdala to a cognitive representation of fear. ***Nat Neurosci*** 2001;(4): 437-41.
179. Sheline YI. 3D MRI studies of neuroanatomic changes in unipolar major depression: the role of stress and medical comorbidity. ***Biol Psychiatry*** 2000;(8): 791-800.
180. den Heijer T, Launer LJ, Prins ND, et al. Association between blood pressure, white matter lesions, and atrophy of the medial temporal lobe. ***Neurology*** 2005;(2): 263-7.
181. Coffey CE, Wilkinson WE, Weiner RD, et al. Quantitative cerebral anatomy in depression. A controlled magnetic resonance imaging study. ***Arch Gen Psychiatry*** 1993;(1): 7-16.
182. Tamburo RJ, Siegle GJ, Stetten GD, et al. Amygdalae morphometry in late-life depression. ***Int J Geriatr Psychiatry*** 2009;(8): 837-46.
183. Sachdev PS, Chen X, Joscelyne A, Wen W, Brodaty H. Amygdala in stroke/transient ischemic attack patients and its relationship to cognitive impairment and psychopathology: the Sydney Stroke Study. ***Am J Geriatr Psychiatry*** 2007;(6): 487-96.
184. Kirton JW, Resnick SM, Davatzikos C, Kraut MA, Dotson VM. Depressive symptoms, symptom dimensions, and white matter lesion volume in older adults: a longitudinal study. ***Am J Geriatr Psychiatry*** 2014;(12): 1469-77.
185. Beekman AT, Copeland JR, Prince MJ. Review of community prevalence of depression in later life. ***Br J Psychiatry*** 1999: 307-11.
186. O'Brien JT. Clinical significance of white matter changes. ***Am J Geriatr Psychiatry*** 2014;(2): 133-7.
187. Alexopoulos GS, Murphy CF, Gunning-Dixon FM, et al. Microstructural white matter abnormalities and remission of geriatric depression. ***Am J Psychiatry*** 2008;(2): 238-44.
188. Taylor WD, MacFall JR, Payne ME, et al. Late-life depression and microstructural abnormalities in dorsolateral prefrontal cortex white matter. ***Am J Psychiatry*** 2004;(7): 1293-6.
189. Shimony JS, Sheline YI, D'Angelo G, et al. Diffuse microstructural abnormalities of normal-appearing white matter in late life depression: a diffusion tensor imaging study. ***Biol Psychiatry*** 2009;(3): 245-52.
190. Lamar M, Charlton RA, Morris RG, Markus HS. The impact of subcortical white matter disease on mood in euthymic older adults: a diffusion tensor imaging study. ***Am J Geriatr Psychiatry*** 2010;(7): 634-42.
191. Sexton CE, Mackay CE, Ebmeier KP. A systematic review of diffusion tensor imaging studies in affective disorders. ***Biol Psychiatry*** 2009;(9): 814-23.
192. Murphy CF, Gunning-Dixon FM, Hoptman MJ, et al. White-matter integrity predicts stroop performance in patients with geriatric depression. ***Biol Psychiatry*** 2007;(8): 1007-10.
193. Colloby SJ, Firbank MJ, Thomas AJ, Vasudev A, Parry SW, O'Brien JT. White matter changes in late-life depression: a diffusion tensor imaging study. ***J Affect Disord*** 2011;(1-3): 216-20.
194. Zhu X, Wang X, Xiao J, Zhong M, Liao J, Yao S. Altered white matter integrity in first-episode, treatment-naive young adults with major depressive disorder: a tract-based spatial statistics study. ***Brain Res*** 2011: 223-9.

195. Association AP. Diagnostic and Statistical Manual of Mental Disorders.: Arlington; 2000.
196. Radloff LS. The CES-D scale: A self report depression scale for research in the general population. ***Applied Psychological Measurement*** 1977: 385-401.
197. Ennis DB, Kindlmann G. Orthogonal tensor invariants and the analysis of diffusion tensor magnetic resonance images. ***Magn Reson Med*** 2006;(1): 136-46.
198. Haringsma R, Engels GI, Beekman AT, Spinhoven P. The criterion validity of the Center for Epidemiological Studies Depression Scale (CES-D) in a sample of self-referred elders with depressive symptomatology. ***Int J Geriatr Psychiatry*** 2004;(6): 558-63.
199. Nestler EJ, Barrot M, DiLeone RJ, Eisch AJ, Gold SJ, Monteggia LM. Neurobiology of depression. ***Neuron*** 2002;(1): 13-25.
200. Rabins PV, Pearlson GD, Aylward E, Kumar AJ, Dowell K. Cortical magnetic resonance imaging changes in elderly inpatients with major depression. ***Am J Psychiatry*** 1991;(5): 617-20.
201. Arnold JF, Zwiers MP, Fitzgerald DA, et al. Fronto-limbic microstructure and structural connectivity in remission from major depression. ***Psychiatry Res*** 2012;(1): 40-8.
202. Liao Y, Huang X, Wu Q, et al. Is depression a disconnection syndrome? Meta-analysis of diffusion tensor imaging studies in patients with MDD. ***J Psychiatry Neurosci*** 2013;(1): 49-56.
203. Glickstein M, Berlucchi G. Classical disconnection studies of the corpus callosum. ***Cortex*** 2008;(8): 914-27.
204. Yang Q, Huang X, Hong N, Yu X. White matter microstructural abnormalities in late-life depression. ***Int Psychogeriatr*** 2007;(4): 757-66.
205. Kieseppa T, Eerola M, Mantyla R, et al. Major depressive disorder and white matter abnormalities: a diffusion tensor imaging study with tract-based spatial statistics. ***J Affect Disord*** 2010;(1-3): 240-4.
206. Taylor WD, MacFall JR, Gerig G, Krishnan RR. Structural integrity of the uncinate fasciculus in geriatric depression: Relationship with age of onset. ***Neuropsychiatr Dis Treat*** 2007;(5): 669-74.
207. Sheline YI, Price JL, Vaishnavi SN, et al. Regional white matter hyperintensity burden in automated segmentation distinguishes late-life depressed subjects from comparison subjects matched for vascular risk factors. ***Am J Psychiatry*** 2008;(4): 524-32.
208. Bunce D, Batterham PJ, Christensen H, Mackinnon AJ. Causal Associations Between Depression Symptoms and Cognition in a Community-Based Cohort of Older Adults. ***Am J Geriatr Psychiatry*** 2014.
209. Crocco EA, Castro K, Loewenstein DA. How late-life depression affects cognition: neural mechanisms. ***Curr Psychiatry Rep*** 2010;(1): 34-8.
210. O'Brien JT, Firbank MJ, Krishnan MS, et al. White matter hyperintensities rather than lacunar infarcts are associated with depressive symptoms in older people: the LADIS study. ***Am J Geriatr Psychiatry*** 2006;(10): 834-41.
211. Vermeer SE, Koudstaal PJ, Oudkerk M, Hofman A, Breteler MM. Prevalence and risk factors of silent brain infarcts in the population-based Rotterdam Scan Study. ***Stroke*** 2002;(1): 21-5.
212. Tomimoto H, Lin JX, Matsuo A, et al. Different mechanisms of corpus callosum atrophy in Alzheimer's disease and vascular dementia. ***J Neurol*** 2004;(4): 398-406.

213. Thomalla G, Glauche V, Koch MA, Beaulieu C, Weiller C, Rother J. Diffusion tensor imaging detects early Wallerian degeneration of the pyramidal tract after ischemic stroke. ***Neuroimage*** 2004;(4): 1767-74.
214. Beaulieu C. The basis of anisotropic water diffusion in the nervous system - a technical review. ***NMR Biomed*** 2002;(7-8): 435-55.
215. Tham MW, Woon PS, Sum MY, Lee TS, Sim K. White matter abnormalities in major depression: evidence from post-mortem, neuroimaging and genetic studies. ***J Affect Disord*** 2011;(1-2): 26-36.
216. Chi S, Wang C, Jiang T, Zhu XC, Yu JT, Tan L. The prevalence of depression in Alzheimer's disease: a systematic review and meta-analysis. ***Curr Alzheimer Res*** 2015;(2): 189-98.
217. Green RC, Cupples LA, Kurz A, et al. Depression as a risk factor for Alzheimer disease: the MIRAGE Study. ***Arch Neurol*** 2003;(5): 753-9.
218. Masters MC, Morris JC, Roe CM. "Noncognitive" symptoms of early Alzheimer disease: A longitudinal analysis. ***Neurology*** 2015.
219. Becker JT, Chang YF, Lopez OL, et al. Depressed mood is not a risk factor for incident dementia in a community-based cohort. ***Am J Geriatr Psychiatry*** 2009;(8): 653-63.
220. Byers AL, Yaffe K. Depression and risk of developing dementia. ***Nat Rev Neurol*** 2011;(6): 323-31.
221. Diniz BS, Butters MA, Albert SM, Dew MA, Reynolds CF, 3rd. Late-life depression and risk of vascular dementia and Alzheimer's disease: systematic review and meta-analysis of community-based cohort studies. ***Br J Psychiatry*** 2013;(5): 329-35.
222. Ownby RL, Crocco E, Acevedo A, John V, Loewenstein D. Depression and risk for Alzheimer disease: systematic review, meta-analysis, and metaregression analysis. ***Arch Gen Psychiatry*** 2006;(5): 530-8.
223. Richard E, Reitz C, Honig LH, et al. Late-life depression, mild cognitive impairment, and dementia. ***JAMA Neurol*** 2013;(3): 374-82.
224. da Silva J, Goncalves-Pereira M, Xavier M, Mukaetova-Ladinska EB. Affective disorders and risk of developing dementia: systematic review. ***Br J Psychiatry*** 2013;(3): 177-86.
225. van Sloten TT, Sigurdsson S, van Buchem MA, et al. Cerebral Small Vessel Disease and Association With Higher Incidence of Depressive Symptoms in a General Elderly Population: The AGES-Reykjavik Study. ***Am J Psychiatry*** 2015: appiajp201414050578.
226. Saczynski JS, Beiser A, Seshadri S, Auerbach S, Wolf PA, Au R. Depressive symptoms and risk of dementia: the Framingham Heart Study. ***Neurology*** 2010;(1): 35-41.
227. Mulsant BH, Pollock BG, Kirshner M, Shen C, Dodge H, Ganguli M. Serum anticholinergic activity in a community-based sample of older adults: relationship with cognitive performance. ***Arch Gen Psychiatry*** 2003;(2): 198-203.
228. Jacobson L, Sapolsky R. The role of the hippocampus in feedback regulation of the hypothalamic-pituitary-adrenocortical axis. ***Endocr Rev*** 1991;(2): 118-34.
229. Butters MA, Young JB, Lopez O, et al. Pathways linking late-life depression to persistent cognitive impairment and dementia. ***Dialogues Clin Neurosci*** 2008;(3): 345-57.
230. Lesser IM, Boone KB, Mehringer CM, Wohl MA, Miller BL, Berman NG. Cognition and white matter hyperintensities in older depressed patients. ***Am J Psychiatry*** 1996;(10): 1280-7.

231. Alexopoulos GS. Frontostriatal and limbic dysfunction in late-life depression. ***Am J Geriatr Psychiatry*** 2002;(6): 687-95.
232. Gudmundsson P, Olesen PJ, Simoni M, et al. White matter lesions and temporal lobe atrophy related to incidence of both dementia and major depression in 70-year-olds followed over 10 years. ***Eur J Neurol*** 2015.
233. Luchsinger JA, Honig LS, Tang MX, Devanand DP. Depressive symptoms, vascular risk factors, and Alzheimer's disease. ***Int J Geriatr Psychiatry*** 2008;(9): 922-8.
234. Wardlaw JM, Smith C, Dichgans M. Mechanisms of sporadic cerebral small vessel disease: insights from neuroimaging. ***Lancet Neurol*** 2013;(5): 483-97.
235. Dumas A, Dierksen GA, Gurol ME, et al. Functional magnetic resonance imaging detection of vascular reactivity in cerebral amyloid angiopathy. ***Ann Neurol*** 2012;(1): 76-81.
236. Peca S, McCreary CR, Donaldson E, et al. Neurovascular decoupling is associated with severity of cerebral amyloid angiopathy. ***Neurology*** 2013;(19): 1659-65.
237. Reijmer YD, Fotiadis P, Martinez-Ramirez S, et al. Structural network alterations and neurological dysfunction in cerebral amyloid angiopathy. ***Brain*** 2015;(Pt 1): 179-88.
238. de Laat KF, van Norden AG, van Oudheusden LJ, et al. Diffusion tensor imaging and mild parkinsonian signs in cerebral small vessel disease. ***Neurobiol Aging*** 2012;(9): 2106-12.
239. Rosenberg GA, Wallin A, Wardlaw JM, et al. Consensus statement for diagnosis of subcortical small vessel disease. ***J Cereb Blood Flow Metab*** 2016;(1): 6-25.
240. O'Sullivan M, Summers PE, Jones DK, Jarosz JM, Williams SC, Markus HS. Normal-appearing white matter in ischemic leukoaraiosis: a diffusion tensor MRI study. ***Neurology*** 2001;(12): 2307-10.
241. Maillard P, Fletcher E, Harvey D, et al. White matter hyperintensity penumbra. ***Stroke*** 2011;(7): 1917-22.
242. Maillard P, Fletcher E, Lockhart SN, et al. White matter hyperintensities and their penumbra lie along a continuum of injury in the aging brain. ***Stroke*** 2014;(6): 1721-6.
243. Reijmer YD, Freeze WM, Leemans A, Biessels GJ, Utrecht Vascular Cognitive Impairment Study G. The effect of lacunar infarcts on white matter tract integrity. ***Stroke*** 2013;(7): 2019-21.
244. Auriel E, Edlow BL, Reijmer YD, et al. Microinfarct disruption of white matter structure: a longitudinal diffusion tensor analysis. ***Neurology*** 2014;(2): 182-8.
245. Duering M, Righart R, Wollenweber FA, Zietemann V, Gesierich B, Dichgans M. Acute infarcts cause focal thinning in remote cortex via degeneration of connecting fiber tracts. ***Neurology*** 2015;(16): 1685-92.
246. Lawrence AJ, Chung AW, Morris RG, Markus HS, Barrick TR. Structural network efficiency is associated with cognitive impairment in small-vessel disease. ***Neurology*** 2014;(4): 304-11.
247. Gons RA, de Laat KF, van Norden AG, et al. Hypertension and cerebral diffusion tensor imaging in small vessel disease. ***Stroke*** 2010;(12): 2801-6.
248. Gons RA, van Oudheusden LJ, de Laat KF, et al. Hypertension is related to the microstructure of the corpus callosum: the RUN DMC study. ***J Alzheimers Dis*** 2012;(3): 623-31.
249. Gons RA, van Norden AG, de Laat KF, et al. Cigarette smoking is associated with reduced microstructural integrity of cerebral white matter. ***Brain*** 2011;(Pt 7): 2116-24.

250. Gons RA, Tuladhar AM, de Laat KF, et al. Physical activity is related to the structural integrity of cerebral white matter. ***Neurology*** 2013;(11): 971-6.
251. Del Bene A, Ciolli L, Borgheresi L, Poggesi A, Inzitari D, Pantoni L. Is type 2 diabetes related to leukoaraiosis? an updated review. ***Acta Neurol Scand*** 2015;(3): 147-55.
252. de Groot M, Cremers LG, Ikram MA, et al. White Matter Degeneration with Aging: Longitudinal Diffusion MR Imaging Analysis. ***Radiology*** 2016;(2): 532-41.
253. Duering M, Gesierich B, Seiler S, et al. Strategic white matter tracts for processing speed deficits in age-related small vessel disease. ***Neurology*** 2014;(22): 1946-50.
254. Duering M, Zieren N, Herve D, et al. Strategic role of frontal white matter tracts in vascular cognitive impairment: a voxel-based lesion-symptom mapping study in CADASIL. ***Brain*** 2011;(Pt 8): 2366-75.
255. O'Sullivan M, Barrick TR, Morris RG, Clark CA, Markus HS. Damage within a network of white matter regions underlies executive dysfunction in CADASIL. ***Neurology*** 2005;(10): 1584-90.
256. Pasi M, Salvadori E, Poggesi A, et al. White matter microstructural damage in small vessel disease is associated with Montreal cognitive assessment but not with mini mental state examination performances: vascular mild cognitive impairment Tuscany study. ***Stroke*** 2015;(1): 262-4.
257. Tuladhar AM, van Dijk E, Zwiers MP, et al. Structural network connectivity and cognition in cerebral small vessel disease. ***Hum Brain Mapp*** 2016;(1): 300-10.
258. Teodorczuk A, Firbank MJ, Pantoni L, et al. Relationship between baseline white-matter changes and development of late-life depressive symptoms: 3-year results from the LADIS study. ***Psychol Med*** 2010;(4): 603-10.
259. Wen MC, Steffens DC, Chen MK, Zainal NH. Diffusion tensor imaging studies in late-life depression: systematic review and meta-analysis. ***Int J Geriatr Psychiatry*** 2014;(12): 1173-84.
260. Brookes RL, Herbert V, Lawrence AJ, Morris RG, Markus HS. Depression in small-vessel disease relates to white matter ultrastructural damage, not disability. ***Neurology*** 2014;(16): 1417-23.
261. Pasi M, Poggesi A, Salvadori E, et al. White matter microstructural damage and depressive symptoms in patients with mild cognitive impairment and cerebral small vessel disease: the VMCI-Tuscany Study. ***Int J Geriatr Psychiatry*** 2016;(6): 611-8.
262. Hollocks MJ, Lawrence AJ, Brookes RL, et al. Differential relationships between apathy and depression with white matter microstructural changes and functional outcomes. ***Brain*** 2015;(Pt 12): 3803-15.
263. de Laat KF, van Norden AG, Gons RA, et al. Diffusion tensor imaging and gait in elderly persons with cerebral small vessel disease. ***Stroke*** 2011;(2): 373-9.
264. Kim SH, Park JS, Ahn HJ, et al. Voxel-based analysis of diffusion tensor imaging in patients with subcortical vascular cognitive impairment: correlates with cognitive and motor deficits. ***J Neuroimaging*** 2011;(4): 317-24.
265. Youn J, Cho JW, Lee WY, Kim GM, Kim ST, Kim HT. Diffusion tensor imaging of freezing of gait in patients with white matter changes. ***Mov Disord*** 2012;(6): 760-4.
266. van der Holst HM, van Uden IW, Tuladhar AM, et al. Cerebral small vessel disease and incident parkinsonism: The RUN DMC study. ***Neurology*** 2015;(18): 1569-77.

267. Reijmer YD, Brundel M, de Bresser J, et al. Microstructural white matter abnormalities and cognitive functioning in type 2 diabetes: a diffusion tensor imaging study. ***Diabetes Care*** 2013;(1): 137-44.
268. Molko N, Pappata S, Mangin JF, et al. Monitoring disease progression in CADASIL with diffusion magnetic resonance imaging: a study with whole brain histogram analysis. ***Stroke*** 2002;(12): 2902-8.
269. Benjamin P, Zeestraten E, Lambert C, et al. Progression of MRI markers in cerebral small vessel disease: sample size considerations for clinical trials. ***J Cereb Blood Flow Metab*** 2015.
270. Ramirez J, McNeely AA, Berezuk C, Gao F, Black SE. Dynamic Progression of White Matter Hyperintensities in Alzheimer's Disease and Normal Aging: Results from the Sunnybrook Dementia Study. ***Front Aging Neurosci*** 2016: 62.
271. 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;(10): 2712-9.
272. Sachdev P, Wen W, Chen X, Brodaty H. Progression of white matter hyperintensities in elderly individuals over 3 years. ***Neurology*** 2007;(3): 214-22.
273. Wardlaw JM, Valdes Hernandez MC, Munoz-Maniega S. What are white matter hyperintensities made of? Relevance to vascular cognitive impairment. ***J Am Heart Assoc*** 2015;(6): 001140.
274. Cho AH, Kim HR, Kim W, Yang DW. White matter hyperintensity in ischemic stroke patients: it may regress over time. ***J Stroke*** 2015;(1): 60-6.
275. Okazaki S, Hornberger E, Griebe M, Gass A, Hennerici MG, Szabo K. MRI Characteristics of the Evolution of Supratentorial Recent Small Subcortical Infarcts. ***Front Neurol*** 2015: 118.
276. Loos CM, Staals J, Wardlaw JM, van Oostenbrugge RJ. Cavitation of deep lacunar infarcts in patients with first-ever lacunar stroke: a 2-year follow-up study with MR. ***Stroke*** 2012;(8): 2245-7.
277. Poels MM, Ikram MA, van der Lugt A, et al. Incidence of cerebral microbleeds in the general population: the Rotterdam Scan Study. ***Stroke*** 2011;(3): 656-61.
278. Lee SH, Lee ST, Kim BJ, et al. Dynamic temporal change of cerebral microbleeds: long-term follow-up MRI study. ***PLoS One*** 2011;(10): e25930.
279. Lambert C, Benjamin P, Zeestraten E, Lawrence AJ, Barrick TR, Markus HS. Longitudinal patterns of leukoaraiosis and brain atrophy in symptomatic small vessel disease. ***Brain*** 2016.
280. Ghafoorian M, Karssemeijer N, Heskes T, et al. Small white matter lesion detection in cerebral small vessel disease. Proceedings of the SPIE Medical Imaging; 2016.
281. Fazekas F, Chawluk JB, Alavi A, Hurtig HI, Zimmerman RA. MR signal abnormalities at 1.5 T in Alzheimer's dementia and normal aging. ***AJR Am J Roentgenol*** 1987;(2): 351-6.
282. Wickham H. ggplot2: Elegant Graphics for Data Analysis: Springer New York; 2009.
283. Bates D, Machler M, Bolker BM, Walker SC. Fitting Linear Mixed-Effects Models Using lme4. ***Journal of Statistical Software*** 2015;(1): 1-48.
284. Prins ND, van Straaten EC, van Dijk EJ, et al. Measuring progression of cerebral white matter lesions on MRI: visual rating and volumetrics. ***Neurology*** 2004;(9): 1533-9.
285. van Uden I, van Leijsen EM, Ghafoorian M, et al. The rise and fall of cerebral small vessel disease; The RUN DMC study. ***Brain (submitted)*** 2016.

286. Rothman KJ, Greenland S. Basic Concepts, Modern Epidemiology; Chapter 2: Causation and Causal Inference. 2nd ed. Philadelphia: Lippincott Williams & Wilkins; 1998.
287. Beekman AT, Deeg DJ, Van Limbeek J, Braam AW, De Vries MZ, Van Tilburg W. Criterion validity of the Center for Epidemiologic Studies Depression scale (CES-D): results from a community-based sample of older subjects in The Netherlands. ***Psychol Med*** 1997;(1): 231-5.
288. Gouw AA, Van der Flier WM, van Straaten EC, et al. Simple versus complex assessment of white matter hyperintensities in relation to physical performance and cognition: the LADIS study. ***J Neurol*** 2006;(9): 1189-96.
289. Malone IB, Leung KK, Clegg S, et al. Accurate automatic estimation of total intracranial volume: a nuisance variable with less nuisance. ***Neuroimage*** 2015: 366-72.
290. Miettinen OS, Cook EF. Confounding: essence and detection. ***Am J Epidemiol*** 1981;(4): 593-603.
291. Staals J, Makin SD, Doubal FN, Dennis MS, Wardlaw JM. Stroke subtype, vascular risk factors, and total MRI brain small-vessel disease burden. ***Neurology*** 2014;(14): 1228-34.
292. Conklin J, Silver FL, Mikulis DJ, Mandell DM. Are acute infarcts the cause of leukoaraiosis? Brain mapping for 16 consecutive weeks. ***Ann Neurol*** 2014;(6): 899-904.
293. White L, Petrovitch H, Hardman J, et al. Cerebrovascular pathology and dementia in autopsied Honolulu-Asia Aging Study participants. ***Ann N Y Acad Sci*** 2002: 9-23.
294. Debette S, Markus HS. The clinical importance of white matter hyperintensities on brain magnetic resonance imaging: systematic review and meta-analysis. ***BMJ*** 2010: c3666.
295. Ikram MA, Vrooman HA, Vernooij MW, et al. Brain tissue volumes in relation to cognitive function and risk of dementia. ***Neurobiol Aging*** 2010;(3): 378-86.
296. Poels MM, Steyerberg EW, Wieberdink RG, et al. Assessment of cerebral small vessel disease predicts individual stroke risk. ***J Neurol Neurosurg Psychiatry*** 2012;(12): 1174-9.
297. Brickman AM, Siedlecki KL, Muraskin J, et al. White matter hyperintensities and cognition: testing the reserve hypothesis. ***Neurobiol Aging*** 2011;(9): 1588-98.
298. Saczynski JS, Rosen AB, McCammon RJ, et al. Antidepressant Use and Cognitive Decline: The Health and Retirement Study. ***Am J Med*** 2015.
299. Bath PM, Wardlaw JM. Pharmacological treatment and prevention of cerebral small vessel disease: a review of potential interventions. ***Int J Stroke*** 2015;(4): 469-78.
300. Poggesi A, Salvadori E, Valenti R, et al. The Florence VAS-COG clinic: a model for the care of patients with cognitive and behavioral disturbances consequent to cerebrovascular diseases. ***J Alzheimers Dis*** 2014: S453-61.
301. Jokinen H, Goncalves N, Vigario R, et al. Early-Stage White Matter Lesions Detected by Multispectral MRI Segmentation Predict Progressive Cognitive Decline. ***Front Neurosci*** 2015: 455.
302. Ryu WS, Woo SH, Schellingerhout D, et al. Grading and interpretation of white matter hyperintensities using statistical maps. ***Stroke*** 2014;(12): 3567-75.
303. Reijmer YD, Fotiadis P, Piantoni G, et al. Small vessel disease and cognitive impairment: The relevance of central network connections. ***Hum Brain Mapp*** 2016;(7): 2446-54.
304. Staals J, Booth T, Morris Z, et al. Total MRI load of cerebral small vessel disease and cognitive ability in older people. ***Neurobiol Aging*** 2015;(10): 2806-11.

Dankwoord

Het voelt vreemd om het afsluitende dankwoord van inmiddels 10 jaar RUN DMC te schrijven. Ik ga het missen. De kennismaking met, en de keuze en liefde voor de neurologie heb ik aan de RUN DMC studie te danken. Dat komt natuurlijk niet door de RUN DMC studie sec, maar door iedereen die mij door dit project heeft geleid, aangehoord, geholpen, begeleid en ondersteund op alle mogelijke manieren.

Allereerst wil ik de deelnemers van het RUN DMC onderzoek bedanken voor hun vrijwillige deelname en inzet tot driemaal toe. Zonder jullie bijdrage was dit proefschrift er helemaal nooit geweest.

Professor H.F. de Leeuw, Frank-Erik, ik had me geen betere promotor kunnen wensen. Vanaf de aller eerste dag (ergens de zomer van 2006) gaf je me het gevoel dat je vertrouwen had in wat ik kwam doen (al had ikzelf geen idee…). Ik kreeg de kans om mee te gaan naar een congres in Bonn (doodeng), en niet lang erna mocht ik als student mee naar VASCOG in San Antonio, Texas (Fantastisch!) om mijn eerste poster te presenteren. Het was niet alleen inspirerend maar ook heel prettig om zo onder jouw vleugels mee te mogen. Zo heb ik je manier van begeleiden kunnen ervaren en de kwaliteit van het onderzoek van dichtbij gezien. Ik heb dan ook geen seconde getwijfeld toen je voorstelde of ik aan het vervolg van het RUN DMC onderzoek wilde deelnemen. Wanneer ik een artikel naar je toestuurde was het eerder regel dan uitzondering dat de volgende ochtend de gecorrigeerde versie weer in mijn mailbox zat. Ook veel dank aan Amanda, dat ze het goed vond dat je 'vrije' avonden en nachten hieraan besteedde. Kers op de taart is dat jij als begeleider nu ook écht mijn promotor bent! Dank je wel voor je vertrouwen en geduld, je (onderzoeks)ideeën (ook al kon ik die soms niet altijd volgen), je oog voor mij als persoon, je teamgevoel, je zorgvuldigheid, je correctheid, jullie vasculaire borrels en natuurlijk de M&M's!
Frank-Erik écht kort van stof zal ik denk ik nooit worden.

Professor C.J.M. Klijn, beste Karin, je hebt 1,5 jaar geleden het UMCU verruild voor het RadboudUMC, en bent mijn mede-promotor geworden. Je heldere , doordachte en duidelijke beslissingen over dilemma's geven rust, dank daarvoor. Als afdelingshoofd en opleider sta je achter me waar nodig.

Dr. E.J. van Dijk, Ewoud, je kritische en waardevolle opmerkingen als co-promotor, met name op methodologisch vlak heb ik erg gewaardeerd. Voor de rest van mijn leven zal je door mijn hoofd flitsen wanneer ik de woorden "confounding by indication", "selection bias" en "reversed causality" hoor. Het was prettig te discussiëren met een ervaringsdeskundige op het gebied van ***delta*** imaging.

Bart Post, als mijn opleider heb je me de ruimte gegeven om mijn promotie, naast mijn opleiding en mijn gezin, in rust af te maken. Hetzelfde geldt voor Arnoud Kappelle de voorgaande jaren. Dank jullie, ik zou mijn opleiding nergens anders willen volgen.

Manuscriptcommissie, professor M.G.M. Olde Rikkert, professor W.M. van der Flier en dr. F.J.A. Meijer, dank dat ik mijn proefschrift aan u ter beoordeling mocht voorleggen.

Ellen van der Holst, Ellen, mijn co-promovendus en RUN DMC maatje, natuurlijk een speciaal plekje in mijn dankwoord voor jou! Ontzettend fijn dat we de perikelen rondom het data verzamelen samen hebben kunnen delen, in je eentje duurt het niet alleen langer maar is het ook een stuk minder gezellig! Samen hebben we ons door verschillende dilemma's geslagen. Het rebooten van een MRI-scanner op zondagochtend (met papieren handleiding), het zoeken van de perfecte RUN DMC kerstkaart, het lokaliseren van 'verdwenen' deelnemers, maar ook het zetten (of drinken….) van koffie zetten bij huisbezoeken. MEMO: bij telefonisch contact volgende keer even direct mededelen van welke parochie je bent a.u.b... Heel erg bedankt voor de samenwerking de afgelopen jaren, en heel fijn dat we het ook samen in 2017 kunnen afronden. Veel succes met je welverdiende plek in het mooie 's-Hertogenbosch, zien we elkaar daar binnenkort?

Anouk van Norden, mijn voorgangster en mentor. Je hebt het bedje gespreid voor me achtergelaten, wat een luxe. Je bent altijd beschikbaar voor overleg, advies en tips, en niet alleen over het onderzoek.
Esther van Leijsen, het is een geruststelling dat ik het stokje juist aan jou heb mogen overdragen. Dank je wel voor al je energie en gezelligheid, je lekkere koken en biertjes en natuurlijk het samen-schrijven van ons laatste stuk de afgelopen maanden.
Anil Tuladhar, van jouw kennis over diffusion tensor imaging heb ik dankbaar gebruik mogen maken. Zonder jou was het onmogelijk geweest om de ingewikkeldste analyses uit te voeren. Extra dank voor de mooie plaatjes die je voor me hebt gemaakt, mag de volgende er dan toch een keer in het roze?
Mohsen Ghafoorian, I would still be segmenting the follow-up scans today, if it wasn't for your semi-automated white matter hyperintensity detection. Thank you for your patience and instant (technical) help whenever I asked. Congratulations and best of luck on the continuation of you project at Harvard University.
Loes Rutten-Jacobs, voor mij ben je echt een statistiek-encyclopedie. Wanneer ik met mijn handen in het haar zat, had jij elke keer weer een oplossing, terecht dat je in Cambridge een plek hebt gevonden. Hopelijk zien we je hier snel weer terug.
Karlijn de Laat, mede RUN DMCer, jij staat samen met Anouk en Rob aan de basis van het RUN DMC onderzoek. Ontzettend fijn dat ik op jouw werk heb mogen voortborduren. Natuurlijk ook dank voor alle gezelligheid op de congressen (San Antonio en Singapore)!

Mayra Bergkamp, jij stopt niet tot het af is. Terecht dat je een plekje binnen onze opleiding hebt weten te bemachtigen. We mogen erg blij zijn met jou als collega op de werkvloer, zeker als je dat net zo gedecideerd (en met altijd goede zin!) aanpakt als het opnieuw segmenteren van de lacunes en microbloedingen.

Alle mede auteurs wil ik bedanken voor hun bijdrage aan data-verzameling, opbouwende kritiek en/of adviezen. Pauline Schaapsmeerders, dank je voor je advies op het neuropsychologisch vlak. Fijn om een hippocampus-collega te hebben! Edo Richard, je bent iets later bij het onderzoek betrokken geraakt, maar van jouw kritische en realistische kijk op de gedragsneurologie heb ik erg veel geleerd. Ik ben het met je eens, over apathie is het laatste woord nog niet gesproken…
Lucas van Oudheusden, via jou ben ik bij het RUN DMC onderzoek terecht gekomen. We hebben elkaar lange tijd niet meer gesproken, dank voor de muziek, de gezelligheid en de goede (studenten)tijd. Roy Kessels, Jurgen Claassen en Indira Tendolkar, jullie specialistische ideeën en commentaren waren zeer waardevol.

Renate Arntz, Lieve Renaat, we waren dan misschien niet 'officieel' een team, maar zo voelde het vaak wel, zeker richting het einde van het promotie-traject. Op persoonlijk vlak zitten we met de kindjes in dezelfde fase, en is het 's ochtends telkens weer een feestje om samen met de kids voor de deur van het dagverblijf te wachten tot hij open gaat (we willen immers niet te laat zijn ;-)). Zonder jouw gezelschap weet ik niet of ik het had kunnen opbrengen om de laatste maanden weer de hokjes van de radiologie in te duiken om alle scans opnieuw in te kleuren. Ook de afrondende fase; het afschrijven van het boekje, het zoeken van de drukker, de feestlocatie, alles is veel leuker wanneer je het samen met een vriendinnetje mag doen, dank je wel!

Speciale dank ook aan de gehele vasculaire groep; Karin, Saskia, Sharon, Annet, Mayte, Merel, Bram, Tessa, Annemieke, Kim, Frank, Liselore, Joyce, Noortje, Nathalie, voor hulp bij dataverzameling en de input op de vasculaire besprekingen. In het bijzonder dank voor de student-assistenten (Willemijn Geense, Heleen van den Berg, Inge van der Holst) voor jullie hulp bij het scannen, telefoneren en compleet maken van de follow-up data. Eline Kooijmans, betrokken als student bij ons onderzoek, naast een prachtige scriptie die je hebt geschreven, ga je door met ons onderzoek naast je co-schappen. We hopen je hierna op de neurologie weer terug te zien! Valerie Lohner, een mooie scriptie en zelfs een presentatie op een internationaal congres ten tijde van je stage, heel knap! Wat een monnikenwerk hebben jullie verzet!

Zonder alle ondersteuning op het Donders Cenctum van Paul Gaalman bij het scannen, en de inventiviteit van Sandra bij de receptie, hadden we het scanwerk niet kunnen doen. Door

het plan-talent en de flexibiliteit van de dames op de polikliniek neurologie hebben we 400 deelnemers in korte tijd op de poli terug kunnen zien.

Ik mag van geluk spreken dat ik mijn opleiding mag volgen in zo'n fijn en collegiaal team. Een speciaal woord van dank voor de 'neuro-ladies' mag absoluut niet ontbreken: Judith van Gaalen (Verbuuuuuuuurg!), Nicolien van der Kolk, Renate Arntz en Ellen van der Holst.
Lieve Juud, direct vanaf het begin van de opleiding zijn we vriendinnen, en beiden getuigen op elkaars bruiloft… Volgens mij zegt dat genoeg, liefs!
Lieve Nico, onze maandelijkse etentjes, initieel met zijn 4en en dadelijk al weer met zijn 8en, eindigen altijd véél te laat! Meeten we volgend jaar weer in ***La France*** ?
Ik vind het geweldig om zulke gezellige, warme en hilarische (en natuurlijk heel knappe) vriendinnen te hebben die ik ook nog bijna dagelijks zie. Dat maakt naar het werk gaan elke dag nóg leuker :-)

Lieve paranimfen, ik ben trots dat juist jullie op deze speciale dag naast mij staan.
Allereerst Dineke Westra, lieve Dien, jij hebt me, toen ik even van het padje af was en ging roeien, weer op het rechte pad gezet en terug naar de hockey gehaald. Je bent een stabiele factor uit mijn studententijd, dat jij in mijn bestuur zat was voor mij als voorzitter een verademing. Daarnaast hebben we fantastisch gefeest! We hebben tegelijkertijd onze kindjes gekregen, en het is heerlijk te zien dat Emma 's ochtends staat te springen als ze naar Margje mag. Dien, dat jij mijn paranimf bent, is niet meer dan logisch.
Roel van Uden, lieve Roelieboelie, lieve broer, allereerst is er iets wat me van het hart moet. Het spijt me dat ik vroeger zo hard fietste tussen die 2 stoplichten langs de N65… ik zal tegen Emma zeggen dat ze dat bij Guus niet mag doen. Als kinderen waren we 2 handen op 1 buik, daar heb ik hele fijne herinneringen aan. Afgelopen jaren hebben we elkaar weer gevonden, dat voelt vertrouwd. Ik ben trots dat je mijn paranimf wil zijn.

Suzanne Claessens-Schraven, Lieve Suzy Q, eigenlijk ben je mijn grote zus; klein en blond (maar wel iets groter). Je vertelt me de waarheid als deze fijn is om te horen, maar ook als ik liever wat anders zou horen. Ik bewonder je oprechtheid en eerlijkheid zonder blad voor de mond, want we denken hetzelfde en stiekem zeg jij vaak, wat ik denk. Je gekheid (soms gewoonweg gestoord), humor, warmte en liefde voor de mensen om je heen zijn geweldig. Samen met Loek, Mathijs en Elise hoop ik dat onze gezinnen de rest van ons leven verbonden blijven én dat we onze 2-jaarlijkse weekenden met Renée en Eelke blijven continueren.

Lieve Saskia, Myrtille en Bianca, lieve co-groepvriendinnen, het lijkt al weer zo lang geleden! Allemaal mama en in opleiding tot specialist, ben best wel trots op ons. Sas, je bent een kanjer, niet alleen gepromoveerd, maar je sleept de subsidies binnen. Hoe doe je dat toch!? Myrt, als ik iets van je kan leren is het wel doorzetten. Er zullen veel baby's en ouders blij

zijn dat jij hun gynaecoloog bent. Bianca, 2e kindje op komst.. zien we je daarna snel in het RadboudUMC?
Lieve Noor, Neel, Siem en Tien als we elkaar zien is het altijd goed en vertrouwd. Dat is echte vriendschap.
Lieve Marijke, een lievere en gezelligere buurvrouw kan ik me niet wensen, we hebben ontzettend veel geluk gehad dat jullie naast ons zijn komen wonen. Ik hoop dat we nog vaak kunnen kletsen, wijn drinken in de tuin, kickfitten en natuurlijk parcoursen bouwen met de boeven (ok, en de mannen)!
Lieve hockeyvriendinnetjes; Anne, Anouk, Chironne, Dineke, Ellen, Eveline, Floor, Franka, Hanneke, Margo, Marie-Louise, Maud, Meike, Muur, Niké, Renee, Saskia, Silke, Sophie, Susanne, Suus, Willemijn en Jouke, de afgelopen 10 jaren hebben we tragische verliezen geleden, fantastische overwinningen behaald, onvergetelijke teamweekenden gehad, onnavolgbare gesprekken en vooral een onvergetelijke tijd. Ik had jullie niet kunnen missen. Wanneer gaan we weer naar 't feest van Ome Joop?

Lieve familie, ik hoef het maar te vragen, en jullie staan voor mij en mijn gezin klaar. In het bijzonder tante Pieternel, dank je wel voor al je 'instant' hulp en je heerlijke jam natuurlijk! Lieve Anke en Chiel, Emma en Guus zijn gek op jullie. Het voelt goed jullie zo dichtbij te hebben. Tante Marion, je bent vanaf jongs af aan mijn 2e mama (wel een hele stoere variant).

Lieve Mieke, dank je voor alle (oppas)hulp, steun en ideeën. Ik hoop dat je gezondheid weer beter wordt en dat we samen met het gezin heel veel leuke dingen kunnen (blijven) doen. Anne, dank je voor je wijsheid en luisterend oor als collega en als schoonzusje. Lieve Ton, dankzij jou (en pap) hebben we een huis, wat nu ook echt voelt als een thuis.

Lieve Bassie, mijn kleinste broertje (inmiddels wel een kop groter), jouw onuitputtelijke enthousiasme en goede zin maken dat ik van jou altijd heel vrolijk wordt. Je bent een keiharde werker, en we zien elkaar nu ook als collega's in het ziekenhuis. Dat maakt een dienst toch weer een stukje leuker!
Lieve broers, Bas en Roel en lieve schoonzusjes Niké en Rianne. Het is zo gewoon, maar ook zo fijn dat we allemaal in Nijmegen wonen en elkaar wekelijks zien. Het voelt altijd als een warme deken als we allemaal bij elkaar zijn, de spelletjesavonden zijn onovertroffen. Ik zou er niks aan willen veranderen.

Lieve pap en mam, zonder jullie was dit boekje er nooit gekomen. Jullie steun en liefde is onvoorwaardelijk. Van kinds af aan hebben jullie mij gestimuleerd en gefaciliteerd om alles uit mezelf te halen en mijzelf te ontwikkelen. Ons thuis is altijd warm en liefdevol geweest en dat is het nog steeds. Dat voel ik, en ook mijn gezin, elke dag. Het is veilig te weten dat jullie er altijd zijn en achter ons staan. Dank jullie wel.. ik hou van jullie.

Aller áller liefste Eric, ik hou zoveel van je. Je denkt in mogelijkheden in plaats van in onmogelijkheden. Je geeft rust als ik onrustig ben. Je creëert tijd (en een huis) als ik denk dat het onhaalbaar is je bent er altijd en doet alles voor mij en ons gezin, je maakt dat ik me bijzonder voel en dat maakt me stil… ik zou nooit meer anders willen.
jbdlvdhwihvj…

Liefste Emma, Liefste Guus, allerliefste boeven… samen met je papa, zijn jullie het beste wat me ooit is overkomen.

Een liedje voor jullie
♫ *In de maneschijn…..* ♪

List of publications

1. van Norden AG, Fick WF, de Laat KF, ***van Uden IW***, van Oudheusden LJ, Tendolkar I, et al. Subjective cognitive failures and hippocampal volume in elderly with white matter lesions. Neurology. 2008;71(15):1152-9.

2. Snaphaan L, Rijpkema M, ***van Uden I***, Fernandez G, de Leeuw FE. Reduced medial temporal lobe functionality in stroke patients: a functional magnetic resonance imaging study. Brain. 2009;132(Pt 7):1882-8.

3. de Laat KF, van Norden AG, Gons RA, van Oudheusden LJ, ***van Uden IW***, Bloem BR, et al. Gait in elderly with cerebral small vessel disease. Stroke; a journal of cerebral circulation. 2010;41(8):1652-8.

4. Gons RA, de Laat KF, van Norden AG, van Oudheusden LJ, ***van Uden IW***, Norris DG, et al. Hypertension and cerebral diffusion tensor imaging in small vessel disease. Stroke; a journal of cerebral circulation. 2010;41(12):2801-6.

5. de Laat KF, van Norden AG, Gons RA, van Oudheusden LJ, ***van Uden IW***, Norris DG, et al. Diffusion tensor imaging and gait in elderly persons with cerebral small vessel disease. Stroke; a journal of cerebral circulation. 2011;42(2):373-9.

6. Gons RA, van Norden AG, de Laat KF, van Oudheusden LJ, ***van Uden IW***, Zwiers MP, et al. Cigarette smoking is associated with reduced microstructural integrity of cerebral white matter. Brain. 2011;134(Pt 7):2116-24.

7. van Norden AG, de Laat KF, Gons RA, ***van Uden IW***, van Dijk EJ, van Oudheusden LJ, et al. Causes and consequences of cerebral small vessel disease. The RUN DMC study: a prospective cohort study. Study rationale and protocol. BMC Neurol. 2011;11:29.

8. ***van Uden IW***, van Norden AG, de Laat KF, van Oudheusden LJ, Gons RA, Tendolkar I, et al. Depressive Symptoms and Amygdala Volume in Elderly with Cerebral Small Vessel Disease: The RUN DMC Study. J Aging Res. 2011;2011:647869.

9. de Laat KF, van Norden AG, Gons RA, ***van Uden IW***, Zwiers MP, Bloem BR, et al. Cerebral white matter lesions and lacunar infarcts contribute to the presence of mild parkinsonian signs. Stroke; a journal of cerebral circulation. 2012;43(10):2574-9.

10. de Laat KF, van Norden AG, van Oudheusden LJ, ***van Uden IW***, Norris DG, Zwiers MP, et al. Diffusion tensor imaging and mild parkinsonian signs in cerebral small vessel disease. Neurobiol Aging. 2012;33(9):2106-12.

11. Gons RA, van Oudheusden LJ, de Laat KF, van Norden AG, ***van Uden IW***, Norris DG, et al. Hypertension is related to the microstructure of the corpus callosum: the RUN DMC study. Journal of Alzheimer's disease : JAD. 2012;32(3):623-31.

12. van Norden AG, de Laat KF, Fick I, ***van Uden IW***, van Oudheusden LJ, Gons RA, et al. Diffusion tensor imaging of the hippocampus and verbal memory performance: the RUN DMC study. Human brain mapping. 2012;33(3):542-51.

13. van Norden AG, de Laat KF, van Dijk EJ, ***van Uden IW***, van Oudheusden LJ, Gons RA, et al. Diffusion tensor imaging and cognition in cerebral small vessel disease: the RUN DMC study. Biochim Biophys Acta. 2012;1822(3):401-7.

14. van Norden AG*, ***van Uden IW****, de Laat KF, van Dijk EJ, de Leeuw FE. Cognitive function in small vessel disease: the additional value of diffusion tensor imaging to conventional magnetic resonance imaging: the RUN DMC study. Journal of Alzheimer's disease : JAD. 2012;32(3):667-76.

15. ***van Uden IW M***, van Norden AGW, Meijer FJA, van Dijk EJ, de Leeuw F-E. Differentiaaldiagnose van witte stofafwijkingen bij volwassenen: een systematische op MRI gebaseerde benadering. 'ABC van witte stofafwijkingen'. Tijdschr Neurol en Neurochirurgie. 2012;113 (3):148-67.

16. Bastiaans DE, ***van Uden IW***, Ruiterkamp RA, de Jong BA. Removal of valproic acid by plasmapheresis in a patient treated for multiple sclerosis. Ther Drug Monit. 2013;35(1):1-3.

17. van der Holst HM, Tuladhar AM, van Norden AG, de Laat KF, ***van Uden IW***, van Oudheusden LJ, et al. Microstructural integrity of the cingulum is related to verbal memory performance in elderly with cerebral small vessel disease: the RUN DMC study. Neuroimage. 2013;65:416-23.

18. van Norden AG*, ***van Uden IW****, de Laat KF, Gons RA, Kessels RP, van Dijk EJ, et al. Cerebral microbleeds are related to subjective cognitive failures: the RUN DMC study. Neurobiol Aging. 2013;34(9):2225-30.

19. Schaapsmeerders P, ***van Uden IW***, Tuladhar AM, Maaijwee NA, van Dijk EJ, Rutten-Jacobs LC, et al. Ipsilateral hippocampal atrophy is associated with long-term memory dysfunction after ischemic stroke in young adults. Human brain mapping. 2015;36(7):2432-42.

20. van der Holst HM, ***van Uden IW***, Tuladhar AM, de Laat KF, van Norden AG, Norris DG, et al. Cerebral small vessel disease and incident parkinsonism: The RUN DMC study. Neurology. 2015;85(18):1569-77.

21. ***van Uden IW****, Tuladhar AM*, de Laat KF, van Norden AG, Norris DG, van Dijk EJ, et al. White matter integrity and depressive symptoms in cerebral small vessel disease: The RUN DMC study. The American journal of geriatric psychiatry : official journal of the American Association for Geriatric Psychiatry. 2015;23(5):525-35.

22. ***van Uden IW***, van der Holst HM, Schaapsmeerders P, Tuladhar AM, van Norden AG, de Laat KF, et al. Baseline white matter microstructural integrity is not related to cognitive decline after 5 years: The RUN DMC study. BBA clinical. 2015;4:108-14.

23. ***van Uden IW***, van der Holst HM, Tuladhar AM, van Norden AG, de Laat KF, Rutten-Jacobs LC, et al. White Matter and Hippocampal Volume Predict the Risk of Dementia in Patients with Cerebral Small Vessel Disease: The RUN DMC Study. Journal of Alzheimer's disease : JAD. 2015;49(3):863-73.

24. van der Kolk NM*, Arts P*, ***van Uden IW****, Hoischen A, van de Veerdonk FL, Netea MG, et al.
Progressive multifocal leukoencephalopathy in an immunocompetent patient. Annals of clinical and translational neurology. 2016;3(3):226-32.

25. ***van Uden IW****, Tuladhar AM*, van der Holst HM, van Leijsen EM, van Norden AG, de Laat KF, et al. Diffusion tensor imaging of the hippocampus predicts the risk of dementia; the RUN DMC study. Human brain mapping. 2016;37(1):327-37.

26. ***van Uden IW***, van der Holst HM, van Leijsen EM, Tuladhar AM, van Norden AG, de Laat KF, et al. Late-onset depressive symptoms increase the risk of dementia in small vessel disease. Neurology. 2016;87(11):1102-9.

27. Pasi M, ***van Uden IW***, Tuladhar AM, de Leeuw FE, Pantoni L. White Matter Microstructural Damage on Diffusion Tensor Imaging in Cerebral Small Vessel Disease: Clinical Consequences. Stroke; a journal of cerebral circulation. 2016;47(6):1679-84.

28. van der Holst HM, ***van Uden IW***, Tuladhar AM, de Laat KF, van Norden AG, Norris DG, et al. Factors Associated With 8-Year Mortality in Older Patients With Cerebral Small Vessel Disease: The Radboud University Nijmegen Diffusion Tensor and Magnetic Resonance Cohort (RUN DMC) Study. JAMA neurology. 2016;73(4):402-9.

29. Tuladhar AM, ***van Uden IW***, Rutten-Jacobs LC, Lawrence A, van der Holst H, van Norden A, et al. Structural network efficiency predicts conversion to dementia. Neurology. 2016;86(12):1112-9.

30. Arntz RM, van den Broek SM, ***van Uden IW***, Ghafoorian M, Platel B, Rutten-Jacobs LC, et al. Accelerated development of cerebral small vessel disease in young stroke patients. Neurology. 2016;87(12):1212-9.

31. Ghafoorian M, Karssemeijer N, ***van Uden IW***, de Leeuw FE, Heskes T, Marchiori E, et al. Automated detection of white matter hyperintensities of all sizes in cerebral small vessel disease. Medical physics. 2016;43(12):6246.

Accepted

32. van der Holst HM, ***van Uden IWM***, Tuladhar AM, de Laat KF, van Leijsen EMC, van Norden AGW et al. Baseline cerebral small vessel disease is not associated with gait decline after 5 years. 2016 Accepted Movement Disorders Clinical Practice

Submitted

33. ***van Uden IWM****, van Leijsen EMC*, Ghafoorian M, Bergkamp MI, Lohner V, Kooijmans ECM et al. The rise and fall of cerebral small vessel disease: the RUN DMC study

34. van der Holst HM*, Tuladhar AM*, Zerbi V, ***van Uden IWM***, de Laat KF, van Leijsen EMC et al. White matter atrophy and loss of white matter integrity are associated with gait decline in cerebral small vessel disease

35. Ghafoorian M, Karssemeijer N, Heskes T, ***van Uden IWM***, Sanchez C, Litjens G et al. Location sensitive deep convolutional neural networks for segmentation of white matter hyperintensities

36. Bergkamp MI, Tuladhar AM, van der Holst HM, van Leijsen EM, Ghafoorian M, ***van Uden IWM*** et al. Increased incidence of parkinsonism in individuals with severe cerebral small vessel disease. A nine year follow-up study

* shared First authorship

Curriculum vitae

Ingeborg Wilhelmina Maria van Uden werd geboren op 23 januari 1985 in 's-Hertogenbosch. Zij is het eerste kind van Jan en Annette van Uden- van Giersbergen en de oudste van 3 kinderen. Inge groeide op in Vught, waarbij zij naast haar middelbare school veel tijd besteedde aan muziek (cello en piano) en hockey. In 2003 behaalde zij haar gymnasiumdiplpoma (Sint Janslyceum).

Zij kon in 2003 direct starten met de geneeskunde opleiding aan de Radboud Universiteit in Nijmegen. Gecombineerd me haar studie heeft zij in 2006 het ***Honours Programma*** aan de Radboud universiteit afgerond en is zij ***praeses*** geweest van de studenten Hockeyvereniging Apeliotes (2005-2006). Gedurende de opleiding is ze enkele jaren student-assistent geweest, bij het anatomie-onderwijs. Haar master-stage heeft Inge gedaan op de afdeling Neurologie van het RadboudUMC, onder begeleiding van prof. H.F. de Leeuw, getiteld ***'amygdala volume and depressive symptoms in cerebral small vessel disease'***. Deze mocht zij presenteren op internationale congressen in Bonn (2006) en op het VASCOG congres in San Antonio, Texas (2007) en Singapore (2009). Tijdens haar co-schappen is zij als student-assistent betrokken gebleven bij de RUN DMC studie, hiermee is de basis gelegd voor haar promotie onderzoek. Haar senior-coschap heeft Inge gelopen op de afdeling neurologie in het RadboudUMC en haar arts-examen behaald in februari 2010. In maart 2010 mocht zij beginnen als ANIOS neurologie in het RadboudUMC, waar zij vanaf augustus 2010 tot heden in opleiding is tot neuroloog (opleider: 2010-2014 prof. G.W.A.M. Padberg, 2014-2015 dr. A.C. Kappelle, 2015-heden. dr. B. Post). In 2012 en 2013 was Inge penningmeester van de assistentenvereniging.

In mei 2011 startte zij haar promotie-traject, onder leiding van prof. H.F. de Leeuw waarvan het resultaat nu voor u ligt, gecombineerd met de opleiding tot neuroloog. Tweemaal won zij de '***Young investigators Award'*** (eenmaal gedeeld auteurschap met Anil Man Tuladhar (2014), en eenmaal gedeeld auteurschap met Esther van Leijsen (2016))**,** voor artikelen die u in dit proefschrift kunt lezen.

Inge hoopt haar specialisatie tot neuroloog te voltooien begin 2020.

Inge is getrouwd met Eric van der Moolen en heeft twee kinderen Emma (6-5-2014) en Guus (31-12-2015), en zijn woonachtig in Nijmegen.

Dissertations of the disorders of movement research group, Nijmegen

Vascular disorders of movement – The Radboud Stroke centre

- Liselore Snaphaan. Epidemiology of post stroke behavioral consequences. Radboud University Nijmegen, 12 March 2010
- Karlijn F. de Laat. Motor performance in individuals with cerebral small vessel disease: an MRI study. Radboud University Nijmegen, 29 November 2011
- Anouk G.W. van Norden. Cognitive function in elderly individuals with cerebral small vessel disease. An MRI study. Radboud University Nijmegen, 30 November 2011
- Rob Gons. Vascular risk factors in cerebral small vessel disease. A diffusion tensor imaging study. Radboud University Nijmegen, 10 December 2012
- Loes C.A. Rutten-Jacobs. Long-term prognosis after stroke in young adults. Radboud University Nijmegen, 14 April 2014
- Noortje A.M.M. Maaijwee. Long-term neuropsychological and social consequences after stroke in young adults. Radboud University Nijmegen, 12 June 2015
- Nathalie E. Synhaeve. Determinants of long-term functional prognosis after stroke in young adults. Radboud University Nijmegen, 28 September 2016
- Anil M. Tuladhar. The disconnected brain: mechanisms of clinical symptoms in small vessel disease. Radboud University Nijmegen, 4 October 2016.
- Pauline Schaapsmeerders. Long-term cognitive impairment after first-ever ischemic stroke in young adults: a neuroimaging study. Radboud Univesity Nijmegen, 24 January 2017.
- Ingeborg W.M. van Uden. Behavioural consequences of cerebral small vessel disease; an MRI approach. Radboud University Nijmegen, 14 Februari 2017.

Parkinson Center Nijmegen (ParC)

- Jasper E. Visser. The basal ganglia and postural control. Radboud University Nijmegen, 17 June 2008
- Maaike Bakker. Supraspinal control of walking: lessons from motor imagery. Radboud University Nijmegen, 27 May 2009
- W. Farid Abdo. Parkinsonism: possible solutions to a diagnostic challenge. Radboud University Nijmegen, 7 October 2009
- Samyra H.J. Keus. Physiotherapy in Parkinson's disease. Towards evidence-based practice. Leiden University, 29 April 2010
- Lars B. Oude Nijhuis. Modulation of human balance reactions. Radboud University Nijmegen, 29 November 2010
- Maarten J. Nijkrake. Improving the quality of allied health care in Parkinson's disease through community-based networks: the ParkinsonNet health care concept. Radboud University Nijmegen, 29 November 2010

- Rick C.G. Helmich. Cerebral reorganization in Parkinson's disease. Radboud University Nijmegen, 24 May 2011
- Charlotte A. Haaxma. New perspectives on preclinical and early stage Parkinson's disease. Radboud University Nijmegen, 6 December 2011
- Johanna G. Kalf. Drooling and dysphagia in Parkinson's disease. Radboud University Nijmegen, 22 December 2011
- Anke H. Snijders. Tackling freezing of gait in Parkinson's disease. Radboud University Nijmegen,4 June 2012
- Bart F.L. van Nuenen. Cerebral reorganization in premotor parkinsonism. Radboud University Nijmegen, 22 November 2012
- Wandana Nanhoe-Mahabier. Freezing of physical activity in Parkinson's disease, the challenge to change behavior. Radboud University Nijmegen, 13 February 2013
- Marlies van Nimwegen. Promotion of physical activity in Parkinson's disease, the challenge to change behavior. Radboud University Nijmegen, 6 March 2013
- Arlène D. Speelman. Promotion of physical activity in Parkinson's disease, feasibility and effectiveness. Radboud University Nijmegen, 6 March 2013
- Tjitske Boonstra. The contribution of each leg to bipedal balance control. University Twente, 6 June 2013
- Marjolein A van der Marck. The Many faces of Parkinson's disease: towards a multifaceted approach? Radboud University Nijmegen, 10 January 2014
- Katrijn Smulders. Cognitive control of gait and balance in patients with chronic stroke and Parkinson's disease. Radboud University Nijmegen, 21 May 2014
- Marjolein B. Aerts. Improving diagnostic accuracy in parkinsonism. Radboud University Nijmegen, 27 June 2014
- Maartje Louter. Sleep in Parkinson's disease. A focus on nocturnal movements. Radboud University Nijmegen, 13 February 2015
- Frederick Anton Meijer. Clinical Application of Brain MRI in Parkinsonism: From Basic to Advanced Imaging, Radboud University Nijmegen, 23 June 2015
- Jorik Nonnekes. Balance and gait in neurodegenerative disease: what startle tells us about motor control, Radboud University Nijmegen, 2 September 2015
- Martijn van der Eijk. Patient-centered care in Parkinson's disease. Radboud University Nijmegen, 1 December 2015
- Ingrid Sturkenboom. Occupational therapy for people with Parkinson's disease: towards evidence-informed care. Radboud University Nijmegen, 11 February 2016
- Merel M. van Gilst. Sleep benefit in Parkinson's disease. Radboud University Nijmegen, 13 April 2016
- Arno M. Janssen. Transcranial magnetic stimulation - measuring and modeling in health and disease. Radboud University Nijmegen, 2 June 2016

Non–Parkinsonian disorders of movement

- Sacha Vermeer. Clinical and genetic characterization of autosomal recessive cerebellarataxias. Radboud University Nijmegen, 5 April 2012
- Susanne T. de Bot. Hereditary spastic paraplegias in the Netherlands. Radboud University Nijmegen, 20 December 2013
- Catherine C.S. Delnooz. Unraveling primary focal dystonia. A treatment update and new pathophysiological insights. Radboud University Nijmegen, 7 January 2014
- Ella MR Fonteyn. Falls, physiotherapy, and training in patients with degenerative ataxias, 29 June 2016

Neuromuscular disorders of movement

- Mireille van Beekvelt. Quantitative near infrared spectroscopy (NIRS) in human skeletal muscle. Radboud University Nijmegen, 24 April 2002
- Johan Hiel. Ataxia telangiectasia and Nijmegen Breakage syndrome, neurological, immunological and genetic aspects. Radboud University Nijmegen, 23 April 2004
- Gerald JD Hengstman. Myositis specific autoantibodies, specificity and clinical applications. Radboud University Nijmegen, 21 September 2005
- M. Schillings. Fatigue in neuromuscular disorders and chronic fatigue syndrome, a neurophysiological approach. Radboud University Nijmegen, 23 November 2005
- Bert de Swart. Speech therapy in patients with neuromuscular disorders and Parkinson's disease. Diagnosis and treatment of dysarthria and dysphagia. Radboud University Nijmegen, 24 march 2006
- J. Kalkman. From prevalence to predictors of fatigue in neuromuscular disorders. The building of a model. Radboud University Nijmegen, 31 October 2006
- Nens van Alfen. Neuralgicamyotrophy. Radboud University Nijmegen, 1 November 2006
- Gea Drost. High-density surface EMG, pathophysiological insights and clinical applications. Radboud University Nijmegen, 9 March 2007
- Maria Helena van der Linden. Pertubations of gait and balance: a new experimental setup applied to patients with CMT type 1a. Radboud University Nijmegen, 6 October 2009
- Jeroen Trip. Redefining the non-dystrophic myotonic syndromes. Radboud University Nijmegen, 22 January 2010
- Corinne G.C. Horlings. A weak balance: balance and falls in patients with neuromuscular disorders. Radboud University Nijmegen, 1 April 2010
- E. Cup. Occupational therapy, physical therapy and speech therapy for persons with neuromuscular diseases, an evidence based orientation. Radboud University Nijmegen, 5 July 2011
- Alide Tieleman. Myotonic dystrophy type 2, a newly diagnosed disease in the Netherlands. Radboud University Nijmegen, 15 July 2011
- Nicol Voermans. Neuromuscular features of Ehlers-Danlos syndrome and Marfan syndrome. Radboud University Nijmegen, 2 September 2011

- Allan Pieterse. Referral and indication for occupational therapy, physical therapy and speech- language therapy for persons with neuromuscular disorders. Radboud University Nijmegen, 13 February 2012
- Bart Smits. Chronic Progressive External Ophthalmoplegia more than meets the eye. Radboud University Nijmegen, 5 June 2012
- Ilse Arts. Muscle ultrasonography in ALS. Radboud University Nijmegen, 31 October 2012
- M. Minis. Sustainability of work for persons with neuromuscular diseases. Radboud University Nijmegen, 13 November 2013
- Willemijn Leen. Glucose transporter – 1 deficiency syndrome. Radboud University Nijmegen, 26 June 2014
- Barbara Jansen. Magnetic Resonance Imaging signature of fascioscpulohumeral muscular dystrophy. Radboud University Nijmegen. 14 September 2015
- Noortje Rijken. Balance and gait in FSHD, relations with individual muscle involvement. Radboud University Nijmegen, 8 December 2015
- Femke Seesing. Shared Medical appointments for neuromuscular patients and their partners. Radboud University Nijmegen, 2 September 2016
- Nicole Voet. Aerobic exercise and cognitive behavioral therapy in fascioscapulohumeral dystrophy: a model based approach. Radboud University Nijmegen , 14 October 2016.

Donders Graduate School for Cognitive Neuroscience

For a successful research Institute, it is vital to train the next generation of young scientists. To achieve this goal, the Donders Institute for Brain, Cognition and Behaviour established the Donders Graduate School for Cognitive Neuroscience (DGCN), which was officially recognised as a national graduate school in 2009. The Graduate School covers training at both Master's and PhD level and provides an excellent educational context fully aligned with the research programme of the Donders Institute.

The school successfully attracts highly talented national and international students in biology, physics, psycholinguistics, psychology, behavioural science, medicine and related disciplines. Selective admission and assessment centres guarantee the enrolment of the best and most motivated students.

The DGCN tracks the career of PhD graduates carefully. More than 50% of PhD alumni show a continuation in academia with postdoc positions at top institutes worldwide, e.g. Stanford University, University of Oxford, University of Cambridge, UCL London, MPI Leipzig, Hanyang University in South Korea, NTNU Norway, University of Illinois, North Western University, Northeastern University in Boston, ETH Zürich, University of Vienna etc.. Positions outside academia spread among the following sectors: specialists in a medical environment, mainly in genetics, geriatrics, psychiatry and neurology. Specialists in a psychological environment, e.g. as specialist in neuropsychology, psychological diagnostics or therapy. Positions in higher education as coordinators or lecturers. A smaller percentage enters business as research consultants, analysts or head of research and development. Fewer graduates stay in a research environment as lab coordinators, technical support or policy advisors. Upcoming possibilities are positions in the IT sector and management position in pharmaceutical industry. In general, the PhDs graduates almost invariably continue with high-quality positions that play an important role in our knowledge economy.

For more information on the DGCN as well as past and upcoming defences please visit:
http://www.ru.nl/donders/graduate-school/donders-graduate/

"Weten wat men weet en weten wat men niet weet, dat is het ware weten."

Confucius, 500 v.Chr.