

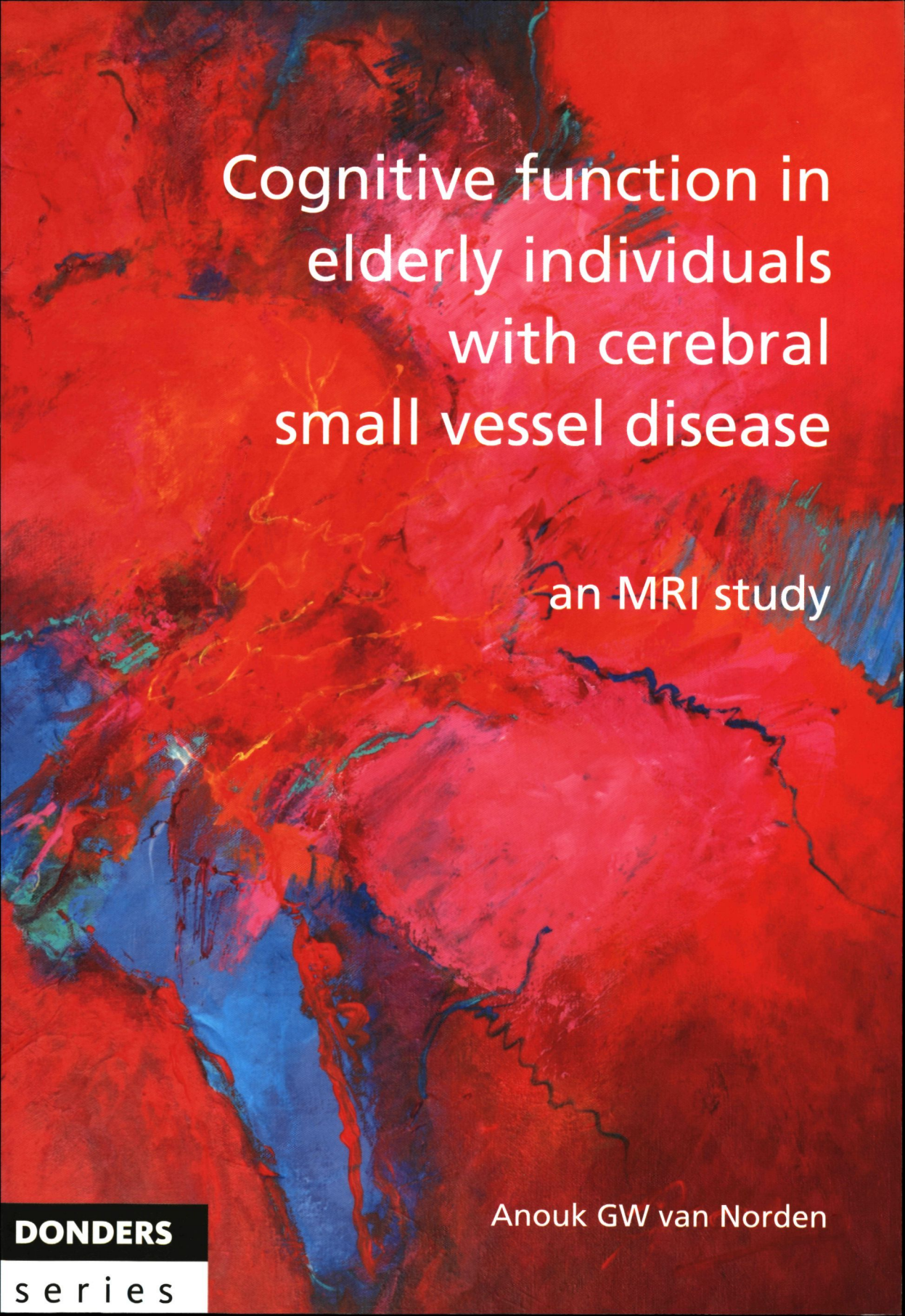
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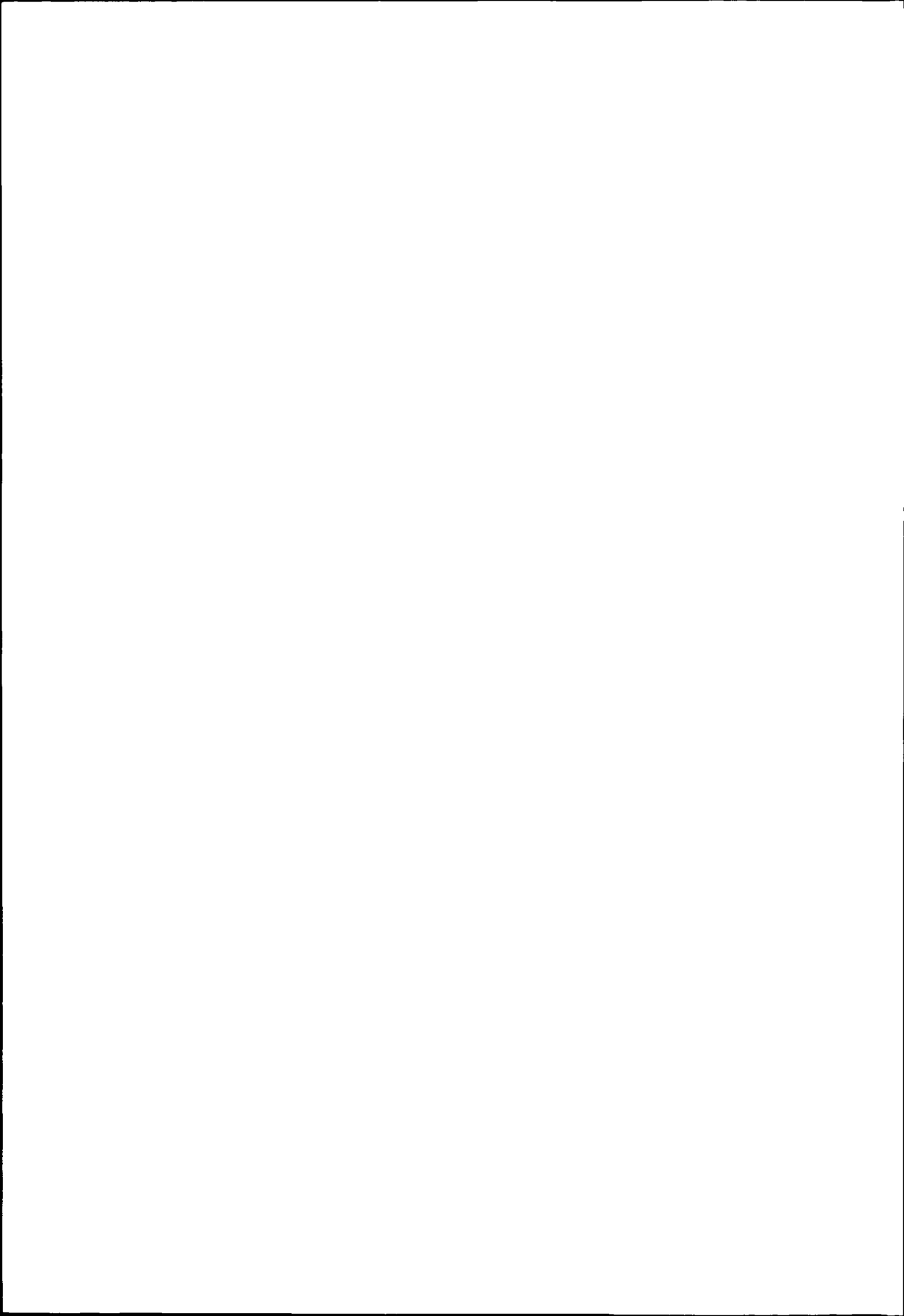
Cognitive function in
elderly individuals
with cerebral
small vessel disease

an MRI study

DONDERS

series

Anouk GW van Norden



**Cognitive function in elderly
individuals with cerebral
small vessel disease**

an MRI study

Anouk G.W. van Norden

The studies presented in this thesis were carried out at the Donders Institute for Brain, Cognition and Behaviour, Centre for Neuroscience, Department of Neurology, Radboud University Nijmegen Medical Centre

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Cognitive function in elderly individuals with cerebral small vessel disease

an MRI study

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van de Medische Wetenschappen

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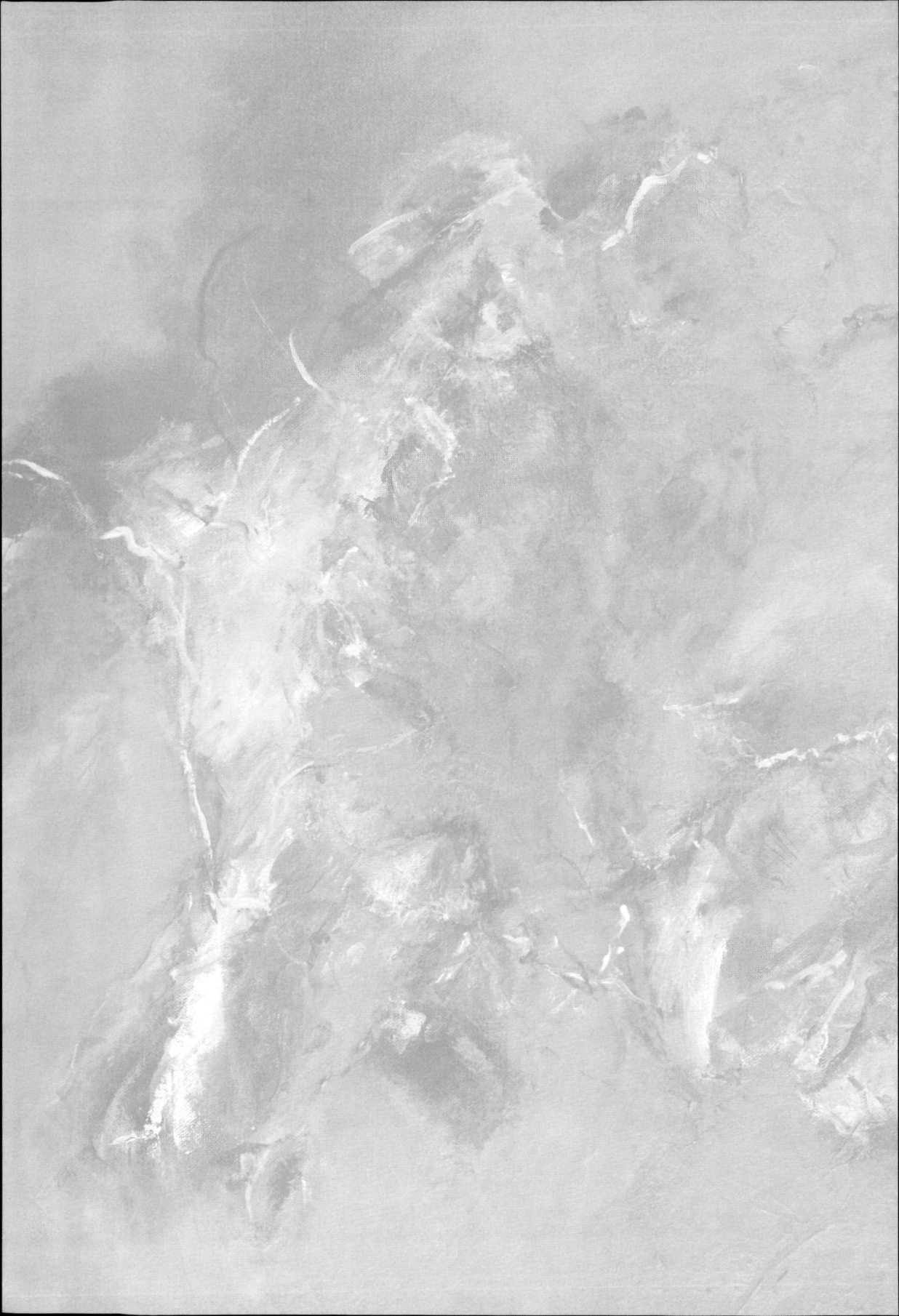
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Chapter 1

General introduction

Cerebral small vessel disease and cognitive performance

Cerebral small vessel disease (SVD) encompasses all the pathological processes that affect the small vessels of the brain, including small perforating end-arteries and arterioles supplying the white matter, deep grey matter nuclei and brainstem. As these small vessels cannot be visualized *in vivo* yet, the parenchymal lesions that are thought to be caused by changes in these vessels have been adopted as an imaging marker of SVD. On magnetic resonance imaging (MRI), cerebral SVD is characterized by small focal areas of infarction (lacunar infarcts), more diffuse areas of incomplete ischemia (white matter lesions (WML)), and perivascular collections of hemosiderin deposits (microbleeds) (Box 1).

Cerebral SVD is common in elderly people, it increases with age and is associated with vascular risk factors, in particular hypertension.^{1,2} The prevalence of SVD varies considerably across studies depending on the population studied and the imaging technique used. In the general population, WML occur in 90%, lacunar infarcts in 20% and MB in 5% of the individuals over 60 years of age.^{3,5} In addition, there is accumulating evidence of SVD related parenchymal involvement, in the 'normal appearing white matter (NAWM)', that is not visible on conventional MRI.

In this thesis we use the term SVD to describe the cerebral lesions related to arterio(lo)sclerosis and vascular risk factors, which is by far the most prevalent form of SVD. Other forms of SVD, as hereditary SVD (e.g. hereditary cerebral amyloid angiopathies and Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy) and inflammatory or infectious SVD (i.e. small vessel vasculitis) will not be discussed in this thesis.

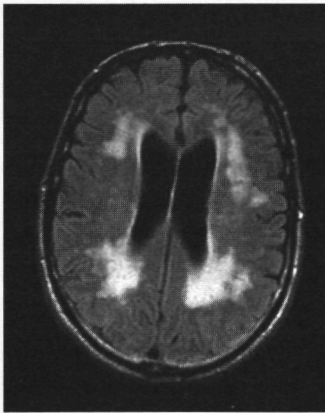
Clinical manifestations of cerebral SVD consist of both acute symptomatology, such as transient ischemic attacks and lacunar syndromes, and of subacute symptoms, such as cognitive, motor (gait) and mood disturbances, and urinary symptoms such as urge incontinence.⁶ The clinical spectrum is heterogeneous ranging from mild symptoms to full blown dementia. Brain changes often exist long before subacute symptoms become evident. Therefore, it has been suggested that in clinical studies the selection of individuals should be based on the more consistent brain imaging features (i.e. based on the presence of SVD).⁷

Both hospital and population-based studies have shown a relation between WML, lacunar infarcts and MB and cognitive impairment.⁸⁻¹¹ In addition WML and (silent) lacunar infarcts have shown to be predictors of cognitive decline and dementia.¹²⁻¹⁵

Box 1. Conventional magnetic resonance imaging

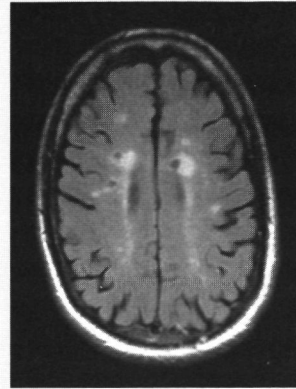
In cerebral SVD, a number of abnormalities can be observed with conventional MRI sequences, encompassing ischaemic and haemorrhagic manifestations.

WMLs are defined as white matter hyperintensities on T2 weighted sequences, such as fluid-attenuated inversion recovery (FLAIR) scans, which are not, or only faintly, hypo-intense on T1 weighted images. They range from small focal lesions to more or less confluent areas that are bilaterally and symmetrically sited in the hemispheric white matter.¹⁶



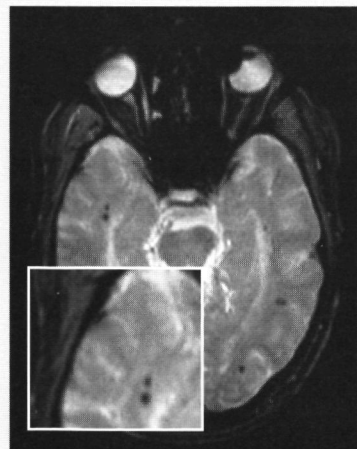
White matter lesions

Lacunar infarcts are defined as foci with low signal intensity on T1 and T2 weighted images, ruling out enlarged perivascular spaces and infraputamina pseudo-lacunae.¹⁶ There is no full consensus on the size of lacunar infarcts; the maximum accepted diameter is 15 mm, because this is the size derived from the original pathological studies.^{17,18} A consensus on the minimum diameter is more difficult to establish; we used a cut-off size of 2 mm.



Lacunar infarcts

Cerebral microbleeds are round or ovoid lesions, seen on gradient echo T2* weighted imaging as black lesions with a blooming effect, representing hemosiderin deposits.¹⁹ They are mostly located in the basal ganglia or cortical-subcortical areas. They must be distinguished from other potential mimics such as iron or calcium deposits, bone or flow voids. Various size cut-off points have been used to classify microbleeds, with a maximum diameter of 5-10 mm.²⁰



Microbleeds

In patients with SVD, cognitive impairment is usually of a characteristic ‘subcortical’ pattern and include psychomotor slowing due to impaired executive function, deficits of attention, planning and set-shifting⁶ Symptoms are thought to be caused by white matter tract disruption and a subsequent ‘disconnection-syndrome’, due to disruption of cortical-cortical and cortical-subcortical connections²¹

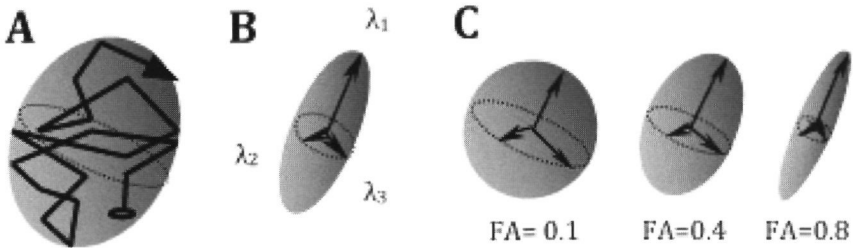
Individuals with virtually identical SVD burden on conventional MRI present with a wide variance of cognitive performance, independent of other factors, such as AD pathology, depressive symptoms and previous cognitive level, that also are known to influence cognitive function The majority of individuals with SVD have no complaints at all, others present with subjective cognitive complaints or objective cognitive impairment, whereas only a relatively small proportion presents with dementia There might be other SVD related changes, apart from those visible on conventional that determine whether identical appearing SVD on conventional MRI leads to cognitive decline in one person, while leaving others more or less unaffected

One of these factors could be the presence cerebral microbleeds (MB), not visible on routine T1 and T2 weighted sequences

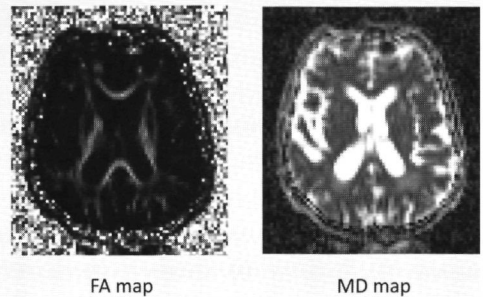
Another factor could be the heterogeneity of white matter lesions itself, as identical appearing WML on conventional MRI are heterogeneous at pathological examination^{22 23} It could be that only the WML with the highest loss of microstructural integrity are related to cognitive impairment and decline Furthermore, it is also important to realize that only a small proportion of the white matter (usually less than a few percent) is affected by SVD, even among individuals with severe SVD²⁴ As conventional MRI is not sensitive to early loss of microstructural integrity in the NAWM, changes in this largest part of the white matter cannot be assessed Damage in this part of the white matter could therefore contribute to cognitive impairment and decline

In the past decades, magnetic resonance imaging has shown to be a powerful non-invasive imaging modality to assess brain changes and recently there has been substantial progress in the development of relatively new MRI techniques to assess these macro- and microstructural abnormalities Diffusion tensor imaging (DTI) allows us to assess the microstructural integrity of the WML and NAWM as it measures the magnitude and direction of water diffusion in the whole white matter²⁵ (Box 2)

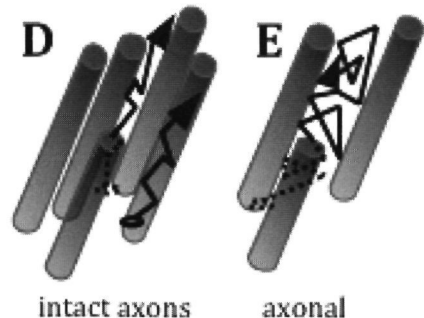
Box 2. Diffusion tensor imaging



Diffusion tensor imaging is an MRI sequence that measures the magnitude and direction of the diffusion of water molecules. In this way, it provides valuable information on the microstructural integrity of the whole-brain white matter. Diffusion is the random thermal motion of molecules and is an intrinsic physical process that is totally independent of the MR effect of the magnetic field. It is a three-dimensional process (Fig A), limited by anatomical boundaries. Hence, molecular mobility in tissues may not be the same in all directions. In and around intact axons for example (Fig D), the diffusion of a water molecule (black line) will be mainly in one direction (i.e. anisotropic). Water diffusion at each voxel is modeled by a tensor, characterized by its three principal eigenvectors and their associated eigenvalues (λ_1 , λ_2 , λ_3) (Fig B). The first is referred to as axial diffusivity, and represents the magnitude of diffusivity parallel to the white matter tracts. The average of λ_2 and λ_3 is termed radial diffusivity, and reflects the magnitude of diffusivity perpendicular to these tracts.



Two different, but complementary, common DTI metrics can be derived from these eigenvalues: the mean diffusivity (MD), which is the average of the three eigenvalues and represents the overall *magnitude* of water diffusion, and the fractional anisotropy (FA), a normalized ratio of diffusion *directionality*.²⁵



The FA ranges between 0 and 1. FA reflects the shape of the tensor, with more isotropic (spherical) tensors having a lower FA value and more anisotropic tensors a higher FA (Fig C). Tissues with intact axons (Fig D) will have a high FA, while tissues with axonal loss or the cerebrospinal fluid for example will have a lower FA.

The MD reflects the magnitude of water diffusion and is expressed in mm^2/s . In intact

axons, diffusion is restricted (Fig D) and the MD will therefore be low. In axonal loss, water molecules have more space to diffuse (Fig E), and the MD will be high. In tissues such as the cerebrospinal fluid, diffusion is not restricted to boundaries and the MD will even be higher. Loss of microstructural integrity is therefore typically reflected by a reduction in FA and increase in MD.²⁶

The increased use of MRI will identify more people with SVD on neuroimaging. More knowledge on the role of SVD in cognitive performance and decline is important, firstly from a pathophysiological point of view. Neuroimaging markers of SVD could both serve as an explanation for cognitive decline and as surrogate markers for cognitive function. In addition, it could help us better predict the prognosis of patients presenting with cognitive disturbances attributable to vascular changes found on MRI. Furthermore, SVD markers on neuroimaging might be of great interest as they may offer more sensitive outcome measures in therapeutic trials on disease-modifying drugs and might reduce the number of patients needed to demonstrate a treatment effect.²⁷

Aim and outline of the thesis

The aim of this thesis was to investigate the relation between conventional and 'new' MRI parameters of cerebral SVD and cognitive performance, in subjects with cerebral SVD.

The studies presented in this thesis are based on the Radboud University Nijmegen Diffusion tensor and Magnetic resonance imaging Cohort (RUN DMC) study, a prospective cohort study among 503 independently living, non-demented elderly, aged between 50 and 85 years, with cerebral SVD. The RUN DMC study was designed to investigate the risk factors and cognitive, motor and mood consequences of functional and structural brain changes, as assessed by MRI.

In **part I** of the thesis we describe the rationale and design of the prospective part of the RUN DMC study (**chapter 3**).

Part II of the thesis describes the relation between brain changes, assessed with conventional MRI, and cognitive performance in individuals with SVD. In **chapter 4** we describe the relation between presence, number and location of MB and cognitive performance, independent of

other manifestations of SVD. Subjective cognitive complaints, have shown to be predictive of dementia^{28,29} and have been related to WML^{30,31}. In **chapter 5** we describe the relation between presence, number and location of MB and subjective cognitive complaints, independent of coexisting SVD and hippocampal volume. In addition, subjective cognitive complaints have been related to hippocampal volume but without taking the degree of WML into account. In **chapter 6** we report on the relation between hippocampal volume and subjective cognitive complaints, independent of WML volume.

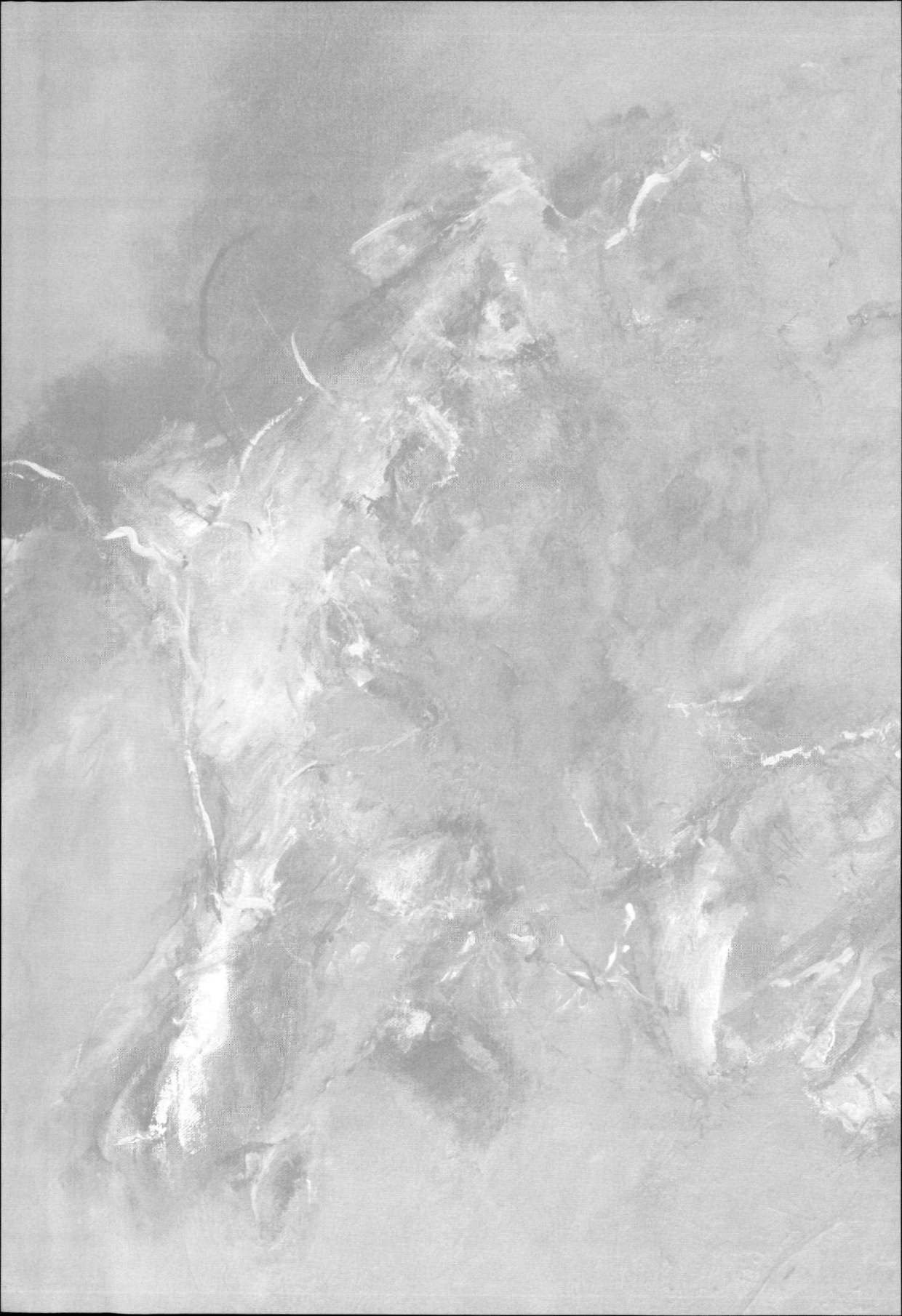
In **part III** of the thesis the relation between brain changes, assessed by DTI, and cognitive function is described. In **chapter 7** we report on the association between the microstructural integrity of both the WML and the NAWM and cognitive performance, this relation was investigated in strata of WML severity. In addition, the microstructural integrity of specific locations in the brain might be important in relation to cognitive performance. In **chapter 8 and 9**, the relation between the microstructural integrity of both the hippocampus and the cingulum bundle and memory performance is described. In **chapter 10** we used Tract-Based-Spatial-Statistics (TBSS) to investigate the relation between white matter integrity and cognitive performance.

In **part IV, chapter 11**, we report on the possible (additional) clinical value of DTI over conventional MRI sequences with respect to cognitive performance.

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Chapter 2

Dementia: Alzheimer pathology and vascular factors: from mutually exclusive to interaction

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Abstract

Alzheimer's disease (AD) is the most common type of dementia. Both its incidence and prevalence are expected to increase exponentially as populations' age worldwide. Despite impressive efforts of research worldwide, neither cure nor effective preventive strategy is available for this devastating disease. Currently there are several hypotheses on what causes AD, with the amyloid hypothesis being the most investigated and accepted hypothesis over the past 20 years. However the exact role of amyloid- β in the onset and progression of AD is not yet fully understood, and even the validity of the amyloid hypothesis itself is still being discussed. This debate is fuelled by the vascular hypothesis, as increasing epidemiological, neuroimaging, pathological, pharmacotherapeutic and clinical studies suggest that vascular pathology plays a key role in the onset and progression of AD. We here will discuss arguments in favor and limitations of both hypotheses within the framework of available literature, but also provide arguments for convergence of both hypotheses. Finally we propose approaches that may aid in unraveling the etiology and treatment of AD.

Introduction

Alzheimer's disease (AD) causes an immense economic burden and an unparalleled social challenge with over 15 million persons affected worldwide together with a decreasing number of potential caregivers in the coming decades¹ Before Alzheimer's publication on the disease in 1907, dementia had been recognized for centuries and was ever since attributed to vascular factors as for example illustrated by Lobstein who coined the term "dementia arteriosclerotica", already as early as in 1833 And even before, the history of vascular dementia dates back to cases of dementia after apoplexia described by Thomas Willis in 1672

In 1906, it was Alzheimer, or actually his teacher Kraepelin, who started confusion with the description of the case of 51-years old Auguste D that resembled "ordinary dementia" cases but who was considerably younger and had neuropsychiatric symptoms² On post mortem neuropathological examination her brain contained senile plaques and neurofibrillary tangles

This presenile disorder affecting patients in their forties and fifties with early symptoms of a severe progressive dementia with focal signs such as amnesia, aphasia, apraxia and agnosia was then named AD In his later work, Alzheimer reported the degeneration of the smaller cerebral blood vessels at a cellular level in a 56 year-old patient, a process also referred to as Alzheimer's sclerosis Alzheimer questioned whether the co-occurrence of plaques and tangles and vascular pathology being typical or atypical for AD, an ever actual issue

After the identification of the plaque's main compound amyloid in the 1960's, much research was devoted on its physiology and pathophysiology, followed by a reappraisal of vascular factors based on the abundance of vascular changes in the brain of patients with cognitive decline and dementia when large scale neuroimaging became available^{3,7}

Currently there are several hypotheses on what causes AD, with the amyloid hypothesis being the most investigated and accepted hypothesis over the past 20 years However the exact role of amyloid- β in the onset and progression of AD is not yet fully understood, and even the validity of the amyloid hypothesis itself is still being discussed This debate is fuelled by the vascular hypothesis, as increasing epidemiological, neuroimaging, pathological, pharmacotherapeutic and clinical studies suggest that vascular pathology plays a key role in the onset and progression of AD We will give a critical appraisal with respect to their causality in AD and provide a framework that enables a convergence of the two hypotheses towards one explanatory model for AD and that will provide clues for future research

The amyloid hypothesis

Observational

The first description of the amyloid composition of the neuritic plaques, that Alzheimer observed decades before, appeared in 1966 by Roth, Tomlinson and Blessed. This British research group quantified the relation between clinical symptoms and pathological changes (plaques and tangles) in the brain.^{8,9} In 1984 the amino-acid sequence of the amyloid-beta peptide was elucidated, that initiated the discussion on its origin.¹⁰ The presence of the amyloid- β protein was first sequenced from (especially leptomeningeal) blood vessels of AD patients and patients with Down syndrome.¹¹ A year later the same peptide was recognized as the primary component of the senile (neuritic) plaques of AD patient brain tissue.¹² These findings resulted in the hypothesis that the blood borne accumulation of amyloid resulted in plaques. The subsequent cloning of the gene encoding for amyloid- β precursor protein (APP) and its localization on chromosome 21 and that of presenilin 1 and 2 that are now known to be involved in splicing the APP suggested that amyloid- β accumulation is the primary event in AD. Mutations in the APP gene were identified causing hereditary cerebral hemorrhage with amyloidosis (Dutch type), this showed that APP mutations could cause amyloid- β deposition, outside the brain parenchyma.^{13,14} The amyloid- β protein is cleaved from APP by the sequential action of β -secretase and γ -secretase respectively, the resulting amyloid protein fragment can be either 40 ($A\beta_{1-40}$) or 42 ($A\beta_{1-42}$) amino acids in length. Mutations in the presenilins result in relative high levels of extracellular $A\beta_{42}$.¹⁵ Many mutations in these genes have now been described, each with an early onset AD as clinical picture, but it should be noted that these mutations represent less than 0.1% of all AD cases.¹⁶

Apolipoprotein E (apoE) is the most consistently associated risk gene for AD, it is involved in the amyloid- β clearance and associated with increased amyloid burden and cholinergic dysfunction. Individuals with two apoE $\epsilon 4$ alleles have a more than seven times increased risk of developing AD.¹⁷

In the late 1980's and begin 90's it was recognized that amyloid- β protein was not only a pathological constituent but could also be part of physiological cellular mechanisms throughout life of healthy humans.^{18,20} Based on these observations the later called "amyloid hypothesis" of AD was formulated (left panel, figure 1).^{21,22} In short, this theory implies that AD is initiated by abnormal cleavage of the APP resulting in a chronically enhanced production and/or decreased clearance of soluble, diffusible amyloid- β . This could lead to a gradual precipitation of aggregated non-diffusible amyloid- β in the form of spherical plaques and vascular deposits in AD. Factors which cause this aggregated amyloid- β include genetic causes like dominant mutations of the genes encoding APP and presenilin 1 and 2.^{23,24}

In addition, tau, a microtubule-associated protein, is the major constituent of neurofibrillary tangles. The "amyloid hypothesis" proposes that an imbalance between amyloid- β

production and amyloid- β clearance resulting in toxic concentrations of amyloid- β which triggers changes in tau and consequent neurofibrillary tangle formation²⁵, although the pathways linking amyloid- β and tau are not fully understood Braak and Braak published the pathological stages of distribution of neurofibrillary tangles that followed a predictable pattern, with initial changes in the entorhinal cortex, than spread into the entorhinal region of the temporal cortex, followed by involvement of the neocortical associated areas²⁶ There is still conflicting evidence between the amyloid burden and the attendant cognitive performance In favor for a role of amyloid pathology in cognitive impairment was the observation that neurotoxic amyloid β soluble oligomers could impair cognitive function in rats^{27 28} These highly organized, low molecular weight oligomeric assemblies precede the formation of amyloid fibrils and they have been identified in the brains of patients suffering from AD as well²⁹

From the viewpoint that amyloid- β is a crucial factor in the etiology of AD it is evident that much effort has been put in its detection during life, ideally within the living brain or, second best, in the cerebrospinal fluid (CSF) Indeed, recent research suggests that a fairly reliable distinction between AD and non-demented can be made on the basis of CSF findings³⁰ However, the results seem less promising when other dementias, as in clinical practice, have to be distinguished, necessitating further research on the role of amyloid- β in the etiology, but also on its use as a diagnostic tool Evidence from a longitudinal study suggests that CSF biomarkers, amyloid- β (1-42), tau and ptau-181, are not sensitive markers for disease progression in Alzheimer's patients [17] Although others do not agree with this finding³¹ and recent reports show a CSF AD profile in subjects with subjective and mild cognitive impairment³² and a relation between increased levels of t-tau and intensity of disease and disease progression³³

In-vivo detection of amyloid- β became possible with the development of the Pittsburg Imaging Compound B (PIB) and recently also other amyloid-probes that enable visualization of amyloid burden using Positron Emission Tomography (PET) scanning³⁴ Positive cortical PIB binding has been associated with low cerebrospinal fluid A β 42 concentrations in AD³⁵ This development offers possibilities for early diagnosis and possibly also tracking progression of disease, although the latter could not be found in a recent small study, in which it appears that anti-amyloid therapies will need to induce a significant decrease in amyloid- β load in order to detect a drug effect with PIB PET³⁶ As PIB is not ideal for commercialization, because of its 20-minute half life, several ¹⁸F-labeled tracers have been developed³⁷ At this time, these tracers are undergoing extensive phase II and III trials Recently, it has been demonstrated that florbetapir-PET imaging was correlated with the presence and density of amyloid- β ³⁸ Next to investigate the effect of novel amyloid- β modifying drugs on a clinical level, these amyloid binding agents may also have the potential to investigate the effects on the presence of amyloid- β in both the parenchyma as vessel wall

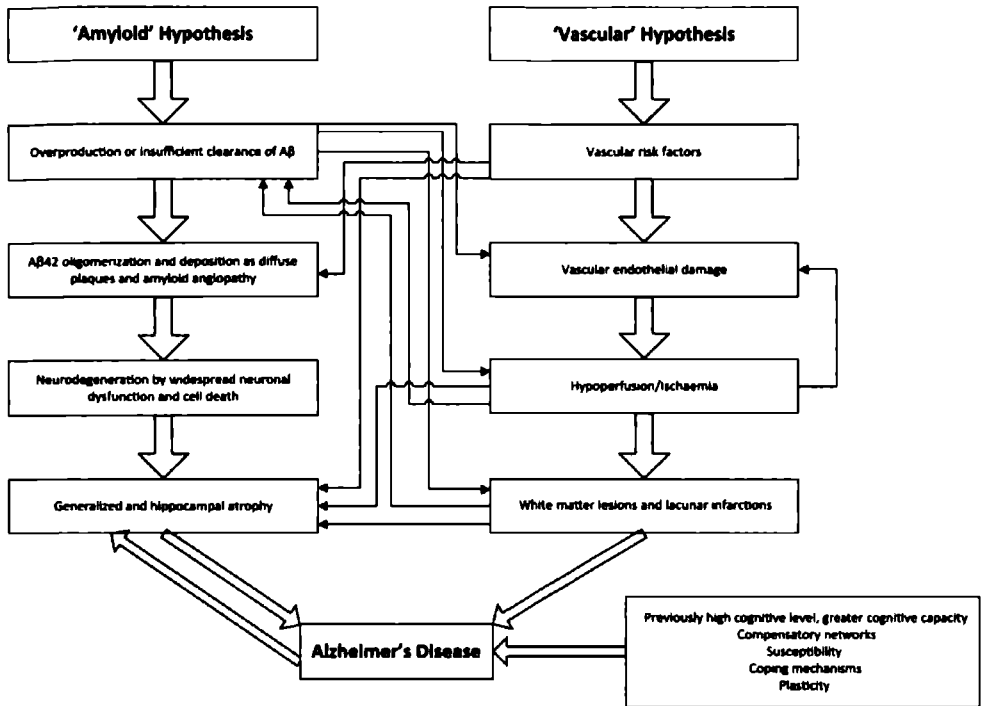


Figure 1 Interaction between the amyloid hypothesis and the vascular hypothesis in the etiology of Alzheimer's Disease

Experimental

The ultimate proof for a role of amyloid- β peptide in AD would be to reduce the brain's amyloid- β burden, using disease modifying therapies and to investigate its effect on cognitive decline. As such the amyloid- β has become a major therapeutic target. Therapeutic strategies include lowering the production of the peptide by 1) prevention of APP transcription 2) inhibiting the enzymes responsible for amyloid- β generation (APP splicing by β - and γ -secretase), 3) preventing aggregation of solid amyloid- β , and 4) increasing the rate of amyloid- β clearance from the brain. Amyloid- β immunotherapy uses anti amyloid- β antibodies, generated following vaccination or introduced passively. Preclinical studies in animal models showed that amyloid- β immunization lowered the plaque burden³⁹ and reversed behavioral deficits.⁴⁰ First phase human clinical trials in AD in which patients were immunized with aggregated human amyloid- β 1-42 to stimulate clearance of amyloid plaques were associated with serious adverse events (meningoencephalitis), the trial had to be terminated prematurely.⁴¹ Later analysis of this interrupted trial showed that some patients ("the antibody responders") had an increased loss of brain volume and no better cognitive performance compared to the placebo group, except for some composite z-score

of the neuropsychological test battery.^{41,42} On the basis of these studies numerous amyloid- β immunotherapy's are currently in human clinical trials

Another approach to decrease levels of amyloid- β is passive immunization with antibodies targeting portions of the amyloid- β molecule. Bapineuzumab is a humanized monoclonal antibody investigated in two trials. In the first trial the amyloid- β burden was decreased, however in the other trial no difference was found in cognitive function between the treatment group and the controls.^{43,44} A safety concern was the occurrence of reversible vasogenic edema.

Another area of drug development involves the targeting of γ secretase, one of the enzymes required for production of amyloid- β from APP. As γ -secretase has many functions in the body, treatment with γ -secretase inhibitors may lead to toxicity. Nevertheless a number of inhibitors have been tested. Recently, a large, multicentre, randomized, double blind, placebo-controlled, phase III trial on Tarenflurbil showed no beneficial effect in the treatment group compared to placebo.⁴⁵

In addition, although several observational studies have concluded that the use of nonselective nonsteroidal anti-inflammatory drugs (NSAIDs) is associated with a reduced risk of AD,⁴⁶ clinical trials of nonselective or COX2-selective NSAIDs in patients with dementia or with mild cognitive impairment have not demonstrated any effect on cognitive performance.^{47,49}

Possible explanations for these negative trials may be because of their use in patients with advanced stages of disease, the pathological processes may start years before the onset of clinical symptoms, this suggests that treatment administration would need to occur much earlier in patients deemed to be at high risk. In addition, it might be because of study design, sample size or choice of treatment dosage. However, it may also be that the negative findings reflect the still incomplete understanding of the pathogenesis of AD, and the role of other (neurodegenerative and vascular) factors.

Discrepancies

Notwithstanding the overwhelming observational data on amyloid and AD, some important issues need to be addressed.

A first discrepancy are the negative results of the trials in which the effect of amyloid- β lowering drugs on disease was studied. In addition, pathological studies on the relation between senile plaques and neurofibrillary tangles (AD pathology) and cognitive performance and clinical AD show various discrepancies. There are numerous reports showing a lack between the degree of amyloid pathology and cognitive function during life,⁵⁰ whereas other studies correlated soluble and fibrillar forms of amyloid- β with cognitive scores.^{51,52} Many cognitively intact, healthy elderly have abundant senile plaques, some even with the same density of senile plaques as patients with AD in their brains without clinical signs of AD.^{53,54} Plaques and tangles are found in cognitively intact individuals after the age of

forty without neuronal loss⁵⁵ This is in line with findings from a large pathology study that found that there is much mixed pathology (cerebrovascular and AD pathology) in healthy individuals from the general population who did not meet the clinical diagnostic criterium of dementia⁵⁶ There are reports on several lines of transgenic mice overexpressing APP in which there is a correlation between the extent of learning and memory deficits and the amount of deposited amyloid- β found in the central nervous system,⁵⁷ however there are also reports on transgenic animal models with high levels of brain amyloid deposits that show no significant neurodegeneration⁵⁸ A recent report shows that neuropathological processes related to AD in persons without dementia were correlated with subtle cognitive dysfunction and that by the age of 80-85 years many non-demented elderly have substantial AD pathology⁵⁹ Furthermore, recent studies in humans showed that clearance of amyloid depositions resulted neither in cognitive improvement nor in a decreased rate of mental deterioration These observations suggests that senile plaques may not be the main cause of neurodegeneration and cognitive decline in AD, accordingly other theories have been established over the past years

A first theory is based on soluble amyloid- β , which proposes that toxic amyloid- β -42 or its oligomers interfere with synaptic plasticity in vitro or with membrane dysfunction and promote cell death^{60,61} However these findings are based on experimental animals models, overexpressing exogenous APP, and may not be applicable to AD Soluble amyloid- β peptides however are normal components of human serum and cerebrospinal fluid and it is not clear under what conditions these peptides become toxic, especially as no significant disease-associated abnormalities have been detected with their production⁶²

A second theory is the presenilin hypothesis which suggests that loss of function of presenilin may be the primary event triggering neurodegeneration in AD Amyloid- β -42 may act primarily to antagonize presenilin dependent functions, possibly by operating as active site-directed inhibitor of γ -secretase, mimicking the effect of presenilin mutations Loss of presenilin activity results in synaptic dysfunction and ultimately leads to progressive neurodegeneration characterized by loss of synapses, dendrites, and neurons, astrogliosis and tau hyperphosphorylation⁶³

Finally, others suggest that tau protein plays a central role in the chain of events leading from amyloid neurotoxicity to tau hyperphosphorylation, microtubular destabilization, disturbed axonal transport and synaptic failure to neurodegeneration In this hypothesis the pathological conversion of normal tau into functionally impaired phosphorylated tau, which forms neurofibrillary tangles and dystrophic tau neuritis and thereby depleting levels of functional microtubules binding and stabilization tau below a critical point that results in depolymerization of microtubules and a disruption of axonal transport These events are thought to culminate in neuronal dysfunction and degeneration, leading to AD⁶⁴

Other mechanisms may also play a role, for example the cognitive reserve theory⁶⁵ The cognitive reserve theory provides at least two explanations of not finding a relation between

the degree of amyloid pathology and cognition. The first is that individuals functioning at a previously high level possibly have a greater cognitive capacity and/or more efficiency and in that way may be less susceptible to disruption compared to the average elderly. Consequently, they may experience a longer subclinical cognitive deterioration, despite the possible presence of amyloid plaques. Another explanation could be the presence of compensatory networks that may compensate for amyloid-based disruption of pre-existing networks.²⁶ This may obscure the relation between amyloid burden and the cognitive decline during the course of AD.

Vascular hypothesis

Observational

The history of vascular dementia can be traced back to cases of dementia postapoplexia described by Thomas Willis in 1672. In 1833 Lobstein introduced the term “dementia atherosclerotica”. In 1894 Binswanger described the subcortical arteriosclerotic encephalopathy, now known as Binswanger disease. Before the CT and MRI era this disease was thought to be very rare.

With the advent of non-invasive imaging of the brain with CT in the 1970’s and 1980’s and MRI in the 90’s it became evident that small vessel disease was not rare at all and a common finding in the general population and in AD patients. The term “leuko-araiosis” was introduced only 25 years ago as a neutral term for deep white matter changes on the basis of vascular disease that were considered to be associated with cognitive impairment.⁶⁶ It was demonstrated that “healthy” elderly with leuko-araiosis performed less on certain cognitive tasks than those without leuko-araiosis and that, in patients with AD, leuko-araiosis was associated with a greater degree of cognitive impairment.^{67,68} Large longitudinal population based cohort studies were then initiated and consistently showed a relation between several vascular risk factors and white matter lesions (WML).^{69,71} These vascular risk factors, hypertension, diabetes, atherosclerosis, homocysteine, smoking and atrial fibrillation have found to be related to AD.^{72,76} Presumably these relations underlie the emergence of vascular (white matter) lesions, as it was demonstrated that individuals with silent brain infarcts, periventricular and subcortical WML have an increased risk of AD.⁴ Furthermore, pathology studies have revealed the presence of ischemic lesions in patients with AD and it was shown that among non-demented people those with coronary heart disease had greater plaque burden in the brain those without heart disease.⁷⁷ This resulted in large population based autopsy studies to unravel the vascular risk factors for cognitive decline in elderly. In a prospective population based cohort study, 209 subjects consented to donate their brain for post-mortem evaluation. Before, they all underwent neuropsychological screening with incident dementia at 2 and 6 years as the primary outcome measure.⁵⁶ The

median age of death was 85 years, at that time 48% of the subjects were demented of which 64% fulfilled the criteria for probable or definite AD. Over one third of the clinically diagnosed AD patients did not have any plaques at all whereas in one third of the clinically non-demented elderly moderate to severe plaques were found. Around 80% of the subjects had vascular abnormalities of the brain, which were significantly more severe in the demented population. The vascular hypothesis emanated from these findings (fig 1, right panel). Vascular risk factors induce neurovascular dysfunction and cause endothelial damage by oxidative stress and inflammation which lead to local hypoperfusion and ischemia which are radiologically represented by WML and silent and/or lacunar brain infarctions.⁷⁸ As discussed earlier, these radiological changes are related to cognitive decline and dementia.⁴

Experimental

Proof of concept for a role of vascular factors in the etiology of dementia could come from intervention trials. In the Syst-Eur trial showed that treatment of isolated systolic hypertension with calcium antagonists among elderly over the age of 60 was associated with a decrease of incident dementia. The absolute risk reduction was 3%.⁷⁹ A 2-year open label extension of this study revealed the same results, long-term antihypertensive therapy reduced the risk of dementia by 55% compared with placebo ($p < 0.001$).⁸⁰ In the Leiden 85-plus study it was also reported that the use of a calcium antagonists prevents the development of dementia, they did not find this for other antihypertensive drugs. They suggested that the protective effect found in the Syst-Eur trial and in their own study was due to the effect of the calcium channel inhibition rather than its blood pressure lowering properties.⁸¹ The PROGRESS study was a randomized, double blind, placebo controlled trial involving 6105 patients with previous stroke or ischemic attack. Participants were assigned to active treatment (perindopril for all participants and indapamine for those with neither an indication for nor a contraindication to a diuretic) or matching placebo(s). After a follow-up of 3.9 years the risk of dementia was reduced from 7.1% to 6.3% (nonsignificant) however the risk of cognitive decline was significantly reduced from 11% to 9.1%. Although, it could be that this benefit was due to the prevention of recurrent stroke.⁸² Preliminary results of the observational OSCAR study support these findings.⁸³

Discrepancies

The previously described intervention studies could not be replicated in several other studies. In the SHEP (systolic hypertension in elderly program) study over 2000 patients were enrolled for treatment with chlortalidone or placebo. Neither the decline in cognitive function nor the incidence of dementia were significantly different between both groups after the treatment period.⁸⁴ In the PROSPER study 6000 individuals with pre-existing vascular disease or raised risk of such disease were randomized to either pravastatin or placebo and followed for a mean of 3.2 years, one of the secondary outcomes was cognitive

decline. There were no significant differences for both groups and several cognitive rating scales.⁸⁵

A Cochrane review with a meta-analysis of four randomized controlled trials (Syst-Eur trial, Scope trial, SHEP trial and the HYVET trial)^{79 84 86 87} on the effects of blood pressure lowering in late-life on the development of cognitive decline and dementia on patients with hypertension without apparent prior cerebrovascular disease. This Cochrane concluded that blood pressure lowering in late-life is not indicated with the aim of prevention of cognitive decline or dementia alone, as only the Syst-Eur trial reported a benefit from treatment but neither the SCOPE, SHEP nor HYVET showed a benefit, and when the trials were entered in a meta-analysis, no statistically significant effect was seen.⁸⁸ As suggested by the authors, primary outcome measures of all trials was cardiovascular disease, cognitive function and dementia were secondary end-points. Trials were terminated once the primary end-points were shown, in that way possible beneficial effects on cognition later in the follow-up can be missed.

One of the possible explanations for this unequivocal role of (treatment of) hypertension could be that the deleterious effects of vascular risk factors in general and hypertension specific already occurs decades before either cardiovascular or cognitive symptoms occur. By the time the risk factors result into disease, the damage is not remediable anymore.

Convergence of the amyloid hypothesis toward the vascular hypothesis

The diagnosis AD is based on the DSM-IV-TR⁸⁹ and the NINCDS-ADRDA criteria⁹⁰. In these criteria, the presence of other systemic or brain diseases that may account for the progressive memory loss and other cognitive deficits, is an exclusion criterion. The criteria for vascular dementia (NINDS-AIREN) include decline from a previously higher level of functioning and manifested by impairment of memory and of two or more other cognitive domains, which also exclude patients systemic or brain disorders (such as AD) that in themselves could account for deficits in memory and cognition.⁹¹ According to these criteria the presence of cerebrovascular disease in a demented individual rules out AD and vice versa.

However, the pathological changes characteristic of AD are observed together with vascular pathology in more than 40% of elderly demented individuals.⁹² Several studies show a variety of underlying neuropathology among clinically diagnosed “probable” AD patients. This pathology includes both Braak and Braaks’ criteria fulfilling amyloid plaques and tau tangles but also vascular lesions such as lacunar infarctions and WML. A study among 102 women between 76-100 years showed that dementia prevalence was highest among those who met the neuropathological criteria for AD and had coexisting vascular pathology.⁹³ In the presence of vascular pathology in the basal ganglia, thalamus and deep white matter

fewer neuropathologic lesions were needed to result in a clinical diagnosis of AD than in those without coexisting vascular pathology, indicating the possible interaction between these factors. In contrast, among those who did not meet the neuropathological criteria for AD, vascular lesions were only weakly associated with poor cognitive function and dementia. The Honolulu Asia Aging Study, a community based study in very old men, of which 29% were demented ante mortem, suggested that the burden of AD and vascular lesions independently contribute to a clinical AD diagnosis, which is compatible with the view of the additive effect that these two types of lesions on cognitive impairment may have.⁹⁴ The combination/interaction of cerebrovascular damage with damage to the brain caused by amyloid- β may result in a lower cognitive threshold of dementia than the two separately.⁹³ The simultaneous double hit by amyloid- β and vascular damage might affect cognitive function more than the sum of parts, their effects may even be synergistic.^{95, 96} These findings are more or less confirmed by the MRC CFAS study that showed an extensive overlap of intermediate Alzheimer type pathology among demented and non-demented older people despite equivalent degrees of vascular pathology.⁵⁶ In addition, hypoxia and ischemia resulting from vascular insufficiency may interact with amyloid- β on a more basic pathophysiological level. They increase the transcription of APP and its cleavage by β -secretase resulting in more amyloid- β production.^{97, 98} Furthermore vascular damage may promote amyloid- β accumulation in the CSF by reducing the vascular clearance of this peptide.^{99, 100} And vascular amyloid- β is a potent vasoconstrictor and impairs fundamental mechanisms regulating cerebral circulation and in that way may induce ischemia.^{101, 102} In that way the interaction between the amyloid and vascular pathway enhances their pathogenic effects and favour the formation of amyloid plaques and cerebral amyloid angiopathy (CAA).¹⁰³ Others. Imaging of in vivo parenchymal and vascular amyloid- β , with PET-radiopharmaceuticals could help in studying this potential self strengthening mechanisms.¹⁰⁴ Next to investigate the effect of novel amyloid- β modifying drugs on a clinical level, PIB-PET may also have the potential to investigate these effects on the presence of amyloid- β .

Neuroimaging studies lend further support for an interaction between amyloid- β features with vascular factors and vice versa. The key radiological hallmark of typical AD is the medial temporal lobe atrophy (MTA). The degree of atrophy was reported to be related to the disease stage defined by Braak and Braak^{105, 106} but a relation between vascular (risk) factors and the severity of MTA has also been observed.^{107, 108} Non-demented patients with diabetes have more MTA on MRI than those without diabetes.¹⁰⁹ At the population level there is a positive association between blood pressure and medial temporal lobe atrophy meaning that increase in blood pressure was related to more severe MTA.¹¹⁰ Among patients with AD a linear relation between systolic blood pressure and the severity of MTA has been observed. WML could be a possible intermediate in this relation as both population based studies and studies among AD patients showed a relation between WML and MTA.^{107, 108} A small prospective study

showed that baseline WML were associated with progression of MTA among 35 clinically defined “probable” AD patients. Stratification on WML indeed revealed that the relation between blood pressure and MTA only existed in AD patients with WML.¹¹¹ In stroke patients it was shown that patients with post-stroke reduced episodic memory function had reduced medial temporal lobe functionality as demonstrated by fMRI.¹¹² Other cardiovascular risk factors than hypertension have been related to AD. Diabetes mellitus doubles the risk of AD corresponding with an increase in microinfarcts and without a corresponding increase in plaques and tangles.¹¹³ Higher total cholesterol, LDL concentrations and a history of diabetes is associated with faster cognitive decline in AD.¹¹⁴ Additionally, in studies on AD patients the presence of atherosclerosis was related to an increased frequency of neuritic plaques and neurofibrillary tangles.^{115 116} Furthermore, treatment of vascular risk factors in patients with AD is associated with slower cognitive decline.¹¹⁷ Conversely, there is increasing evidence for a role of the amyloid metabolism on presumed cerebral vascular lesions and its effects on cerebral blood vessels. Cerebral microbleeds might be a missing link between the amyloid and vascular hypothesis. Cerebral microbleeds in the cortico-subcortical regions might be a marker of CAA.¹¹⁸ CAA is characterized by deposition of amyloid- β peptide within the walls of the small and medium sized cerebral arteries,¹¹⁹ and is associated with AD.¹²⁰ ApoE plays a crucial role in lipid metabolism and neuronal repair.¹²¹ The apoE $\epsilon 4$ allele is not only associated with cognitive impairment and dementia, but also with vascular risk factors for WML.¹²² $\epsilon 4$ Homozygotes exhibit more extensive WML than other genotypes.¹²³ It was hypothesized that the $\epsilon 4$ allele was related to cognitive impairment because of the presence of WML. Large population based studies showed interactions between hypertension and apoE $\epsilon 4$ allele with regard to subcortical WML, and relations between plasma amyloid- β levels and WML and lacunar infarcts in people who carry a apoE $\epsilon 4$ allele.^{124 125} However, studies in demented populations and other population based studies could not find these relations.^{126,127} The $\epsilon 4$ allele modulates the severity of amyloid- β deposits in animal models¹²⁸ and in humans¹²⁹, especially in patients with WML.¹³⁰ In a sample of autopsy proven AD patients apoE $\epsilon 4$ was associated with small vessel arteriosclerosis, microinfarcts, neuritic senile plaque density and CAA.¹³¹ On the other hand cerebral microbleeds in the deep grey matter and infratentorial regions are related to hypertensive vasculopathy and might be a result of microangiopathy, such as arteriosclerosis or ischemia.¹³²

In addition, experimental findings suggest cytotoxic effects of amyloid- β on the vascular smooth muscle cells, resulting in prolonged and intensified endothelium dependent vasoconstriction that could possibly result in hypoperfusion.¹⁰¹ In a study, based within the same Rotterdam Study, in-vivo support for these observations has been obtained. High plasma levels of amyloid- β assessed 6.5 years before transcranial Doppler investigations were related to impaired CO₂ enhanced transcranial Doppler assessed vasomotor reactivity.¹³³ Next to this classical approach, novel developments, including those from other areas, may help in elucidating the cause of AD. One of these could be the systems approach to the

networks of aging as proposed by West.¹³⁴ This approach emphasizes the multiplicative effect that damage in network systems such as the vascular tree may have on organ functions in general. In this way the cerebrovascular damage, probably highly related to amyloid plaque depositions in the vascular wall, may act in a synergistic way with the amyloid plaques, leading to a further compromised neuronal function. In addition, this theory makes use of the fundamental physiological properties of different species as they age with molecular and cellular changes, analyzing a system from both a top-down (a system decomposing into parts), but also from a bottom-up approach.

Future perspectives

Most likely there is no single one-risk factor based explanatory model for AD as more and more evidence suggests that it is not a single cause disease entity, but a variety of causes, including neurodegenerative (amyloid- β), vascular and probably more.

None of these risk factors alone are proven to be necessary risk factors (which means that the disease (AD) must always be preceded by this risk factor) nor sufficient risk factors (i.e. the risk factor always leads to the disease). Most likely the factors described here are contributory risk factors (the risk factor is sometimes succeeded by AD and the other way around). In addition to this interaction between several of these factors may also play a role in the final development of the clinical syndrome of AD as we call it today. (Fig. 1)

Dementia is a very complex disease in the elderly with a large heterogeneity in phenotype and genotype, with a heterogeneous life-time exposure to a lot of different frequently interacting vascular, amyloid and/or other damaging risk factors.

In future research more distinctive and reliable biomarkers might play an important role, yet they are part of the revised research criteria for AD, proposed to allow earlier and more specific AD diagnosis.¹³⁵

Although some discrepancies as discussed earlier, CSF markers may be a potentially promising approach for pathology *in vivo*, as they can distinguish between AD and non-demented patients and it was shown that subjects with subjective and mild cognitive impairment have a AD CSF profile, and this profile was predictive of AD-type dementia.^{30,32} are related to. If amyloid deposition plays an important role in the etiology of AD, development of anti-amyloid- β therapies, including amyloid- β immunotherapy, that reduce the plaque burden or even prevent the development of amyloid plaques is one of many future approaches to reduce the ever-growing incidence of AD.

Imaging can be used as another biomarker. With the advance of imaging techniques that are now available, repeated imaging with thinner slices in combination with a valid automated lesion detection tool could quantify the total lesion volume more precisely. Furthermore, as identical appearing WML on conventional MRI are actually histopathologically

heterogeneous,¹³⁶ it could be that only WML with the highest loss of structural integrity are related to cognitive impairment. It is also important that only a small portion of the white matter (usually less than a few percent) is affected by WML. As conventional MRI is not sensitive to early loss of microstructural integrity in the normal appearing white matter, possible changes in this largest part of the white matter cannot be assessed. The integrity of the normal appearing white matter might be an important factor for a better understanding of the relation between white matter integrity and cognitive function and decline. These limitations of conventional MRI can potentially be overcome with the use of novel imaging techniques as Diffusion Tensor Imaging (DTI) which allows the assessment of the microstructural integrity of the whole white matter.¹³⁷ DTI may particularly be suited to detect subtle white matters' structural abnormalities at even earlier moments in time (at least before detection with conventional FLAIR imaging). And as such these early signs of microstructural loss can be found before actual disabling cognitive consequences may have occurred and as such may aid in the selection of patients who may be at risk for future development of "leuko-araiosis" and the possible attendant cognitive decline. Even more stringent treatment of vascular risk factors may, although unproven until now, possibly postpone future cognitive deterioration. In future it could be possible to use these new imaging techniques to visualize neural compensation networks. Therapeutic strategies may then be developed that aim at stimulating the formation of such networks.

Analogous to the amyloid theory much needs to be done to establish a causal role for vascular factors in the etiology of dementia, as most of the data are cross-sectional or of non-replicated intervention studies. In future research on the etiology of AD and the possible role vascular factors in this etiology, the phenotype of the study population should be critically defined. This phenotype should be defined by the clinical presentation, exposure to risk factors and age of the study population. In etiologic research, the phenotype should not be based on the absence or presence of biomarkers as these are the etiologic factors you want to investigate and when part of the diagnosis, obviously there will be a relation between the biomarker and disease. It should be a very large study sample in order to be able to define subgroups to investigate interactions between these groups. In prognostic or intervention trials biomarkers can be used in the definition of disease, although there should be consensus on the definition of these biomarkers. For example, the presence of WML, there can be rated semi-quantitatively, by location, by volume estimation or by automated or manually segmented total volume, and with or without taking silent brain infarcts or lacunar infarcts into consideration or by using new imaging techniques which assess the microstructural integrity of the whole white matter. Dementia should be among the endpoints in any cardiovascular intervention trial, with an extended, open-label follow up to be able to detect incident dementia occurrence after the primary vascular outcome events.

Conclusion

In 1907, Alois Alzheimer described a clinical syndrome, now known as AD, characterized by progressive behavioral abnormalities with prominent focal symptoms and an impaired memory in a relatively young woman

Nowadays, patients that are being diagnosed with AD are usually much older than Auguste D and the clinical symptoms differ Alzheimer most likely described a presenile dementia based on amyloid- β pathology without, or with only few concomitant (vascular) risk factors Although some clinical similarities between Auguste D's dementia and the senile dementia exist, the latter is probably being caused by accumulated cerebral damage caused by life-time exposure to vascular, amyloid and/or damaging factors that probably differs in terms of pathophysiology from Auguste D Vascular factors as a contributing cause to the development of AD have come into play again, next to affecting the large arteries at the skull's base as one thought in the 19th century, the small vessels (arterioles) deep within the brain parenchyma also play a role The presence of small vessel cerebrovascular disease in AD is very common, cerebrovascular disease and AD share the same risk factors, coexisting cerebrovascular disease and AD results in more severe cognitive impairment, either by direct damage of the neural pathway or indirect by worsening the impact of AD pathology As hypothesized by others, this suggests that cerebrovascular disease may be in the causal pathway for development of AD or interacts synergistically with AD pathology^{103 138}

Future studies on the etiology of AD should preferably start with proper diagnostic research criteria in order to exactly define the disease investigated instead of etiologic research on merely a complex of symptoms rather than a single disease entity A well described phenotype and large numbers of patients are important factors to enable unraveling the complex interacting pathologies, best in etiological studies in vivo using MRI, CSF markers and PET¹³⁵ Once that has been accomplished studies on etiology should be large, prospective and presumably already start during mid-life or even before Treatment of risk factors found by this approach should then be further evaluated in intervention studies with dementia as a primary end point We should also keep an open eye towards innovative approaches including those derived from systems biology, a field which focuses on complex interactions of biological systems,¹³⁴ that may possibly speed up the quest for the etiology of AD

Imaging and biomarkers have improved dramatically over the last ten years Identifying the individuals at risk before clinical onset of symptoms, might help us to treat these individuals and to reduce the ever growing incidence of AD

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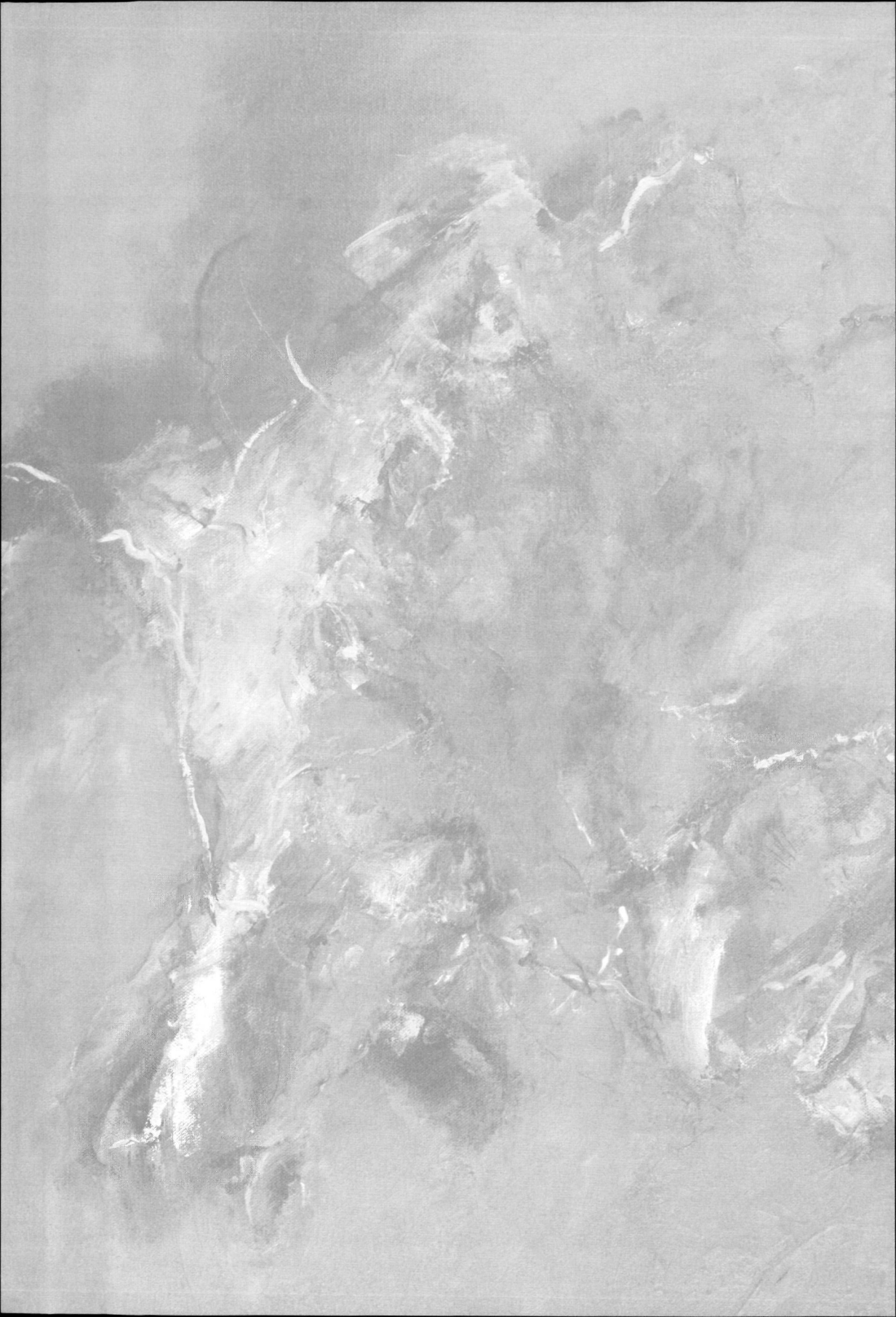
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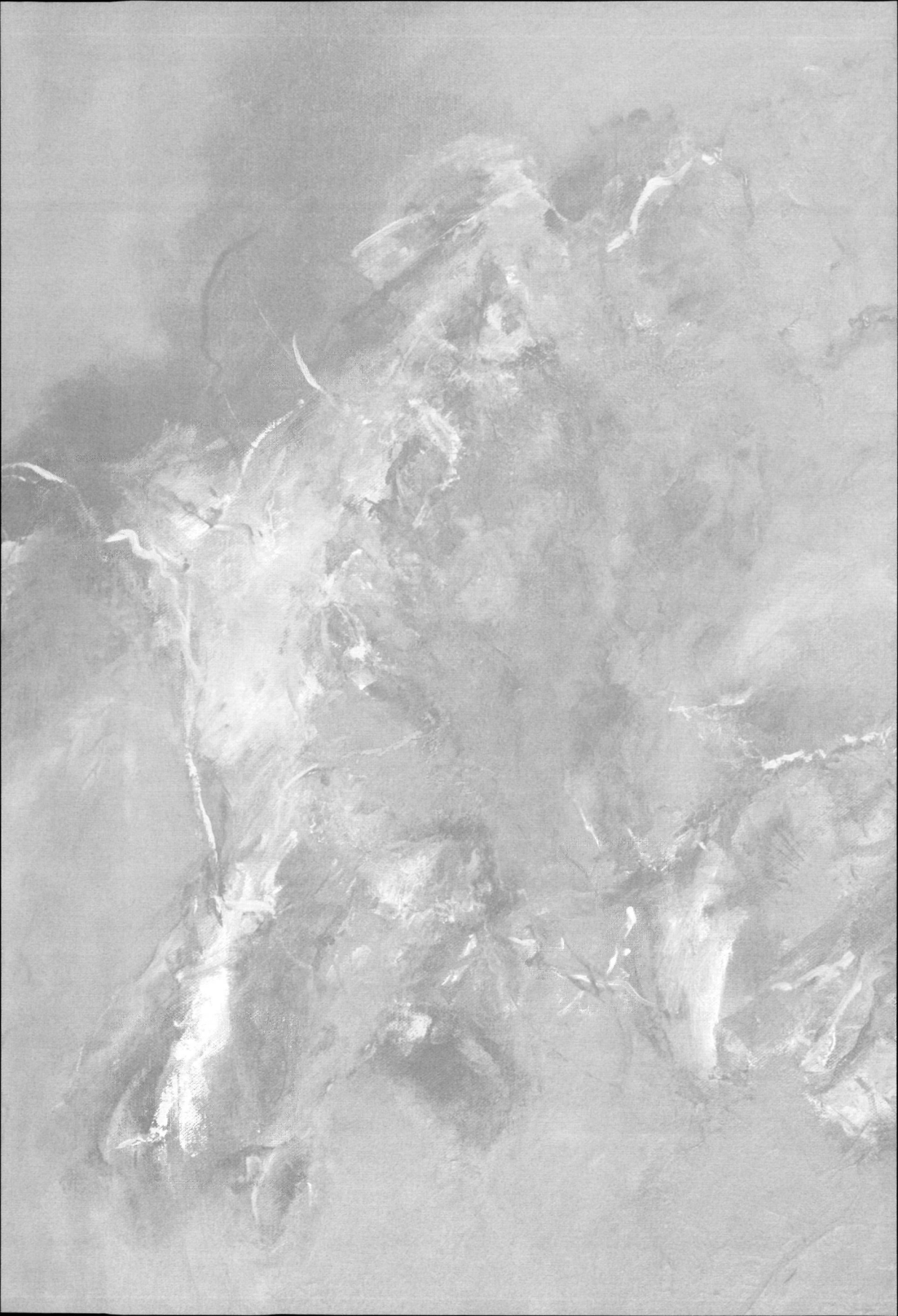
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PART I

Study design and rationale



Chapter 3

The Radboud University Nijmegen Diffusion Tensor and Magnetic resonance imaging Cohort (RUN DMC) study: a prospective cohort study: rationale and study design

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Abstract

Background Cerebral small vessel disease (SVD) is a frequent finding on CT and MRI scans of elderly people and is related to vascular risk factors and cognitive and motor impairment, ultimately leading to dementia or parkinsonism in some. In general, the relations are weak, and not all subjects with SVD become demented or get parkinsonism. This might be explained by the diversity of underlying pathology of both white matter lesions (WML) and the normal appearing white matter (NAWM). Both cannot be properly appreciated with conventional MRI. Diffusion tensor imaging (DTI) provides alternative information on microstructural white matter integrity. The association between SVD, its microstructural integrity, and incident dementia and parkinsonism has never been investigated.

Methods The RUN DMC study is a prospective cohort study on the risk factors and cognitive and motor consequences of brain changes among 503 non-demented elderly, aged between 50-85 years, with cerebral SVD. First follow up is being prepared for July 2011. Participants alive will be included and invited to the research centre to undergo a structured questionnaire on demographics and vascular risk factors, and a cognitive, and motor assessment, followed by an MRI protocol including conventional MRI, DTI and resting state fMRI.

Discussion The follow up of the RUN DMC study has the potential to further unravel the causes and possibly better predict the consequences of changes in white matter integrity in elderly with SVD by using relatively new imaging techniques. When proven, these changes might function as a surrogate endpoint for cognitive and motor function in future therapeutic trials. Our data could furthermore provide a better understanding of the pathophysiology of cognitive and motor disturbances in elderly with SVD. The execution and completion of the follow up of our study might ultimately unravel the role of SVD on the microstructural integrity of the white matter in the transition from “normal” aging to cognitive and motor decline and impairment and eventually to incident dementia and parkinsonism.

Background

Cerebral small vessel disease (SVD) includes white matter lesions (WML) and lacunar infarcts and is a frequent finding on computer tomography (CT) and magnetic resonance imaging (MRI) scans of elderly people.¹ It is associated with vascular risk factors, such as hypertension, atherosclerosis, diabetes mellitus and atrial fibrillation.^{2,4} In cerebral SVD symptoms are due to either complete (lacunar syndromes) or incomplete infarction (WML) of subcortical structures leading to accompanying complaints including the lacunar syndromes, cognitive, motor (gait) and/or mood disturbances.⁵ The prevalence of WML and lacunar infarcts varies considerably across studies from 5-95% and 8-28% respectively, depending on the population studied and the imaging technique used.^{1,6} There is evidence of an increased risk of cognitive decline, dementia, gait and balance disturbances and parkinsonism among individuals with SVD, although prospective studies are scarce.⁷⁻¹⁰

However, individuals with a virtually identical WML burden on conventional FLuid Attenuated Inversion Recovery (FLAIR) imaging present with a wide variance in cognitive and motor performance ranging from no complaints at all to subjective cognitive complaints and mild parkinsonian signs to dementia and parkinsonism. Apparently there are other factors that determine whether identical appearing WML on FLAIR lead to for example cognitive or motor decline in one person, while leaving others unaffected.

One of the other factors could be the presence the coexisting manifestations of cerebral SVD on conventional MRI such as lacunar infarcts and cerebral microbleeds which might influence the cognitive and motor performance.¹¹

As identical appearing WML on conventional MRI are actually histopathologically heterogeneous¹², it could be that only the WML with a high loss of microstructural integrity are related to cognitive and motor impairment. It is also important to realize that only a small proportion of the white matter (usually less than a few percent) is affected by SVD, even among individuals with severe SVD.¹³ As conventional MRI is not sensitive to early loss of microstructural integrity in the normal appearing white matter (NAWM), possible changes in this largest part of the white matter cannot be assessed.^{14,15} These limitations of conventional MRI can potentially be overcome with the use of Diffusion Tensor Imaging (DTI) which allows us to assess the microstructural integrity of the whole white matter.¹⁶ DTI, amongst others, provides two parameters; mean diffusivity (MD), a measure of the magnitude of diffusion of water in the white matter, and fractional anisotropy (FA), which provides information about the directionality of water diffusion. Damage to the white matter is supposedly accompanied roughly by an increase in MD and a decrease in FA.¹⁷

Another explanation for the clinical diversity due to WML could be the efficiency of compensation mechanisms that prevent further cognitive and motor (gait) deterioration. Support for the existence of compensatory mechanisms comes from a study among young carriers of a pre-senilin mutation (at risk for genetically determined Alzheimer's' disease

(AD), but still without cognitive impairment) who showed altered functional connectivity (assessed with fMRI) compared with controls.¹⁸ With innovative resting state fMRI techniques the strength of functional connectivity between brain regions can be investigated.¹⁹ In that way it might be that these compensation mechanisms also play a role in the variety of clinical presentation of individuals with SVD.

In the RUN DMC (Radboud University Nijmegen Diffusion tensor and Magnetic resonance imaging Cohort) study we prospectively investigate the effect of SVD on the transition from non-demented, independently living elderly people with cerebral SVD between 50 and 85 years towards cognitive and motor (gait) decline, and ultimately dementia and parkinsonism in a population with cerebral SVD. The primary objective of the RUN DMC study is to prospectively investigate the risk factors for and cognitive and motor (gait) consequences of longitudinal functional and structural changes in the integrity of the cerebral white matter as assessed by DTI, resting state fMRI and conventional structural MRI. To the best of our knowledge there are no other prospective cohort studies investigating the development of incident dementia and parkinsonism using these novel imaging techniques. Here we describe the study design and protocol of the RUN DMC study.

Patients and Methods

Study population

Cerebral SVD is characterized on neuroimaging by either WML or lacunar infarcts. Symptoms of SVD include acute symptoms, such as transient ischemic attack (TIA) or lacunar syndromes, but also subacute manifestations such as cognitive and motor (gait) disturbances.⁵ 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.²⁰

Accordingly, in 2006, consecutive individuals referred to the Department of Neurology between October 2002 and November 2006, were selected for possible 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 and subacute clinical symptoms of SVD were assessed by standardized structured assessments (a questionnaire for TIA and stroke²¹; for cognition the Cognitive Failures Questionnaire²²; for gait the Falls Questionnaire²³ and the Freezing of Gait Questionnaire²⁴) Subjects who were eligible because of a lacunar syndrome were included only > 6 months after the event to avoid acute effects on the outcomes.

To be able to detect incident dementia and parkinsonism we applied the following exclusion criteria: (a) presence of dementia²⁵ and (b) parkinson(-ism)^{26,27}. In addition patients with (c) intracranial hemorrhage; (d) life expectancy of less than six months; (e) intracranial space

occupying lesion, (f) (psychiatric) disease interfering with cognitive testing or follow-up, (g) recent or current use of acetylcholine-esterase inhibitors, neuroleptic agents, L-dopa or dopa-a(anta)gonists, (h) non-SVD related WML (e.g. multiple sclerosis), (i) prominent visual or hearing impairment, (j) language barrier, (k) MRI contraindications or known claustrophobia were excluded. All participants signed an informed consent form. The Medical Review Ethics Committee region Arnhem-Nijmegen approved the study.

Follow-up

After 5 and 10 years all participants alive will be contacted for the prospective assessment of possible outcome events. This evaluation is currently being prepared for July 2011.

Between 2006 and 2011 we contacted all participants every year by letter for an update on their address information and telephone number and for their survival status.

In 2011 all participants alive will be invited by letter and subsequently contacted by telephone to visit our research centre. During their visit to the research centre a cognitive, gait, balance and parkinsonian signs assessment, a structured interview, physical examination, neurological examination, and an extensive MRI protocol, an electrocardiogram and an ultrasonography of the carotid arteries will be performed. All tests will be performed by the same two trained neurology residents and all MRI scans will take place on the same scanner.

Outcome events

Primary measures of outcome of the study are incident dementia and parkinsonism according to international diagnostic criteria^{25,27}, as well as all-cause mortality and death from all vascular causes, non-fatal stroke, and non-fatal myocardial infarction.

Secondary outcome measures are defined as change from baseline examination in cognitive function, gait and balance and parkinsonian signs.

Incident outcome events are to be identified by three different approaches:

- 1 During the follow-up a structured questionnaire on the possible occurrence on these outcome events is administered to each participant. When an incident event is suspected the treating physician will be contacted for the most recent information on that particular outcome event.
- 2 When a participant died before follow-up, the general practitioner will be contacted for the most recent information on the cause of death and presence of primary outcome events. In case of presence of primary outcome events the treating physician will be contacted for the most recent information available.
- 3 When during follow-up assessment participants' test results are suggestive for incident dementia or parkinsonism, subjects will be referred to our outpatient clinic. In case the diagnosis is established according to the international criteria, this will be considered an incident case.

All outcome events will be adjudicated independently by two specialised physicians, if the two classifications differ, the outcome event will be discussed and consensus will be made

Assessment of cognitive and motor outcomes

Two trained residents in neurology will administer the complete outcome assessment

Cognitive assessment We will use an extensive neuropsychological test battery that encompasses items from other large scale epidemiological studies that cover virtually all cognitive domains^{10,28} A measurement of global cognitive function will be assessed by the Mini Mental State Examination (MMSE)²⁹ The verbal memory function will be assessed by the three-trial version of the Rey Auditory Verbal Learning Test (RAVLT), a test used to evaluate the ability to acquire and retain new verbal information³⁰ Visuospatial memory will be administered by the Rey's Complex Figure Test (RCFT), that consists of three subtasks: the copy trial, the immediate recall trial, within 3 minutes and the delayed recall trial, after 30 minutes³¹ To evaluate speed of mental processes four tests will be used, the Stroop test (three subtasks)³², the Paper and Pencil Memory Scanning Task (four subtasks)³³, the Symbol-Digit Substitution Task, which is a modified version of the Symbol Digit Modalities Test³⁴ and a verbal fluency task in which as many animals as possible have to be named within 60 seconds, followed by as many professions within 60 seconds To evaluate attention, the verbal series attention test (VSAT) will be used³⁵ To register subjective cognitive failures we will administer the Cognitive failures questionnaire (CFQ)²² The tests will be carried out in quiet rooms and a stopwatch will be used in timed tests

Assessment of gait, balance and parkinsonian signs All participants will perform a tandem walk by walking ten steps heel to toe (registering intact, one side step, more side steps, impossible) A quantitative gait analysis will be performed with a 5.6-meter long, 0.89-meter wide electronic walkway (GAITrite® MAP/CIR Inc., Havertown, PA) with sensor pads (12.7 mm apart from each other) connected to a computer This system has strong concurrent validity and test-retest reliability, also in older people³⁶ The participants walk twice at self-selected gait speed on low-heeled shoes They start two meters before the carpet and walk until two meters behind it in order to measure steady-state walking

We will use a widely used modified version of the original Tinetti test with 17 items: 9 for body balance (score 0-16) and 8 for gait (score 0-12), with a maximum score of 28³⁷ It grades balance while sitting, standing with eyes open and closed, nudging and turning, gait initiation, stride length and width and symmetry Functional mobility will be classified by using the widely-used TUG-test which is a timed test during which the participant is asked to rise from a standard armchair, walk 3 m, turn, walk back and sit down again³⁸ Each participant will perform the test three times To evaluate parkinsonian signs we apply the Unified Parkinson's Disease Rating Scale (UPDRS), the motor score³⁹ Finally disease severity

will be assessed with the Hoenhn and Yahr stage assessing⁴⁰ For the evaluation of gait and balance we will also administer the Freezing of gait questionnaire (FOG), a questionnaire consisting of 16 items regarding gait and falls and the Falls questionnaire^{23 24}

Assessment of other variables

Assessment of activities of daily living As a measure of disability the Barthel Index will be used⁴¹ The activities of daily living will be assessed by the instrumental activities of daily living questionnaire⁴²

Demographics and life style Standardized questionnaires on demographics, education (classified using 7 categories, 1 being less than primary school and 7 reflecting an academic degree)⁴³, marital status, living conditions, and life style habits (alcohol consumption, smoking, exercise) will be administered Alcohol consumption is defined as units per day and the age at which alcohol consumption had started (and if stopped) was noted Cigarette smoking behaviour is defined as the number of pack-years, calculated as the number of packs of cigarettes smoked per day multiplied by the number of years a participant had smoked Exercise is expressed in the metabolic equivalent value (MET) according to accepted standards, where 1 MET is proportional to the energy expended while sitting quietly⁴⁴

Vascular risk factors and cardiovascular disease With the aid a of structured, standardized questionnaire each participant will be asked for a history of hypertension, diabetes mellitus, atrial fibrillation, TIA, stroke, myocardial infarction, coronary artery bypass graft, per-cutaneous transluminal coronary angiography, aortic prothesis, vascular prothesis, carotid endarterectomy^{2 4 21} and migraine⁴⁵ The presence of a family history of myocardial infarction, cerebrovascular disease and diabetes mellitus in next of kin will be recorded

Current medication Current medication use will be noted and classified according to the Anatomical therapeutic chemical (ATC) classification system (World Health Organization, WHO Collaborating Centre for drug statistics and methodology, <http://www.whocc.no/atcddd/>)

Depressive symptoms A standardized structured questionnaire used in previous large scale epidemiological studies will be used to assess for the history of depressive symptoms, normal reactions to stressful events or normal grief will carefully be excluded⁴⁶ In case of a depressive episode, age of onset, the medical advice and medication use will be registered We defined 'depression' as those depressive episodes that have required attention of a general practitioner, psychologist, or psychiatrist This definition includes minor depression, as well as more severe depression syndromes such as major depression and bipolar depression⁴⁶

In addition participants will be screened for depressive symptoms by means of the Mini International Neuropsychiatric Interview (MINI), part A, which is a short diagnostic structured interview based on the DSM IV⁴⁷ Additionally, presence of actual depressive symptoms will be assessed by two self report questionnaires, the Center of Epidemiologic Studies Depression Scale (CES-D)⁴⁸ and the Hospital Anxiety and Depression Scale (HADS)⁴⁹

Sleep problems For the assessment of sleep disorders we will use the SCOPA-Sleep scale⁵⁰ and for fatigue the Checklist on Individual Strength (CIS20R)⁵¹

Quality of life The overall health status (quality of life) will be assessed with the Short Form 36 (SF-36)^{52 53}

Physical Examination Height and weight will be measured without shoes in light clothing The body mass index (BMI) is calculated as weight divided by height (in meters) squared The maximal waist circumference will be measured without shirt, in standing position, between the lowest rib and the iliac crest, at the end of normal expiration⁵⁴ Blood pressure and pulse rate will be measured in triplicate in supine position after 5 minutes rest Subsequently one measurement is performed after 1 minute in upright position³

Primary reflexes The presence of the glabella, snout and grasp reflex, the applause sign⁵⁵ and the plantar response will be registered

Muscle strength The strength of the biceps, hand grip, iliopsoas, quadriceps and foot extensor muscles on both sides will be measured by the medical research council scale (MRC) and by a dynamometer (Citec® hand-held dynamometer)⁵⁶

Sensory system will be assessed by a quantitative measurement by vibration tuning fork (Rydel-Seiffer®) on both first toes and both medial malleolus, also registering ankle oedema and ankle jerks

MRI scanning and processing

MRI scanning will be performed on a 1.5-Tesla Magnetom scanner (Siemens, Erlangen, Germany) The scanning protocol includes whole brain 3D T1 magnetization-prepared rapid gradient-echo (MPRAGE) sequence (TR/TE/TI 2250/3 68/850ms, flip angle 15°, voxel size 1 0x1 0x1 0mm), FLAIR pulse sequences (time repetition [TR]TE/TI 9000/84/2200 ms, voxel size 1 0x1 2x6 0mm (including slice gap of 1 mm), transversal T2* weighted gradient echo sequence (TR/TE 800/26ms, voxel size 1 3x1 0x6 0mm (including slice gap of 1 0 mm), DTI (TR/TE 10100/93ms, voxel size 2 5x2 5x2 5mm, 4 unweighted scans, 30 diffusion weighted scans, with non co-linear orientation of the diffusion-weighting gradient, and b value 900s/

mm²) and resting state imaging using a gradient echo EPI (TR/TE 2400/40ms, voxel size 3.5x3.5x4 mm (including slice gap of 0.4mm)) During resting state, subjects will be told not to concentrate on any particular subject, but just to relax with their eyes closed. The complete scanning protocol takes 31 minutes.

White matter lesions All images will be evaluated without prior notice of any clinical parameter. WML are 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 WML.⁵⁷ Total WML volume is calculated by an in-house developed, validated technique.

Brain volumetry Normalization parameters to the ICBM152 linear template (as provided with SPM5, Wellcome Department of Cognitive Neurology, University College London, UK) and gray and

white matter tissue and cerebrospinal fluid probability maps is computed by using SPM5 unified segmentation routines on the T1 MPRAGE images.⁵⁸ Total grey and white matter volumes are calculated by summing all voxel volumes that have a $p > 0.5$ for belonging to the tissue class. Total brain volume is taken as the sum of total grey- and total white matter volume. Co-registration parameters of the FLAIR image to the T1 image are computed (SPM5 mutual information co-registration) and used to bring both the FLAIR and WML segmentation images into the subject's (anatomical) reference frame. Transformed images will visually be checked for co-registration errors. Subsequently, the WML segmentations are resampled to and combined with the white matter maps to yield to a WML map (the intersection of WML and white matter) and NAWM map (the complement of WML in white matter) in the T1 reference space. Total brain volume is taken as the sum of total gray and white matter.

Lacunar and territorial infarcts Lacunar infarcts are defined as hypo-intense areas > 2 mm and ≤ 15 mm on FLAIR and T1, ruling out enlarged perivascular spaces (≤ 2 mm, except around the anterior commissure, where perivascular spaces can be large) and infraputamina pseudolacunae.^[57] Territorial infarcts are defined as hypointense lesions on FLAIR and on T1 images > 15 mm.⁵⁷

Microbleeds Microbleeds are defined as small, homogeneous, round foci of low signal intensity on T2* weighted images of less than 10 mm in diameter.⁵⁹ Microbleeds are counted per hemisphere separately. In addition they are classified as cortical/subcortical including the periventricular white matter and deep portions of the centrum semiovale (frontal, parietal, occipital and temporal separately), in the basal ganglia, including caudate nucleus, internal and external capsule, globus pallidus, thalamus and putamen, infratentorial including the

cerebellar hemispheres, pons and medulla oblongata⁵⁹ Lesions are not considered to be microbleeds when they are symmetric hypointensities in the globus pallidus, most likely calcifications or iron deposits, flow voids artifacts of the pial blood vessels or hyposignals in T2* inside a lesion compatible with an infarct, likely to be hemorrhagic transformation⁵⁹

Diffusion tensor imaging The diffusion weighted images of each participant are realigned on the unweighted image using mutual information based Matlab (The Mathworks, Inc) routines from SPM5 Then, the diffusion tensor and its eigenvalues are computed using an SPM5 add-on ([http //sourceforge net/projects/spmtools](http://sourceforge.net/projects/spmtools))⁶⁰ Unphysical spurious negative eigenvalues of the diffusion tensor were set to zero, after which the tensor derivatives the FA and MD are calculated⁶¹ The mean unweighted image is used to compute the co-registration parameters to the anatomical T1 image (SPM5 mutual information co-registration), which are then applied to all diffusion weighted images and results All images are visually checked for motion artefacts and coregistration errors

Electrocardiogram

An electrocardiogram (ECG) will be performed and evaluated by a standardized assessment by an experienced cardiologist, registering frequency, cardiac rhythm, cardiac ectopias, cardiac axis, conduction time over the PQ, QRS and QTC intervals, conduction disturbances, left ventricle hypertrophy, pathologic Q's, infarction, repolarisation disturbances and acute ischemia A final diagnosis is defined as normal, abnormal without clinical significance, abnormal with clinical consequences or pathologic ECG with immediate consultation of a cardiologist when necessary

Ultrasonography of the carotid arteries

All ultrasound measurements will be performed by three experienced and specific trained clinical neurophysiology technicians A carotid ultrasound assessment at which the intima media thickness (IMT) is measured in the distal left and right carotis communis, near the bulbous, will be performed All measurements will be performed using a phased array real-time scanner (Philips i-u22, The Netherlands) with a 17-5 MHz broadband linear transducer Two-dimensional ultrasound imaging of the carotid artery will be performed to measure the IMT The IMT will be automatically measured by QLab® qualification software (V 4 2 1) An edge detection algorithm identified the lumen/intima and the media/adventitia interfaces within a region of interest over a 10mm long segment and calculated the average thickness⁶²

The same cognitive, motor, gait and balance assessment, structured interview and assessment of other variables and the same ancillary investigation were performed at baseline in 2006

Statistical analysis

Sample size calculation. Based on the literature we expect about 60 incident dementia cases during the five year follow up (absolute risk 4-5%/year), as about half of our study population has a relatively high degree of WML.⁶³ We expect that each SD increase in MD increases this absolute risk of dementia by 2% per year. To detect this increased risk with a high probability of 90% at the 5% significance level we will need 380 participants at the end of the follow up, so therefore we included 500 participants at baseline and hope to end up with 400 participants at follow-up protocol (taking into account an expected loss to follow up of about 20%).

Analysis of primary outcome measures. We will analyze mean baseline MD and FA and change in MD and FA on follow up imaging in relation to incident dementia and parkinsonism by Cox proportional hazard models adjusted for age, sex, education, depressive symptoms, total brain volume, white matter lesion volume and lacunar infarcts, where appropriate.

Discussion

The RUN DMC study is a large prospective cohort study on causes and consequences of structural and functional changes in the integrity of the cerebral white matter (in both the WML and the NAWM) as assessed by conventional MRI as well as new techniques, such as DTI and resting state fMRI, among elderly with cerebral SVD, starting to include participants for the follow-up protocol in July 2011.

Numerous studies have shown that WML observed on conventional MRI are related to vascular risk factors and have reported associations with cognitive and motor decline and found these relations to be rather weak.^{3 4 8 10,46} To the best of our knowledge there are no prospective cohort studies on individuals with cerebral SVD investigating the development of incident dementia and parkinsonism in relation to white matter changes assessed by DTI and resting state fMRI.

Strengths of the RUN DMC study include the prospective fashion of the study in which all vascular risk factors, clinical and imaging measures will be followed up after five years, and the large and well-established protocol used to explore demographics, vascular risk factors, and cognitive and motor function. The tests chosen are furthermore widely accepted and have been proven specific and sensitive in this population with structural brain changes.

Another strength is the fact that it is a single centre study. Moreover, the complete study protocol will take place in one research centre with the use of a single scanner and only two investigators performing all investigations.

In conclusion, the RUN DMC study has the potential to further unravel the causes and consequences of changes in white matter integrity in elderly with cerebral SVD by using

new imaging techniques, DTI and resting state fMRI. When proven, changes in white matter integrity assessed by these techniques might function as a surrogate endpoint for cognitive and motor function in future therapeutic trials of vascular risk factors in SVD.

The execution and completion of the follow-up of our study will ultimately unravel the role of SVD on the microstructural integrity of the white matter in the transition from “normal” aging to cognitive and motor decline and impairment and eventually to incident dementia and parkinsonism.

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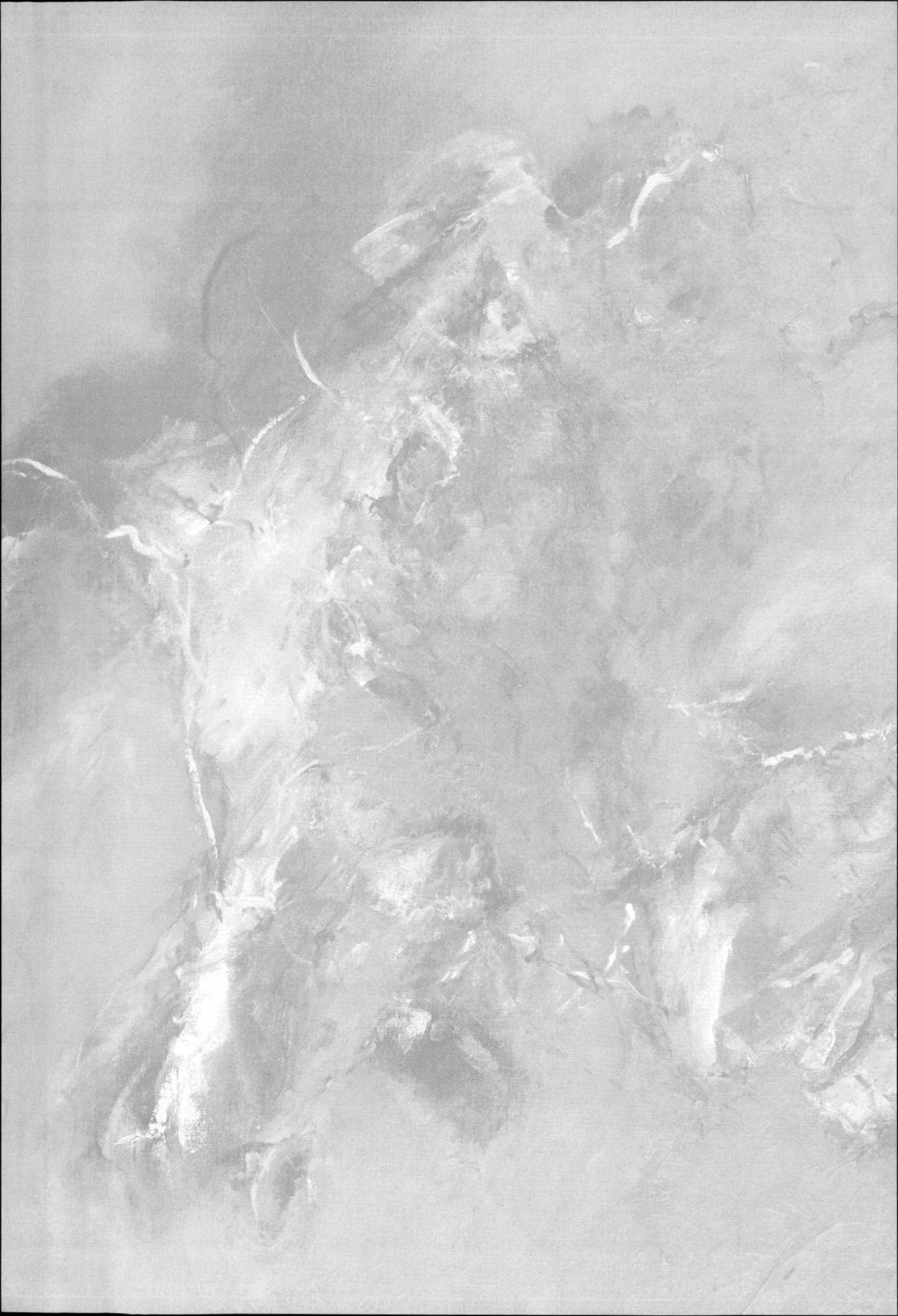
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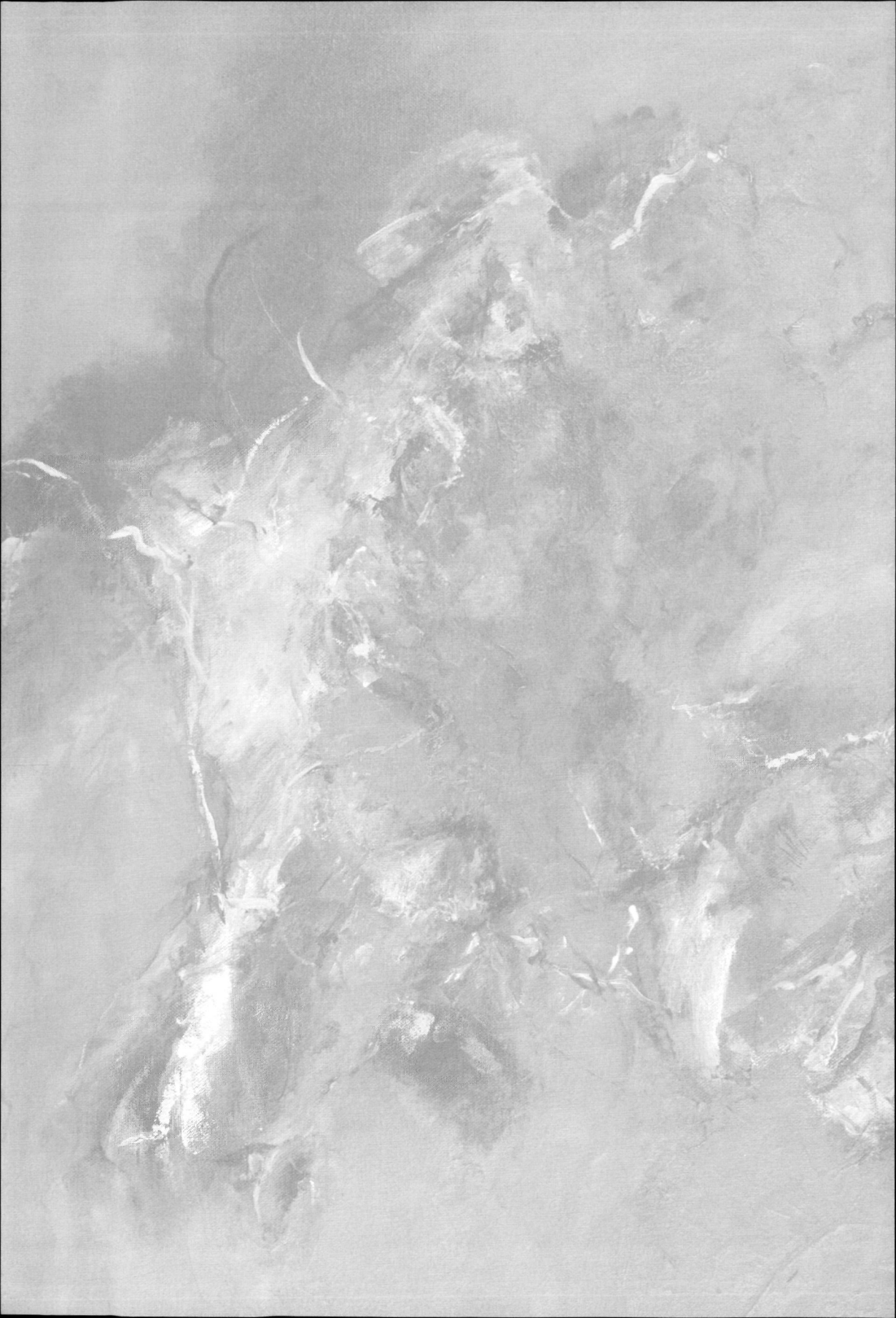
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PART II

A conventional MRI approach



Chapter 4

Presence, number and location of cerebral microbleeds are associated with cognitive function

Published as:

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Abstract

Background Cerebral small vessel disease (SVD), including white matter lesions (WML) and lacunar infarcts, is related to cognitive impairment. Cerebral microbleeds (MB) are increasingly being recognized as another manifestation of SVD and are also related to cognitive function. However, it remains unclear whether this relation is independent of WML and lacunar infarcts, and if location of MB plays a role. We investigated the relation between the presence, number, and location of MB and cognitive performance, adjusted for WML and lacunar infarcts.

Methods Presence, number and location of MB were rated on a gradient echo T2*-weighted MRI in 500 non-demented elderly with SVD. Cognitive performance was assessed in different domains. Analyses were adjusted for age, sex, education, depressive symptoms, total brain volume, WML volume, lacunar and territorial infarcts.

Results Mean age was 65.6 years (SD 8.8) and 57% were male. MB were present in 10.4% of the participants. Subjects with MB were significantly older, had a higher WML volume and more lacunar infarcts ($p < 0.01$). Presence and number of MB were related to global cognitive function ($\beta = -10, p = 0.08$, $\beta = -20, p = 0.02$), psychomotor speed ($\beta = -10, p = 0.12$, $\beta = -19, p = 0.06$) and attention ($\beta = -10, p = 0.2$, $\beta = -205, p = 0.01$). The relations with cognitive performance were mainly driven by frontal, temporal and strictly deep located MB.

Conclusions Frontal and temporal located MB correlate with cognitive performance in non-demented elderly independent of coexisting other SVD related lesions. MB are clinically not silent and may help to understand the role of vascular disease in cognitive decline.

Introduction

Cerebral small vessel disease (SVD) related lesions, including white matter lesions (WML) and lacunar infarcts, are common in the elderly. Cerebral SVD is an important cause of cognitive impairment in elderly, eventually leading to dementia in some.^{1,2} Cerebral microbleeds (MB) are increasingly recognized as another manifestation of SVD.^{3,4} Radiologically they are characterized as small, homogeneous, round foci of low signal intensity on gradient echo (GRE) T2* sequences. Histopathological analysis shows that these are perivascular collections of hemosiderin deposits.⁵ Accumulating evidence suggests that the distribution of MB may reflect the underlying pathological changes, lobar MB are presumably attributable to cerebral amyloid angiopathy (CAA), whereas MB in the deep/infratentorial regions are considered to represent hypertensive microangiopathy.^{4,6,7}

Although considered to be clinically silent, MB have been related to cognitive impairment.^{8,10} This has predominantly been investigated in memory clinic patients, usually without taking location of MB into account and without quantitative WML adjustment.¹¹ As MB virtually always coexist with WML and lacunar infarcts, its relation with cognitive performance should be assessed independent of manually segmented WML and number of lacunar infarcts, at different lobar and deep locations. Due to the typical profile of 'subcortical' cognitive impairment in patients with SVD with psychomotor slowing due to impaired executive function, deficits of attention, planning and set-shifting, and episodic memory disturbances¹², we hypothesized that MB in frontal and temporal regions have the strongest relation with cognitive function. Therefore we wanted to investigate the relation between the presence, number, and location of MB and cognitive performance, independent of coexisting WML and lacunar infarcts.

Materials and Methods

Study population

The Radboud University Nijmegen Diffusion tensor and Magnetic resonance Cohort (RUN DMC) study prospectively investigates the risk factors and clinical consequences of brain changes as assessed by MRI among 503, 50-85 year old non-demented elderly with cerebral SVD.¹³

On the basis of established research criteria SVD was defined as the presence of lacunar infarcts and/or WML.¹⁴ Symptoms of SVD include acute symptoms, such as TIAs or lacunar syndromes, or subacute manifestations, such as cognitive, motor (gait) disturbances and/or depressive symptoms.¹² 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 features¹⁴ Accordingly, 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 (WML and/or lacunar infarcts)¹²

Exclusion criteria were (a) dementia¹⁵ (b) parkinson(-ism)¹⁶ (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/current use of acetylcholine-esterase inhibitors, neuroleptic agents, L-dopa or dopa-a(anta)gonists, (g) WML or SVD 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 for participation by a three step approach After reviewing medical records, 1004 individuals were invited by letter, 727 were eligible after contact by telephone of whom 525 agreed to participate During these three steps, patients excluded because of dementia were diagnosed according to the Diagnostic and Statistical Manual for Mental Disorders, version IV¹⁵, with formal cognitive assessment First, patients were excluded based on the clinical information and cognitive assessment obtained by reviewing the medical records Second, during contact by telephone, patients were excluded when a close informant told us that the eligible person was admitted to a nursing home because of dementia, diagnosed by a neurologist or geriatrician Third, during their visit to our research center, all subjects underwent extensive cognitive assessment and questionnaires about their social functioning, they were excluded because of dementia if they fulfilled the previously mentioned criteria (n=4) In 18 individuals one of the other exclusion criteria were found during their visit to our research center, yielding a response of 71.3% (503/705) All participants signed informed consent The Medical Review Ethics Committee region Arnhem-Nijmegen approved the study

MRI

All subjects underwent a 1.5T MRI The protocol included, among other sequences, 3-D T1 MPRAGE (TR/TE/TI 2250/3.68/850ms, flip angle 15°, voxel size 1.0x1.0x1.0mm), FLAIR pulse sequences (TR/TE/TI 9000/84/2200ms, voxel size 1.0x1.2x5.0mm, interslice gap 1mm) and transversal T2*-weighted gradient echo sequences (TR/TE 800/26ms, voxel size 1.3x1.0x6.0mm, interslice gap 1mm)⁷

MRI analysis

White matter lesions, lacunar and territorial infarcts White matter signal hyperintensities on FLAIR images, which were not, or only faintly, hypo-intense on T1-weighted images, were considered WML, except for gliosis surrounding infarcts¹⁷ WML were manually segmented on FLAIR images by two trained raters Total WML volume was calculated by summing the segmented areas multiplied by slice thickness Lacunar infarcts were defined as hypo-intense

areas with a diameter $>2\text{mm}$ and $<15\text{mm}$ with low signal intensity on T1 and FLAIR, ruling out enlarged perivascular spaces and infraputaminar pseudolacunae.¹⁷ All imaging analyses were performed by raters blinded to clinical information. In a random sample of 10%, inter-rater variability for total WML volume yielded an intra-class correlation coefficient of 0.99; intra- and inter-rater reliability for the lacunar infarcts yielded a weighted kappa of 0.80 and 0.88. Territorial infarcts are defined as hypointense lesions on FLAIR and on T1 images $>15\text{mm}$.¹⁷

Microbleeds. MB are defined as small, homogeneous, round focal areas of very low signal intensity on T2*-weighted images of less than 10 mm in diameter.^{4,8} They were categorized in lobar (cortical gray and subcortical or periventricular white matter), deep (deep gray matter, including the basal ganglia and thalamus, the white matter of the corpus callosum, internal, external and extreme capsule), and infratentorial (brainstem and cerebellum).^{4,8} Lesions are not considered to be MB when they are symmetric hypointensities in the globus pallidus, most likely calcifications or iron deposits, flow voids artifacts of the pial blood vessels or hyposignals in T2* inside a lesion compatible with an infarct, likely to be hemorrhagic transformation.^{4,8} MB were rated by two trained raters, in a 10% random sample, intra- and interrater reliability yielded an intraclass correlation coefficient of 0.79 and 0.99 for total number of MB and 0.94 and 1.00 for individual locations.

Total brain volume and intracranial volume. Gray (GM) and white matter (WM) tissue and cerebrospinal fluid (CSF) probability maps were computed using SPM5 unified segmentation routines on the T1 MPRAGE images (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. Total brain volume (TBV) was taken as the sum of total GM and WM. Intracranial volume (ICV) was a summation of all tissue classes, i.e. total GM, WM and CSF volume. To normalize for head size, TBV was expressed as percentage of total ICV.

Measurement of cognitive function

Cognitive function was measured with a neuropsychological test battery that proved to be sensitive and suitable for this purpose in other, large epidemiological studies.¹ The tests used are described in detail elsewhere.¹³ In short, we calculated compound scores for seven cognitive domains. Global cognitive function was evaluated by the Mini Mental State Examination 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 Rey Auditory Verbal Learning Test (RAVLT) and the delayed recall of this last test.¹ Verbal memory

is a compound score of the mean of two z-scores from the RAVLT, one for the added scores of the three learning trials of this test, and one for the delayed recall of this test. Visuospatial memory is 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. 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.¹ Fluency was calculated from the mean of the z-scores of both verbal fluency tasks. Concept shifting was calculated as the z-score of the third subtask of the Stroop. Attention is a compound score of the z-score of the total time of the Verbal Series Attention Test.¹

Other measurements

Age, sex, education, depressive symptoms, WML volume, lacunar infarcts, territorial infarcts and normalized TBV were considered possible confounders. Depressive symptoms were present if a subject had a score ≥ 16 on the Center of Epidemiologic Studies on Depression Scale (CES-D) and/or the present use of anti-depressive medication.¹⁸

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 $\geq 140/90$ mmHg and/or use of anti-hypertensive medications), diabetes (treatment with antidiabetic medications), hypercholesterolemia (treatment with lipid-lowering drugs) and smoking status.

Statistical Analysis

Baseline characteristics were presented as means \pm standard deviation (SD) and for the skewed distributed parameters the median and interquartile range was calculated. Baseline characteristics were compared between subjects with and without MB by age- and sex-adjusted ANCOVA.

The relation between the presence and number of MB and cognitive performance was investigated using multiple linear regression analysis adjusted for age, sex, education, depressive symptoms, WML volume, number of lacunar infarcts, territorial infarcts and TBV normalized for head size.¹³

Second, the relation between the location of MB and cognitive performance was studied both in the previously described model and next, with adjustments for MB at other locations. Bonferroni corrections were applied.

The relation between location and cognitive performance was also investigated with the subjects divided into 4 groups (no MB, strictly lobar, deep/infratentorial and mixed) using analysis of covariance adjusting for the same covariates.

Table 1 Baseline Characteristics

Participants	n=500	Microbleeds		p-value‡
		Yes (n=52)	No (n=448)	
Demographics				
Age, yr	65.6 (8.8)	69.8 (8.0)	65.1 (8.7)	< .001
Male*	284 (56.8)	32 (61.5)	252 (56.2)	.47
MMSE	28.1 (1.6)	28.0 (1.7)	28.1 (1.6)	.63
Only primary education*	49 (9.8)	4 (7.7)	45 (10.0)	.18
Depressive symptoms*	168 (33.6)	19 (36.5)	149 (33.3)	.64
Hypertension*	367 (73.4)	43 (82.7)	324 (72.3)	.57
Diabetes mellitus*	74 (14.8)	7 (13.5)	67 (15.0)	.50
Hypercholesterolemia*	236 (47.2)	26 (50.0)	210 (46.9)	.98
Smokers, current*	75 (15.0)	6 (11.5)	69 (15.4)	.76
Smokers, former*	277 (55.4)	33 (63.5)	244 (54.5)	.67
Neuroimaging				
TBV, mL	1093.7 (120.8)	1108.0 (127.9)	1092.0 (120.1)	.25
ICV, mL	1677.6 (156.7)	1709.2 (178.9)	1672.8 (153.3)	.09
WML volume, mL	7.1 (3.4, 18.1) [†]	25.2 (12.1, 45.5)	6.4 (3.3, 15.1)	< .001
Lacunar infarcts*	171 (34.2)	38 (73.1)	133 (29.7)	< .001
Territorial infarcts*	59 (11.8)	6 (11.5)	53 (11.8)	.52

Data represent N of subjects*(%), mean(SD), or median (interquartile range)[†]

‡Analysis of covariance, adjusted for age and sex

MMSE Mini Mental State Examination, TBV total brain volume, ICV intracranial volume, WML white matter lesions

Results

For the present study three subjects were excluded because of MRI artifacts, resulting in a final study population of 500 subjects. MB were present in 10.4% of the population, 48.1% had one MB, 21.1% had two MB, 15.4% had 3 to 5 MB and 15.4% had >5 MB. Thirty-one (59.6%) individuals had strictly lobar MB, seven (13.5%) had isolated deep/infratentorial MB. Subjects with MB were significantly older ($p < .001$), had a higher WML volume ($p < .001$) and a higher proportion of subjects with MB had lacunar infarcts compared to those without MB ($p < .001$) (Table 1).

The presence of MB was related to the cognitive index, psychomotor speed and attention. A higher number of MB was independently related to a lower performance on cognitive performance (Table 2).

Table 2 Relation between presence and number of microbleeds (MB) and cognitive performance

	Presence of MB	Number of MB
MMSE	- .03	- .05
Cognitive index	-.10 **	- .20 **
Verbal memory	- .08	-.17*
Visuospatial memory	- .07	-.12
Psychomotor speed	- .10*	- .19 **
Fluency	-.01	- .13
Concept shifting	-.06	- .09
Attention	- .10*	- .25 **

Data are standardized β values, adjusted for age, sex, education, depressive symptoms, WML volume, lacunar infarcts, territorial infarcts and TBV

* $p < .05$, ** $p < .01$

The relation between MB and cognitive performance was mainly driven by frontal and temporal located MB. Deep MB were related to global cognitive function, psychomotor speed and attention. (Table 3) Additional adjustment for MB at other locations did not alter the magnitude of the relations.

Only those with strictly deep and mixed MB performed worse on global cognitive function ($p=.013$), psychomotor speed ($p=.002$) and attention ($p=.001$) compared to those without MB.

Table 3 Relation between location of microbleeds and cognitive performance

Microbleeds		Cognitive performance							
		Global cognitive function		Memory function		Executive function			
		N	Cognitive index	Verbal memory	Visuospatial memory	Psychomotor speed	Fluency	Concept shifting	Attention
Location	osubjects	MMSE							
Lobar	45	- .06	- .17*	-.16*	- .17*	- .16*	- .13	- .09	-.24*
Frontal	23	- .04	-.18*	- .14*	-.26*	- .18*	-.12	-.06*	- .30*
Parietal	20	-.02	- .13*	-.10	- .10	-.12*	-.04	- .05	- .08
Occipital	13	-.05	- .13	-.12	- .08	-.10	-.13	- .04	- .03
Temporal	16	- .09	- .14*	-.14*	- .19*	-.11	-.14	- .13	-.22*
Deep	13	- .08	-.14*	- .11	-.06	- .13*	-.10	-.04	-.28*
Basal ganglia	8	-.02	- .08	-.06	- .04	-.06	- .06	-.02	-.35*
Thalamus	8	- .06	- .08	- .08	- .05	-.05	-.02	-.01	-.11
Infratentorial	2	-.05	- .08	-.08	- .03	- .06	- .05	-.03	-.06

Data are standardized β values, adjusted for age, sex, education, depressive symptoms, WML volume, lacunar infacts, territorial infacts and TBV

* Significant after Bonferroni correction $p < .05$

Discussion

The presence and number of MB, especially those located in the frontal and temporal lobe, but also strictly deep located MB, interfere with cognitive performance, independent of other manifestations of SVD

The relation between MB and cognitive performance has been demonstrated before in population based cohorts, memory clinic cohorts and a cohort from a neurovascular clinic¹¹ Strong elements of our study are that we examined for the first time the effect of MB at different lobar and at deep locations, independent of quantitatively assessed WML, rather than a semi-quantitative assessment, in which a ceiling effect of this other parameter of SVD could result in residual confounding Furthermore we extensively adjusted for other possible confounders We intentionally did not adjust for vascular risk factors such as hypertension as they were considered to be an earlier part of the causal chain between MB and cognitive performance Finally, other major strengths of our study included the large sample size, the extensive assessment of cognitive function, the single centre design, the use of a single scanner use and the high response rate

A limitation is the cross-sectional nature of the study, which prevented us from proving causality. Furthermore, MRI techniques on the detection of MB have improved, which may have resulted in underestimation of the actual number of MB in our population, which may have affected the effect size, but not the association with cognitive performance.³ However, a recent study on the clinical relevance of the improved detection techniques for MB in terms of associations with clinical characteristics, vascular risk factors and other MRI markers of SVD, did not find stronger relations (or more variance explained) between cognition and MB detected with susceptibility-weighted imaging (SWI) compared to T2*-weighted imaging¹⁹ We found that MB were related to cognitive performance, independent of WML and lacunar infarcts Furthermore location played a role in the associations These findings, together with results from pathological studies showing that MB are frequently characterized by surrounding microstructural damage²⁰, suggest that they have direct effect on cognitive performance rather than simply reflecting the presence of other markers of SVD, as our findings are independent of WML volume and lacunar infarcts

It is hypothesized that the distribution of MB reflects the underlying etiology Lobar MB have been attributed to CAA, whereas MB in the deep/infratentorial regions (with or without lobar MB) have been associated with hypertensive microangiopathy.^{4 6 7} We found that the relation between MB and lower cognitive performance was mainly driven by MB located in the frontal and temporal lobes A recent report on the distribution of lobar MB taking lobar volumes and clustering effects into account found that lobar MB are significantly more often located in the temporal lobe.²¹ This suggests that the relation observed between temporal located MB and lower cognitive performance might, at least in part, be explained by the higher number of MB in this region, compared to other regions if one would take the volume

of the temporal lobe into account. As the temporal lobe is known to be more affected by CAA, our findings are therefore suggestive of CAA as underlying etiology. However, we also found a relation between the strictly deep located MB and a lower cognitive performance, favoring multiple causes rather than a single cause for cognitive impairment in patients with MB (and SVD).

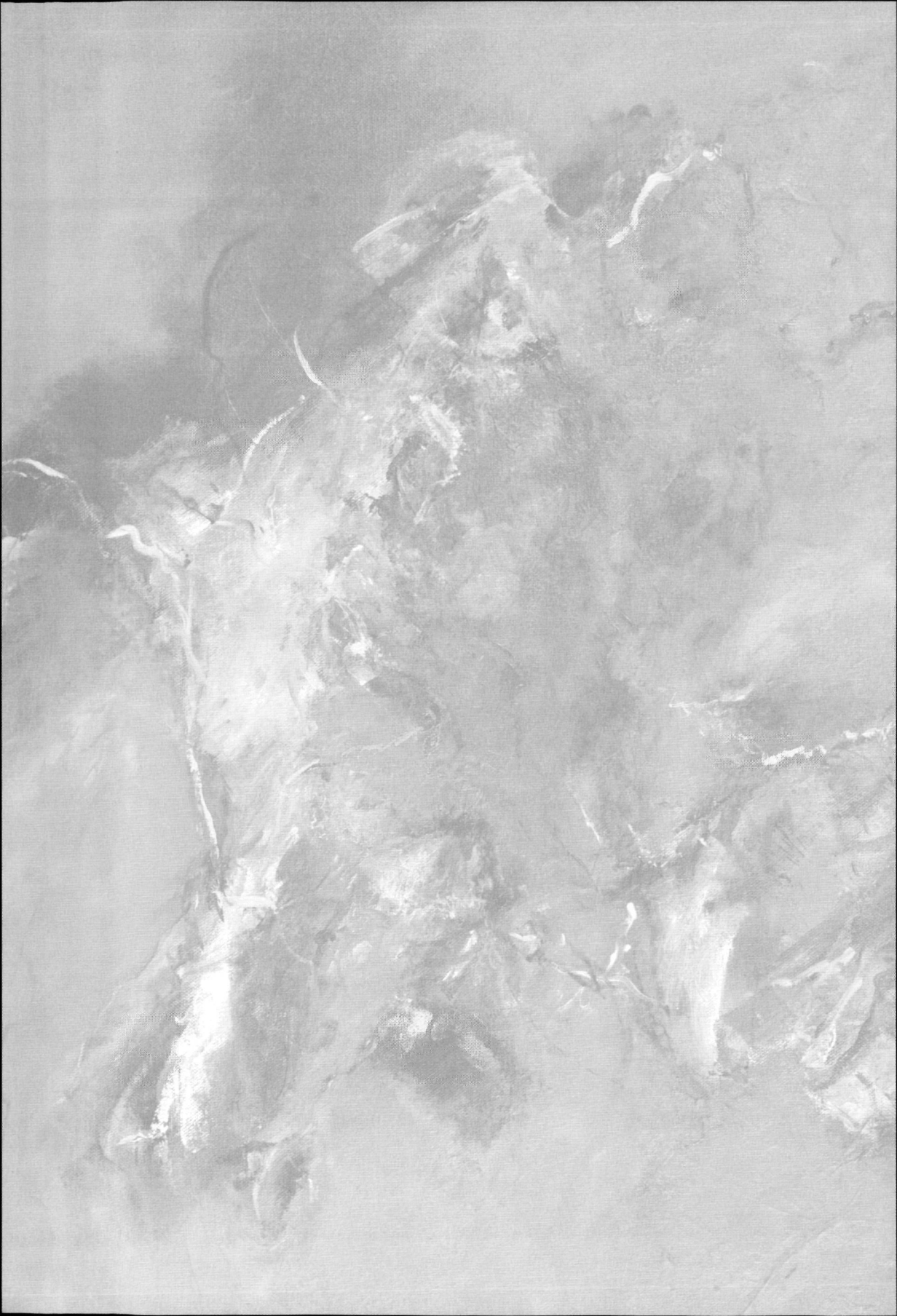
The underlying mechanisms of the pathologic association between MB and cognitive function are unknown. However, histopathologic studies have shown that the presence of MB indicate widespread damage of arterioles by hypertension or by amyloid deposition, as well as surrounding gliosis or even frank necrosis or infarction, resulting in microstructural damage of the surrounding white matter.^{5,20} In this way MB may disrupt white matter tracts relevant for cognitive function leading to damage to the neural networks, superimposed to the effects of often co-occurring WML and lacunar infarcts. This tissue damage, not visible on conventional MRI, can be assessed in future studies with rather new techniques such as diffusion tensor imaging or resting state MRI.

In conclusion, we found that presence, number of cerebral MB, independent of coexisting WML and lacunar infarcts, correlate with cognitive performance in non-demented elderly, this relation is mainly driven by the frontal and temporal located MB, but also by strictly deep located MB. These results suggest that MB are clinically not as silent as they are considered to be and in that way might help us to understand the role of vascular disease in cognitive decline. Follow-up should identify whether the presence at baseline and/or increase of MB over time predict future cognitive decline and development of dementia, and whether location of these MB plays a role in this relation.

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Chapter 5

Presence, number and location of cerebral microbleeds are associated with subjective cognitive complaints

Submitted as:

van Norden AGW, de Laat KF, Gons RAR, Kessels RPC, van Dijk EJ, de Leeuw F-E. Cerebral microbleeds are related to subjective cognitive failures. The RUN DMC study.

Abstract

Background Cerebral small vessel disease (SVD), including white matter lesions (WML) and lacunar infarcts, is related to objective cognitive impairment but also to subjective cognitive failures (SCF). SCF have reported to be an early predictor of dementia. Cerebral microbleeds (MB) are another manifestation of SVD and have been related to cognitive impairment, but the role of MB in SCF has never been studied. We therefore investigated whether MB are related to SCF among non-demented elderly with SVD, independent of coexisting WML and lacunar infarcts.

Methods The RUN DMC study is a prospective cohort study among 503 older people with cerebral SVD aged between 50-85years. All participants underwent FLAIR and T2* scanning. SCF, subjective memory (SMF) and subjective executive failures (SEF) were assessed. The relation between SCF and the presence, number and location of MB was assessed by linear regression analyses adjusted for age, sex, education, depressive symptoms, cognitive function, total brain volume, territorial infarcts, WML and lacunar infarcts.

Results MB were present in 11%. We found a relation between the presence, total number and lobar located MB and SCF, SMF and SEF and the reported progression of these failures, especially in participants with good objective cognitive function.

Conclusion MB are related to SCF independent of coexisting WML and lacunar infarcts, especially in those with good objective cognitive performance. These results suggest that MB are associated with the earliest manifestations of cognitive impairment. MB may help us understand the role of the ever expanding spectrum of SVD in cognitive impairment.

Introduction

Subjective cognitive failures (SCF) including memory complaints are frequently reported by older people. Despite considerable controversy whether these complaints predict objective cognitive failures^{1,2}, some have shown these complaints to be predictive of dementia^{3,4}. Cerebral SVD, which includes WML and lacunar infarcts, is very common in the elderly. Both hospital- and population-based studies have shown that SVD is related to cognitive impairment, and in some may ultimately lead to dementia^{5,6}. In some studies, SCF have been related to SVD, although these relations are rather weak^{7,10}.

Cerebral microbleeds (MB) are increasingly recognized as another manifestation of SVD^{11,12}. Although generally considered to be clinically silent, a study in a neurovascular clinic population first showed a relation between MB and cognitive impairment¹³. More recent studies found the same relation in population based- and memory clinic cohorts^{14,15}. It might be that MB, as they virtually always coexist with WML and lacunar infarcts, are related to SCF as well^{11,12}. We therefore wanted to investigate whether MB are associated with SCF in a non-demented SVD population, in the absence of detectable cognitive impairment and independent of other manifestations of SVD (WML and lacunar infarcts). As SCF have been strongly related to depressive symptoms^{18,9} and associated with hippocampal volume^{10,16}, we wanted to investigate this relation independent of these covariates.

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 with cerebral SVD. The selection procedure of the participants and study protocol were described in detail previously¹⁷.

In short, on the basis of established research criteria SVD was defined as the presence of lacunar infarcts and/or WML¹⁸. Symptoms of SVD include acute symptoms, such as TIAs or lacunar syndromes, or subacute manifestations, such as cognitive, motor (gait) disturbances and/or depressive symptoms¹⁹. Accordingly, 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 (WML and/or lacunar infarcts). The main exclusion criteria were dementia²⁰, (psychiatric) disease interfering with cognitive testing or follow-up, WML or SVD mimics and MRI contraindications or known claustrophobia. All participants signed an informed consent form. The Medical Review Ethics Committee region Arnhem-Nijmegen approved the study.

Conventional MRI scanning protocol

All participants underwent a 1.5-T MRI. The protocol included, among other sequences, the following whole brain scans: 3D T1 MPRAGE imaging, FLAIR pulse sequences and a transversal T2* weighted gradient echo sequence.¹⁷

Conventional MRI analysis

WML volume, lacunar infarcts and MB White matter signal hyperintensities on FLAIR images, which were not, or only faintly, hypo-intense on T1-weighted images, were considered WML, except for gliosis surrounding infarcts.²¹ WML were manually segmented on FLAIR images, total WML volume was calculated by summing the segmented areas multiplied by slice thickness. Lacunar infarcts were defined as hypo-intense areas with a diameter >2mm and <15mm with low signal intensity on T1 and FLAIR.²¹ All imaging analyses were performed by raters blinded to clinical information. In a random sample of 10%, inter-rater variability for total WML volume yielded an intra-class correlation coefficient of 0.99, intra- and inter-rater reliability for the lacunar infarcts yielded a weighted kappa of 0.80 and 0.88. Territorial infarcts were defined as hypointense lesions on FLAIR on T1 images >15mm.²¹

MB were defined as small, homogeneous, round focal areas of very low signal intensity on T2* weighted images of less than 10 mm in diameter.^{12,14} They were categorized into lobar, deep, and infratentorial.^{12,14} Lesions were not considered to be MB when they were symmetric hypointense in the globus pallidus, calcifications or iron deposits, flow voids artifacts or hyposignals in T2* inside a lesion compatible with an infarct, likely to be hemorrhagic transformation.^{12,14}

Hippocampus and intracranial volumetry One experienced investigator, blinded to clinical data, manually segmented the left and right hippocampus on the MPRAGE image using the interactive software program "ITK-SNAP".²² Anatomical boundaries were determined in coronal sections with the aid of neuroanatomical atlases.^{23,24} actual segmentation was performed using a previously published protocol²⁵ in which segmentation was performed from posterior to anterior.¹⁷ Inter-rater studies on a random sample of 10% showed an intra-class correlation coefficient for the left hippocampus of 0.73, and for the right hippocampus of 0.79. Intra-rater studies on this sample showed an intra-class correlation coefficient for the left and right hippocampus of 0.97 and 0.96, respectively.

For the same image, gray (GM) and white matter (WM) tissue and cerebrospinal fluid (CSF) probability maps were computed using SPM5 unified segmentation routines on the T1 MPRAGE images (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. Intracranial volume (ICV) was a summation of all tissue classes, i.e. total GM, WM and CSF volume.

Hippocampal volume (HV) measurements were normalized to the total ICV. The normalized

hippocampal volume (NHV) is defined as $NHV = ICV_m \times HV_p / ICV_p$, where ICV_m is the average total ICV of all participants, ICV_p the ICV of the participant and the HV_p the hippocampal volume of the participant²⁶

Subjective Cognitive Failures

Information on SCF was assessed by a 15-item semi-structured interview,¹⁶ which is an adaption of the Cognitive Failures Questionnaire²⁷ Responses were added to a sumscore for SCF with a maximum of 25 According to previous studies, subjective failure in remembering, word finding, planning, concentration, or slowness in thought had a higher weight in the sumscores (score range 0-3, none, mild, moderate, severe), than the 10 other items (0-1) SCF were considered present when a participant reported at least one moderate problem (score 2 or higher) on an item having a score range of 0-3 or a score of 1 on dichotomous items¹⁶ In addition we assessed whether participants reported progression in failures of remembering, word finding, planning, concentration, or slowness of thought over the past 5 years¹⁶ SCF were subdivided into subjective memory failures (SMF) and subjective executive failures (SEF) SMF were considered present if failures were reported in 1 or more of the 10 items concerning memory problems, SEF were considered present if failures in planning, concentration, and slowness of thought were reported¹⁶

Objective Cognitive Performance

Cognitive performance was assessed by a standardized neuropsychological test battery that has been described in detail elsewhere¹⁷ Performance across tests was made comparable by transforming raw test scores into z-scores We used the Mini-Mental State Examination (MMSE) and a compound score for global cognitive function, this was calculated as the mean of the z-scores of the one-letter subtask of the Paper-and-Pencil Memory Scanning task, the reading subtask of the Stroop test, the Letter-Digit Substitution Task, the added score of the three learning trials of the three-trial version of the Rey Auditory Verbal learning test, and the delayed recall of this test^{5,17}

Other measurements

Age, sex, education, depressive symptoms (score ≥ 16 on the Center of Epidemiologic Studies on Depression Scale (CES-D) and/or the present use of anti-depressive medication), WML volume, lacunar infarcts, territorial infarcts and normalized hippocampal volume were considered possible confounders²⁸ 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 $\geq 140/90$ mmHg and/or use of anti-hypertensive medications), diabetes (treatment with antidiabetic medications), hypercholesterolemia (treatment with lipid-lowering drugs) and smoking status

Table 1 Baseline Characteristics

	Total (n=497)	Subjective cognitive failures		p-value*
		Yes (n=450)	No (n=47)	
Demographics				
Age (y)	65.6 (8.8)	66.1 (8.6)	61.1 (9.0)	< .001
Male	281 (56.5)	251 (55.7)	30 (63.8)	.26
Subjects with only primary education	47 (9.5)	45 (10.0)	2 (4.3)	.65
Subjects with depressive symptoms†	166 (33.4)	160 (35.6)	6 (12.8)	.003
Subjects with hypertension*	365 (73.4)	334 (74.2)	31 (66.0)	.91
Subjects with diabetes mellitus‡	73 (14.7)	70 (15.6)	3 (6.4)	.13
Subjects with hypercholesterolemia§	234 (47.1)	219 (48.7)	15 (31.9)	.06
Smokers, current	74 (14.9)	65 (14.4)	9 (19.1)	.58
Smokers, former	276 (55.5)	249 (55.3)	27 (57.4)	.41
Cognition				
MMSE	28.1 (1.6)	28.1 (1.6)	28.4 (1.3)	.24
Neuroimaging				
Subjects with microbleeds	52 (10.5)	47	5	.53
Lobar	45 (9.1)	40	5	.36
Deep	13 (2.6)	12	1	.92
Strictly lobar	31 (6.2)	27	4	.33
Strictly deep	7 (1.4)	7	0	.54
Mixed	14 (2.8)	13	1	.88
Intracranial volume, mL	1677.9 (157.1)	1676.0 (155.7)	1695.4 (168.1)	.38
Hippocampal volume, mL	6.8 (1.0)	6.7 (1.0)	7.2 (1.1)	.002
White matter lesions volume, mL	7.2 (3.4-18.2)	7.1 (3.4-19.0)	8.0 (4.5-13.5)	.89
Subjects with lacunar infarcts	170 (34.2)	155	15	.61
Subjects with territorial infarcts	58 (11.7)	56	2	.20

Data represent mean (SD), number (%) or median (interquartile range)*Analysis of covariance, adjusted for age and sex, where appropriate †Defined as CES-D score≥16 and/or the use of antidepressive medication *Blood pressure ≥ 140/90 mm Hg and/or use of antihypertensive drugs ‡Using oral glucose-lowering drugs or insulin §Using lipid-lowering drugs

Statistical Analyses

Baseline characteristics were presented as mean \pm standard deviation (SD) and the median and interquartile range for skewed distributed parameters. Demographic characteristics were compared between subjects with and without SCF by age and sex adjusted analysis of covariance (ANCOVA).

The relation between presence and total number of MB (and by lobar and deep MB) and the sumscore of SCF, SMF and SEF was investigated using multiple linear regression analysis adjusted for age, sex, education, depressive symptoms, MMSE, WML volume, number of lacunar infarcts, territorial infarcts and normalized hippocampal volume. In addition we analyzed whether progression of failures was related to number of MB. In a second model we adjusted for MB at other locations.

Participants who reported SCF were considered having severe SCF, when in the upper tertile of the SCF score, the others were classified as mild. SMF and SEF were categorized accordingly.

To investigate whether the level of objective cognitive performance modified the association between number of MB and severity of SCF, SMF and SEF, we analyzed (ANCOVA) this relation stratified by objective performance, according to tertiles of the cognitive index, the lowest tertile reflecting the worst cognitive performance, adjusted for all previous confounders.

Results

The final sample consisted of 503 participants (response 71.3%) , three were excluded because of MRI artifacts and three because of an incomplete SCF interview. Demographic characteristics are shown in Table 1. SCF were reported by 91% of the participants (n=450), 88% reported SMF (n=439) and 61% reported SEF (n=303). Participants reporting SCF were older ($p < .001$), had more depressive symptoms ($p = .003$) and a smaller hippocampal volume ($p = .002$), than those without, independent of age and sex.

Table 2 Relation between number of microbleeds and subjective failures (sumscores) and progression of failures

	Number of Microbleeds		
	Total (n=497)	Lobar	Deep
Subjective cognitive failures	20 (.010)	20 (.010)	13 (.102)
Progression of SCF	22 (.010)	17 (.049)	23 (.006)
Subjective memory failures	16 (.050)	18 (.028)	07 (.418)
Progression of SMF	28 (.001)	25 (.004)	24 (.005)
Subjective executive failures	22 (.006)	20 (.011)	18 (.025)
Progression of SEF	43 (.000)	38 (.000)	37 (.000)

Data represent standardized β values (p -value) adjusted for age, sex, education, depressive symptoms, cognitive function (MMSE), WML volume, number of lacunar infarcts, territorial infarcts and normalized hippocampal volume

SCF and SMF were associated with lower objective cognitive performance ($p < .05$ and $p = .02$, respectively), independent of age, sex, education, depressive symptoms, WML volume, lacunar infarcts, hippocampal volume and MB.

The presence of MB was, independent of objective cognitive function and coexisting WML and lacunar infarcts, related to SCF ($\beta = 0.14$, $p < .05$) and SEF ($\beta = 0.15$, $p < .05$) and the progression of these failures. We found no relation with the presence of MB and SMF.

Table 2 illustrates the relation between the total number of MB and SCF, SMF and SEF, independent of other manifestations of SVD. Lobar MB were related to SCF, SMF and SEF, especially those in the temporal ($\beta = 0.18$, $p = .02$; $\beta = 0.15$, $p < .05$; $\beta = 0.20$, $p < .01$, respectively) and frontal lobe ($\beta = 0.19$, $p = .02$; $\beta = 0.14$, $p = .09$; $\beta = 0.20$, $p = .01$, respectively). Deep MB were related to SEF ($\beta = 0.18$, $p = .025$). Progression of failures was related to total number of MB. Additional adjustment for MB at other locations did not change the magnitude of the associations.

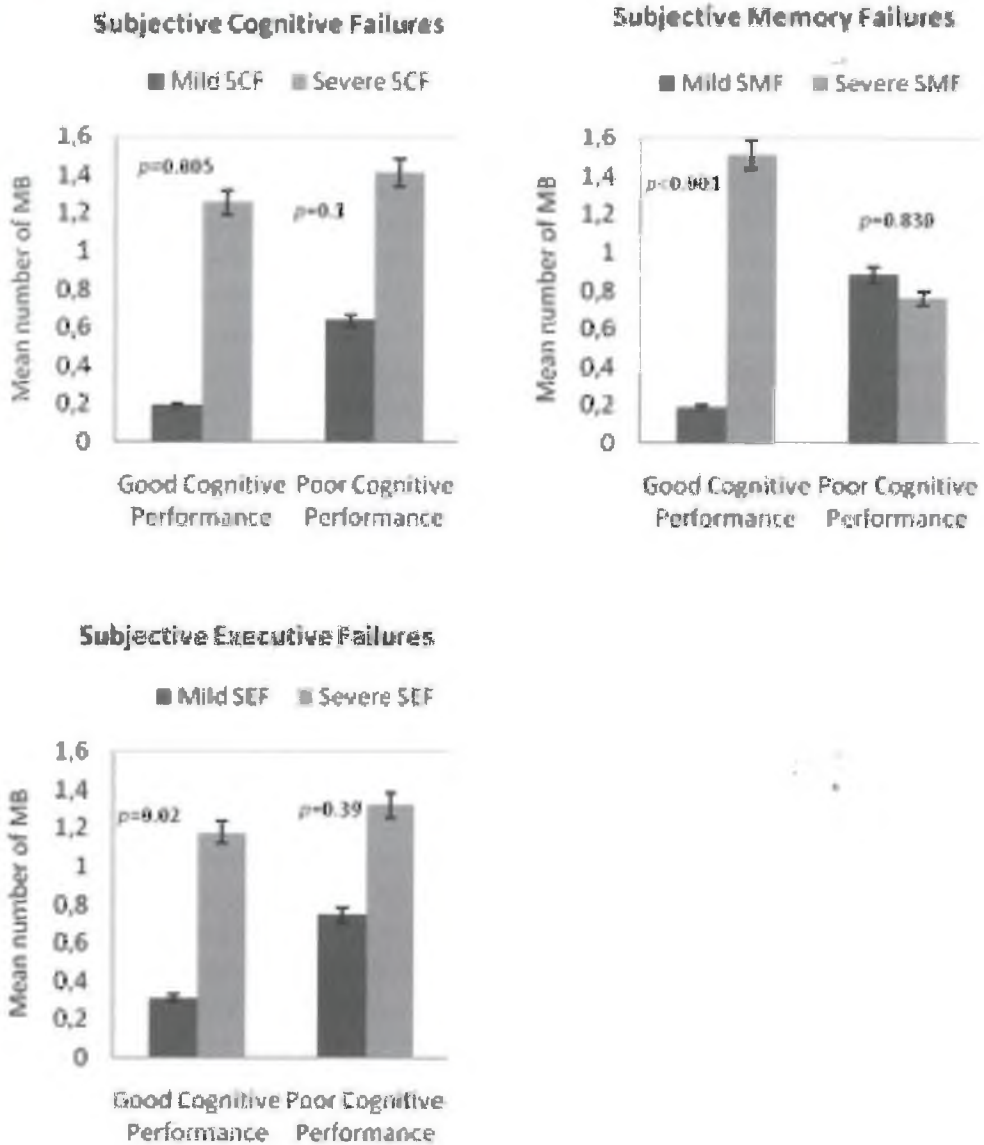


Figure 1 Mean number of microbleeds for participants with mild and severe subjective failures. Mean number of microbleeds for participants with mild and severe subjective cognitive, memory and executive failures. Participants are dichotomized in good and poor objective cognitive performance. Analysis are adjusted for age, sex, education, depressive symptoms, white matter lesion volume, number of lacunar infarcts, territorial infarcts and normalized hippocampal volume

Figure 1 shows the mean number of MB in strata of objective cognitive performance for participants with mild and severe failures. In participants with good cognitive performance those with severe SCF had a higher number of MB compared to those with mild failures ($p=0.005$). The same was found for participants with severe SMF ($p<0.001$) and severe SEF ($p=0.02$). In subjects with poor cognitive performance no relations were found between those with mild or severe subjective failures and number of MB.

Discussion

We found that cerebral MB, independent of other manifestations of SVD, WML and lacunar infarcts, were related to SCF, in elderly with cerebral SVD. This relation was most evident in participants with good objective cognitive performance, and independent of depressive symptoms and hippocampal volume.

Major strengths of this study included its large sample size and single-center design, with a high response rate, extensive assessment of cognitive function by only two investigators and the use of a single MR scanner. We were able to investigate the effect of MB on SCF independent of WML, lacunar and territorial infarcts and hippocampal volume and in the absence of objective cognitive impairment. We intentionally did not adjust for vascular risk factors such as hypertension as they were considered to be an earlier part of the causal chain between MB and SCF.

A limitation is the cross-sectional design of our study. Although we included questions on progression of SCF over the past 5 years and demonstrated relations between progression of failures and presence and number of MB, the cross-sectional nature prevents us from proving causality. However, the RUN DMC study has a longitudinal design and follow-up assessments on SVD and cognitive performance are already planned.¹⁷ Another limitation might be the MRI technique we used to detect the MB. As MRI techniques on the detection of MB have improved¹¹, we might have underestimated the actual number of MB in our population. This may have affected the effect size, but not the direction of the association with SCF. However, a recent study on the clinical relevance of the improved detection techniques for MB in terms of associations with clinical characteristics, vascular risk factors and other MRI markers of SVD, did not find stronger relations (or more variance explained) for MB detected with susceptibility weighted images (SWI) compared to T2* weighted imaging.²⁹

Another issue is the relatively high proportion of reported SCF (91%) compared to other studies.^{7,8,30} This might be explained by the method we used to determine SCF. Instead of spontaneous reporting complaints we actively asked for it, which potentially results in higher prevalence of complaints. Another explanation could be that we included subjects with SVD, which is characterized by, among other things, subacute cognitive symptoms¹⁹, and as a

result are more likely to express complaints. A third explanation might be the relatively high prevalence of depressive symptoms (33%) in our cohort, which was significantly higher in those with complaints (36%) compared to those without (12%) ($p=0.003$).

The relation between WML and SCF has been investigated before and was found to be weak and mainly demonstrated in people with severe WML^{7 10 16}, whereas the relation between MB and SCF has not been investigated before. This study offers the first indication that another manifestation of cerebral SVD, i.e. cerebral MB, contributes to SCF, independently of WML and lacunar infarcts. Therefore our finding that a higher number of MB was related to severe SCF compared to subjects with only mild SCF is of interest. These results, together with those from pathology studies showing that MB are frequently characterized by surrounding microstructural damage³¹, suggest that they independently contribute to SCF rather than simply reflecting the presence of other markers of SVD, as our findings are independent of WML volume and lacunar infarcts. The underlying mechanisms of the pathologic association between MB and cognitive function are unknown. Histopathologic studies have shown that MB indicate widespread damage of arterioles by hypertension or by amyloid deposition, as well as surrounding gliosis and infarction, resulting in microstructural damage.^{31 32} MB may disrupt white matter tracts that are important for cognitive function, i.e. in the temporal lobe, where we found a relation with SCF and which is no predilection site for WML.³³ This tissue damage, not visible on conventional MRI, may be investigated using relatively new techniques as DTI or resting state MRI.

Importantly, the relation between MB and severe SCF was especially demonstrated in those participants with a good objective cognitive performance. This supports the hypothesis that SCF may be a prelude to subtle cognitive deterioration that may, in the end, even progress to dementia^{2 3}, but that in its preclinical stage is difficult to assess using available neuropsychological instruments. In that way SCF may serve as an early marker of cerebral pathology in participants with SVD as they may be a sensitive tool and early predictor to identify those at risk for dementia, in the absence of objective cognitive impairment. The prevalence of MB was found to increase after follow-up assessment and MB present at baseline predict the development of new MB.³⁴ Possibly, the presence and increase of MB over time influence the relation between SCF and the development of dementia years later, although this hypothesis has to be investigated in a longitudinal study design.

We found no relation between mean number of MB and SCF in participants with poor objective cognitive performance. This could be due to a type II error, or due to the fact that true objective cognitive impairment obscures reporting of subjective cognitive disturbances. It is suggested that strictly lobar-located MB are attributable to amyloid angiopathy, whereas MB in the deep/infratentorial regions (with or without lobar MB) reflect hypertensive microangiopathy.^{12 35} Except for SEF we did not find relations between deep located MB and cognitive complaints, although this could be due to a power problem, as only seven participants had strictly deep MB. A recent report on the distribution of lobar MB, taking

lobar volumes and clustering effects into account, found that lobar MB are significantly more often located in the temporal lobe, a region known to be more affected by amyloid angiopathy.³⁶ Especially MB in the frontal and temporal lobe were related to SCF. Our findings are therefore in agreement with the notion that amyloid angiopathy may be the underlying etiology.

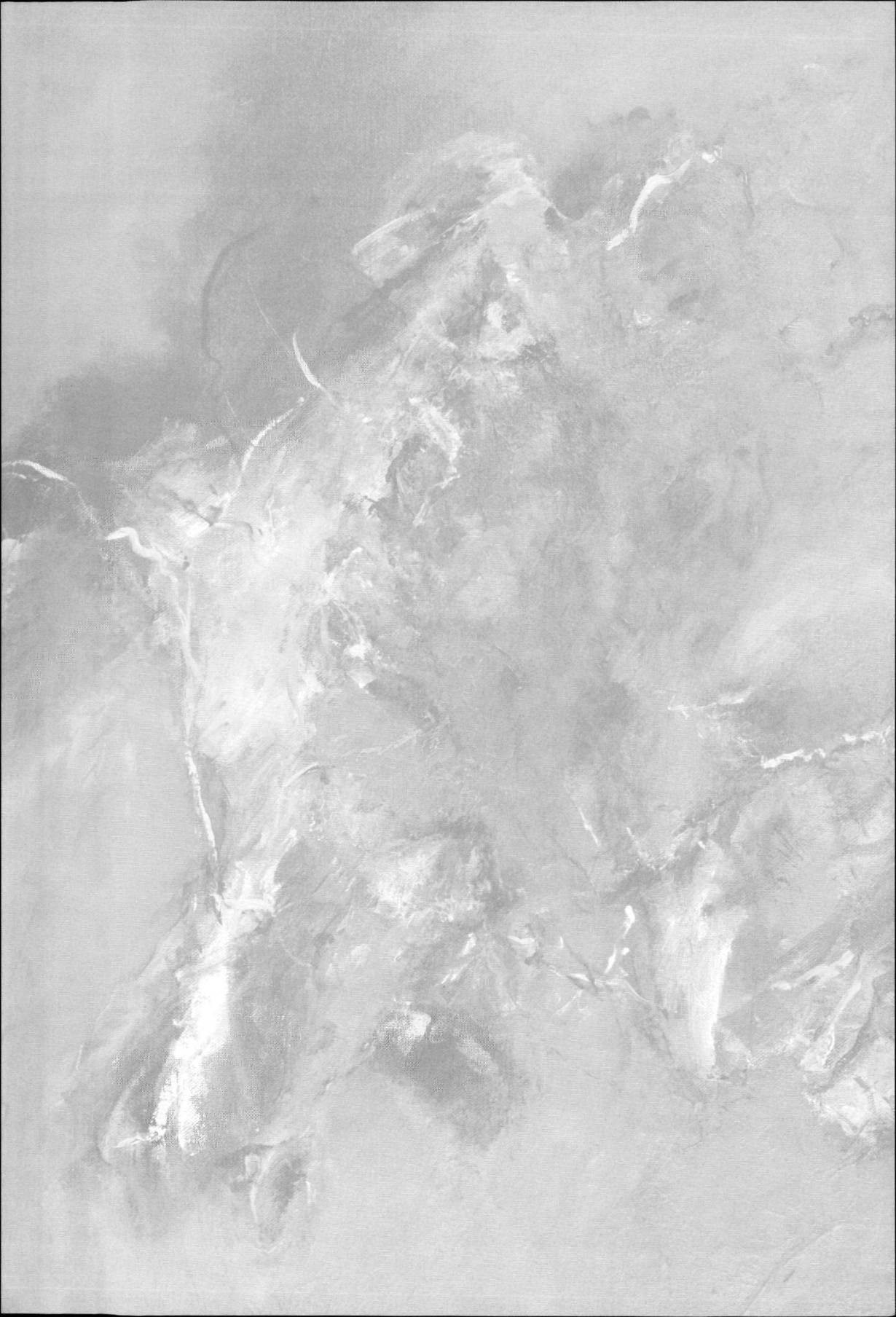
In conclusion, we found that cerebral MB are related to SCF in non-demented older people with SVD, independent of coexisting WML and lacunar infarcts. These results suggest that MB are clinically not as silent as they are usually considered to be and in that way might help us to understand the role of vascular disease in cognitive decline. Follow-up examination should identify whether progression of failures coincides with these radiologic findings of SVD manifestations and whether this parallels objective cognitive impairment.

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Chapter 6

Hippocampal volume is associated with subjective cognitive complaints

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Abstract

Background Subjective cognitive failures (SCF) and subjective memory failures (SMF) have reported to be an early predictor of Alzheimer's disease (AD) and have been attributed to white matter lesions (WML). Surprisingly, the relation with hippocampal atrophy has only sparsely been investigated, which would have been obvious since AD is characterized by hippocampal degeneration. Previous studies on this are rare, limited in sample size and did not adjust for WML.

Objective To determine the relation between SCF and hippocampal volume in strata of objective cognitive performance among non-demented elderly with incidental WML.

Methods The RUN DMC study is a prospective cohort study among 503 subjects with WML aged between 50 and 85 years. All subjects underwent FLAIR and T1 MRI scanning. The amount of SCF and SMF was rated by the Cognitive Failure Questionnaire. Cognitive function was assessed by a cognitive screening battery. Volumetric measures of hippocampus and WML were manually performed. We assessed the relation between hippocampal volume and SCF and SMF adjusted for age, sex, education, depression, total brain volume and WML volume.

Results Subjects with SCF and SMF had lower hippocampal volumes than those without ($p = .01$ and $p = .02$). This was most noteworthy in subjects with good objective cognitive performance ($p_{\text{trend}} = .007$ and $p_{\text{trend}} = .03$), and not in those with poor objective cognitive performance.

Conclusion SCF are associated with lower hippocampal volume, even in subjects without objective cognitive impairment and independent of WML. SCF has a radiologically detectable pathological-anatomical substrate.

Introduction

Clinicians are often confronted with elderly who report subjective cognitive complaints including memory complaints such as forgetting appointments in the near future or recent occurrences. Despite considerable controversy,^{1,2} several studies have found a relation between these subjective cognitive complaints and objective cognitive impairment, such as loss of executive and memory function as assessed by neuropsychology in non-demented elderly.^{3,4} More noteworthy, subjective complaints in individuals with normal cognition as assessed by formal neuropsychological testing predict Alzheimer's disease (AD) years after initial subjective complaints.⁵ Hence, these subjective complaints, distinguished in memory complaints and executive complaints, could be the earliest sign of cognitive dysfunction and might be a sensitive tool to identify those at risk for AD.

Early in its course, AD is characterized by a slowly progressive memory deficit due to gradual hippocampal degeneration, ultimately resulting in hippocampal atrophy.⁶ The memory deficit is often accompanied by loss of executive function, presumably caused by frontal grey matter atrophy, but also by white matter lesions (WML).^{7,8} Neuropathological studies indicate that hippocampal atrophy and WML may be present years or even decades before cognitive symptoms become detectable.^{9,11}

Depressive symptoms are also often present during early AD. There is an ongoing debate whether these symptoms influence or even cause cognitive complaints or whether a pre-stage of Alzheimer pathology is responsible for the cognitive complaints, despite spared cognition during formal testing.^{1,2,4,12} However, the presence of depressive symptoms is usually not taken into account in studies that assessed the relation between cognitive complaints, objective cognitive function and their underlying pathological substrate.

We hypothesized that subjective memory complaints and subjective executive complaints are already neuroradiologically characterized by hippocampal atrophy and WML, even in subjects without any objective cognitive impairment. Only a few studies have addressed this issue. Two large population-based studies found that WML were related to cognitive complaints, but did not adjust for hippocampal volume.^{4,12} Besides that, WML were rated semi-quantitatively or by automated segmentation, potentially leading to underestimation of the actual WML burden.¹³ Conversely, other studies related hippocampal volume to subjective memory complaints, without taking the degree of WML into account.^{12,14,15} In addition, most studies did not adjust for depressive symptoms, being a potential confounder. We therefore wanted to investigate whether subjective cognitive complaints on both the memory and executive domain were associated with hippocampal and WML volume in 503 independent living healthy elderly with incidental WML.

Methods

Study population

This study is embedded within the Radboud University Nijmegen Diffusion tensor and Magnetic resonance imaging Cohort (RUN DMC) study. The RUN DMC study prospectively investigates causes and cognitive and motor consequences of longitudinal functional and structural changes in the brain of individuals with incidental WML. Baseline investigations took place in 2006. Consecutive subjects, aged between 50–85 years, who visited the neurology outpatient clinic between October 2002 and October 2006 and who underwent routine diagnostic brain imaging, for reasons not related to the cognitive and motor study outcome (including collapses, mild traumatic brain injury, vertigo, chronic head pain or cranial nerve palsy), were eligible for participation. Exclusion criteria were any abnormality on the routine diagnostic brain imaging, that could interfere with the outcome (space occupying lesions, hemorrhages, large-artery infarcts), MRI-contraindications and prevalent dementia. Subjects were selected in strata of age (5 years), sex and WML severity according to the ARWMC scale.¹⁶ Upon agreement to participate in the study they underwent an extensive MRI protocol as part of the study on which all MRI measures reported here were based (see below). In 2006, 1004 subjects were selected for possible participation. On the basis of MRI contra-indications and the other exclusion criteria 299 subjects were excluded. The final sample consisted of 705 subjects of whom 503 agreed to participate (response 71.3%). Each participant signed an informed consent. The Medical Review Ethics Committee region Arnhem-Nijmegen approved the study.

MRI acquisition

All subjects underwent a 1.5 Tesla MRI scanning on the same Magnetom Sonata scanner (Siemens, Erlangen, Germany). The scanning protocol included whole brain T1 3D MPRAGE imaging (TR/TE/TI 2250/3.68/850 ms; flip angle 15°; voxel size 1.0x1.0x1.0 mm) and FLuid Attenuated Inversion Recovery (FLAIR) image (TR/TE/TI 9000/84/2200 ms; voxel size 1.0x1.2x6.0 mm (including gap of 1 mm); NEX=2).

Hippocampus and intracranial volume

One experienced investigator blinded to clinical data, total WML volume and FLAIR images (IVU), manually segmented left and right hippocampus on the MPRAGE image using the interactive software program “ITK-SNAP”.¹⁷ Anatomical boundaries were coronally determined with neuroanatomical atlases^{18,19} and actual segmentation was performed using a previously published protocol.²⁰ In short: segmentation was performed from posterior to anterior. The posterior border of the hippocampus was identified in the slice before the level in which the crurae fornices appeared in full view. The anterior border of the hippocampus was defined as the slice in which the hippocampus was no longer present, and the amygdala

fully covered the hippocampus^{20,22} The superior border was the inferior horn of the lateral ventricle, the inferior border was determined by the white matter The lateral border was defined by the temporal horn of the lateral ventricle and the white matter adjacent to the hippocampus

Volumes were calculated for the left and right hippocampus separately by summing all voxel volumes of the segmented areas Intra-rater studies on a random sample of 50 MRI scans showed an intra-class correlation coefficient for the left hippocampus of 0.73, and for the right hippocampus of 0.79

For the same image, gray (GM) and white matter (WM) tissue and cerebrospinal fluid (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 $p > 5$ for belonging to the tissue class Intra-cranial volume (ICV) was taken as the sum of total GM, WM and CSF¹³

WML volume

WML were manually segmented on transversal FLAIR images WML were defined as hyper-intense lesions on FLAIR MRI and not cerebrospinal fluid like hypo-intense lesions on T1-weighted image WML volume was calculated in the same fashion as for the hippocampus Gliosis surrounding lacunar and territorial infarctions was not considered to be WML^{16,23} Two trained raters (lvU, LvO) segmented all scans For the total WML volume the inter-rater intra-class correlation coefficient was 0.98 in a random sample of 50 scans

Subjective Cognitive Failures

Information on Subjective Cognitive Failures (SCF) was assessed by a 15 items semi-structured interview based on the Cognitive Failure Questionnaire^{4,24} (Table 1) Responses were added to a sum-score for SCF with a maximum of 25 According to previous studies, subjective failure in remembering, word finding, planning, concentration, or slowness in thought had a higher weight in the sum-scores (score range of 0-3 none-mild-moderate-severe), than the ten other items (0-1) SCF were considered present when a subject reported at least one moderate problem (score 2 or higher) on an item having a score range of 0-3 or a score of 1 on dichotomous items⁴ In addition we assessed whether subjects reported progression of remembering, word finding, planning, concentration or slowness of thought over the past 5 years (part of the Subjective Cognitive Failures Questionnaire, Table 1) Progression was defined as obvious progression of at least one failure or little progression on more than one of these failures⁴

SCF were subdivided in subjective memory failures (SMF) and subjective executive failures (SEF) SMF were considered present if failures were reported in one of the 10 items concerning memory problems, SEF were considered present if failures in planning, concentration and slowness in thought were reported⁴

Table 1 Subjective Cognitive Failures Questionnaire (n=500)

Question	Score range	% with problems
<i>Memory problems</i>		
Do you consider yourself as forgetful?	0-3	54 2%
Have you experienced any progression on this item?*	0-2	56 0%
Do you experience word-finding problems?	0-3	61 8%
Have you experienced any progression on this item?*	0-2	26 7%
Do you ever forget names of family members or friends?	0-1	59 2%
Do people tell you that you tell stories twice?	0-1	39 2%
Do you ever forget occurrences of the past one or two days?	0-1	32 2%
Do you worry about forgetfulness?	0-1	42 2%
Do you experience hinder in everyday-life because of forgetfulness?	0-1	37 8%
Do you ever misplace items at odd locations, leave the stove burning, or forget how to use everyday appliances?	0-1	11 2%
Do you ever forget appointments?	0-1	21 2%
Do you ever lose your way in your neighbourhood or do not recognise a person that is actually well acquainted?	0-1	10 2%
<i>Related executive problems</i>		
Have you experienced problems with planning of activities?	0-3	15 2%
Have you experienced any progression on this item?*	0-2	41 7%
Do you have concentration problems?	0-3	39 6%
Have you experienced any progression on this item?*	0-2	48 1%
Do you think or act more slowly than you used to?	0-3	47 4%
Have you experienced any progression on this item?*	0-2	52 1%
<i>Remaining problems</i>		
Do you feel more exhausted than you used to?	0-1	30 2%
Do you ever feel so depressed that you lose interest in life?	0-1	22 8%

* Percentage of participants **with** subjective failure on the accompanying item who reported little (score 1) or obvious (score 2) progression over the past 5 years on that particular item

Objective cognitive performance

Cognitive performance was assessed by a standardized neuropsychological test battery that has been used in other large-scale epidemiological studies of cognition in healthy elderly.^{8 25 26} The tests were administered by two trained investigators (AvN, KdL) and included the Mini Mental State Examination (MMSE),²⁷ a 15 words verbal learning test,²⁸ an abbreviated Stroop test consisting three subtasks,²⁹ the Paper and Pencil Memory Scanning Task,³⁰ a verbal fluency task in which as many animals as possible had to be named within 60

seconds³¹ and the Symbol-Digit Substitution Task, which is a modified version of the Symbol Digit Modalities Test³²

Performance across tests was made comparable by transforming the raw test scores into z-scores as described elsewhere³³ z-scores of the tests that had higher scores representing worse performance, like the speed score, were inverted (-z) From these z-scores we calculated compound scores for cognitive function, memory function and executive function, as described previously⁸ In short compound score for cognitive function was calculated as the mean of the z-scores of the 1-letter subtask of the Paper-and-Pencil Memory Scanning Task, the reading subtask of the Stroop test, the Letter-Digit Substitution Task, the added score of the three learning trials of 15 words verbal learning test and the delayed recall of this test⁹ A compound score for memory function was calculated by taking the mean of two z-scores of the 15 words verbal learning test, one for the added scores on the three learning trials, and one for the delayed recall⁸ The compound score for executive function was calculated as the mean of the z-scores of the verbal fluency test, the Symbol-Digit Substitution Task and the interference subtask of the Stroop test

If the test assistant encountered problems, a code was given for test status and the result was not used in the calculation of the z-scores Separate codes were given for lack of motivation (2.7%), presence of a physical handicap (1.2%), or deviation from the instructions (2.9%) For 465 subjects (93%) reliable compound scores could be calculated

Other measurements

The following characteristics were considered as possible confounders age, sex, education (according to Verhage)³⁴ and depressive symptoms Depressive symptoms were considered present when a subject had a score ≥ 16 on the Center of Epidemiologic Studies on Depression Scale (CES-D)³⁵ and/or the use of anti-depressive medication³⁶ ICV was also considered as a potential confounder

Statistical analysis

Statistical analyses were performed with the use of SPSS 14.0 for Windows Demographic characteristics were compared between subjects with or without SCF by sex- and age-adjusted analysis of covariance (by so called "forced entry") (ANCOVA) (Table 2)

Mean hippocampal and WML volume, and the proportion of subjects with severe WML (highest quintile)⁴ were calculated for subjects with or without SCF, SMF or SEF by ANCOVA (Table 3) All assumptions for ANCOVA were verified for each analysis

Next, we calculated the mean hippocampal and WML volume in subjects with and without SCF by severity of the failures (ANCOVA) Subjects who reported no SCF, as defined earlier, were considered having "No SCF" The group with SCF was dichotomized at the upper tertile of the SCF-score, which reflected "Severe SCF", the lower two tertiles were defined as "Moderate SCF" SMF and SEF were categorized accordingly

To investigate whether the level of objective cognitive performance modified the association between hippocampal or WML volume and severity of SCF, we analyzed this relation stratified by objective performance, according to tertiles of the compound score for cognitive function (poor, moderate or good).⁴

Finally, we investigated whether progression of failures was related to hippocampal or WML volume.

For the trend analysis of the ANCOVA results, groups of severity of failures (none, moderate, severe) were considered as a continuous variable in a multiple linear regression model. Age, sex, education, depressive symptoms, ICV and where appropriate WML volume and hippocampal volume were put together into the model (so called “forced entry”).

Table 2 Characteristics of the 500 subjects who completed the SCF Questionnaire with or without subjective cognitive failures

Characteristic	Total	Subjective cognitive failures		p*
	(n=500)	Yes (n=453)	No(n=47)	
Age (years)	65.6 (8.8)	66.1 (8.7)	61.1 (8.7)	< .001
Sex (male/female)	281/219	251/202	30/17	.244
Subjects with only primary education	47 (9.4%)	45 (9.9%)	2 (4.3%)	.654
MMSE	28.2 (1.6)	28.1 (1.6)	28.4 (1.6)	.249
CES-D	10.9 (9.4)	11.4 (9.3)	6.1 (9.4)	< .001
Subjects with depressive symptoms †	166 (33%)	160 (35%)	6 (13%)	.003
Subjects with CES-D ≥16	139 (28%)	135 (30%)	4 (9%)	.003
Subjects with anti-depressive medication	62 (12%)	59 (13%)	3 (6%)	.271
Subjective cognitive failures (SCF)				
Sum-score on the SCF questionnaire	8.5 (5.7)	9.3 (5.2)	0.5 (5.2)	< .001
Subjects with SCF	453 (91%)			
Sum-score of sub-questionnaire on SMF	5.4 (3.5)	5.9 (3.1)	0.4 (3.1)	< .001
Subjects with SMF	442 (88%)			
Sum-score of sub-questionnaire on SEF	2.5 (2.3)	2.8 (2.3)	0.2 (2.2)	< .001
Subjects with SEF	304 (61%)			
Subjects with SCF reporting its progression	194 (43%)			

Values are means (SD) or n (%) * ANCOVA adjusted for age and sex, where appropriate † Defined as CES-D scores ≥16 and/or the use of anti-depressive medication (n=498) MMSE Mini Mental State Examination, CES-D Centre of Epidemiological Studies on Depression Scale, SCF Subjective Cognitive Failures, SMF Subjective Memory Failures, SEF Subjective Executive Failures

Results

Of the 503 subjects one was excluded because of an automatic segmentation problem and two because of an incomplete SCF questionnaire. The presence of depressive symptoms was assessed in 498 subjects. Demographic characteristics are shown in Table 2. Subjects reporting SCF were older ($p < .001$) and had more depressive symptoms ($p = .003$) adjusted for age and sex. Subjective failures on all three domains (SCF, SMF and SEF) were associated with reduced objective performance, independent of depressive symptoms, WML and hippocampal volume ($p < .01$).

Table 3 illustrates that subjects with SCF had a lower hippocampal volume than those without ($p = .01$). Subsequent analysis showed that this was also evident in subjects with SMF ($p = .01$) and SEF ($p = .07$). Additional adjustment for WML volume did not significantly change the effect. No relation was found between SCF and WML volume ($p > .1$), except for subjects reporting SEF who more often had severe WML (25%) than those who did not (15%; $p = .05$).

Table 3 Mean hippocampal and WML volume (SD) for subjects with and without subjective cognitive failures (SCF), subjective memory failures (SMF) and subjective executive failures (SEF).*

		Hippocampal volume	WML volume	n (%) severe WML
SCF	Present	6.7 (0.9)	14.4 (19.2)	94 (21%)
	Absent	7.1 (1.1) [†]	14.6 (12.0)	6 (13%)
SMF	Present	6.7 (1.0)	14.4 (19.4)	91 (21%)
	Absent	7.0 (1.0) [†]	14.3 (11.6)	9 (16%)
SEF	Present	6.7 (0.9)	15.1 (19.8)	71 (24%)
	Absent	6.9 (1.0)	13.4 (16.5)	29 (15%) [†]

* Numbers represent milliliters, adjusted for age, sex, education, depressive symptoms, intracranial volume, WML volume and hippocampal volume where appropriate. [†] $p < .02$, [†] $p = .05$, WML White matter lesions

There was also a relation of decreasing hippocampal volume by increasing severity (no, moderate and severe failures) of SCF ($p_{\text{trend}}=.024$), SMF ($p_{\text{trend}}=.01$) and SEF ($p_{\text{trend}}=.07$). In figure 1 hippocampal volumes are given in strata of actual cognitive performance for subjects with severe, moderate and no SCF. The difference in hippocampal volume across these different groups with SCF was most apparent in subjects with a good cognitive performance ($p_{\text{trend}}=.005$). This relation was also found for SMF ($p_{\text{trend}}=.024$; figure 2), with borderline significance for subjects with moderate memory performance ($p_{\text{trend}}=.076$). Additional adjustment for WML did not change the magnitude of the associations. These relations were not found for WML volume, in neither of the objective cognitive strata, nor in the whole group analysis. Progression of failures was not associated with hippocampal volume, nor with WML volume.

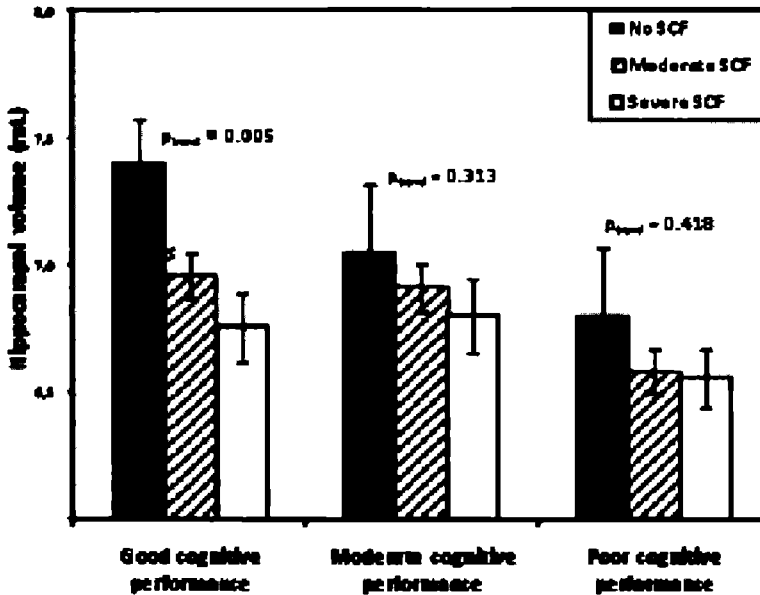


Figure 1 Mean hippocampal volume for subjects without, moderate and severe Subjective Cognitive Failures (SCF), in strata of objective cognitive performance (tertiles of compound score for cognitive function). Adjusted for age, sex, education, depressive symptoms, ICV and WML volume no SCF (good n=26; moderate n=11; poor n=10); moderate SCF (good n=90; moderate n=100; poor n=91); severe SCF (good n=40; moderate n=44; poor n=54)

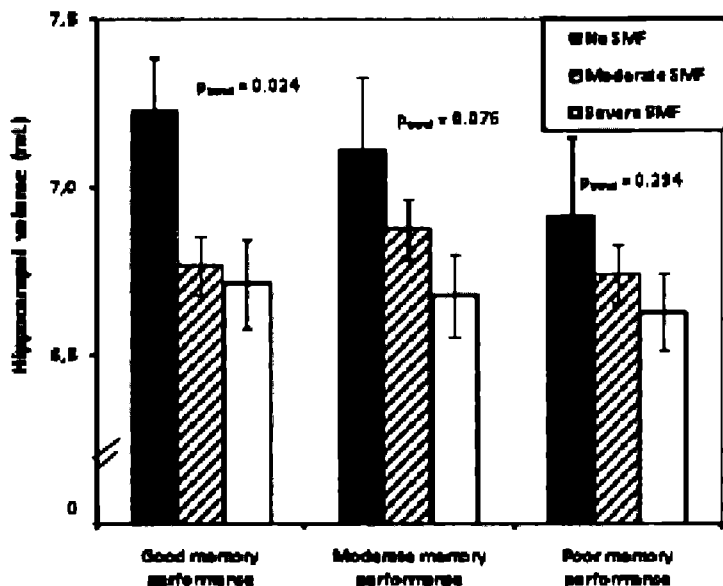


Figure 2 Mean hippocampal volume for subjects without, moderate and severe Subjective Memory Failures (SMF), in strata of objective memory performance (tertiles of compound score for memory function). Adjusted for age, sex, education, depression, ICV and WML volume no SMF (good n=28; moderate n=15; poor n=15); moderate SMF (good n=97; moderate n=95; poor n=94); severe SMF (good n=39; moderate n=52; poor n=54)

Discussion

We found that SCF were associated with lower hippocampal volume, independent of depressive symptoms and WML volume, in elderly with incidental WML. This relation was most evident in subjects with intact cognition as revealed by neuropsychological testing.

In our study the proportion of subjects reporting SCF (91%) and SMF (88%) was higher than in other studies, which reported percentages between 26 and 72%.^{3,4,12} A possible explanation for our relatively high frequency might be the method used to determine SCF. SCF were not identified through spontaneous reporting, but by actively asking for it, potentially resulting in a higher degree of reported failures. Another explanation could be that all subjects were recruited through our outpatient clinic, rather than being community-based. Consequently, we have included people who once sought medical help and who therefore are more likely to express complaints simply because they had visited an outpatient clinic. Nonetheless, our study cohort represents a typical outpatient population that was being investigated for reasons that frequently lead to the discovery of WML among healthy, independent living

elderly. We intentionally included subjects with incidental WML as advising these subjects reflect every day clinical practice. These advices have currently been limited by the fact that most studies on this topic have a different design. It is important to realize that most knowledge on causes and consequences of WML is based on either population-based studies or patients with more advanced stages of dementia, that may not be applicable to patients with incidental WML. These studies have improved our understanding on the etiology and consequences of WML, but results may not be applicable to individuals who visit an outpatient clinic. Acquiring more data on causes and consequences of WML in this particular group is an important goal of the RUN DMC study.

A third explanation for the difference in SCF reporting, is that the high percentage of SCF may be related to the relatively high proportion of subjects reporting depressive symptoms (33%) in our cohort compared to percentages reported in a systematic review (0.4-35%),³⁷ and we were able to adjust for that.

The strength of this study includes the fact that it is a large, single centre study with a high response; its structured and extensive assessment of cognitive functioning performed by only two investigators; the use of a single MR scanner; the use of a reliable and sensitive volumetric assessment of WML instead of a visual rating scale;¹³ the use of a single expert who segmented the hippocampus with high inter-rater agreement, blinded to all clinical and radiological data; and the opportunity of extensive adjustment for possible confounders.

Our results are in agreement with two recent studies, which reported reduced hippocampal volumes in subjects with memory complaints as compared to controls.^{12 14 15} The unique aspect of our study was the inclusion of depressive symptoms and WML volume as covariates, since they are related to SCF,^{2 4,25} and to hippocampal atrophy.³⁸

Importantly, the relation between lower hippocampal volume and SCF and SMF was demonstrated in subjects with good objective performance. This may provide an underlying patho-anatomical explanation for the frequently observed SCF and may therefore function as an early marker for the development of diseases characterized by memory loss, including AD and its attendant neuropathological substrates such as hippocampal atrophy.^{4 14 15} Since memory impairment is mainly related to hippocampal neurodegeneration,^{6 39} it is conceivable that SCF and SMF are also primarily related to hippocampal atrophy rather than WML. As lower hippocampal volume is associated with future conversion to AD,⁴⁰ SCF can serve as an early predictor of AD, even in the absence of objective cognitive impairment.

In our study, SCF and SMF were not related to WML volume. This in accordance with another study,⁴ whereas other studies did report an association between WML and cognitive complaints.^{4,12 25} However, some methodological considerations of these studies should be discussed. Most importantly, they did not adjust for hippocampal volume, which obviously appears to be related to SCF and SMF. In addition, the first study²⁵ had a relatively small sample (n=60) and in the other study⁴ the severity of WML was scored semi-quantitatively, potentially leading to an underestimation of the actual WML burden. This effect may

especially be present among those with severe WML, because of ceiling effects in semi-quantitative rating scales. Yet, in our study severe WML were more frequently observed in subjects reporting SEF than those who did not. This is in line with previous studies that found WML to be primarily related to executive functions and speed/motor control domains.^{7,36}

We found no relation between hippocampal atrophy and SCF or SMF in subjects with moderate or poor objective cognitive performance, this may be due to a type II error. A previous study found a similar association for WML in relation to reported progression of SCF.⁴ It has been suggested that the relation between SCF and objective performance may be disproportional in subjects with poor cognitive performance, simply because of their cognitive impairment and inability to judge their own cognitive function.

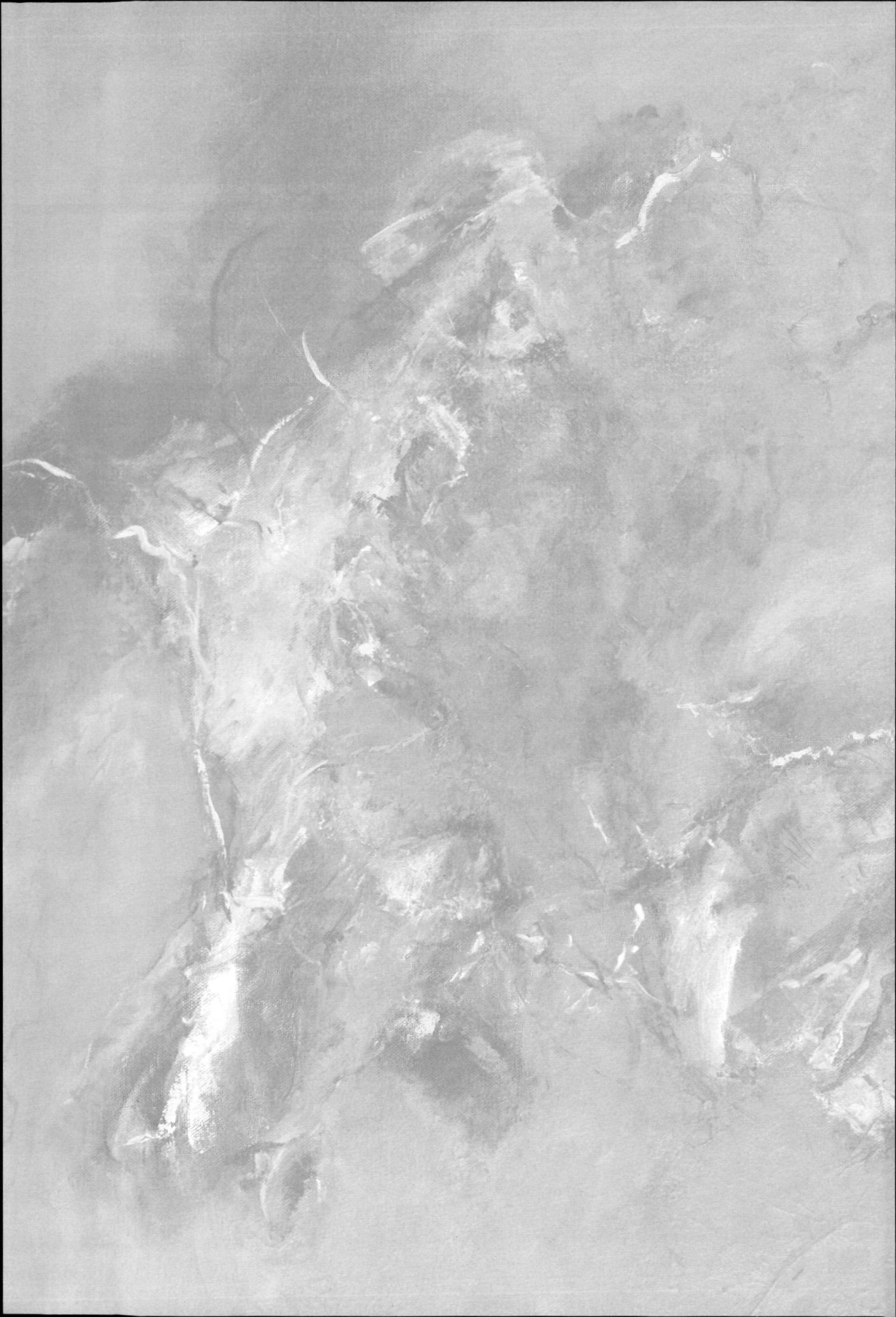
Subjective cognitive failures may already have a radiological fingerprint with an underlying patho-anatomical explanation. Future studies should identify whether progression of failures coincides with these radiological observations and whether this parallels objective cognitive decline.

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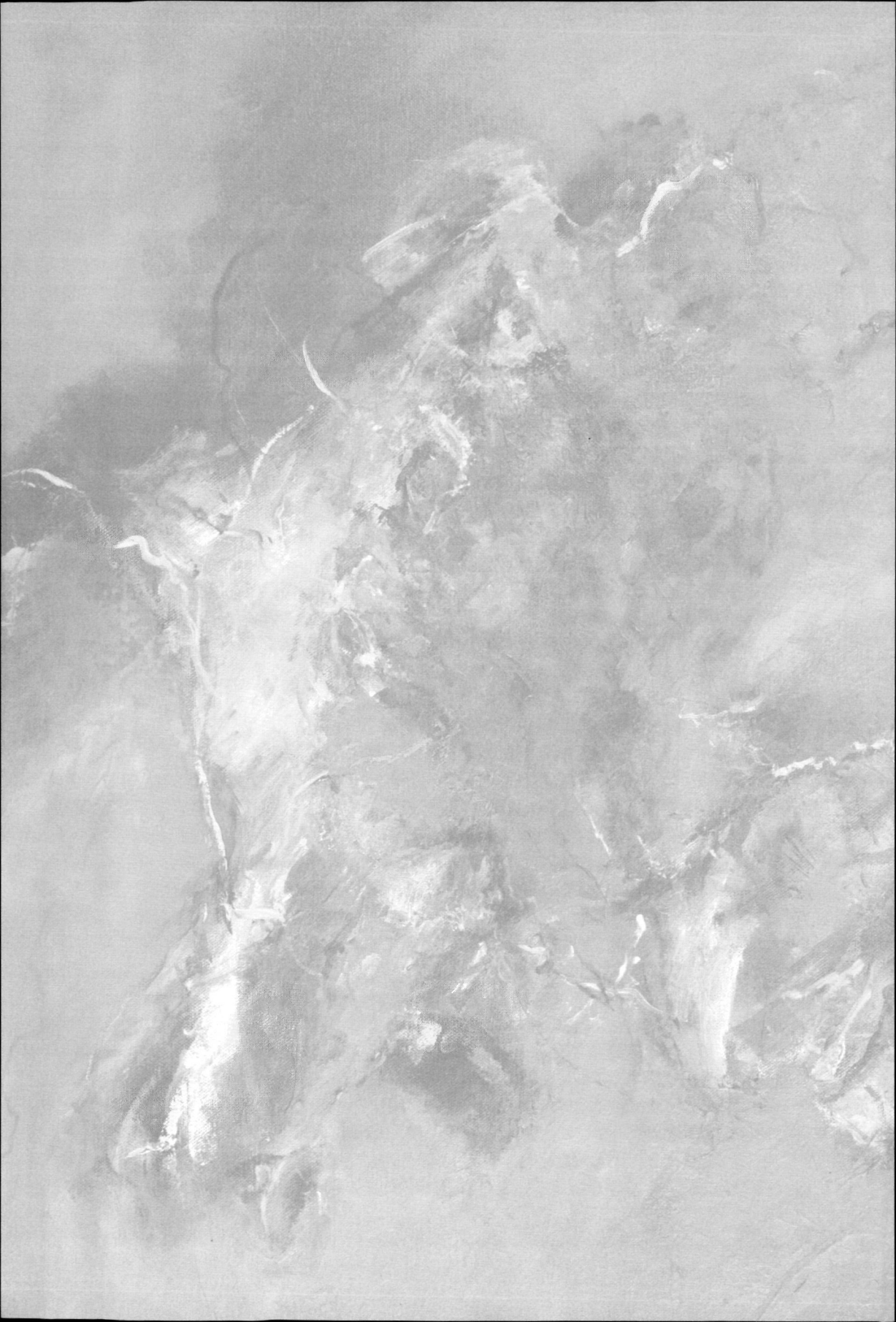
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PART III

A diffusion tensor imaging approach



Chapter 7

Loss of white matter integrity is associated with cognitive function

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Abstract

Background Cerebral small vessel disease (SVD) is very common in elderly and related to cognition, although this relation is weak. This might be because the underlying pathology of white matter lesions (WML) is diverse and cannot be properly appreciated with conventional FLAIR MRI. In addition, conventional MRI is not sensitive to early loss of microstructural integrity of the normal appearing white matter (NAWM), which might be an important factor. Diffusion tensor imaging (DTI) provides alternative information on microstructural white matter integrity and we have used this to investigate the relation between white matter integrity, in both WML and NAWM, and cognition among elderly with cerebral SVD.

Methods The RUN DMC study is a prospective cohort study among 503 independently living, non-demented elderly with cerebral SVD aged between 50-85 years. All subjects underwent MRI and DTI scanning. WML were segmented manually. We measured mean diffusivity (MD) and fractional anisotropy (FA), as assessed by DTI in both WML and NAWM.

Results Inverse relations were found between MD in the WML and NAWM and global cognitive function ($\beta = -0.11$, $p < 0.05$, $\beta = -0.18$, $p < 0.001$), psychomotor speed ($\beta = -0.15$, $p < 0.01$, $\beta = -0.18$, $p < 0.001$), concept shifting ($\beta = -0.11$, $p < 0.05$, $\beta = -0.10$, $p < 0.05$) and attention ($\beta = -0.12$, $p < 0.05$, $\beta = -0.15$, $p < 0.001$). In subjects with severe WML, DTI parameters in the WML and NAWM are related to cognitive performance.

Conclusion DTI parameters in both WML and NAWM correlate with cognitive performance, independent of SVD. DTI may be a promising tool in exploring the mechanisms of cognitive decline and could function as a surrogate marker for disease progression in therapeutic trials.

Introduction

Cerebral small vessel disease (SVD) includes white matter lesions (WML) and lacunar infarcts and is very common in the elderly. Both patient and population based studies have shown that cerebral SVD is an important cause of cognitive impairment, and may ultimately lead to dementia in some.^{1,2} Despite the high prevalence of WML and lacunar infarcts in the population over 50 years of age (90% and 20% respectively),^{3,4} surprisingly few individuals develop cognitive decline or dementia.² Apparently there are other factors apart from the SVD visible on conventional MRI that determine the transition from intact cognitive performance to cognitive decline in some, while leaving the cognition unaffected in most. As identical appearing WML on conventional MRI are actually histopathologically heterogeneous⁵, it could be that only WML with the highest loss of structural integrity are related to cognitive impairment. It is also important to realize that only a small proportion of the white matter (usually less than a few percent) is affected by SVD, even among individuals with severe SVD. As conventional MRI is not sensitive to early loss of microstructural integrity in the normal appearing white matter (NAWM), possible changes in this largest part of the white matter cannot be assessed. The integrity of the NAWM might be an important factor for a better understanding of the relation between white matter integrity and cognitive performance and decline.

These limitations of conventional MRI can potentially be overcome with the use of Diffusion Tensor Imaging (DTI) which allows the assessment of the microstructural integrity of the whole white matter.^{6,7} DTI provides two scalar parameters: mean diffusivity (MD), a measure of the magnitude of diffusion of water averaged in all spatial directions, and fractional anisotropy (FA) measure, which provides information about the directionality of water diffusion. A reduction in FA and increase in MD are believed to represent reduced microstructural integrity in SVD.⁸ We therefore hypothesized a relation between the degree of structural integrity of WML and NAWM and cognitive performance.

Studies on CADASIL and multiple sclerosis showed a relation between DTI parameters in WML and NAWM and cognitive function,^{9,10} whereas only a few studies investigated this relation in individuals with SVD.¹¹⁻¹⁴ Most of these studies had small sample sizes ($n < 105$) and did not adjust for possible confounders. In addition, most studies addressed only few cognitive domains of the spectrum observed in WML related cognitive decline. Recently a population based cohort study demonstrated a relation between microstructural integrity of both the WML and NAWM and cognitive function.¹⁵

In this study we report on the relation between various domains of cognitive function and the structural integrity of both the WML and NAWM, as assessed by DTI, in 503 independently living, non-demented elderly with cerebral SVD. We hypothesized that a higher MD and lower FA in the WML as well as in the NAWM are related to cognitive function. The second aim was to determine whether these associations in the NAWM are independent of WML and lacunar infarcts.

Methods

Study population

The Radboud University Nijmegen Diffusion tensor and Magnetic resonance imaging Cohort (RUN DMC) study, is a prospective cohort study that investigates risk factors and clinical 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 of SVD include acute symptoms, such as TIAs or lacunar syndromes, or subacute manifestations, such as cognitive, motor (gait) disturbances and/or depressive symptoms¹⁶. 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 features¹⁷. Accordingly, 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 (WML and/or lacunar infarcts). Subsequently, the above mentioned acute or subacute 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) dementia¹⁸ (b) parkinson(-ism)¹⁹ (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/current use of acetylcholine-esterase inhibitors, neuroleptic agents, L-dopa or dopa-antagonists, (g) WML or SVD mimics (e.g. multiple sclerosis and irradiation induced gliosis), (h) prominent visual or hearing impairment, (i) language barrier, (j) MRI contraindications or known claustrophobia.

From 1004 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 center, yielding a response of 71.3% (503/705).

All participants signed an informed consent form. The Medical Review Ethics Committee region Arnhem-Nijmegen approved the study.

Conventional MRI scanning protocol

All participants underwent a 1.5-Tesla MRI scanning on the same Magnetom scanner (Siemens, Erlangen, Germany). The protocol included the following whole brain scans: 3D T1 MPRAGE imaging (TR/TE/TI 2250/3.68/850ms, flip angle 15°, voxel size 1.0x1.0x1.0mm), FLAIR pulse sequences (TR/TE/TI 9000/84/2200ms, voxel size 1.0x1.2x5.0mm, interslice gap 1mm), DTI (TR/TE 10100/93ms, voxel size 2.5x2.5x2.5mm, 4 unweighted scans, 30 diffusion weighted scans with b-value 900s/mm²). The complete protocol took 31 minutes.

Conventional MRI analysis

White matter signal hyperintensities in both supra and infratentorial regions on FLAIR scans, which were not, or only faintly, hypo-intense on T1 weighted images, were considered WML, except for gliosis surrounding infarcts.²⁰ WML were manually segmented on FLAIR images by two trained raters. Total WML volume was calculated by summing the segmented areas multiplied by slice thickness. Lacunar infarcts were defined as hypo-intense areas with a diameter >2mm and <15mm with low signal intensity on T1 and FLAIR, ruling out enlarged perivascular spaces and infraputamina pseudolacunes.²⁰ All imaging analyses were performed by raters blinded to clinical information. In a random sample of 10%, inter-rater variability for total WML volume yielded an intra-class correlation coefficient of 0.99; intra- and inter-rater reliability for the lacunar infarcts yielded a weighted kappa of 0.80 and 0.88. Normalization parameters to the ICBM152 linear template (as provided with SPM5; Wellcome Department of Cognitive Neurology, University College London, UK) and gray (GM) and white matter (WM) tissue and cerebrospinal fluid (CSF) probability maps were computed by using SPM5 unified segmentation routines on the T1 MPRAGE images.²¹ 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 (TBV) 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, TBV was expressed as percentage of total ICV.

Co-registration parameters of the FLAIR image to the T1 image were computed (SPM5 mutual information co-registration) and used to bring both the FLAIR and WML segmentation images into the subject's (anatomical) reference frame. Transformed images were visually checked for co-registration errors. Subsequently, the WML segmentations were resampled to and combined with the white matter maps to yield a WML map (the intersection of WML and white matter) and NAWM map (the complement of WML in white matter) in the T1 reference space.

DTI analysis

Affine distortion in our DW images from residual eddy-currents were minimized during MR acquisition and did not require further correction using post-processing methodology.²² 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 tensor⁶ 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.²³ 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 derivatives. All images were visually checked for motion

artefacts and co registration errors, which resulted in a final sample of 499 subjects because of the exclusion four due to technical artefacts. The mean MD and FA were then calculated in both the WML and NAWM.

Measurement of cognitive function

Cognitive function was measured with a neuropsychological test battery that proved to be sensitive and suitable for this purpose in other, large epidemiological studies.[1] The tests included the Mini Mental State Examination (MMSE)²⁴, and the Rey Auditory Verbal Learning Test (RAVLT)²⁵. To evaluate speed of mental processes we used the Stroop test²⁶, the Paper-Pencil Memory Scanning Task²⁷, the Symbol-Digit Substitution Task²⁸ and a verbal fluency task in which as many animals followed by as many professions as possible had to be named within 60 seconds. Attention was measured by the verbal series attention test (VSAT).²⁹ To evaluate visuospatial memory we used the Rey's Complex Figure Test (RCFT).³⁰ Performance across tests was made comparable by transforming the raw test scores into z-scores as described elsewhere.¹

Subsequently, we calculated compound scores for seven cognitive domains. Global cognitive function was evaluated by the MMSE 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.¹ Verbal memory is a compound score of the mean of two z-scores from the RAVLT; one for the added scores of the three learning trials of this test, and one for the delayed recall of this test. Visuospatial memory is a compound score of the mean of the z-scores of the immediate recall trial and the delayed recall trial of the RCFT. 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.¹ Fluency was calculated from the mean of the z-scores of both verbal fluency tasks. Concept shifting was calculated as the z-score of the third subtask of the Stroop. Attention is a compound score of the z-score of the total time of the VSAT.¹

Other measurements

Age, sex, level of education³¹, depressive symptoms, lacunar infarcts and TBV were considered possible confounders. Depressive symptoms were present if a subject had a score ≥ 16 on the Center of Epidemiologic Studies on Depression Scale (CES-D) and/or the present use of anti-depressive medication.³² The living conditions of every participant were administered.

Statistical Analysis

Baseline characteristics were presented as mean \pm standard deviation (SD) and for the skewed distributed parameters the median and interquartile range were calculated.

The relation between DTI parameters in WML and NAWM, WML volume and lacunar infarcts with cognitive performance was assessed by means of linear regression analysis. Regression coefficients are presented as standardized beta's.

To investigate if the burden of WML was an intermediate in this relation we analyzed this in strata (tertiles) of WML volume. All analyses were adjusted for age, sex, education, depressive symptoms, TBV normalized for head size, lacunar infarcts and WML whenever appropriate. All data were analyzed using SPSS statistical software, version 16.0.

Table 1 Baseline characteristics of the RUN DMC study population

<i>Demographic and clinical characteristics</i>	
Number of participants	499
Age at admission (yrs)	64.2 \pm 8.8
Age at enrolment (yrs)	65.6 \pm 8.8
50-60 yrs (number, %)	161 (32)
60-70 yrs (number, %)	161 (32)
70-85 yrs (number, %)	177 (36)
Disease duration (yrs)[†]	1.4 \pm 1.1
Sex (% male)	56.5
% only primary education	10
Depressive symptoms (number, %)[‡]	168 (34)
<i>Neuro-imaging characteristics</i>	
Total brain volume (ml)	1093.1 (121.1)
Total white matter volume (ml)	464.4 (66.5)
Total white matter lesion volume (ml)	7.1 (3.4, 18.1)
Total normal appearing white matter volume (ml)	450.3 (70.3)
Presence lacunar infarcts (number, %)	171 (34)

Numbers represent means (SD), percentages or medians (inter-quartile range) [†]Defined as age at enrolment-age at admission [‡] Depressive symptoms defined as CES-D (Center of Epidemiologic Studies Depression Scale) \geq 16 and/or present use of anti depressants

Results

Demographics and neuro-imaging characteristics of 499 subjects are shown in Table 1. Mean age of the population was 65.6 years (SD 8.8) and 56.5% were male. In Table 2 the test scores of the cognitive test battery are shown. The mean MMSE of the study population was 28.1 (SD 1.6).

In the WML, MD was related to global cognitive function (MMSE: $\beta=-.12, p=.02$; cognitive index: $\beta=-.11, p=.01$) and executive function (psychomotor speed: $\beta=-.15, p<.01$; concept shifting: $\beta=-.11, p=.02$; attention: $\beta=-.12, p=.02$). (Table 3) MD in the WML did not significantly correlate with either of the memory domains and fluency. FA in the WML was only related with concept shifting and attention ($\beta=.09, p=.03$; $\beta=.11, p=.01$). In the NAWM, MD was related to all cognitive domains independent of the WML load (Table 3). The FA in the NAWM was related with the cognitive index ($\beta=.11, p=.002$), verbal memory ($\beta=.11, p=.01$), psychomotor speed ($\beta=.12, p<.001$), concept shifting ($\beta=.10, p=.03$) and attention ($\beta=0.16, p=.001$), again independent of WML volume.

Stratification on tertiles of WML severity revealed a relation between the MD in the WML with all cognitive domains, except for verbal memory performance and fluency, in subjects with severe WML, not in those with mild or moderate WML. (Table 4) The FA in the WML was related to the MMSE ($\beta=.17, p<.05$), the cognitive index ($\beta=.12, p<.05$), visuospatial memory ($\beta=.16, p<.05$), psychomotor speed ($\beta=.16, p<.01$), concept shifting ($\beta=.18, p<.05$), and attention ($\beta=.27, p<.001$) in subjects with severe WML, whereas no significant relations were found in subjects with mild or moderate WML. Identical correlations were found in the NAWM between MD and FA and cognitive performance in subjects with severe WML, except for concept shifting and the cognitive index.

WML volume was related to MMSE ($\beta=-.11, p=.02$), visuospatial memory ($\beta=-.14, p=.004$), psychomotor speed ($\beta=-.09, p=.05$), concept shifting ($\beta=-.09, p=.04$) and attention ($\beta=-.13, p=.003$). We did not find a relation between cognitive performance and presence or number of lacunar infarcts.

Table 2 Cognitive test scores

Mini Mental State Examination (range 0-30)	28.1 (1.6)
15 word verbal learning task (no. of words recalled)	
Immediate recall trial 1	5.1 (1.7)
Immediate recall trial 2	7.3 (2.2)
Immediate recall trial 3	8.7 (2.5)
Total trial 1-3	21.1 (5.8)
Delayed recall	6.0 (3.1)
Delayed recognition	27.0 (3.2)
Stroop test (time in sec.)	
trial 1 (words)	25.8 (6.3)
trial 2 (colours)	33.2 (7.7)
trial 3 (concept-shifting)	63.8 (22.1)
Paper and Pencil memory scanning task (time in sec.)	
1 character	45.0 (13.4)
2 characters	62.3 (19.7)
3 characters	77.4 (26.5)
Symbol digit substitution task (no. in 60 sec.)	27.1 (9.7)
Fluency (no. in 60 seconds)	
Animals	22.0 (6.5)
Jobs	16.5 (5.3)
Verbal series attention test	
total time (sec.)	90.6 (32.9)
total faults (no.)	2.1 (2.7)
Rey Complex Figure Test (range 0-36)	
Copy trial	33.5 (3.4)
Immediate recall trial	18.2 (6.8)
Delayed recall trial	18.1 (6.6)

Numbers represent means (SD)

Table 3 The relation between DTI parameters in both the white matter lesions and the normal appearing white matter and cognitive performance.

	White Matter Lesions		Normal appearing white matter	
	Mean Diffusivity	Fractional Anisotropy	Mean Diffusivity	Fractional Anisotropy
Global cognitive function				
MMSE	-.12 (-.21 to -.01) *	.06 (-.02 to .15)	-.17 (-.29 to -.07)*	.06 (-.21 to .16)
Cognitive Index	-.11 (-.16 to -.02) *	.03 (-.03 to .09)	-.18 (-.22 to .06)**	.11 (.04 to .17)**
Memory				
Verbal memory	-.08 (-.17 to .02)	.04 (-.04 to .12)	-.18 (-.28 to -.06)**	.11 (.01 to .19)*
Visuospatial memory	-.09 (-.16 to .01)	.03 (-.05 to .10)	-.13 (-.21 to .02)*	.10 (-.01 to .19)
Executive Function and Attention				
Psychomotor Speed	-.15 (-.21 to -.06)**	.02 (-.04 to .09)	-.18 (-.24 to -.06)**	.12 (.04 to .18)**
Fluency	-.09 (-.18 to .01)	.03 (-.05 to .11)	-.11 (-.21 to -.02)*	.07 (-.03 to .15)
Concept Shifting	-.11 (-.20 to -.02)*	.09 (.01 to .17)*	-.10 (-.16 to -.06)*	.10 (.01 to .19)*
Attention	-.12 (-.22 to -.02)*	.11 (.02 to .20)*	-.15 (-.27 to -.03)**	.16 (.06 to .25)**

Numbers represent standardized β 's and are adjusted for age, sex, education, depressive symptoms, total brain volume normalized for head size, lacunar infarcts and in the NAWM also for white matter lesions

* $p < .05$, ** $p < .01$

Discussion

In this large cohort of patients with cerebral SVD, we found that the microstructural integrity, of both WML and NAWM, as assessed by DTI, is related to global cognitive function, memory and executive function. In the NAWM this relation persisted even after controlling for presence of SVD on conventional MRI.

In contrast to previous DTI studies we measured MD and FA using a whole brain voxel-based analysis differentiating between NAWM and WML, whereas others investigating the relation between cognitive decline and WML used whole brain histogram approach, not accounting for the absence or presence of WML, or region of interest (ROI) analyses.^{9,33} A ROI provides a local estimate of possible changes in the microstructure within the brain, not necessarily reflecting the structural integrity of the remainder of the white matter. However, this whole-brain analysis does not allow dissociating between specific brain areas in relation to cognitive performance. As cognitive function is the resultant of the integrated action of many parts of the brain we wanted to investigate the structural integrity of the global white matter with respect to cognitive performance, rather than a ROI approach. In addition an overall assessment is less prone to partial volume artefacts.

The co-registration of different image types and their co-registration to standard space could be a methodological limitation of our study. Two co-registration steps should be discerned, i.e. from the FLAIR to T1 and from the mean-b0 to T1.

Furthermore, two sources of error should also be discerned, i.e. contrast differences between the source and target image and geometric distortion differences between source and target. The contrast source of error in our study should be minimal, as normalized mutual information was used a cost function to compute the optimal co-registration parameters. The distortions in the FLAIR and T1 images are both minimal and of no concern in general. There can be significant distortion differences between the DW images and the T1 and these will increase the variability of our analyses. Ideally one would therefore like to first correct the distortions in the DW images before co-registering them to the T1, which would require one to have information on these distortions, e.g. from measured field maps. We did not acquire such field maps, but we did visually inspect all co-registrations for optimality and found no apparent inaccuracies or errors. These local misalignments will, if anything, underestimate the strengths of the relations found. Major strengths of this study included its large sample size and use of novel imaging techniques, such as DTI. Furthermore, our study is a single centre study, with a high response rate and all subjects examined by only two investigators. Another strength is the manual segmentation of the WML without prior knowledge of the clinical data.

The relation between DTI parameters and cognitive function was investigated with extensive adjustments for possible confounders as education, depressive symptoms, TBV, and SVD on FLAIR.

Adjustment for SVD visible on conventional MRI is important in order to determine whether DTI, on top of conventional MRI measures, explains more of the variance in the relation between DTI measures and cognitive performance or whether it represents the same degree of white matter damage. There are only a few studies adjusting for WML volume, two adjusted for WML as measured by a visual scale,^{12,33} two other studies adjusted for WML volume but had a small sample sizes (n=36 and n=42) and did not adjust for depressive symptoms, another well known confounder in the relation between white matter pathology and cognitive performance.^{11,14,33}

A limitation is the cross-sectional nature of our study, which prevents us from proving causality. The RUN DMC study had a longitudinal design and follow-up is already planned to evaluate the effect of progression of SVD on (changes in) cognition.³⁴

Our findings are in agreement with other studies relating DTI parameters to cognitive function in individuals with symptomatic SVD, however these studies had a small sample size and depressive symptoms were not taken into account.^{11,13,14} Recently a population based cohort study demonstrated a relation between microstructural integrity of both the WML and NAWM and cognitive function.¹⁵ They did not adjust for depressive symptoms. A longitudinal study of normal aging demonstrated a correlation between MD and working memory and showed that DTI is sensitive to detect microstructural change over a 2-year period.³⁵

The relation between DTI parameters of the WML and NAWM and cognitive performance has also been investigated in individuals with WML other than sporadic vascular WML, such as multiple sclerosis, CADASIL and auto immune mediated WML.^{9,10,36,37} Our results are in accordance with their results, however, they had small sample sizes (n < 105) and did not, or rather selective adjust for possible confounders.

The relation between DTI parameters and cognitive performance has also been studied in subjects with mild cognitive impairment (MCI) and Alzheimer's Disease (AD). In most of these studies, subjects with SVD were excluded.^{38,19} Although the assessment of cognitive performance was limited and the sample sizes were relatively small, they all demonstrated a significant increase in MD and decrease in FA in cognitively impaired subjects. Two studies did not exclude subjects with WML.^{40,41} One study found a clear inverse relation between MD in the posterior cingulate gyrus and the MMSE. However, they did not report whether MD was measured in WML or NAWM and did not adjust for WML volume.⁴⁰

The relation between DTI parameters and cognitive performance has also been investigated in healthy elderly without severe WML.^{42,43} They demonstrated a relation between lower FA in the pericallosal frontal region and in the genu of the corpus callosum and the relationship between perceptual speed and episodic retrieval reaction time and FA in the frontoparietal white matter and reaction time.^{42,43}

In our study, the relation between cognitive performance was less pervasive for FA than for MD. This could be due to the fact that the value of MD is relatively independent of

Table 4 The relation between DTI parameters in white matter lesions and the normal appearing white matter and cognitive performance in subjects with mild, moderate and severe white matter lesions (defined as tertiles of the distribution).

	White Matter Lesions					
	Mean Diffusivity			Fractional Anisotropy		
	Mild WML	Moderate WML	Severe WML	Mild WML	Moderate WML	Severe WML
Global Cognitive Function						
MMSE	04 (- 17 to 25)	-.04 (- 16 to .25)	- 18 (- 39 to - 01)*	.01 (- 12 to 15)	00 (- 17 to 17)	17 (01 to .35)*
Cognitive Index	03 (- 11 to 17)	-.08 (-.22 to .07)	- 19 (- 29 to - 06) **	.02 (- 07 to .11)	- 03 (-.15 to 09)	12 (01 to .23)*
Memory						
Verbal memory	11 (- 10 to .32)	-.11 (- 30 to 09)	- 13 (- 30 to 04)	.03 (-.11 to .16)	01 (- 15 to 17)	.10(-.05 to .26)
Visuospatial memory	03 (- 15 to 20)	-.05 (- 23 to .13)	- 23 (- 41 to - 06)**	- 03 (- 14 to 08)	- 02 (- 18 to 13)	.16 (01 to 32)*
Executive Function and Attention						
Psychomotor speed	- 07 (- 23 to 08)	- 07 (- 23 to 10)	-.23 (-.35 to - 08)**	- 01 (-.11 to .09)	- 06 (- 21 to 08)	16 (04 to .27)**
Fluency	- 10 (- 32 to 10)	01 (-.19 to 21)	- 07 (- 23 to .09)	.08 (- 06 to 22)	- 06 (- 23 to .11)	02 (- 12 to 16)
Concept Shifting	- 03 (-.19 to .13)	03 (-.16 to 23)	- 24 (-.45 to - 07)**	.06 (-.05 to 16)	06 (- 10 to 23)	18 (.02 to 35)*
Attention	04 (- 16 to 26)	- 05 (- 26 to 16)	- 28 (-.50 to - 12)**	11 (- 03 to 25)	- 02 (-.19 to .15)	32 (16 to .49)**
	Normal appearing white matter					
	Mean Diffusivity			Fractional Anisotropy		
	Mild WML	Moderate WML	Severe WML	Mild WML	Moderate WML	Severe WML
Global Cognitive Function						
MMSE	-.03 (- 28 to 22)	- 05 (- 17 to .26)	- 24 (- 44 to - 04)*	03 (- 16 to 23)	- 02 (-.18 to 15)	.13 (-.03 to .29)
Cognitive Index	-.19 (- 35 to - 01)*	- 10 (- 25 to 07)	- 17 (- 27 to - 02) **	11 (-.02 to 24)	11 (- 02 to 22)	11 (00 to .20)
Memory						
Verbal memory	- 08 (- 34 to 16)	- 15 (- 38 to 01)	-.16 (- 32 to -.02)*	12 (- 07 to 32)	15 (- 01 to 30)	.07 (-.07 to 21)
Visuospatial memory	- 08 (- 29 to 13)	- 09 (-.16 to 10)	- 22 (- 39 to - 05)*	13 (- 02 to 29)	05 (- 19 to 10)	.16 (01 to 29)*
Executive Function and Attention						
Psychomotor speed	-.19 (- 37 to - 02)*	- 07 (- 18 to 24)	-.23 (- 35 to - 08)**	08 (- 06 to 22)	08 (-.05 to .22)	.17 (05 to .27)**
Fluency	- 07 (- 33 to 17)	- 03 (- 18 to 22)	- 16 (- 32 to - 01)*	22 (.04 to 43)*	- 05 (- 21 to 11)	15 (02 to 30)*
Concept Shifting	-.07 (- 26 to 13)	- 03 (- 17 to .22)	- 12 (- 32 to 07)	11 (- 04 to 26)	09 (- 07 to 24)	15 (- 01 to 31)
Attention	.12 (- 10 to .40)	08 (- 11 to 32)	-.36 (- 56 to -.19)**	03 (- 16 to 23)	09 (- 08 to 25)	27 (.12 to 42)**

Numbers represent standardized β 's and are adjusted for age, sex, education, depressive symptoms, total brain volume normalized for head size and lacunar infarcts * p< .05, ** p< .01

the location measured in the brain whereas FA values very much depend on the degree of white matter organisation and thus on the location in which it is measured³⁶ In addition, because of the voxel size in which the DTI parameters are measured multiple fibres are present within a single voxel, that may all have different destinations Due to this intervoxel incoherence the measured FA within a voxel may be low and does not necessarily reflect an underlying lower structural integrity⁷ These factors are likely to be important in the strength of the relation between FA and cognitive performance In addition, correlations in the WML may be harder to demonstrate as WML constitute a very small proportion ($\pm 1\%$) of the whole white matter

Our findings suggest that the structural integrity of the NAWM should also be taken into account when investigating the relation between SVD and cognitive function, as microstructural pathology extends in the NAWM beyond the detection limit of conventional FLAIR imaging In addition our findings show that especially in those with severe WML, damage to the microstructural integrity of the NAWM is related to impairment of cognitive performance This suggests a positive relation between WML load and severity of damage to the NAWM, which is demonstrated before⁴⁴ This is in line with our understanding of the underlying neuropathological changes that occur in the “NAWM”, corresponding with mild tissue changes, with lower myelin density and a looser but largely preserved axonal network and glial cell density⁴⁵ Microstructural changes in the NAWM are postulated to reflect remote effects in tracts damaged by the WML themselves and/or white matter changes that have not yet become visible on conventional MRI This is supported by the finding of an increased blood-brain barrier permeability in the NAWM in subjects with SVD⁴⁶ This suggests that the microstructural changes of the white matter detected by DTI have important clinical consequences

Another issue is that other studies have shown that particularly lacunar infarcts drive the association between WML and cognitive performance and incident dementia² We tried to overcome this issue to the best of our ability by rating all lacunar infarcts and adjusting for them in the statistical analysis We found a relation between the structural integrity of both NAWM and WML and cognitive performance, independent from SVD visible on FLAIR MRI DTI could therefore serve as an additional tool to conventional MRI in order to investigate cognitive dysfunction The relation of DTI parameters within the WML with cognitive performance and impairment may explain why subjects with identical WML on FLAIR differ in cognitive performance Future studies should prospectively investigate the predictive value of DTI parameters for incident cognitive decline or dementia When proven, DTI could possibly be a surrogate marker for disease progression, and can as such be used in therapeutic trials

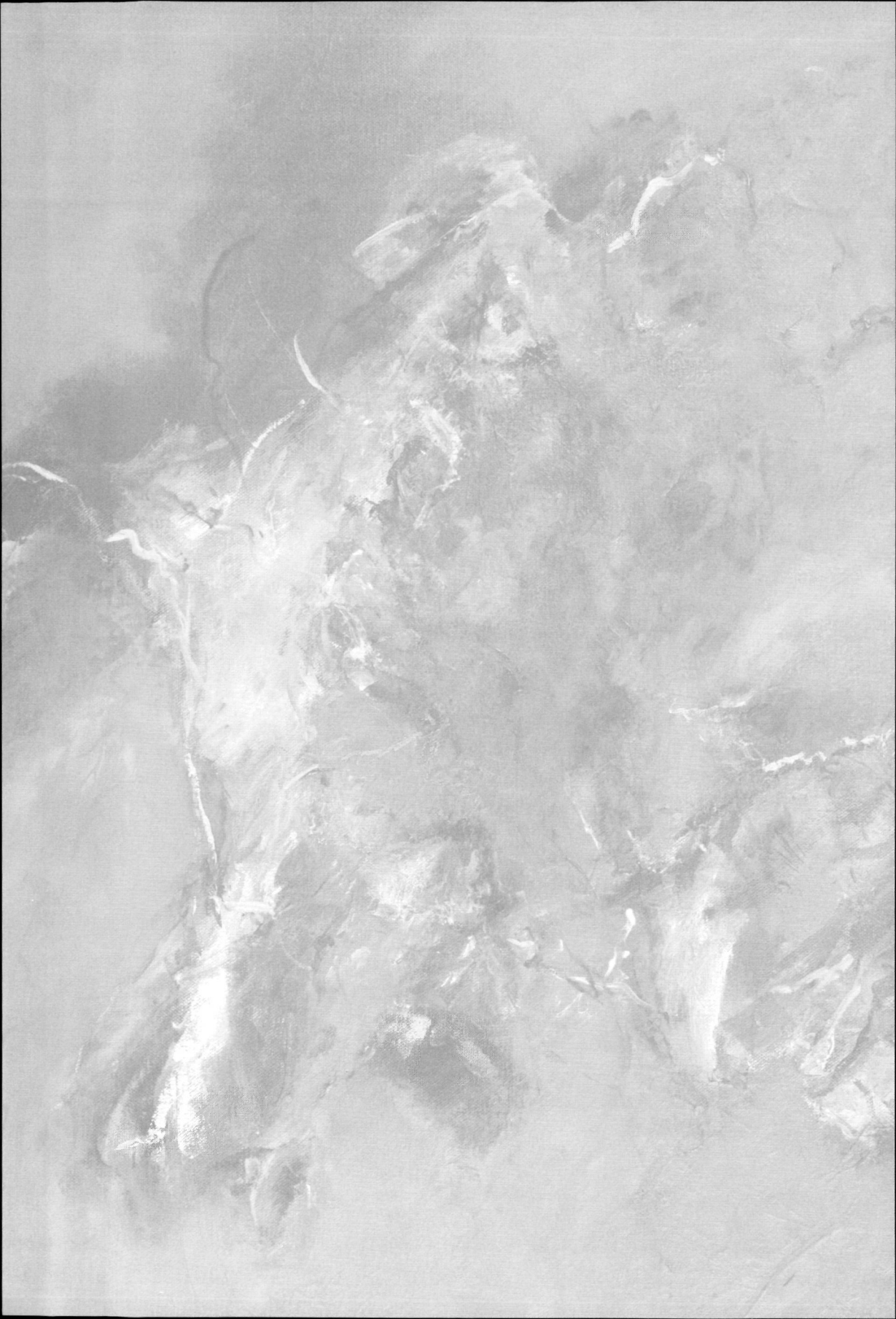
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Chapter 8

Loss of hippocampal integrity is associated with memory performance

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Abstract

Background Cerebral small vessel disease (SVD) and hippocampal atrophy are related to verbal memory failures, and may ultimately result in Alzheimer's disease (AD). However, verbal memory failures are often present before structural changes on conventional MRI appear. Changes in micro-structural integrity of the hippocampus, which cannot be detected with conventional MRI, may be the underlying pathological substrate. With DTI we investigated the relation between the microstructural integrity of the hippocampus and verbal memory performance in 503 non-demented elderly with SVD.

Methods The RUN DMC study is a prospective cohort study among 503 non-demented elderly with cerebral SVD aged between 50 and 85 years. All participants underwent T1 MPRAGE, FLAIR and DTI scanning and the Rey Auditory Verbal Learning Test. After manual segmentation of the hippocampi, we calculated the mean diffusivity (MD) and fractional anisotropy (FA) in both hippocampi. The relation between memory performance and hippocampal DTI parameters was adjusted for age, sex, education, depressive symptoms, hippocampal and WML volume and lacunar infarcts.

Results We found inverse relations between hippocampal MD and verbal memory performance ($\beta = -0.22, p < 0.001$), immediate recall ($\beta = -0.22, p < 0.001$), delayed recall ($\beta = -0.20, p < 0.001$), and forgetting rate ($\beta = -0.13, p = 0.025$), most outspoken in participants with a normal hippocampal volume.

Conclusion Microstructural integrity of the hippocampus assessed by DTI is related to verbal memory performance in elderly with SVD, also in participants with an intact appearing hippocampus. Changes in hippocampal micro-structure may be an early marker of underlying neurodegenerative disease, before macro-structural (i.e., volumetric) changes occur.

Introduction

Cerebral small vessel disease (SVD) includes white matter lesions (WML) and lacunar infarcts. The prevalence of SVD in the general population over 60 years is high.^{1,2} Both patient and population based studies have shown that cerebral SVD is an important cause of verbal memory failure and may ultimately result in cognitive decline associated with Alzheimer's disease (AD) in some.^{3,5}

In brains of patients with AD, atrophy of the hippocampus is one of the first observed changes.⁶ Interestingly, the progression of hippocampal atrophy is related to the presence and progression of cerebral SVD.^{7,8} Generally, its presence is supportive for the diagnosis of AD and indicative for future development of AD in patients with mild cognitive impairment (MCI).^{9,10} Hippocampal atrophy underlies the profound deficit in the consolidation of memory in AD¹¹, i.e. long-term encoding and storage of relevant new information.

However, these macroscopic structural hippocampal changes on conventional MRI occur at a relatively late stage and are usually preceded by clinical symptoms including subjective cognitive failures, verbal memory decline and problems in other cognitive domains.¹² Conceptually, there must be changes in the microstructural integrity of the hippocampus before macroscopic loss of volume occurs. Diffusion tensor imaging (DTI) can provide more detailed information on the micro-structural integrity of the hippocampus. DTI provides amongst other possibilities, two scalar parameters: mean diffusivity (MD), a measure of the magnitude of diffusion of water averaged in all spatial directions, and fractional anisotropy (FA), which provides information about the directionality of water diffusion.^{13,14} DTI is usually used to assess microstructural integrity of the white matter in which damage to the white matter has found to be accompanied by an increase in MD and a decrease in FA,^{15,16} but there are a number of reports showing its usefulness in the assessment of (predominantly) gray matter structures.^{17,20} There is some evidence of a higher diffusion in the hippocampus in MCI and AD patients that is independent of hippocampal atrophy and white matter lesions (WML).^{17,19}

More importantly, two studies have shown that hippocampal MD measures were superior to volume measures in predicting clinical progression to dementia in MCI patients.^{18,20} However, to date the role of hippocampal DTI measurements has never been investigated in the light of specific processes of verbal memory function in non-demented elderly, focusing on encoding, storage and retrieval. And to detect early changes in hippocampal microstructure even before macrostructural changes occur and the relation with early impairment of verbal memory.

In the present study the relation between hippocampal DTI measures and verbal memory processes is examined, independent of hippocampal volume and SVD, in 503 non-demented, independently living elderly with cerebral SVD, aged between 50 and 85 years.

Methods

Study population

The Radboud University Nijmegen Diffusion tensor and Magnetic resonance imaging Cohort (RUN DMC) study is a prospective cohort study that was designed to investigate risk factors and cognitive, motor and mood consequences of functional and structural brain changes as assessed by structural MRI, DTI and resting state fMRI among independently living non-demented elderly with cerebral SVD

In people with cerebral SVD symptoms are due to either complete (lacunar syndromes) or incomplete infarction (WML) of subcortical structures, that might lead to acute symptoms as transient ischaemic attacks (TIAs) or lacunar syndromes, or subacute manifestations as cognitive, motor (gait) and/or mood disturbances²¹ As the onset of cerebral SVD is often insidious, clinically heterogeneous, and typically with mild symptoms, it has been suggested that the selection of people with cerebral SVD in clinical studies should be based on the more consistent brain imaging features²²

Accordingly, in 2006, consecutive individuals who visited the department of neurology between October 2002 and November 2006 were selected for possible participation Inclusion criteria were (a) age between 50 and 85 years, (b) cerebral SVD on neuroimaging Subsequently, the above mentioned acute and subacute 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,^{23 25} (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(anta)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

From 1004 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 center (14 with unexpected claustrophobia, one died before MRI scanning, one was diagnosed with multiple sclerosis, in one there was a language barrier, one participant fulfilled the criteria for Parkinson's disease and four met the dementia criteria),^{23 25} yielding a response of 71.3% (503/705) These 503 individuals had symptoms of TIA or lacunar syndrome (n=219), cognitive disturbances (n=245), motor disturbances (n=97), depressive symptoms (n=100) or a combination thereof All participants signed an informed consent form The Medical Review Ethics Committee region Arnhem-Nijmegen approved the study

Conventional MRI scanning protocol

All participants underwent a 1.5-Tesla MRI scanning on the same Magnetom scanner (Siemens, Erlangen, Germany). The protocol included the following whole brain scans: T1 3D MPRAGE imaging (TR/TE/TI 2250/3 68/850ms, flip angle 15°, voxel size 1.0x1.0x1.0mm), Fluid-attenuated inversion recovery (FLAIR) pulse sequences (TR/TE/TI 9000/84/2200ms, voxel size 1.0x1.2x5.0mm, with an interslice gap of 1 mm), Diffusion Tensor Imaging (DTI) (TR/TE 10100/93ms, voxel size 2.5x2.5x2.5mm, 4 unweighted scans, 30 diffusion weighted scans with b-value 900s/mm²). The complete protocol took 31 minutes.

Conventional MRI Analysis

Hippocampus and intracranial volumetry One experienced investigator, blinded to clinical data (lvU), manually segmented the left and right hippocampus on the MPRAGE image using the interactive software program "ITK-SNAP"²⁶. Anatomical boundaries were determined in coronal sections with the aid of neuroanatomical atlases,^{27,28} actual segmentation was performed using a previously published protocol²⁹ in which segmentation was performed from posterior to anterior. The posterior border of the hippocampus was identified in the slice before the level in which the crurae fornices appeared in full view. The anterior border of the hippocampus was defined as the slice in which the hippocampus was no longer present, and the amygdala fully covered the hippocampus (General Brain Segmentation Method and Utilization, Version 3. Center for Morphometric Analysis, 2004).^{29,30} The superior border was the inferior horn of the lateral ventricle, the inferior border was determined by the white matter boundary. The lateral border was defined by the temporal horn of the lateral ventricle and the white matter adjacent to the hippocampus.

Volumes were calculated for the left and right hippocampus separately by summing all voxel volumes of the segmented areas. Inter-rater studies on a random sample of 10 percent showed an intra-class correlation coefficient for the left hippocampus of 0.73, and for the right hippocampus of 0.79. Intra-rater studies on this sample showed an intra-class correlation coefficient for the left and right hippocampus of 0.97 and 0.96, respectively.

For the same image, gray (GM) and white-matter (WM) tissue and cerebrospinal fluid (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. Intracranial volume (ICV) was taken as the sum of total GM, WM and CSF volume.

Hippocampal volume (HV) measurements were normalized to the total ICV. The normalized hippocampal volume (NHV) is defined as $NHV = ICV_m \times HV_p / ICV_p$, where ICV_m is the average total ICV of all participants, ICV_p is the ICV of the participant and the HV_p is the hippocampal volume of the participant.³¹

WML volumetry WML were manually segmented on transversal FLAIR images. WML were defined as hyper-intense lesions on FLAIR and not CSF like hypo-intense lesions on T1-weighted image.³² Two trained raters, blinded for all clinical, cognitive and DTI information, segmented all scans. WML volume was calculated as lesion surface multiplied by slice thickness. Inter-rater variability was determined in a random sample of ten percent and yielded an intra-class correlation coefficient of 0.99 for total WML volume. The FLAIR image was used to compute the co-registration parameters to the anatomical T1 image, which were then applied to the segmentation results. All images were visually checked for co-registration errors.

Lacunar infarcts Lacunar infarcts were defined as hypo-intense areas $> 2\text{mm}$ and $\leq 15\text{mm}$ on FLAIR and T1, ruling out enlarged perivascular spaces ($\leq 2\text{ mm}$, except around the anterior commissure, where perivascular spaces can be large) and infraputaminial pseudolacunae.³² Evaluation of infarcts was performed by a resident in neurology blinded to all clinical data 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.

Diffusion Tensor Imaging analysis

The diffusion weighted images of each patient were realigned on the unweighted image using mutual information based co-registration routines from SPM5. Then, the diffusion tensor¹³ and its eigenvalues were estimated using linear regression (spurious negative values were set to zero), after which the tensor derivatives MD and FA were calculated.³³ 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 weighted images and derivatives. The mean MD and FA were then calculated in both hippocampi. All images were visually checked for motion artifacts and co-registration errors, especially for not including peri-hippocampal CSF.

Measurement of cognitive function

Cognitive function was assessed by two trained investigators (AvN, KdL). For this sub-study, the Mini Mental State Examination (MMSE) (range 0-30)³⁴ was used as an index of overall cognitive performance. The three-trial version of the Rey Auditory Verbal Learning Test (RAVLT)³⁵ was administered to examine episodic memory formation.

We defined five memory indices based on RAVLT performance (e.g., immediate recall, learning rate, forgetting rate, delayed recall and delayed recognition), as described previously.³⁶ Immediate recall was calculated by the mean of the total number of words remembered in the three learning trials of the RAVLT. Learning rate was also determined by the three learning trials of the RAVLT, in which a learning curve was estimated with the following formula: $((\text{trial 2} - \text{trial 1}) / \text{trial 1}) + ((\text{trial 3} - \text{trial 2}) / \text{trial 2}) / 2$. Delayed recall was

the number of words recalled thirty minutes after the learning trials. Forgetting rate, as a measure of decay over time, was obtained by the scores in the delayed recall trial corrected for the score obtained in the third learning trial ($\text{delayed recall-trial3/trial3}$). The delayed recognition score was calculated by computing the total of each correctly recognized word (the 15 target words among 15 new distracter items) thirty minutes after the learning trials. Performance across tests was made comparable by transforming the raw test scores into z-scores as described elsewhere,³ for which the assumption of normality of the distribution was examined. For data reduction purposes, a compound score for global verbal memory function was calculated, as described previously,³ by taking the mean of two z-scores from the RAVLT, one for the added scores on three learning trials of this test, and one for the delayed recall of this test. If the test assistant encountered problems, a code was given for test status and the result was not used in the calculation of the z scores. Separate codes were given for lack of motivation (0.8%) or not following the instructions (0.9%). For 98% of all participants reliable compound scores for global verbal memory performance could be calculated without any recording of test problems.

Other measurements

The following characteristics were considered possible confounders: age, sex, educational level (classified using 7 categories, 1 being less than primary school and 7 reflecting an academic degree)³⁷ and depressive symptoms. Depressive symptoms were defined as a score ≥ 16 on the Center of Epidemiologic Studies on Depression Scale (CES-D)^{38,39} and/or current use of anti-depressive medication.³⁹

Statistical analysis

Statistical analyses were performed with SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA). Baseline characteristics were summarized as means (standard deviations, SD) or proportions, for skewed variability parameters the median and the inter-quartile range was calculated. We used multiple linear regression analyses to investigate the relation between hippocampal DTI (FA/MD) parameters (left and right separately) and global verbal memory performance as well as with sub-processes of verbal memory performance. Adjustments were made for potential confounders including age, sex, educational level, depressive symptoms, hippocampal and WML volume and the presence of lacunar infarcts. Next, we calculated estimated mean scores of global verbal memory performance per tertile of hippocampal MD by analysis of covariance (ANCOVA), adjusted for the same potential confounders. In order to identify whether these microstructural changes precede macroscopic hippocampal volume loss we investigated the relation (linear regression analysis) between hippocampal DTI measures and global verbal memory performance stratified on low (lowest tertile of the distribution) versus normal hippocampal volume (upper two tertiles) adjusting for the same confounders as the previous analysis. If so, we would expect a significant relation between

hippocampal DTI measures and global verbal memory performance, particularly in the group with still intact (normal) hippocampal volumes. Regression coefficients are presented as standardized beta-values.

All assumptions for ANCOVA analysis were tested and verified for all measures. For the trend analysis of the ANCOVA results, tertiles of hippocampal MD were considered as a continuous variable in a multiple linear regression model.

Table 1 Baseline characteristics of the 499 participants

Demographic characteristics	
Age (years)	65.6 (8.8)
Sex (male, n (%))	282 (56.5%)
Participants with only primary education	49 (9.8%)
CES-D	11.0 (9.4)
Participants with depressive symptoms *	166 (33%)
Cognitive function	
MMSE	28.1 (1.6)
Rey auditory verbal learning test - number of words recalled	
Immediate recall	
Trial 1	5.1 (1.8)
Trial 2	7.2 (2.3)
Trial 3	8.6 (2.6)
Total trial 1-3	20.9 (6.0)
Learning rate	0.36 (0.24)
Delayed recall	5.8 (3.1)
Forgetting rate	-0.34 (0.25)
Delayed recognition	26.7 (3.5)
Neuroimaging characteristics	
WML volume	7.1 (3.4, 18.1)
Normalized hippocampal volume	6.8 (0.9)
Left normalized hippocampal volume	3.5 (0.5)
Right normalized hippocampal volume	3.3 (0.5)
Presence of lacunar infarcts (n, %)	171 (34)

Values are means (SD), n (%), medians (inter-quartile range) or ml for the neuroimaging characteristics

* Defined as CES-D scores ≥ 16 and/or the current use of anti-depressive medication. MMSE: Mini Mental State Examination. CES-D: Centre of Epidemiological Studies on Depression Scale. WML: White matter lesions.

Results

Of the 503 participants three were excluded because of DTI motion artefacts and one because of an automatic segmentation problem that could not be solved manually. Demographic and neuroimaging characteristics of 499 participants are shown in table 1. Mean age of the population was 65.6 years (SD 8.8), 56.5% were male and mean MMSE was 28.1 (SD 1.6). In an univariate analysis all confounding variables were related to global memory performance and hippocampal DTI parameters ($p < .05$). The relation between left and right hippocampal DTI parameters and the performance on global verbal memory as well as on the sub-processes of memory is shown in Table 2.

Table 2 The relation between hippocampal DTI parameters and the sub-processes of verbal memory function as measured with the Rey Auditory Verbal Learning Test (RAVLT).

	Hippocampus					
	Left		Right		Total	
	MD	FA	MD	FA	MD	FA
Global verbal memory performance	-0.18**	0.10*	-0.21**	0.04	-0.22**	0.07
Immediate memory						
<i>Trial 1</i>	-0.16**	0.03	-0.12*	0.01	-0.16**	0.03
<i>Trial 2</i>	-0.16**	0.07	-0.18**	0.03	-0.19**	0.06
<i>Trial 3</i>	-0.18**	0.12*	-0.20**	0.09*	-0.21**	0.12*
<i>Total trials 1-3</i>	-0.19**	0.09*	-0.19**	0.06	-0.21**	0.08
Learning rate	-0.08	0.02	-0.01	0.05	0.04	0.04
Delayed recall	-0.16**	0.09*	-0.20**	0.03	-0.20**	0.06
Forgetting rate***	-0.10	0.04	-0.15*	0.03	-0.14*	0.01
Delayed recognition	-0.17**	0.03	-0.14*	0.01	-0.17**	0.02

Numbers represent regression coefficients (standardized beta's), adjusted for age, sex, educational level, depressive symptoms, hippocampal and WML volume and the presence of lacunar infarcts * $p < .05$, ** $p < .01$ *** Higher test scores reflect a less negative forgetting rate, i.e. a better performance

In this analysis we did not find a relation between global verbal memory performance and hippocampal volume ($p > .05$). Adding hippocampal MD in the analysis showed a significant relation with global memory performance (left $p = .002$; right $p < .001$; total $p = .002$). MD in the left and right hippocampus showed a significant relation with the compound score of the global verbal memory performance ($\beta = -0.18$, $p = .001$ and $\beta = -0.21$, $p < .001$). Analyses of the specific components of the RAVLT demonstrated significant relations with immediate recall ($\beta = -0.19$, $p = .001$ and $\beta = -0.19$, $p < .001$), delayed recall ($\beta = -0.16$, $p = .005$ and $\beta = -0.20$, $p < .001$)

Table 3 The relation between hippocampal DTI parameters and global verbal memory performance in participants with low and normal hippocampal volume

	Hippocampus					
	Left		Right		Total	
	MD	FA	MD	FA	MD	FA
Global verbal memory performance						
Low hippocampal volume (n=166)	-0.20 (.044)	0.11 (.169)	-0.13 (.162)	0.03 (.706)	-0.19 (0.073)	0.07 (.368)
Normal hippocampal volume (n=333)	-0.15 (.020)	0.08 (.108)	-0.24 (<.001)	0.05(.390)	-0.21 (0.002)	0.07 (.194)

Numbers represent regression coefficients standardized beta's (*p* values), adjusted for age, sex, educational level, depressive symptoms, hippocampal and WML volume and the presence of lacunar infarcts. Low hippocampal volume is defined as the lowest tertile of the hippocampal volume distribution. Normal hippocampal volume is defined as the upper two tertiles of the hippocampal volume distribution.

and delayed recognition ($\beta=-0.17$, $p=0.003$ and $\beta=-0.14$, $p=0.013$) and the MD of the left and right hippocampus, independent of the confounders. The MD in the right hippocampus and total hippocampus showed a relation with forgetting rate ($\beta=-0.15$, $p=0.013$ and $\beta=-0.14$, $p=0.027$). The relation between hippocampal FA values and memory performance was less evident, but were most outspoken for the left hippocampus (Table 2). We did not find essential differences in standardized betas and p-values between men and women separately performing the same analyses. We could not demonstrate a sex difference in lateralization of verbal memory performance. There was no relation between hippocampal DTI parameters and learning rate.

When stratified on tertiles of hippocampal MD, higher MD values, represented by a higher MD tertile, for both left and right hippocampus were related to worse performances on immediate recall ($p_{\text{trend}}=0.001$ and $p_{\text{trend}}<0.001$), delayed recall ($p_{\text{trend}}=0.006$ and $p_{\text{trend}}=0.001$) and forgetting rate ($p_{\text{trend}}=0.057$ and $p_{\text{trend}}=0.035$) after adjusting for possible confounders (Figure 1). Again, no significant relation was found for the learning rate.

Table 3 shows the relation between hippocampal DTI parameters and global verbal memory performance in participants with a low or normal hippocampal volume.

The relation was most striking for MD values in participants with normal hippocampal volumes for left ($\beta=-0.15$, $p=0.02$), right ($\beta=-0.24$, $p<0.001$) and total ($\beta=-0.21$, $p=0.002$) hippocampus, independent of earlier mentioned confounders. This relation was also found for MD in the left hippocampus in participants with a low hippocampal volume. The FA of the hippocampus in participants with low or normal hippocampal volume did not correlate with global memory function.

Figure 1a Mean scores on sub-processes of memory performance per tertile of hippocampal MD adjusted for age, sex, educational level, depressive symptoms, hippocampal and WML volume and the presence of lacunar infarcts.

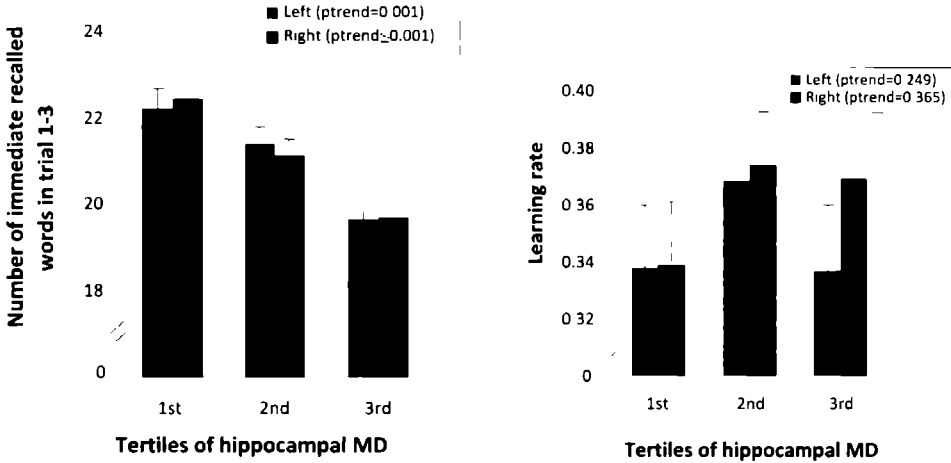
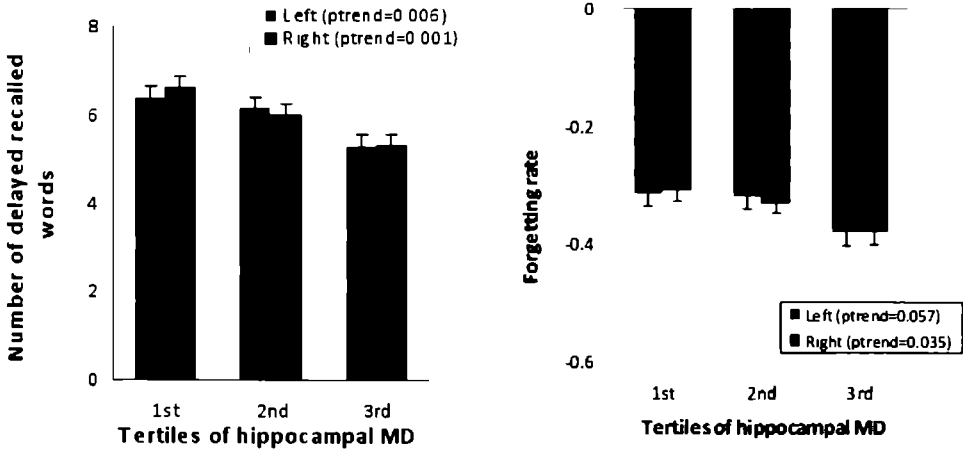


Figure 1b Mean scores on sub-processes of memory performance per tertile of hippocampal MD adjusted for age, sex, educational level, depressive symptoms, hippocampal and WML volume and the presence of lacunar infarcts.



Discussion

Our findings demonstrate that early changes in hippocampal integrity are related to reduced global verbal memory function, specifically immediate and delayed recall, delayed recognition and forgetting rate, independent of depressive symptoms, hippocampal and WML volume and lacunar infarcts, in 499 participants with cerebral SVD. These findings are in line with the notion that the hippocampus is crucial for the acquisition of new knowledge that has to be recalled (i.e., episodic memory formation). Our data demonstrate that micro-structural integrity of the hippocampus beyond the detection limit of conventional MRI is related with episodic memory formation. The findings clearly show episodic memory formation – specifically the encoding and storage of new information – are related to hippocampal integrity. Although it is difficult to isolate encoding as a process using a standard word-list paradigm, the encoding stage is most prominently reflected in the immediate recall index of the RAVLT^{36,40}. We demonstrate that hippocampal integrity is related to immediate recall, which is in line with neuroimaging studies showing that the hippocampus is implicated in verbal encoding, predicting later successful recall⁴¹. Storage of information is reflected by various indices on the RAVLT³⁶. In general, successful recall after learning depends on adequate storage of information. However, the forgetting rate (delayed free recall performance compared to the amount of previously learned information) may also be confounded by a retrieval deficit, i.e. the inability to access previously stored information. Cued-recall paradigms have been developed to facilitate access to previously acquired information (e.g., the recognition trial on the RAVLT). Our findings show a relation between hippocampal integrity and free delayed recall, forgetting rate and delayed recognition, clearly showing a crucial role for the hippocampus in the storage of information that is independent from a retrieval deficit. These findings support recent neuroimaging evidence indicating that the hippocampus is crucial for successful performance on recognition memory paradigms^{42,42}. With respect to lateralization effects, our results suggest a mild lateralization for hippocampal DTI measures and verbal memory performance. This is in line with previous findings from functional neuroimaging studies and lesion studies⁴¹. Additional analyses on possible sex differences in lateralization of verbal memory performance showed no significant findings related to sex. Research investigating the effects of sex on lateralization of verbal (memory) function shows some mixed results, with some studies demonstrating sex differences in which men have a left predominance and women a more bilateral representation⁴⁴. However, these findings have not been replicated in an extensive meta-analysis demonstrating no consistent evidence for more bilateral representation of verbal function in women than men⁴⁵ as well as in a functional MR imaging study⁴⁶. Some methodological issues need to be considered. Although our data are derived from the largest DTI study thus far, they are cross-sectional in nature preventing us from drawing conclusions with respect to causality. Follow-up examination of this cohort is already

planned, to investigate whether early microstructural changes in the hippocampus correlate with the development of hippocampal atrophy, MCI and eventually AD

We found that the relation between FA and memory performance is less strong than for MD. This may be due to the fact that FA is a measure reflecting the dominant directionality of diffusion of water. Because of the size of our region of interest (the hippocampus), multiple fibers are present that may all have different directions, which influence the FA. Due to that intra-hippocampal fiber incoherence, low FA may not necessarily reflect underlying lower structural integrity.¹⁴ In contrast, MD is affected by fiber crossing to a lesser extent because it reflects magnitude of water diffusion which is not influenced by direction. Subsequently, MD remains relatively constant.¹⁴ This might be the explanation for the lack of finding with FA but not with MD. These complications in the analyses of FA in the hippocampus were also reported by others looking at MCI and AD patients.¹⁷ In that study, they tried to take this into account by considering smaller regions of interest in the hippocampus, but still found a low sensitivity for FA values in contrast to MD.¹⁷

The strengths of our study include its large size with a high response of over 70%. In addition, it is a single-centre study in which MRI data were identically acquired on a single scanner and the hippocampus and WML were assessed volumetrically in a reliable, sensitive and objective way by two trained experts, who were blinded to all clinical data. Furthermore, we used extensive adjustment for possible confounders such as educational level, depressive symptoms, a well known confounder in cognitive performance in elderly with SVD³⁹, hippocampal and WML volume and lacunar infarcts.

To the best of our knowledge, there are no other studies investigating the relation between hippocampal DTI parameters and verbal memory function in non-demented elderly. A few studies have assessed DTI measurements in the hippocampus of MCI and AD patients.^{20, 47} In line with these studies, we found support for the notion that the structural integrity change already produces decrements in verbal memory performance before hippocampal volume does. DTI parameters in the hippocampus were found to discriminate between early phases of cognitive decline (MCI) and normal aging. Patients with more advanced cognitive decline (i.e., AD) could only be discriminated based on their lower hippocampal volume compared to controls and not by differences in diffusivity.¹⁹ The same investigators also found that high diffusivity in the hippocampus and low hippocampal volume were related to an increased risk for conversion of MCI to AD, with diffusivity being a stronger predictor than volume.¹⁸ In these studies, additional adjustments were made only for age, sex and education. Specific processes of episodic memory performance as in our study, which represent a more sensitive measure for cognitive decline than a clinical diagnosis of MCI or AD based on clinical diagnostic criteria^{24, 48} were not taken into account. A further difference with our study was the mean age of their study group that was around 80 years¹⁸. Furthermore, they demonstrated that baseline hippocampal MD is associated with conversion to AD rather than hippocampal volume.⁴⁷ Others demonstrated a higher sensitivity of hippocampal

DTI parameters than hippocampal volume measures in the diagnosis of MCI²⁰ However, participants with relevant cerebrovascular disease (e.g. cortical infarcts, multiple lacunar infarcts and leukoaraiosis) were excluded in these studies in contrast to our study. Consequently, these may have a limited external validity as over 50% of AD patients have WML to some extent⁴⁹ In our study population, SVD was present in some degree in all participants, making our findings more representative.

Interestingly, the relation between hippocampal integrity and verbal memory function was also found in participants without loss of hippocampal volume as assessed by structural MRI. While there is abundant evidence that episodic memory formation is impaired by patients with hippocampal lesions or atrophy, such as early AD, and functional neuroimaging studies also indicate a crucial role for the medial temporal lobe including the hippocampus¹¹, this is the first study to demonstrate, using DTI, such a relationship in older people without apparent hippocampal pathology using DTI. This may indicate that microstructural changes assessed by DTI in the hippocampus indeed precede structural changes as assessed by conventional MRI, this must be unraveled by the follow up examination.

The relation between DTI parameters and verbal memory function was less striking in participants with low hippocampal volumes. This could be because at the time that atrophy occurs, the structural changes (atrophy) are more prominently correlated with verbal memory performance than the microstructural changes. This line of reasoning is in agreement with the results of others demonstrating that diffusion parameters of the hippocampus can differentiate between MCI and controls, but could not classify AD (19), as well as studies showing that hippocampal DTI parameters in patients with MCI correlated with disease progression to AD^{18,47}.

Future studies should prospectively investigate the predictive value of DTI parameters of the hippocampus before the development of hippocampal atrophy and incident cognitive decline and dementia. To extrapolate our results future studies should include participants from the general population. When proven, DTI of the hippocampus could possibly play a role as a surrogate marker for disease progression, and could as such be used in therapeutic trials.

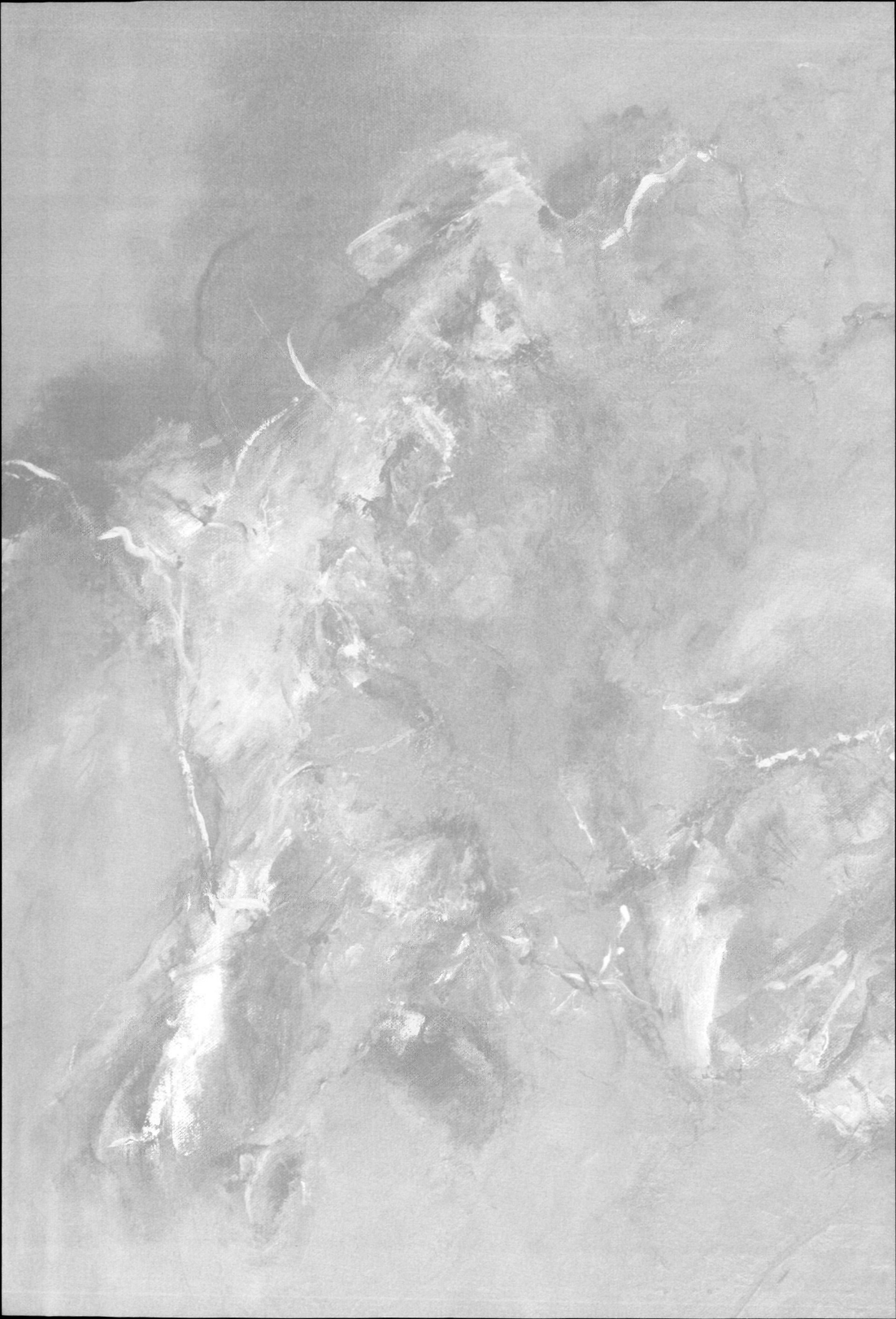
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Chapter 9

Loss of cingular integrity is associated with memory performance

Submitted as:

van der Holst HM, van Norden AGW, de Laat KF, van Uden IWM, van Oudheusden LJB, Zwiers MP, Norris DG, Kessels RPC, de Leeuw FE. Microstructural integrity of the cingulum is related to verbal memory performance in elderly with cerebral small vessel disease. The RUN DMC study.

Abstract

Background Cerebral small vessel disease (SVD) is related to verbal memory failures. An important structure for verbal memory is the mid and posterior part of the cingulum bundle. Verbal memory failures are often present before structural changes on conventional MRI are seen. Changes in the microstructural integrity of the cingulum assessed with diffusion tensor imaging (DTI) beyond detection with conventional MRI may precede macrostructural changes and be related with verbal memory failures.

Objective To investigate the relation between cingular microstructural integrity and verbal memory performance in 503 non-demented elderly with cerebral SVD.

Methods The RUN DMC study is a prospective cohort study in elderly (50-85 years) with cerebral SVD. All participants underwent T1 MPRAGE, FLAIR and DTI scanning and the Rey Auditory Verbal Learning Test. Mean diffusivity (MD) and fractional anisotropy (FA) were assessed in six different cingular regions of interests (ROIs). Linear regression analysis was used to assess the relation between verbal memory performance and cingular DTI parameters, with appropriate adjustments.

Results We found inverse relations between MD of the left mid and posterior cingulum (superior-posterior and inferior-posterior ROI) and overall verbal memory ($\beta=-.13$, $p=.006$; $\beta=-.10$, $p=.029$ and $\beta=-.12$, $p=.004$), immediate recall ($\beta=-.13$, $p=.008$; $\beta=-.13$, $p=.008$ and $\beta=-.10$, $p=.015$) and delayed recognition ($\beta=-.20$, $p<.0005$; $\beta=-.18$, $p<.0005$ and $\beta=-.14$, $p=.002$), independent of confounders. This relation remained significant in participants with an intact hippocampal structural integrity.

Conclusion Microstructural integrity of the mid and posterior cingulum assessed by DTI is related to verbal memory performance in elderly with SVD, especially in participants with intact hippocampal structural integrity.

Introduction

Cerebral small vessel disease (SVD) includes white matter lesions (WML) and lacunar infarcts and is a frequent finding on magnetic resonance imaging (MRI) scans of elderly people.¹ Several, patient- and population based studies, have shown that cerebral SVD is related to verbal memory failure and may eventually result in cognitive decline and dementia in some.^{2,3} Cerebral SVD is also associated with hippocampal atrophy,^{4,5} one of the first signs observed on conventional MRI scans of patients with (Alzheimer's) dementia. However hippocampal atrophy as seen on conventional MRI is usually preceded by clinical symptoms of cognitive decline. More recent studies also showed involvement of the white matter in cognitive impairment.^{6,7} Specifically, the cingulum bundle, a white matter bundle which connects the medial temporal lobe (MTL) structures (e.g. hippocampus) and the posterior cingulate cortex, is an important structure for verbal memory performance.^{8,10} It is expected that macroscopic changes of the cingulum are preceded by changes in the microstructural integrity of this white matter bundle. This can be revealed using a noninvasive MRI technique, diffusion tensor imaging (DTI), which provide detailed information on the microstructural integrity of brain structures.^{11,12} Two DTI parameters are of special interest: mean diffusivity (MD), a measure of water diffusion averaged in all spatial directions, and fractional anisotropy (FA), which provides information about the directionality of water diffusion. Loss of microstructural integrity is accompanied by an increase in MD and a decrease in FA.¹³ There is increasing evidence that DTI parameters are an earlier marker of cognitive decline in comparison to volume measures.^{14,15} Many DTI studies in patients with cognitive decline and dementia have shown loss of microstructural integrity of the cingulum.^{9,16,19} The relation between loss of structural integrity of the cingulum and gray matter structures as the hippocampus and cognitive decline has not been clarified. Some studies have suggested that hippocampal atrophy is related to cingulum integrity in (Alzheimer's) dementia (AD),^{10,20,22} whereas others did not find this relation.²³ The majority of previous DTI studies focused on the cingulum adjacent to the posterior cingulate cortex. Most of them found that loss of structural integrity of the cingulum begins near medial temporal structures and then progresses to the posterior cingulum with AD progression, supporting the posterior-anterior hypothesis of progression of AD pathology.²⁴ Furthermore, a recent study showed that posterior cingulum integrity was associated with cognitive function (including verbal and visuospatial memory, as well as executive functioning) in older adults without dementia.¹⁶ Less or no relations were found for the anterior cingulum integrity and cognitive function,^{16,25} probably because the anterior cingulum is not directly part of the posterior neuronal network that subserves episodic memory.²⁶ To the best of our knowledge the role of cingular DTI measurements and the specific processes of verbal memory function, focusing on encoding, storage and retrieval of verbal information, have never been investigated in non-demented elderly with cerebral SVD.

We hypothesized that loss of microstructural integrity of the mid and posterior cingulum is related to impaired verbal memory performance. We examined this in six different regions from the posterior to the anterior cingulum in order to get information about regional distribution patterns in respect to verbal memory. Furthermore, we investigated the influence of hippocampal structural integrity on this relation. This study is part of the Radboud University Nijmegen Diffusion tensor and Magnetic resonance imaging Cohort (RUN DMC) study that included 503 non-demented, independently living elderly with cerebral SVD, aged between 50 and 85 years.

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 with cerebral SVD. The selection procedure of the participants and study protocol were described in detail previously²⁷.

In short, on the basis of established research criteria SVD was defined as the presence of lacunar infarcts and/or WML²⁸. Accordingly, 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 (WML and/or lacunar infarcts). The main exclusion criteria were dementia (American Psychiatric Association, 2000), (psychiatric) disease interfering with cognitive testing or follow-up, WML or SVD mimics and MRI contraindications or known claustrophobia. All participants signed an informed consent form. The Medical Review Ethics Committee region Arnhem-Nijmegen approved the study.

Conventional MRI Scanning Protocol

All participants underwent a 1.5-T MRI scanning. The protocol included, among other sequences, the following whole brain scans: T1 3D MPRAGE imaging, fluid-attenuated inversion recovery (FLAIR) pulse sequences, DTI²⁷.

Conventional MRI Analysis

Hippocampus and intracranial volumetry One experienced investigator, blinded to clinical data (lvU), manually segmented the left and right hippocampus on the MPRAGE image using the interactive software program "ITK-SNAP"²⁹. Anatomical boundaries were determined in coronal sections with the aid of neuroanatomical atlases,^{30,31} and actual segmentation was performed using a previously published protocol³² in which segmentation was performed

from posterior to anterior²⁷ Interrater studies on a random sample of 10% showed an intraclass correlation coefficient for the left hippocampus of 0.73 and for the right hippocampus of 0.79. Intrarater studies on this sample showed an intraclass correlation coefficient for the left and right hippocampus of 0.97 and 0.96, respectively. For the same image, gray (GM) and white-matter (WM) tissue and cerebrospinal fluid (CSF) probability maps were computed using SPM5 unified segmentation routines (Wellcome Department of Cognitive Neurology, University College London, UK)³³ 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. Intracranial volume (ICV) was taken as the sum of total GM, WM, and CSF volume. Hippocampal volume (HV) measurements were normalized to the total ICV. The normalized hippocampal volume (NHV) is defined as $NHV = ICV_m \times HV_p / ICV_p$, where ICV_m is the average total ICV of all participants, ICV_p is the ICV of the participant, and the HV_p is the HV of the participant³⁴.

WML volumetry WML was manually segmented on transversal FLAIR images. WML were defined as hyperintense lesions on FLAIR, which were not, or only faintly, hypointense on T1 weighted images, except for gliosis surrounding infarcts³⁵ WML volume was calculated as lesion surface multiplied by slice thickness. Two trained raters, blinded for all clinical, cognitive, and DTI information, segmented all scans. Interrater variability was determined in a random sample of ten percent and yielded an intraclass correlation coefficient of 0.99 for total WML volume. The FLAIR image was used to compute the co-registration parameters to the anatomical T1 image, which was then applied to the segmentation results. All images were visually checked for co-registration errors.

Lacunar infarcts Lacunar infarcts were defined as hypointense areas >2 mm and ≤ 15 mm on FLAIR and T1, ruling out enlarged perivascular spaces (≤ 2 mm, except around the anterior commissure, where perivascular spaces can be large) and infraputaminial pseudolacunes³⁵ Evaluation of infarcts was performed by a trained rater blinded to all clinical data with good intrarater variability with a weighted κ of 0.80. In 10% of the scans, interrater variability was calculated with a weighted κ of 0.88.

DTI Analysis

The diffusion-weighted images of each participant were realigned on the unweighted image using mutual information based co-registration routines from SPM5. Then, the diffusion tensor¹¹ and its eigenvalues were estimated using linear regression (spurious negative values were set to zero), after which the tensor derivatives MD and FA were calculated³⁶ The mean unweighted image was used to compute the co-registration parameters to the anatomical T1 image (SPM5 mutual information co-registration), which was then applied to all diffusion-weighted images and derivatives.

MD and FA values of the cingulum were measured in six bilateral regions of interest (ROIs) in the cingulum bundle as illustrated in figure 1. An experienced neurologist (FE_dL) blinded to subject information used FSLView³⁷ to overlay an histological atlas (Juelich Histological Atlas)³⁸; over the MNI152 standard brain, in order to manually mark out the center coordinates of the ROIs in the different locations of the cingulum and the parahippocampal region (based on neuroanatomy data from the literature)³⁹

We marked out 5 ROIs on a sagittal slice of the cingulum. The cingulum was divided in 4 parts: anterior, middle, posterior and parahippocampal section.⁴⁰ The center of ROI C was placed at the centre of the middle curve of the cingulum fibers, just above the body of the corpus callosum: this is the middle cingulate region. We placed the center of the 2 anterior ROIs (ROI A and B) before the middle curve of the cingulum (around the rostrum and genu of the corpus callosum) and the 2 posterior ROIs (ROI D and E) nearby the dorsal curve of the cingulum fibers (around the splenium of the corpus callosum). The center of the sixth ROI (ROI F) was placed in the parahippocampal cingulum on the medial temporal portion of the cingulum fibers. The center coordinates of each ROI were mirrored with respect to the mid-sagittal plane to obtain these same regions in both cerebral hemispheres. The ROI center coordinates were mapped back to the individual DTI space of each subject using the inverse of the SPM5 unified T1 normalization parameters.³³ ROIs were defined as 6 mm diameter spheres (volume of 0.056 mL) around the DTI-space center coordinates. We used a modest ROI size to ensure that all ROIs included only white matter (which was visually checked). FA and MD values within each ROI were averaged. Furthermore, the mean MD was calculated in both hippocampi. All images were visually checked for motion artifacts and co-registration errors, especially for not including perihippocampal CSF.

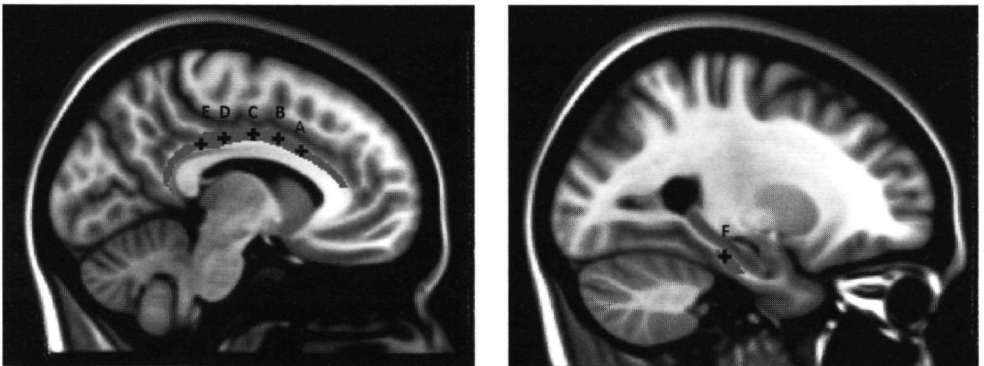


Figure 1 Location of the six (A-F) regions of interest (ROIs) in the left cingulum bundle and parahippocampal cingulum in a sagittal plane.

Measurement of cognitive function

Cognitive function was assessed by two trained investigators (AvN and KdL). For this substudy, the Mini-Mental State Examination (MMSE) (range, 0–30)⁴¹ was used as an index of overall cognitive performance. The three-trial version of the Rey Auditory Verbal Learning Test (RAVLT)⁴² was administered to examine episodic memory formation. To evaluate speed of mental processes we used the Stroop test⁴³, the Paper-Pencil Memory Scanning Task⁴⁴, the Symbol-Digit Substitution Task⁴⁵. The Rey's Complex Figure Test (RCFT)⁴⁶ was included as an index of visuospatial construction (copy trial) and incidental learning (immediate and delayed recall).

We defined four memory indices based on RAVLT performance (overall verbal memory performance, immediate recall, delayed recall, and delayed recognition), as described previously^{47,48}. Immediate recall was calculated by the mean of the total number of words remembered in the three learning trials of the RAVLT. Delayed recall was the number of words recalled 30 min after the learning trials. The delayed recognition score was calculated by computing the total of each correctly recognized word (the 15 target words among 15 new distracter items) 30 min after the learning trials. Performance across tests was made comparable by transforming the raw test scores into z-scores as described elsewhere for which the assumption of normality of the distribution was examined⁴⁸. For data reduction purposes, a compound score for overall verbal memory performance was calculated, as described previously² by taking the mean of two z-scores from the RAVLT, one for the added scores on three learning trials of this test and one for the delayed recall of this test.

For visuospatial memory and psychomotor speed we also calculated compound scores. Visuospatial memory is a compound score of the mean of the z-scores of the immediate recall trial and the delayed recall trial of the Rey's Complex Figure Test. 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 Task⁴⁸.

Other measurements

The following characteristics were considered possible confounders: age, sex, educational level and depressive symptoms. (49) Depressive symptoms were defined as a score ≥ 16 on the Center of Epidemiologic Studies on Depression Scale and/or current use of antidepressive medication.^{50,51}

Structural quality of the hippocampus

As it has been demonstrated that a combination of high diffusivity in the hippocampus and low hippocampal volume is related to conversion to AD in MCI patients,¹⁴ we composed a score for hippocampal quality using both parameters. Both diffusivity and volume were given a score ranging from 1 (poor: lowest tertile of the volume/highest tertile of MD

distribution) to 3 (good; highest tertile of the volume/lowest tertile of the MD distribution) leading to a maximum score between 2 (lowest tertile of the volume + highest tertile of the MD distribution (1+1): worst hippocampal quality) and 6 (highest tertile of the volume + lowest tertile of the MD distribution (3+3):best hippocampal quality). A good hippocampal quality was defined as a score of ≥ 4 , while poor quality was defined as a score of 2 or 3.

Statistical analysis

Statistical analyses were performed with SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA). Baseline characteristics were summarized as means (with standard deviations; SD) or proportions, for skewed variability parameters the median and the inter-quartile range were calculated.

The relation between the cingular DTI (MD/ FA) parameters of the six ROIs and the RAVLT indices as well as with the compound scores for overall verbal memory performance, psychomotor speed, visuospatial memory and MMSE, were analyzed by multiple linear regression analysis. Adjustments were made for potential confounders including age, sex, educational level, depressive symptoms, normalized hippocampal volume and WML volume and the presence of lacunar infarcts.

To assess whether the quality of the hippocampus modified the relation between cingular DTI parameters and performance in verbal memory, psychomotor speed, visuospatial memory and MMSE, we performed the previously described linear regression analyses stratified in two groups of hippocampal quality (good vs. poor); adjustments were made for the same confounders as mentioned above. Regression coefficients are presented as standardized β -values. Bonferroni corrections were applied.

Table 1 Baseline characteristics

	Participants n=499
Demographics	
Age	65.6 (8.8)
Male	282 (56.5)
Participants with only primary education (n, %)	49 (9.8)
CES-D	11.0 (9.4)
MMSE	28.1 (1.6)
Participants with depressive symptoms (n, %) *	168 (33.8)
Neuroimaging characteristics	
Normalized hippocampal volume (mL)	6.8 (0.9)
Normalized cingular volume (mL)	4.9 (0.4)
Left normalized cingular volume (mL)	2.5 (0.2)
Right normalized cingular volume (mL)	2.4 (0.2)
WML volume (mL)	7.1 (3.4,18.1)
Presence of lacunar infarcts (n, %)	171 (34)

Numbers represent means (SD), percentages or medians (inter-quartile range)

CES-D: Centre of Epidemiological Studies on Depression Scale, WML: White Matter Lesions

* Defined as CES-D scores ≥ 16 and/or the current use of anti-depressive medication

Results

The total study population consisted of 503 participants. Three participants were excluded because of visible motion induced signal loss in the DTI data and one because of an automatic segmentation problem that could not be solved manually. Table 1 shows the baseline characteristics of 499 participants. The mean age of the population was 65.6 years (SD 8.8) and 56.5% were male. In table 2 the test scores of the cognitive test battery are shown. The mean MMSE score was 28.1 (SD 1.6).

Table 2 Cognitive test scores

Mini Mental State Examination (range 0-30)	28.1 (1.6)
RAVLT (no. of words recalled)	
Immediate recall trial 1	5.1 (1.7)
Immediate recall trial 2	7.3 (2.2)
Immediate recall trial 3	8.7 (2.5)
Total trial 1-3	21.1 (5.8)
Delayed recall	6.0 (3.1)
Delayed recognition	27.0 (3.2)
Stroop test (time in sec.)	
trial 1 (words)	25.8 (6.3)
trial 2 (colours)	33.2 (7.7)
trial 3 (concept-shifting)	63.8 (22.1)
Paper and Pencil memory scanning task (time in sec.)	
1 character	45.0 (13.4)
2 characters	62.3 (19.7)
3 characters	77.4 (26.5)
Symbol digit substitution task (no. in 60 sec.)	27.1 (9.7)
Rey Complex Figure Test (range 0-36)	
Copy trial	33.5 (3.4)
Immediate recall trial	18.2 (6.8)
Delayed recall trial	18.1 (6.6)

Numbers represent means (SD) RAVLT Rey Auditory Verbal Learning Test

Table 3 Mean MD and FA of the ROIs in the left and right cingulum and hippocampus

	Mean diffusivity	Fractional anisotropy
ROI A: anterior cingulum		
Left	8.48 (0.91)	0.40 (0.10)
Right	8.38 (0.73)	0.38 (0.07)
ROI B: anterior cingulum		
Left	8.45 (1.06)	0.40 (0.11)
Right	8.34 (0.89)	0.41 (0.09)
ROI C: mid cingulum		
Left	7.90 (0.69)	0.51 (0.10)
Right	7.97 (0.69)	0.50 (0.09)
ROI D: superior-posterior cingulum		
Left	7.94 (0.57)	0.53 (0.07)
Right	7.88 (0.53)	0.50 (0.07)
ROI E: inferior-posterior cingulum		
Left	7.86 (0.48)	0.45 (0.07)
Right	8.09 (0.58)	0.39 (0.07)
ROI F: parahippocampal cingulum		
Left	8.73 (0.78)	0.25 (0.06)
Right	8.93 (0.87)	0.26 (0.07)
Hippocampus		
Left	12.00 (1.11)	0.17 (0.02)
Right	12.01 (1.13)	0.17 (0.02)

Numbers represent means (SD). MD: Mean Diffusivity, expressed as 10^{-4} mm²/s. FA: Fractional Anisotropy

Table 3 shows the mean MD and FA values of the six bilateral ROIs and the hippocampus. In none of the participants was more than 5% of the ROIs in the cingulum affected by WML. The relations between the MD and FA of the six ROIs in left and right cingulum and the different cognitive tasks are shown in tables 4 and 5.

Table 4 Relation between the MD values of the six ROIs in the left and right cingulum and verbal memory performance and cognitive performance

	to anterior ←		mid cingulum		→ to posterior		parahippocampal					
	MD ROI A		MD ROI B		MD ROI C		MD ROI D		MD ROI E		MD ROI F	
	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right
Overall memory performance¹	-.02	.02	-.05	-.01	-.13*	-.04	-.10**	-.03	-.12*‡	-.10**	-.05	-.05
Immediate memory	-.05	.01	-.07	-.01	-.13*	-.03	-.13*	-.03	-.10**	-.10**	-.03	-.06
Delayed recall	.01	.03	-.02	-.01	-.12*	-.05	-.07	-.02	-.13*‡	-.08	-.06	-.03
Delayed recognition	-.08	-.05	-.02	-.08	-.20*†	-.10**	-.18*†	-.07	-.14*‡	-.09**	-.08	-.07
Psychomotor speed¹	-.05	-.05	-.07	-.05	-.11**	-.09**	-.13*‡	-.08**	-.05	-.09	.03	-.04
Visuospatial memory¹	.05	.03	-.05	.01	-.04	-.06	-.06	-.01	-.03	.01	.01	-.05
MMSE	-.01	.03	-.05	.03	-.12**	-.05	-.05	.01	-.08	-.05	-.05	-.09**

Numbers represent regression coefficients (standardized betas), adjusted for age, sex, educational level, depressive symptoms, hippocampal volume, WML volume and the presence of lacunar infarcts *p< 01, **p<.05; After Bonferonni correction: †p< 01; ‡p< 05; ¹Compound scores

Significant relations were found between the MD values in the left mid cingulum (ROI C) and in the posterior part of the left cingulum (ROI D and E) and overall verbal memory performance ($\beta = -13$, $p = 006$, $\beta = -10$, $p = 029$ and $\beta = -12$, $p = 004$), immediate recall ($\beta = -13$, $p = 008$, $\beta = -13$, $p = 008$ and $\beta = -10$, $p = 015$) and delayed recognition ($\beta = -20$, $p < 0005$, $\beta = -18$, $p < 0005$ and $\beta = -14$, $p = 002$), independent of confounders. Furthermore MD in the left mid (ROI C) and posterior cingulum (ROI E) also showed a significant relation with delayed recall ($\beta = -12$, $p = 01$ and $\beta = -13$, $p = 002$)

Table 5 shows almost the same distribution for the relation between the FA values of the six ROIs and (subprocesses of) verbal memory. However these relations were less strong than for the MD values.

When stratified on good and poor hippocampal quality, the relations between the MD of the left mid (ROI C) and posterior cingulum (ROI D and E) and verbal memory performance were most outspoken in participants with a good hippocampal quality (intact structural integrity) ($n=338$)(table 6). In participants with a poor hippocampal quality ($n=161$) almost no significant relations were found between the MD values of the six cingular ROIs and verbal memory, numbers are not shown.

Table 5 Relation between the FA values of the six ROIs in the left and right cingulum and verbal memory performance and cognitive performance

	to anterior ←		mid cingulum		→ to posterior		parahippocampal					
	FA ROI A		FA ROI B		FA ROI C		FA ROI D		FA ROI E		FA ROI F	
	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right
Overall memory performance¹	.03	-.01	.05	-.01	.11*	.01	.11*	.12*‡	.09**	.10**	-.03	-.01
Immediate memory	.05	-.01	.06	-.02	.13*‡	.01	.15*‡	.12*	.08**	.10**	-.03	-.05
Delayed recall	.01	-.01	.04	-.01	.08	.01	.07	.11**	.08	.08**	-.03	.03
Delayed recognition	.04	.03	.06	.05	.15*‡	.09**	.09**	.13*‡	.08	.08	-.01	-.04
Psychomotor speed¹	-.01	.05	.07	.01	.07	.07	.09**	.12*‡	.03	.03	-.04	.02
Visuospatial memory¹	-.02	-.07	.03	-.09**	.01	-.04	.04	.05	-.01	.01	-.03	-.04
MMSE	-.04	-.04	.01	-.04	.05	.06	.03	.06	.04	.06	-.05	.03

Numbers represent regression coefficients (standardized betas), adjusted for age, sex, educational level, depressive symptoms, hippocampal volume, WML volume and the presence of lacunar infarcts *p< 0.1, **p< 0.05 After Bonferroni correction †p< 0.1, ‡p< 0.05 ¹Compound scores

Table 6 Relation between the MD values of the six ROIs in the left and right cingulum and verbal memory performance and cognitive performance in participants with a good hippocampal quality (n= 338)

Good hippocampal quality	to anterior ←				mid cingulum		→ to posterior				parahippocampal	
	MD ROI A		MD ROI B		MD ROI C		MD ROI D		MD ROI E		MD ROI F	
	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right
Overall memory performance¹	- 03	06	- 02	- 01	- 13**	- 03	- 10	- 03	- 16*‡	08	03	05
Immediate memory	- 04	06	- 03	- 01	- 12**	- 02	- 11**	02	- 14*	- 10	01	- 05
Delayed recall	- 01	06	- 01	- 01	- 13**	- 04	- 07	- 03	- 16*‡	- 05	- 06	- 03
Delayed recognition	- 09	01	05	01	- 21*†	- 05	- 05	- 04	16*‡	- 07	- 05	- 01
Pasychomotor speed¹	- 06	- 07	- 02	- 03	- 06	- 03	- 07	- 07	- 08	- 11**	03	- 05
Visuospatial memory¹	06	04	07	06	02	- 06	03	- 05	- 05	- 01	- 03	- 02
MMSE	- 03	07	01	06	- 11	01	01	01	- 09	- 04	- 07	- 04

Numbers represent regression coefficients (standardized betas), adjusted for age, sex, educational level, depressive symptoms, hippocampal volume, WML volume and the presence of lacunar infarcts *p< 01, **p< 05 After Bonferonni correction †p< 01, ‡p< 05 ¹Compound scores

Discussion

Our results show that the microstructural integrity of the mid and posterior cingulum, as assessed by DTI, is related to verbal memory performance, especially to delayed recall and delayed recognition, independent of hippocampal volume and coexisting SVD. Furthermore, we found that this relation was most outspoken in participants with intact hippocampal structural integrity compared to those with a poor integrity.

To the best of our knowledge, this is the first study investigating the relation between the microstructural integrity of the cingulum and verbal memory function in a large group of non-demented, independently living elderly with cerebral SVD. Our data show that the microstructural integrity of the cingulum beyond the detection limit of conventional MRI is related to episodic memory formation. On conventional MRI, only less than 5% of the ROIs in the cingulum of our study population, showed the presence of visible WML, strengthening our assumption that we indeed investigated the earliest structural changes in the cingulum bundle integrity. This finding is in line with the results of previous studies in patients with mild cognitive impairment (MCI) or dementia, showing that loss of structural integrity of the cingulum is associated to impaired verbal memory performance^{9, 10, 16, 21, 52}. Furthermore, we found this relation in people with an intact hippocampal volume and with the highest hippocampal microstructural integrity. There is still much debate whether, in general, WM pathology is related to, or independent of, GM pathology. There are two main hypotheses, one suggesting that microstructural WM changes occur as a result of Wallerian degeneration⁵³, meaning that WM pathology is preceded by GM pathology. In contrast, the retrogenesis theory proposes that loss of WM integrity is the result of myelin breakdown that occurs in the reverse order to myelogenesis^{54, 55}. Our results suggest that loss of microstructural integrity of the white matter starts in the cingulum in respect to verbal memory, before micro- and macrostructural changes in the hippocampus occur, and possibly provide some evidence for the last mentioned hypothesis.

We found almost no significant relation between verbal memory performance and cingulum integrity in participants with a poor hippocampal quality. A possible explanation might be that by the time both the microstructural integrity as well as the volume of the hippocampus is impaired, these changes in the hippocampus are more prominently related to verbal memory than the microstructural changes in the cingulum, even in our sample of participants with a relatively good hippocampal quality. A recent study⁵⁶ showed that microstructural integrity of the hippocampus is strongly related to verbal memory performance in older people, also in participants with an intact appearing hippocampus.

We found that the microstructural integrity of the mid and, especially, the posterior part of the cingulum is related to impaired verbal memory, probably suggesting that loss of structural integrity starts in these parts, and maybe further spreading to the parahippocampal parts with progression of verbal memory impairment and ultimately dementia. Our results further

indicate that the structural integrity of the anterior cingulum is not related to episodic memory. Only a few other studies have assessed ROIs at different locations in the cingulum in MCI and AD patients^{19,25,40,52,57}. Most of them found that loss of microstructural integrity of the cingulum begins near medial temporal structures, which is in line with our findings. One study also reported FA reduction in the mid cingulum in AD patients⁵⁸ and one study reported FA reduction in the anterior cingulum in MCI and AD patients⁵². However, these studies were underpowered and their study populations differ in disease severity compared to our study, therefore future studies are needed to describe the possible spread over time of the pathological process throughout the cingulum. A follow-up of the RUN DMC study is already being executed, to further investigate this.

Our findings show that cingulum integrity is specifically related to verbal episodic memory. We only found weak or no relations between cingulum integrity and psychomotor speed, visuospatial memory and MMSE. Although a recent study did find associations between (posterior) cingulum integrity and attention/executive functioning and visuospatial memory,¹⁶ this discrepancy may be due to the limited additional adjustments performed in the latter study. Furthermore, their study population differed from ours. Of their 220 participants, 149 had MCI, while our study population had a mean MMSE of 28.1. As a result, it is likely that more damage to the structural integrity of the cingulum and MTL structures like the hippocampus has occurred in that study population. The reason for not finding a relation between DTI parameters and visuospatial memory in our study, may be due to the fact that memory performance based on the Rey's Complex Figure Test may in part rely on motor learning, and as such, cannot be regarded as a pure measure of spatial memory as part of episodic memory, which as a result may be less dependent of the hippocampal memory circuit. While it has been suggested that the medial temporal lobe also mediates executive functioning,⁵⁹ the prefrontal lobes are predominantly involved in executive functioning,⁶⁰ in agreement with our present lack of correlation between cingulum integrity and executive function.

The MD, which reflects the magnitude of the diffusion of water, within the ROIs in the cingulum was found to be more strongly related to verbal memory performance than the FA, a measure reflecting the dominant directionality of the diffusion of water. This finding is in line with a recent study showing that MD is more sensitive than FA in detecting differences between AD and control participants⁶¹. This might be due to the different underlying pathophysiology that both parameters (MD and FA) reflect. Hypothetically, it has been suggested that WM degeneration starts with demyelination of axons, resulting in an increase of water diffusion in all directions (increased MD). Presumably, at this early stage the dominant diffusion direction remains unchanged as will the FA.²¹ We would expect that progression of white matter degeneration of the cingulum bundle leads to disruption of axons, resulting in changes in MD as well as FA. With respect to lateralization effects, our study showed that the strongest relation to verbal memory was found in the cingular ROIs.

C, D and E in the left hemisphere, as would be expected for a verbal task.

Still, a few methodological considerations need to be addressed. Although our data are derived from the largest DTI study on the cingulum thus far, our study design is cross-sectional, which prevents us from drawing conclusions with respect to causality. Follow-up of this cohort has already been planned in 2011 to further investigate microstructural changes in cingulum integrity in relation with macrostructural changes in the cingulum and medial temporal lobe structures and in the development of cognitive decline and dementia.²⁷ Furthermore, we used an atlas to identify the cingulum bundle, and manually placed the ROIs in order to avoid GM and WM of other tracts to blur our DTI measures. Although the placement of the atlas was visually checked and the placement of the ROIs was performed by an experienced neurologist, blinded to clinical data, this method can be somewhat subjective and make interpretation between studies difficult.

A major strength of our study is the fact that it is a large, single-centre study with a response rate of over 70%. All MRI data were acquired on a single scanner in a similar way, and the hippocampus and WML were assessed volumetrically in a reliable, sensitive and objective way by two trained experts, who were blinded to all clinical data. Furthermore, extensive adjustments were made for possible confounders, such as educational level, depressive symptoms (a well known confounder in cognitive performance in elderly with SVD),⁵¹ hippocampal volume, WML volume and lacunar infarcts. Moreover, cognitive function was assessed by only two investigators using a standardized cognitive battery.

In conclusion, this study demonstrates that microstructural integrity of the mid and posterior cingulum is related to episodic memory function, notably verbal memory, in elderly with SVD, and this is especially true for participants with an intact hippocampal structural integrity. Consequently, our findings show that DTI is a sensitive diagnostic tool to detect early microstructural changes in the cingulum before macrostructural changes in the cingulum and hippocampus can be detected on conventional MRI. Future studies should prospectively investigate the predictive value of DTI parameters of the cingulum in relation to cognitive decline and incident dementia, even before macrostructural changes of both the cingulum and medial temporal lobe can be detected. If the predictive value is proven, DTI of the cingulum could possibly be a surrogate marker for future development of cognitive decline and dementia and could be a starting point for therapeutic trials aiming to prevent disease progression.

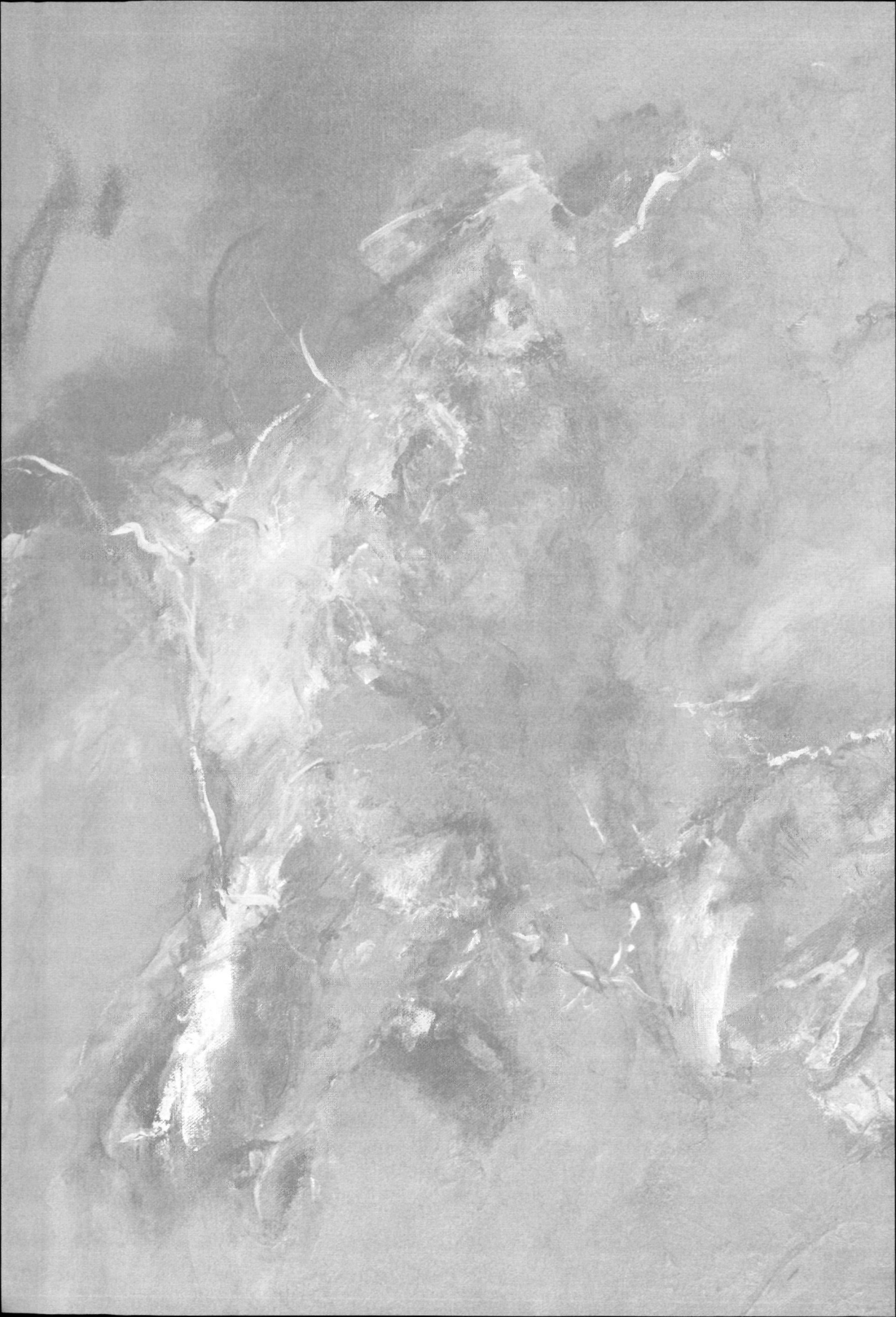
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Chapter 10

Loss of white matter integrity is associated with cognitive performance: A Tract-Based-Spatial-Statistics study

Submitted as:

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Loss of white matter integrity is associated with cognitive disturbances in elderly with small vessel disease.*

Abstract

Cerebral small vessel disease (SVD), including white matter lesions (WML) and lacunar infarcts bleeds (MB), is common in elderly people and related to cognitive impairment and dementia. Possibly cognitive impairment is caused by disruption of white matter tracts that connect regions involved in cognitive functions. Pathological and imaging studies have shown abnormalities in the white matter that appear normal on conventional MRI. The loss of microstructural integrity in the normal appearing white matter (NAWM) may play a role in the cause of cognitive disturbances. As conventional MRI is not sensitive for the microstructural integrity of both the WML and the NAWM, we used tract-based spatial statistics (TBSS) to assess the relation between the white matter integrity and cognitive function.

The RUN DMC study is a prospective cohort study among 503 independently living, non-demented elderly with cerebral SVD aged between 50 and 85 years. All subjects underwent MRI and DTI scanning and an extensive neuropsychological assessment.

Loss of white matter integrity, as indicated by a lower fractional anisotropy and higher mean diffusivity was related to cognitive performance. This relation was found in numerous regions mostly in the NAWM. The microstructural integrity in the genu and splenium showed the highest significant relation with general cognitive function, in the cingulum bundle with verbal memory performance and in the frontal white matter with psychomotor speed. Associations between DTI parameters and most cognitive domains remained present after adjustment for SVD visible on conventional MRI. In conclusion, our study showed that cognitive disturbances in elderly with cerebral SVD are attributable to loss of microstructural integrity of multiple white matter fibers connecting different cortical and subcortical regions. DTI could be of added value to conventional MRI parameters in investigating the pathophysiology of cognitive impairment in cerebral SVD.

Introduction

Cerebral small vessel disease (SVD) includes white matter lesions (WML) and lacunar infarcts and is very common in the elderly population. Both hospital- and population-based studies have shown that SVD is related to cognitive impairment, and in some may ultimately lead to dementia.^{1,2} This is supposedly due to the disruption of the white matter tracts. Despite the high prevalence of WML and lacunar infarcts in the population over 60 years of age (90% and 20% respectively),^{3,4} relatively few develop evident cognitive decline or dementia.²

Other factors apart from SVD-related lesions on conventional MRI may determine the transition from intact cognitive performance to cognitive decline in some, while leaving the cognition unaffected in most. One of these factors could be the structural integrity of normal appearing white matter (NAWM) surrounding WML. It is important to realize that only a small proportion of the white matter (usually less than a few percent of its volume) is visibly affected by SVD on FLAIR MR images, even in individuals with severe SVD. As conventional MRI is not sensitive to loss of microstructural integrity in the NAWM, possible changes in this largest part of the white matter cannot be assessed. Pathological studies have demonstrated that abnormalities in this part of the white matter may, nevertheless, be present.⁵

Diffusion tensor imaging (DTI) is a non-invasive MRI technique that provides valuable information on the microstructural organization of the white matter. By measuring the local water diffusion profiles, several common DTI-derived indices can be measured: the fractional anisotropy (FA), which represents a normalized ratio of diffusion directionality, the mean diffusivity (MD), which reflects the overall magnitude of water diffusion, axial diffusivity, which reflects the diffusivity parallel to the white matter tracts and radial diffusivity, which is the diffusivity perpendicular to these tracts.⁶ Loss of microstructural integrity is typically reflected by a reduction in FA and/or an increase in MD,⁷ which can result from different combinations of changes in axial and radial diffusivity. Investigating these four DTI measures conjointly provides more information about the possible changes of white matter microstructure than FA and MD alone. Studies showed that DTI can be used to assess the SVD-induced changes in the white matter and that areas with WML could have quite different characteristics on DTI.⁸

A few studies in patients with cerebral SVD demonstrated a relation between DTI parameters and cognitive function, especially executive function.⁹⁻¹³ All of these studies had small sample sizes ($n \leq 47$), and did not or only limited adjust for possible confounders. A large population-based cohort demonstrated a relation between microstructural integrity in both the WML and the NAWM and cognitive function.¹⁴ Most of these studies used either a region-of-interest (ROI) approach or voxel-based morphometry (VBM)-style analysis. Both methods are prone to methodological constraints hindering analysis and interpretation of DTI data. Tract-based spatial statistics (TBSS) is a relatively new method that mitigates the limitations

of VBM analyses¹⁵ Using TBSS, the analysis is restricted to those white matter voxels that constitute the skeleton (core) of the brain's connective architecture. This skeleton can be matched more accurately (compared to whole-brain normalization) across subjects, enabling robust voxelwise analysis of the microstructural integrity of the white matter across subjects. We hypothesized that cognitive disturbances in elderly with cerebral SVD would not only be related to loss of microstructural integrity of the white matter within the WML, but also of the NAWM. We conducted TBSS to investigate the location of microstructural white matter loss related to cognitive disturbances. In addition, we examined whether the associations in the white matter were primarily explained by SVD.

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 with cerebral SVD. The selection procedure of the participants and the study rationale and protocol were described previously in detail¹⁶

In short, on the basis of established research criteria SVD was defined as the presence of lacunar infarcts and/or WML on neuroimaging.¹⁷ Symptoms of SVD include acute symptoms, such as TIAs or lacunar syndromes, or subacute manifestations, such as cognitive, motor (gait) disturbances and/or depressive symptoms.¹⁸ Accordingly, 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 (WML and/or lacunar infarcts). The main exclusion criteria were dementia, (psychiatric) disease interfering with cognitive testing or follow-up, WML or SVD 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 center, yielding a response of 71.3% (503/705). For the present study, 63 subjects were additionally excluded because of territorial infarcts (n=55), inadequate quality of the MRI image (n=4) and four subjects because of incomplete data on cognitive test scores. All participants signed an informed consent form. The Medical Review Ethics Committee region Arnhem-Nijmegen approved the study.

Measurement of cognitive function

Cognitive function was assessed by a standardized neuropsychological test battery and has been described in detail elsewhere¹⁶ Performance across tests was made comparable by transforming raw test results in z-scores We calculated compound scores for seven cognitive domains Global cognitive function was evaluated by the Mini Mental State Examination (MMSE) 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 test¹ Verbal memory is a compound score of the mean of two z-scores from the Rey Auditory Verbal Learning Test, one for the added scores of the three learning trials of this test, and one for the delayed recall of this test Visuospatial memory is a compound score of the mean of the z-scores of the immediate recall trial and the delayed recall trial of the Rey's Complex Figure Test 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 Task¹ Fluency was calculated from the mean of the z-scores of both verbal fluency tasks Concept shifting was calculated as the z-score of the third subtask of the Stroop Attention is a compound score of the z-score of the total time of the Verbal Series Attention Test¹

Magnetic resonance imaging scanning protocol

MRI scans of all participants were acquired on a single 1.5-T MRI The protocol included, among other sequences, the following whole brain scans 3D T1 magnetization-prepared rapid gradient echo (MPRAGE) sequence (TR/TE/TI 2250/3.68/850ms, flip angle 15°, voxel size 1.0x1.0x1.0mm), a fluid-attenuated inversion recovery (FLAIR) sequence (TR/TE/TI 9000/84/2200ms, voxel size 1.0x1.2x5.0mm, interslice gap 1 mm) and a DTI sequences (TR/TE 10100/93ms, voxel size 2.5x2.5x2.5mm, 4 unweighted scans, 30 diffusion weighted scans with b-value 900s/mm²)¹⁶

Conventional magnetic resonance imaging analysis

WML were manually segmented on FLAIR images, and the number of lacunar infarcts was rated according to a standardized protocol¹⁶ All imaging analyses were performed by two trained raters blinded to clinical information In a random sample of 10%, interrater variability for total WML volume yielded an intra-class correlation coefficient of 0.99, intra and interrater reliability for the lacunar infarcts yielded a weighted kappa of 0.80 and 0.88 We computed gray (GM) and white matter (WM) tissue and cerebrospinal fluid (CSF) probability maps using Statistical Parametric Mapping 5 unified segmentation routines on the T1 MPRAGE images (SPM5, Wellcome Department of Cognitive Neurology, University College London, UK)¹⁹ Total GM, WM and CSF volumes were calculated by summing all

voxel volumes that had a $p > .5$ for belonging to that tissue class. Total brain volume (TBV) 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, TBV was expressed as percentage of total ICV.

Diffusion tensor imaging analysis

Diffusion data were first preprocessed to detect and correct head and cardiac motion artifacts, using an in-house developed iteratively re-weighted-least-squares algorithm named 'PATCH' (www.ru.nl/neuroimaging/diffusion).²⁰ Corrections of Eddy current and motion artifacts from affine misalignment are performed simultaneously by minimization of the residual diffusion tensor errors.²¹ Next, FA, MD, axial and radial diffusivity images were calculated using a DTIFit within the Functional MRI of the Brain diffusion toolbox, which were then fed into the TBSS pipeline.¹⁵ The thinning procedure was conducted on the mean FA image to create a common skeleton, which represents the core-structure of the white matter tract. Subsequently this skeleton was thresholded at FA-value 0.3 to include the major white matter tracts and to account for the inter-subject variability. All normalized FA data were then projected onto this skeleton. These skeleton projection factors were then applied to the mean, axial and radial diffusivity images. During the normalization procedures, the images were not modulated with the Jacobian of the spatial transformations (i.e. not corrected for the brain volume). These data were then fed into the voxel-wise cross-subject statistics.

In addition, we obtained the mean FA, MD, axial and radial diffusivity for three parts of the corpus callosum by performing regions-of-interest analyses. We created the masks for genu, body and splenium of the corpus callosum by applying the white matter atlas (John Hopkins University white matter labels, provided by Functional MRI of the Brain Software Library (FSL)) on the mean FA skeleton. The masks were visually inspected and miscellaneous voxels that belonged to other regions, such as the cingulum bundle, were excluded.

Other measurements

Age, sex and level of education²², depressive symptoms and normalized TBV were considered possible confounders. Depressive symptoms were assessed using the Center of Epidemiologic Studies on Depression Scale (CES-D).²³ Functional independence was assessed using the Barthel Index (range 0-20).²⁴ 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 $\geq 140/90$ mmHg and/or use of anti-hypertensive medications),²⁵ diabetes (treatment with antidiabetic medications), hypercholesterolemia (treatment with lipid-lowering drugs) and smoking status.

Statistical analysis

Baseline characteristics were presented as mean \pm standard deviation (SD) and for the skewed parameters the median and interquartile range were calculated.

For the TBSS analyses, we assessed voxel-wise correlations between the skeletal DTI parameters (FA and MD) and cognitive performance on the several cognitive domains, while adjusting for age, sex, education, depressive symptoms and normalized TBV. To test whether these associations were attributable to SVD (i.e. WML and lacunar infarcts), we adjusted for SVD in a second model. For the voxel-wise statistical analyses, we applied permutation-based statistical interference tool for non-parametric approach as a part of the Functional MRI of the Brain Software Library, with number of permutation tests set to 5000.^{15,26} Significant clusters were identified using the threshold-free cluster enhancement with a p-value $< .05$, corrected for multiple comparisons.²⁷ In addition, we performed the same analysis with a threshold at $p < .01$.

For the region-of-interest analyses, we computed regression coefficients of the mean FA, MD, axial and radial diffusivity of the three regions-of-interest in the corpus callosum (genu, body and splenium) with cognitive performance, while adjusting for age, sex, education, depressive symptoms and normalized TBV. Regression coefficients were presented as standardized β -values. Bonferroni corrections were applied.

Table 1 Baseline characteristics of the study population

Characteristics	n =440
Demographics	
Age	65.2 (8.9)
Male*	239 (54.3)
Only primary education*	41 (9.3)
MMSE	28.2 (1.6)
CES-D scale	11.1 (9.4)
Barthel Index	19.7 (0.7)
Hypertension*	316 (71.8)
Diabetes*	60 (13.6)
Hypercholesterolemia*	193 (43.9)
Smokers, current*	66 (15.0)
Smokers, former*	240 (54.5)
Neuroimaging	
TBV, ml	1098.6 (119.0)
ICV, ml	1674.7 (157.4)
WML volume, ml†	6.5 (3.2; 17.9)
White matter volume, ml	466.8 (66.9)
Lacunar infarcts*	135 (30.7)

Data represent N of subjects*(%), mean (SD), or median† (interquartile range)

MMSE Mini Mental State Examination, CES-D Center of Epidemiologic Studies on Depression,

TBV total brain volume, ICV intracranial volume; WML white matter lesions

Results

Demographic and neuroimaging characteristics are shown in Table 1. Mean age of the population ($n=440$) was 65.2 years (SD 8.9) and 54.3% were male. Mean WM volume was 466.8ml (SD 66.9), the largest part of the WM consisted of NAWM, with a median percentage of 98.5 (IQR 96.0-99.2).

Figure 1 shows the relation between the voxel-wise analysis of the FA and the cognitive domains tested ($p < .05$, corrected for multiple comparisons). FA in almost all regions was positively related to the cognitive index and verbal memory performance and negatively associated with psychomotor speed and concept shifting.

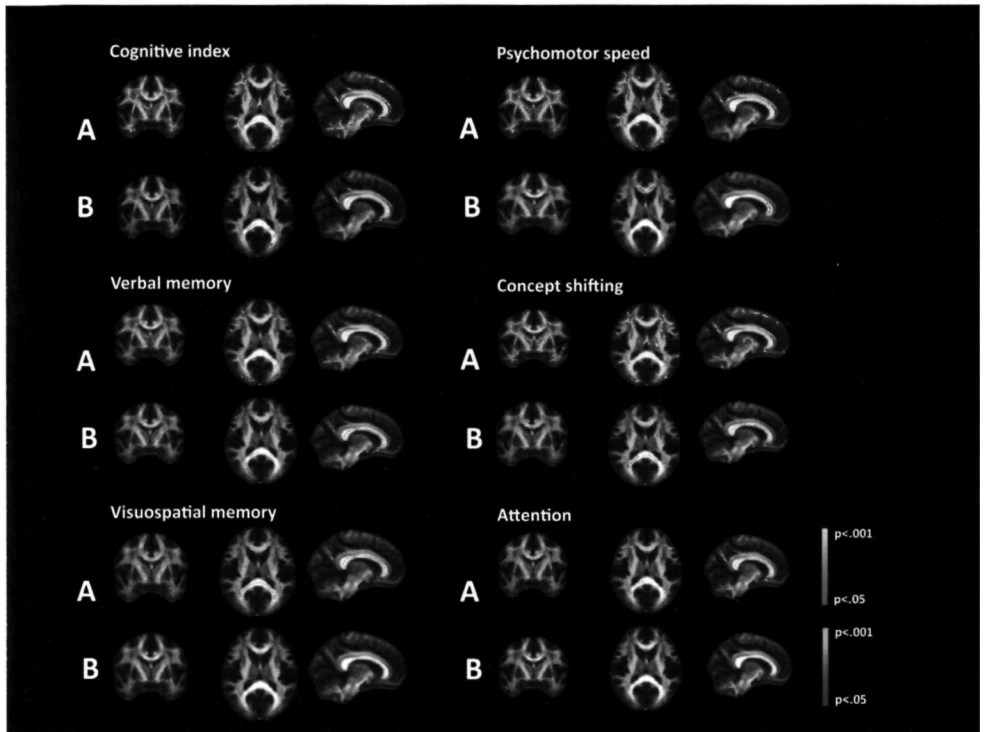


Figure 1 Voxel-wise analysis of the fractional anisotropy positively associated with cognitive index, verbal memory and visuospatial memory performance and negatively associated with psychomotor speed, concept shifting and attention. Adjusted for age, sex, education, depressive symptoms and normalized TBV (A) and additional adjustment for white matter lesions and lacunar infarcts (B). Thresholded at $p < 0.05$ and corrected for multiple comparisons. The statistical maps are superimposed onto the spatially normalized (Montreal Neurological Institute stereotactic space) and averaged ($n=440$) fractional anisotropy map

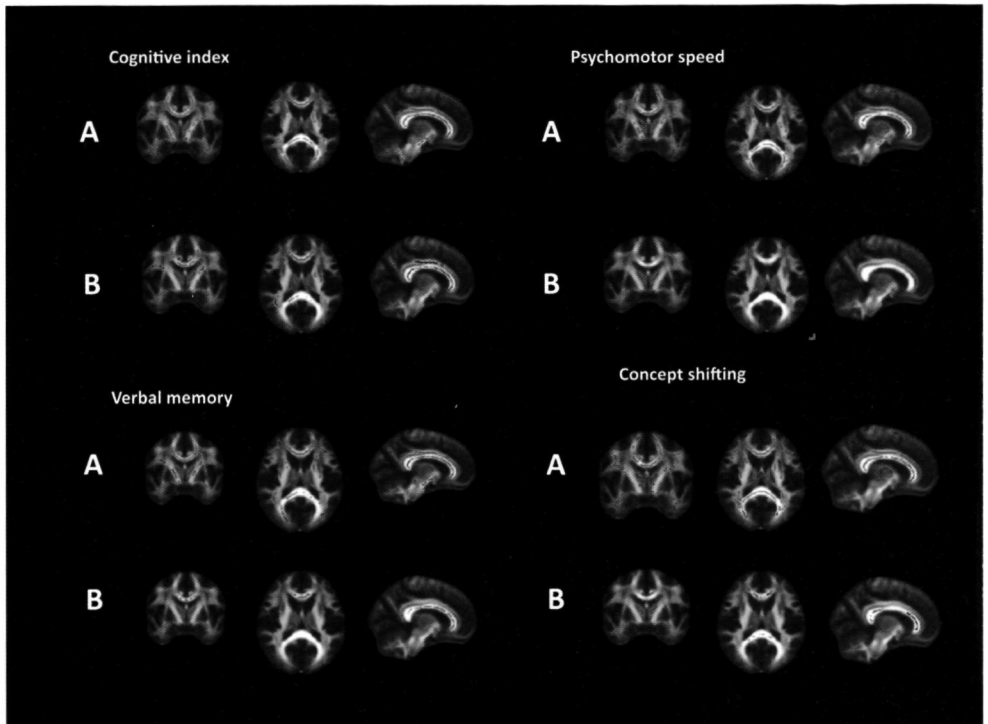


Figure 2 Voxel-wise analysis of the fractional anisotropy positively associated with cognitive index and verbal memory and negatively associated with psychomotor speed and concept shifting. Adjusted for age, sex, education, depressive symptoms and normalized TBV (A) and additional adjustment for white matter lesions and lacunar infarcts (B). Thresholded at $p < 0.01$ and corrected for multiple comparisons. The statistical maps are superimposed onto the spatially normalized (Montreal Neurological Institute stereotactic space) and averaged ($n=440$) fractional anisotropy map

By contrast, fewer and less significant relations were found with visuospatial memory performance and attention. We found a similar distribution for the inverse association with MD and the cognitive domains, although for visuospatial memory no relations were found.

With regard to MMSE and fluency, no significant associations were found with FA and MD. Additional adjustment for WML and lacunar infarcts (Figure 1B) weakened the relations found between the FA and cognitive index, verbal memory and concept shifting, however the associations remained present ($p < .05$). For psychomotor speed, the relation with FA of the body of the corpus callosum remained present after adjustment. With regard to visuospatial memory and attention, no associations were found after additional adjustment

for WML and lacunar infarcts. We found no associations between MD and visuospatial memory, psychomotor speed, concept shifting and attention after additional adjustment for WML and lacunar infarcts.

Figure 2 shows the relation between the voxel-wise analysis of the FA and the cognitive domains thresholded at $p < 0.1$ (corrected for multiple comparisons). A high significant association was identified in almost all voxels of the skeleton in the relation between FA and cognitive index, psychomotor speed and concept shifting. In verbal memory, these voxels with the highest significant association ($p < 0.1$) in the relation with FA were located in the corpus callosum along its complete course and the cingulum bundle, the same was found for MD. Additional adjustment for WML and lacunar infarcts (Figure 2B) showed the strongest significance for FA in relation to cognitive index, verbal memory and concept shifting remained located in the corpus callosum ($p < 0.1$). The voxels with the strongest significance ($p < 0.01$) in the relation between MD and psychomotor speed were located in the frontal lobe. For the cognitive index they were located in the frontal lobe and the corpus callosum (data not shown). With a threshold at $p < 0.1$ we did not find significant relations for FA and MD and MMSE, visuospatial memory, fluency and attention.

We found that microstructural integrity of the genu and splenium of the corpus callosum related to cognitive index. The microstructural integrity of the genu and body was associated with verbal memory, whereas the microstructural integrity of the splenium was related to visuospatial memory. The DTI parameters in the genu and splenium are related to the executive domains, psychomotor speed and concept shifting. We found no relations between microstructural integrity in the corpus callosum and attention, MMSE and fluency. The associations with FA and MD and cognitive performance were mainly driven by changes in radial diffusivity, and not by changes in axial diffusivity.

Table 2 Association between the microstructural integrity of the corpus callosum and cognitive performance

Microstructural integrity of the corpus callosum	Cognitive Domains					
	Cognitive index	Verbal memory	Visuospatial memory	Psychomotor speed	Concept shifting	Attention
Genu						
fractional anisotropy	.14*	.13	.11	.16*	.14*	.09
mean diffusivity	-.19*	-.19*	-.07	-.16*	-.13*	-.07
axial diffusivity	-.10	-.11	-.05	-.04	-.01	-.01
radial diffusivity	-.22*	-.20*	-.14	-.22*	-.19*	-.12
Body						
fractional anisotropy	.10	.13*	.07	.07	.11	.08
mean diffusivity	-.11	-.15*	-.01	-.06	-.07	-.09
axial diffusivity	-.04	-.05	-.09	-.01	-.05	-.02
radial diffusivity	-.17*	-.19*	-.11	-.14*	-.16*	-.14*
Splenium						
fractional anisotropy	.12*	.09	.14*	.14*	.14*	.09
mean diffusivity	-.09	-.09	-.06	-.09	-.10	-.08
axial diffusivity	-.04	-.04	-.08	-.02	-.01	-.01
radial diffusivity	-.17*	-.12	-.19*	-.21*	-.19*	-.13*

Standardized β values adjusted for age, sex, education, depressive symptoms and normalized TBV*Significant after Bonferroni correction $p < .05$

Discussion

In this study we examined the relation between the microstructural integrity of the white matter and cognitive performance in subjects with cerebral SVD, using whole-brain TBSS analysis in an unbiased manner. We demonstrated that a lower FA and a higher MD in multiple regions of the white matter skeleton were associated with a lower cognitive performance. The corpus callosum showed the highest significant relation with cognitive function, especially in the genu and splenium. The microstructural integrity in the cingulum bundle showed the highest significant relation with verbal memory performance and in the frontal white matter with psychomotor speed.

Several issues should be addressed in this study. First, a limitation is the cross-sectional nature of our study, which limits causal inferences. The RUN DMC study has a longitudinal design and follow-up being executed to evaluate the effect of progression of SVD on (changes in) cognitive performance.¹⁶ Second, in moderate and severe stages of SVD, WML are diffusely distributed throughout the white matter, probably resulting in diffuse loss of microstructural integrity. However, WML or microstructural loss in one region might be associated with those in other regions. This prevents us from drawing conclusions about the importance of the microstructural integrity at a specific location in relation to cognitive disturbances. Finally, WML and lacunar infarcts are widely accepted signs of SVD. However, lacunar infarcts may also result from non-SVD related mechanisms, for example due to an embolism from the heart or proximal arteries.²⁸ We therefore cannot rule out that some lacunar infarcts have been misclassified as symptoms of SVD. However, as the majority of lacunar infarcts is SVD related²⁸ and all lacunar infarcts in our study were accompanied by some degree of WML, favoring an underlying SVD mechanism,²⁹ we think that misclassification would be rather small and did not greatly influence our results.

Major strengths of the study included the large sample size, the single center design, the use of a single scanner and the high response rate. Furthermore, we manually segmented the WML, the extensive assessment of cognitive function was performed by only two investigators and all analyses were extensively adjusted for potential 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 SVD and cognitive performance. The structural abnormality of the white matter tracts is a nondiscernible finding in neurodegenerative diseases, such as Alzheimers' disease and mild cognitive impairment.^{30, 33} Also, age-related structural changes in the NAWM have been observed in a population based cohort, which were explained by the presence of white matter atrophy and WML³⁴ and in a longitudinal study on 84 healthy adults, age-related DTI changes throughout the brain were demonstrated in a two-year follow-up assessed by TBSS.³⁵ However, these studies did not investigate the relation with cognitive performance and so far, these studies have not yet been conducted in subjects with SVD related cognitive impairment using TBSS.

In patients with SVD, clinical manifestations of cognitive impairment are usually of a characteristic and fairly homogeneous 'subcortical' pattern and include psychomotor slowing due to impaired executive function, deficits of attention, planning and set-shifting and forgetfulness¹⁸ In our study, the white matters' structural integrity was associated with a lower performance on cognitive index, verbal memory, psychomotor speed and concept shifting We found no relations between microstructural integrity of the white matter and fluency and MMSE This might be explained by the fact that the MMSE is a too crude measure not designed for subcortical damage related function loss and hence not sensitive to detect subtle cognitive changes that correlate with macro- and microstructural SVD changes

A strong significant relation between microstructural integrity and verbal memory performance was located in the cingulum bundle This bundle, which connects the medial temporal lobe and the posterior cingulate cortex, is an important structure in verbal memory performance³⁶ DTI studies in patients with cognitive decline and dementia have shown loss of microstructural integrity of the cingulum³⁷ Here, we demonstrated that this relation also occurs in SVD related early cognitive decline With regard to psychomotor speed, we found that a strong significant relation with microstructural integrity located in the frontal lobe The pre-frontal-subcortical circuits are known to be involved in executive function, which affects the psychomotor speed¹⁸ The corpus callosum has been related to global cognitive status and, based on the topographical organization, the genu has been related to frontal-lobe-mediated executive function and attention,³⁸ whereas the splenium is associated with visuospatial construction³⁹ We demonstrated that global cognitive function (cognitive index) was associated with the microstructural of all three parts of the corpus callosum Verbal memory was associated with the anterior parts of the corpus callosum, whereas visuospatial memory was related to the splenium, corresponding with its connection with the parietal, temporal and occipital regions⁴⁰

The majority of the relations between microstructural integrity and cognitive performance were located in the NAWM, as this is 98.5% of the total white matter This suggests that the microstructural integrity of the NAWM has an important role in the cognitive disturbances in our subjects with SVD The presence of WML and lacunar infarcts weakened the relation between the microstructural integrity and cognitive performance This, together with the knowledge that the severity of damage in the NAWM is positively associated with the WML load,⁴¹ suggests that the damage to this NAWM might partially be SVD related Several explanations could therefore be proposed by which SVD may be related to the associations between microstructural integrity of the NAWM and cognitive performance First, it may be that these associations were, at least in part, explained by the coexisting WML, and not so much by the loss of integrity of the NAWM Second, the structural changes of the NAWM could be caused by direct additional damage to the NAWM in some, by the same risk factors that are involved in the development of SVD, such as hypertension^{42,43} This hypothesis is supported by the finding of increased blood-brain barrier permeability in the NAWM in

subjects with SVD⁴⁴ Finally, the observed association between the microstructural integrity of the NAWM and cognitive performance could reflect indirect damage to the NAWM by distant effects of functionally the most severe WML by means of anterograde (Wallerian) or retrograde degeneration^{45 46} The pattern of a low FA, high MD, and a high radial diffusivity, and to a lesser extent axial diffusivity was related to impaired cognitive performance This pattern of change may provide some additional information on the underlying mechanisms of loss of white matter integrity in our study, as this pattern is believed to be mainly a result of myelin breakdown⁴⁷ and axonal damage and atrophy⁷ resulting in an increase in extra-axonal fluid and lower membrane density⁷ Both the direct effect of SVD and the chronic Wallerian degeneration can mirror this DTI pattern However, the exact (combination of) histopathological processes leading to changes in the diffusion tensor in humans and the interaction between these measures is still not fully understood

Using TBSS we were able to assess the microstructural integrity of the whole-brain white matter in an unbiased manner Regions-of-interest (ROI) analysis has the disadvantage of revealing only changes in the preselected regions that might not necessarily correspond to areas that are affected in cognitive impairment In addition, ROI analysis is an observer dependent analysis in which partial volume effect can confound the results by including different tissue types (GM and CSF), with a modest reliability and reproducibility Furthermore, TBSS analysis allowed us to identify regions with the strongest association with specific cognitive domains, that overcomes some limitations of voxel-based morphometry style analysis, which is prone to residual misalignment and in which the results are affected by the amount of spatial smoothing¹⁵ In addition, compared to ROI analyses, TBSS is sufficiently sensitive for detection of longitudinal regional microstructural integrity changes³⁵ This latter issue might be of importance during follow-up examination, which should identify whether changes in white matter integrity over time predict future cognitive decline and dementia

In conclusion, our study showed that cognitive disturbances in elderly with cerebral SVD are attributable to loss of microstructural integrity of multiple white matter fibers connecting different cortical and subcortical regions Especially damage to the callosal fibers of the genu and the splenium, projecting to the prefrontal areas and parietal, temporal and occipital regions showed the highest significant relations with cognitive function The microstructural integrity in the cingulum bundle showed to play an important role in verbal memory performance and in the frontal white matter with psychomotor speed DTI, by using TBSS, reveals functionally relevant damage to the white matter integrity which is related to cognitive disturbances, not detected by conventional MRI DTI could therefore serve as an additional tool to conventional MRI in order to investigate the cognitive consequences of cerebral SVD Future studies should prospectively investigate the predictive value of microstructural damage assessed by DTI for incident cognitive decline or dementia

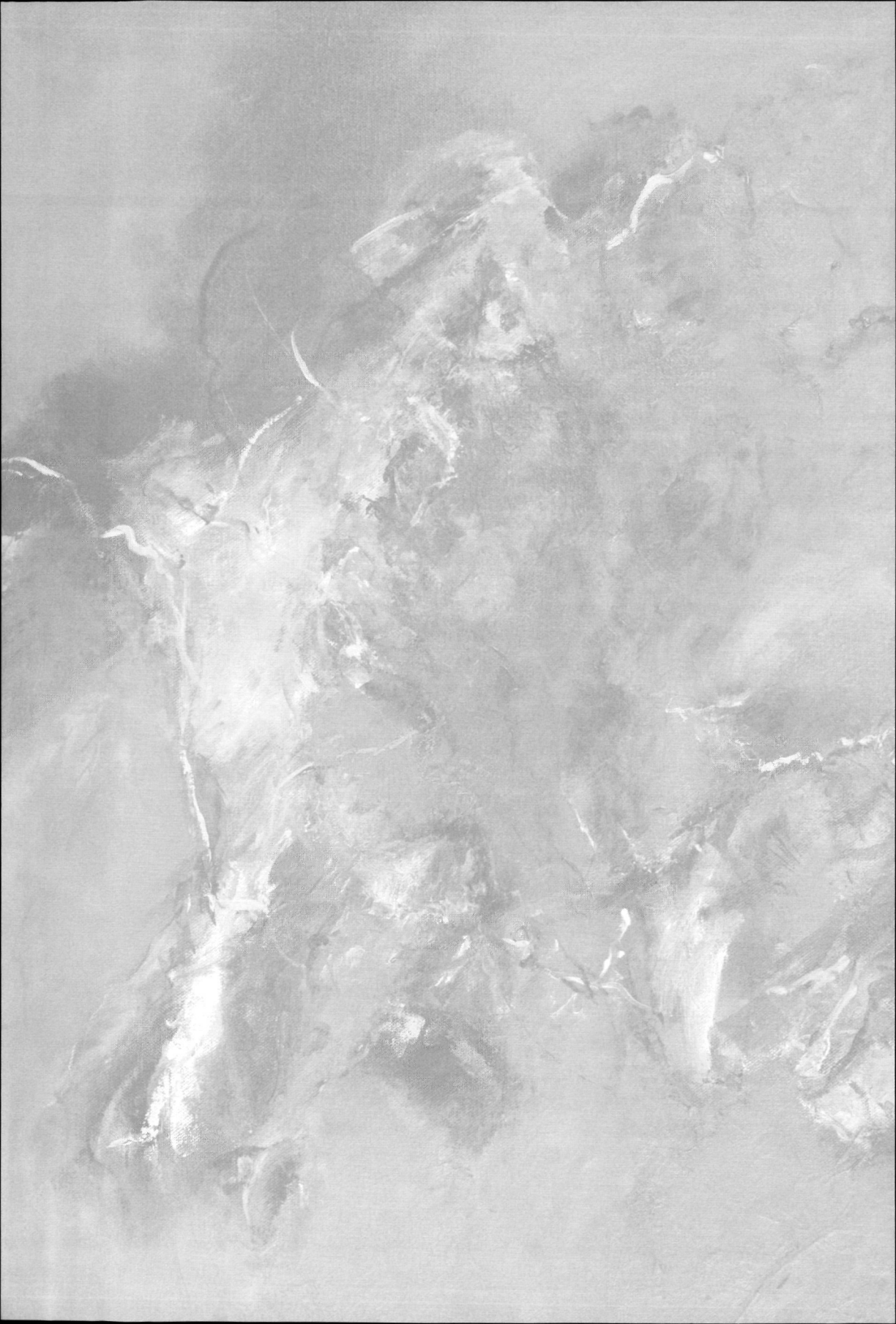
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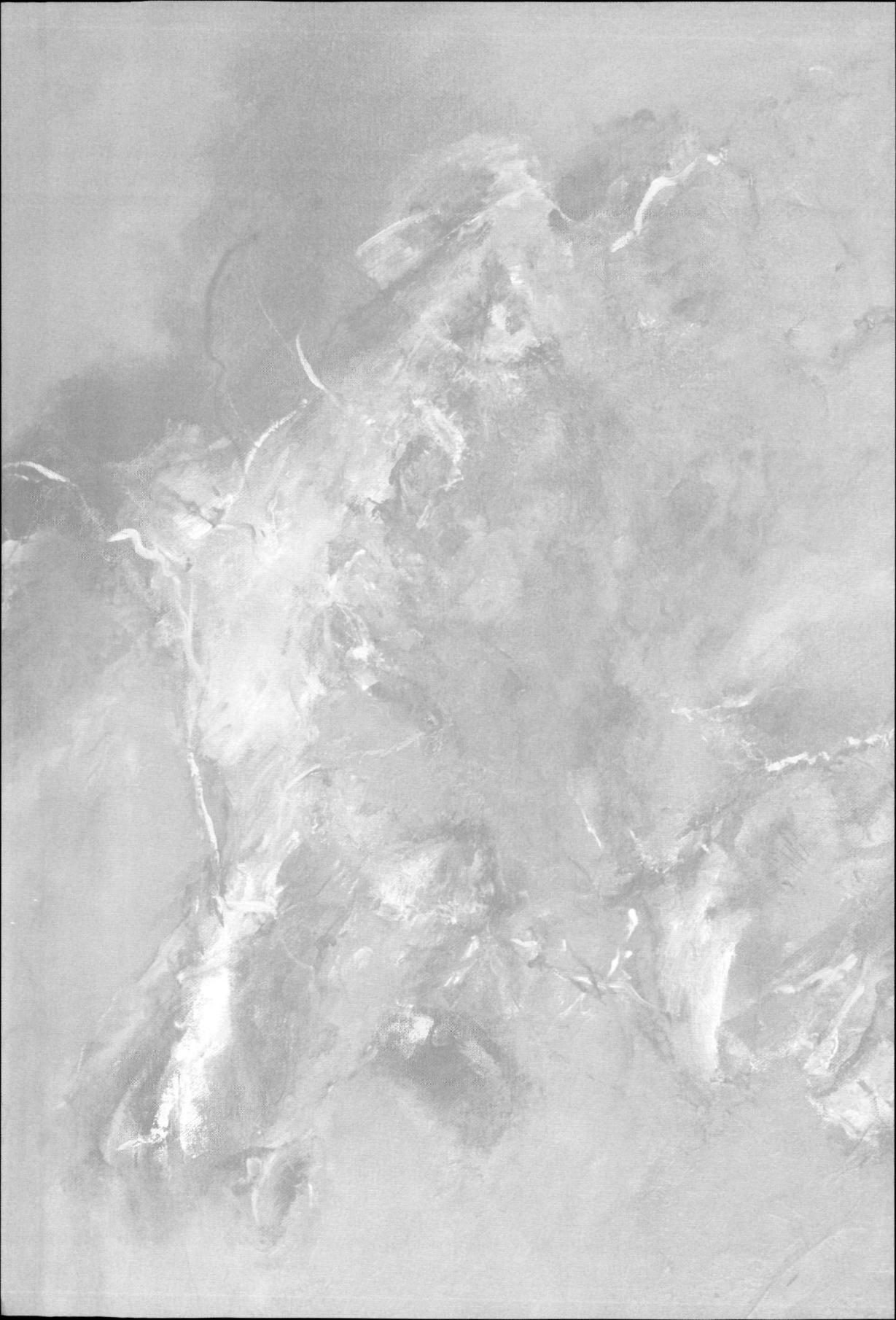
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PART IV

**Conventional MRI compared to
diffusion tensor imaging**



Chapter 11

The association between cerebral small vessel disease and cognitive function: a comparison between conventional MRI and diffusion tensor imaging

Submitted as:

van Norden AGW, van Uden IWM, de Laat KF, Norris DG, Zwiers MP, van Dijk EJ, de Leeuw FE. Cognitive function in small vessel disease: the additional value of diffusion tensor imaging to conventional MRI. The RUN DMC study.

Abstract

Objective Diffusion tensor imaging (DTI), which measures the integrity of the white matter in both the white matter lesions (WML) and normal appearing white matter (NAWM), is an innovative imaging technique, that is suggested to be of added value in the explanation of cognitive dysfunction in cerebral small vessel disease (SVD) We investigated the value of DTI of NAWM and WML on top of conventional MRI parameters in the assessment of cognitive function in subjects with SVD

Methods 499 individuals with SVD, 50-85 years, without dementia, underwent MRI scanning Grey (GM), white matter (WM), and WML volume, number of microbleeds, lacunar and territorial infarcts, 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 and sex

Results All MRI parameters together accounted for 3-6% of the variance in cognitive function on top of 10-23% already explained by age and sex When considered separately, GM and WM volume, and MD of the NAWM had the strongest association with cognitive performance However, mean MD of the NAWM did not substantially contribute to the assessment of cognitive function, to that already explained by conventional MRI parameters

Conclusion DTI of NAWM and WML has limited additional value to conventional MRI parameters in explaining variance in cognitive function among individuals with SVD

Introduction

Conventional MRI plays an essential role in the diagnosis of cerebral small vessel disease (SVD), 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 lead to dementia.¹⁻³ Despite the high prevalence of SVD in the population over 60 years of age,⁴⁻⁶ only few individuals with SVD ultimately develop cognitive decline.

Apparently the SVD/WML in these patients differs from those without cognitive decline. WML on conventional MRI merely reflect increased water content, and conventional MRI does not differentiate between mildly or more severely damaged areas while the latter results in (more outspoken) disruption of white matter tracts.⁷ Another potential factor that may contribute to the differential cognitive decline may be the integrity of the white matter surrounding the SVD. SVD on conventional MRI usually affects only a few percent of the total white matter (WM),⁸ leaving the remainder of the normal appearing white matter (NAWM) unassessed.

Diffusion tensor imaging (DTI) has proven beneficial in assessing the microstructural integrity of both the WML and the NAWM.⁹⁻¹⁰ However, the relative importance of each of these MRI and DTI parameters in explaining the variance of cognitive performance in patients with SVD, and with that the value of DTI in clinical practice, is not clear. It is unknown which (combination of) 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) MRI parameter(s) accounted for the largest portion of variance 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 SVD, and other brain lesions, including territorial infarcts and grey and white matter 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 with cerebral SVD. The selection procedure of the participants and the study rationale and protocol were described in detail previously.¹¹

In short, on the basis of established research criteria SVD was defined as the presence of

lacunar infarcts and/or WML on neuroimaging¹² Accordingly, 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 (WML and/or lacunar infarcts) The main exclusion criteria were dementia, (psychiatric) disease interfering with cognitive testing or follow-up, WML or SVD 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 center, 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 artifacts

MRI scanning protocol

Standardized T1- and T2-weighted and gradient echo T2*-weighted sequences on a single 1.5 Tesla scanner, were acquired as earlier described¹¹ 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 >15mm in known arterial territories) and microbleeds rated according to a standardized protocol Grey (GM) and white matter (WM) and cerebrospinal fluid (CSF) probability maps were obtained by automated segmentation using Statistical Parametric Mapping 5 unified segmentation routines on the T1 MPRAGE images (SPM5, Wellcome Department of Cognitive Neurology, University College London, UK)¹³ 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 (TBV) 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, TBV was expressed as percentage of total ICV Mutual information coregistration (SPM5) was used to align WML maps to the T1 image and to yield a NAWM map (the complement of WML in WM)

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 calculated¹⁴ 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 derivatives. All images were visually checked for motion artifacts 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 and has been described in detail elsewhere.¹¹ In short, we calculated compound scores for seven cognitive domains; 1) Global cognitive function was evaluated by the 1a) Mini Mental State Examination (MMSE) and 1b) 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.¹ 2) Verbal memory is a compound score of the mean of two z-scores from the Rey auditory verbal learning test; 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 is 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.¹ 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 is a compound score of the z-score of the total time of the verbal series attention test.¹

Other measurements

Level of education was assessed (classified using 7 categories; 1 being less than primary school and 7 reflecting an academic degree).¹⁵ 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 $\geq 140/90$ mmHg and/or use of antihypertensive medications,¹⁶ diabetes as treatment with antidiabetic 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 \pm standard deviation (SD) and for the positively skewed WML volume distribution, the median and interquartile 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, and their mutual correlations.

Second, to establish which MRI parameter (conventional MRI or DTI parameter) explained most of the variance in the different cognitive domains, we performed a multiple linear regression analysis (with age, sex 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 SVD-related MRI abnormalities and are related to cognition as well.⁸

Third, we assessed which combination of MRI parameters (MD and FA of both WML and NAWM, and all conventional MRI parameters which showed significant correlations when analyzed separately in step two) accounted for the largest variance in cognitive performance. For each cognitive domain, a multiple regression analysis was performed with the introduction of age, sex and education prior to the MRI parameters. These MRI parameters were 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 added value of DTI over conventional MRI. We used a multiple linear regression model in which age, sex 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) were added.

Collinearity diagnostic procedures (tolerance statistics) did not reveal collinearity between any of the MRI parameters in relation to cognitive performance.

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 mm ² /s	0.89 (0.04)
Mean MD in WML * 10 ⁻³ mm ² /s	0.10 (0.67)
Mean MD in total WM * 10 ⁻³ mm ² /s	0.89 (0.05)
Mean FA in NAWM mm ² /s	0.33 (0.02)
Mean FA in WML mm ² /s	0.34 (0.03)
Mean FA in total WM mm ² /s	0.33 (0.02)

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

Results

Demographics and neuroimaging characteristics of 499 subjects are shown in Table 1, four subjects were excluded because of MRI artifacts. 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 correlations between the distinct MRI parameters. Almost all MRI parameters were significantly correlated to each other, apart from some correlations with territorial infarcts and microbleeds. Mean FA and MD in the NAWM and total WM 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, $p < .001$; for MD between 0.62 and 0.66, $p < .001$) and age.

In table 3 we determined the amount of variance in cognitive performance explained by each MRI parameter separately, after adjustment for age, sex and education. Most of the MRI parameters were significantly associated with all cognitive domains, except for FA and MD in the WML, WM volume and territorial infarcts in relation to some cognitive domains. Mean MD in the NAWM and in the total WM explained most variance of the MMSE, cognitive index, verbal memory performance and attention. Additional to age, sex 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 mean MD in the NAWM and total WM. In all cognitive domains, DTI parameters accounted for only slightly more variance in cognitive performance than the conventional SVD parameters (WML volume, number of lacunar infarcts and number of MB).

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.	Terr 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< 05, **p< 001

WML: white matter lesions, NAWM: normal-appearing white matter, MD: mean diffusivity, FA: fractional anisotropy

Table 3 Value of each MRI parameter separately in the assessment of cognition

Step	MRI parameter	MMSE		Cognitive index		Verbal memory		Psychomotor speed		Attention	
		total expl. variance	Stand. beta	total expl. variance	Stand. beta	total expl. variance	Stand. beta	total expl. variance	Stand. beta	total expl. variance	Stand. beta
1	age, sex, education	21.7		35.7		24.3		35.0		27.9	
	Conventional MRI parameters										
2	GM volume, ml	22.6	0.12*	36.6	0.12*	24.7	0.07	36.2	0.14*	28.6	0.11*
2	WM volume, ml	21.8	0.05	36.8	0.13*	24.6	0.07	37.6	0.20**	28.7	0.11*
2	WML volume, ml†	22.9	-0.13*	36.4	-0.10*	24.4	-0.04	35.9	-0.11*	28.4	-0.09*
2	Microbleeds	22.1	-0.07	37.3	-0.13**	25.3	-0.10*	36.7	-0.13**	29.5	-0.16**
2	Lacunar infarcts	23.1	-0.12*	36.8	-0.11*	24.9	-0.08*	36.4	-0.12*	28.6	-0.09*
2	Territorial infarcts	21.7	-0.03	36.5	-0.09*	24.4	-0.04	37.0	-0.14**	27.9	-0.02
	DTI parameters										
2	NAWM, mean MD	23.1	-0.16*	37.6	-0.19**	25.7	-0.15*	37.2	-0.20**	30.3	-0.13**
2	WML, mean MD	22.8	-0.13*	37.1	-0.14*	24.9	-0.09	37.1	-0.18**	30.2	-0.12*
2	WM, mean MD	23.2	-0.17*	37.8	-0.19**	25.7	-0.15*	37.4	-0.20**	30.3	-0.14**
2	NAWM, mean FA	22.7	0.11*	37.0	0.13*	25.2	0.10*	36.5	0.13**	28.6	0.12*
2	WML, mean FA	22.3	0.08	36.3	0.09*	24.6	0.06	35.3	0.05	29.0	0.11*
2	WM, mean FA	22.7	0.11*	37.0	0.13*	25.2	0.10*	36.5	0.13**	28.9	0.13*

Age, sex and education were introduced in the model prior to one MRI parameter. Total explained variance (%)

†log transformed, * $p < .05$, ** $p < .001$, GM: grey matter, WM: white matter, WML= white matter lesions, NAWM: normal appearing white matter, WM: white matter, MD: mean diffusivity, FA: fractional anisotropy,

Table 4 The value of a combination of MRI parameters in the degree of variance explained for the various cognitive domains

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

Age, sex and education were introduced in the model prior to MRI parameters. 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.

Table 5 Contribution of DTI on top of 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, sex and education were introduced in the model prior to conventional MRI parameters. 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, \dagger log transformed.

We furthermore determined which combination of MRI parameters best explained the variance in cognitive performance, after controlling for age, sex and level of education, which accounted for 21.7%-35.7% already (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, sex and education ranged from 1-6%.

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 another 1.0% to 4.2% over age, sex 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 percent. This was not found for MMSE, visuospatial memory performance, fluency and concept shifting (for latter three data not shown). 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% additional variance in performance of the different cognitive domains to the 22-36% variance already explained by age, sex 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 rate, 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. Another strength is the manual segmentation of the WML without prior knowledge of the clinical data and the assessment of microbleeds as another manifestation of SVD. A limitation is the cross-sectional analysis of this part of the study, which prevents us

from drawing conclusions about the predictive value of DTI parameters in cognitive decline. However, the RUN DMC study has a longitudinal design and follow-up is currently being executed.¹⁷ Second, cognitive function also is influenced by several factors for which we intentionally did not account in this study such as medial temporal lobe atrophy, as wanted to investigate the effect of SVD 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. 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 SVD (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, 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 SVD, as both have been related to SVD and cognitive performance as well.^{18,19} Furthermore, it might be that age influenced the degree of variance explained. Selective survival may have led to this due to a decreased susceptibility to the effect of SVD among survivors. Moreover there is probably a selective non-participation of survivors with a higher degree of SVD and more cognitive disturbances. Another explanation for the relative small contribution of SVD in the variance of cognitive performance may be due 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 SVD patients that have shown a relation between cognitive function and markers of cerebral atrophy,²⁰ such as atrophy of the corpus callosum,²¹ gray matter volume and hippocampal volume.²²

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,^{23,24} 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 white matter.²⁵ 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 parameters into 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 in the total WM and NAWM are highly correlated and explained the same amount of 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 SVD related lesions visible on conventional MRI. This might be explained by the fact that the cognitive tests used are not sensitive enough to detect these subtle changes in microstructural integrity. Another explanation might be 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 studies²⁶

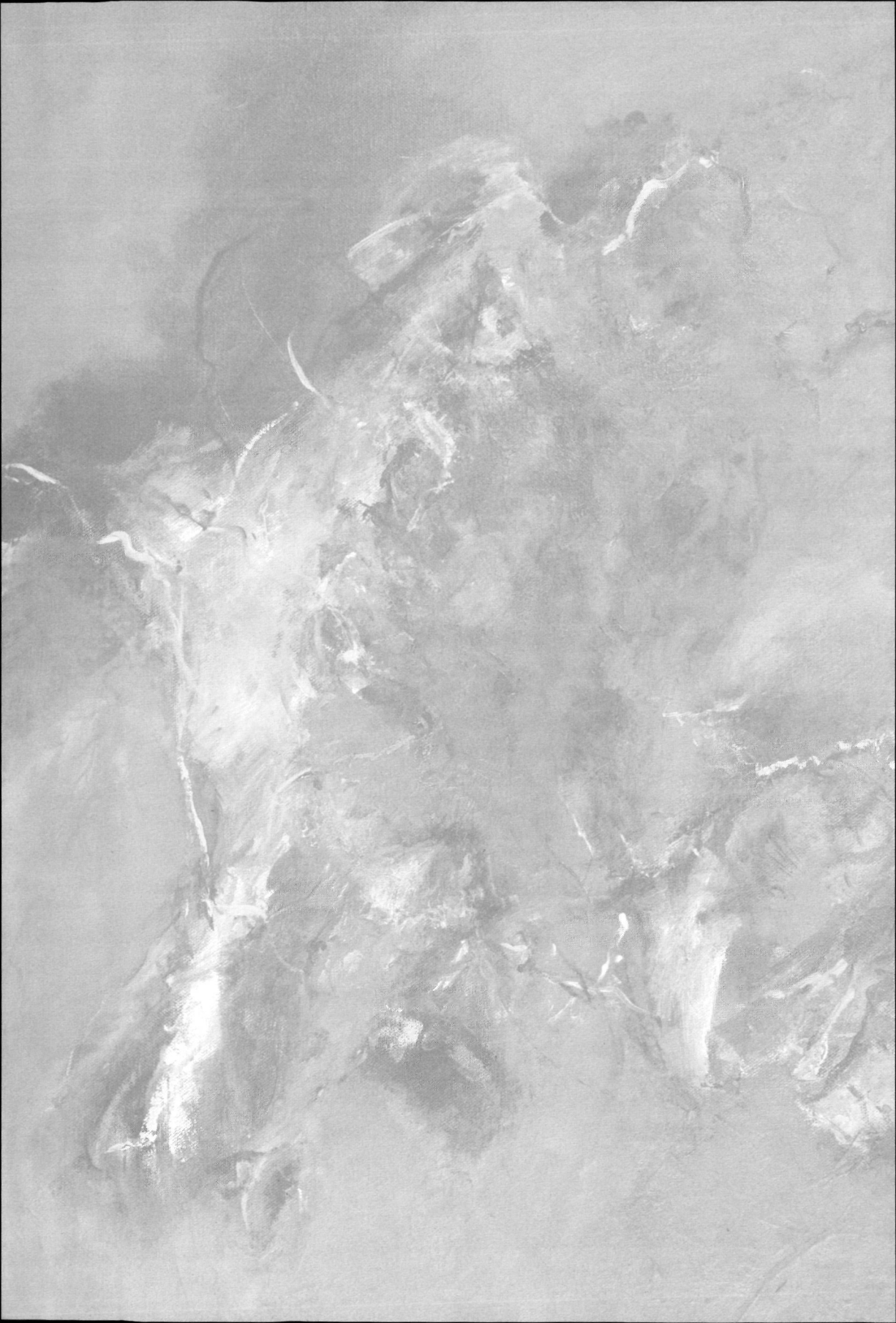
Our results show that DTI does not seem necessary in clinical practice in order to understand cognitive impairment in patients with SVD. However, despite the small contribution of DTI parameters in the variance of cognitive performance at the cross-sectional level, DTI parameters may be of additional value in longitudinal studies and clinical trials in which there is interest in a surrogate marker for progression of cognitive disturbances and in the evaluation of potential therapeutic effects. 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 studies²⁷. 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^{23, 28, 29}. 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, in clinical practice, DTI of the white matter (NAWM, WML or total WM) does not seem to be of added value to the conventional MRI parameters, in explaining the variance of cognitive impairment in subjects with SVD. 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 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 SVD.

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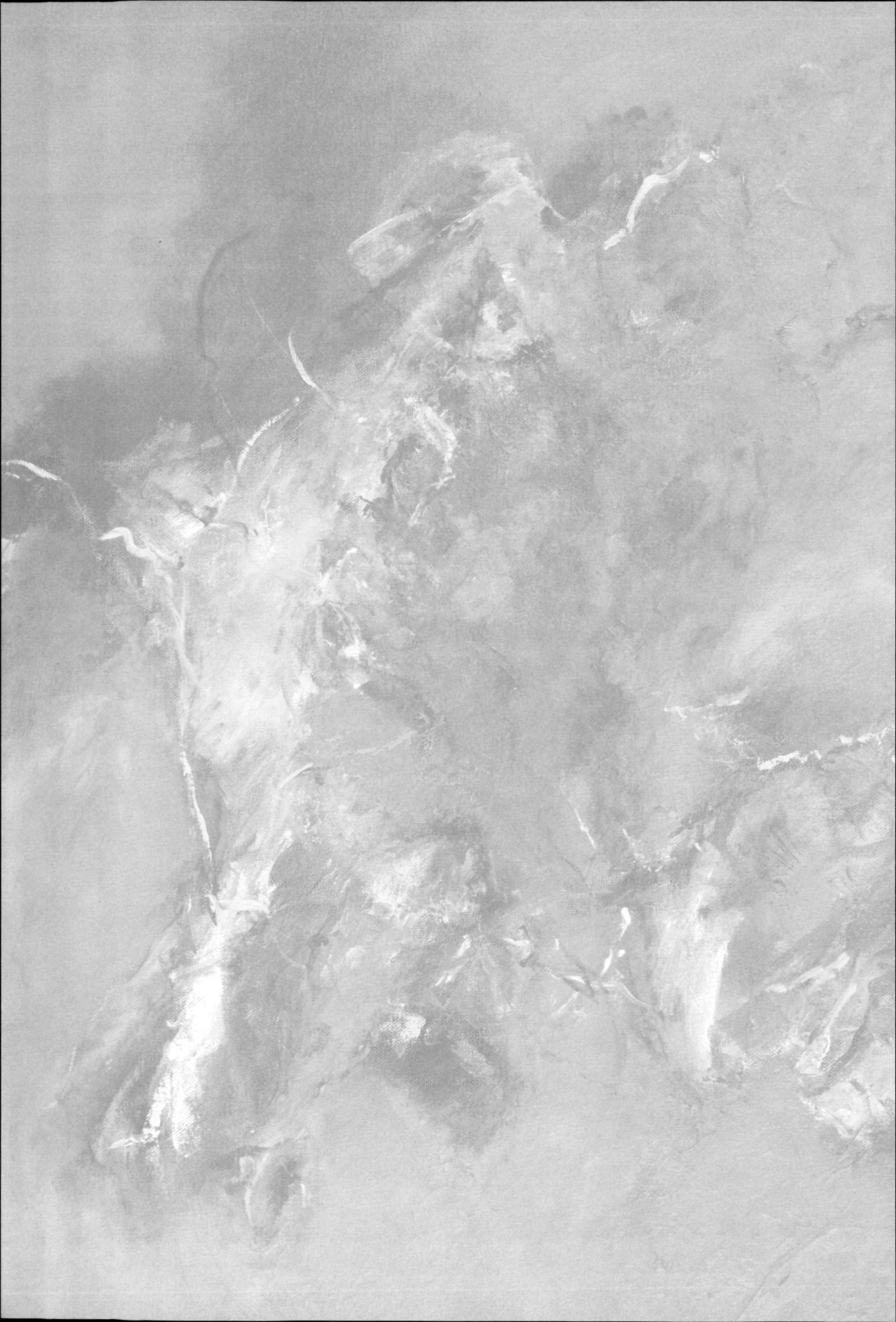
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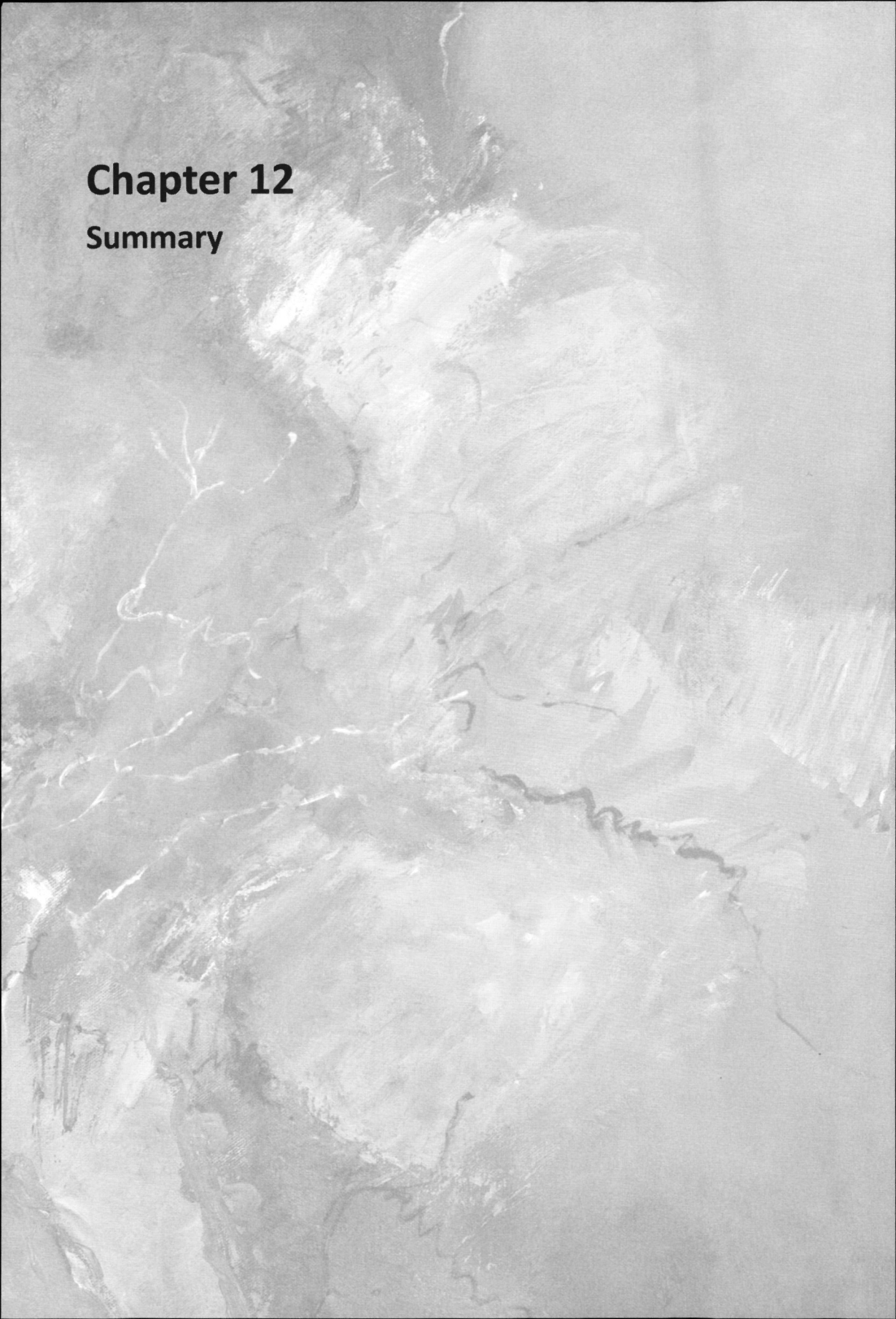
PART V

Summary and Discussion



Chapter 12

Summary



On magnetic resonance imaging (MRI), cerebral small vessel disease (SVD) is characterized by white matter lesions (WML), lacunar infarcts and microbleeds (MB). Cerebral SVD is common in the elderly population, increases with age and is associated with vascular risk factors and is an important cause of cognitive decline and dementia. However, individuals with a virtually identical SVD burden on conventional, routine T1 and T2 images present with a wide variance in cognitive performance ranging from no complaints at all to overt dementia, independent of other factors as Alzheimer's disease pathology. Other factors apart from SVD related lesions on conventional MRI may determine whether identical appearing SVD on routine imaging leads to cognitive decline in one person, while leaving others unaffected. One of these factors could be the microstructural integrity of the white matter and the WML, as assessed with DTI.

In this thesis we describe the associations between different SVD related brain changes and cognitive performance with the use of a conventional MRI approach and a diffusion tensor imaging (DTI) approach.

In **part I** of the thesis we describe the rationale and design of the RUN DMC study, a prospective cohort study on the risk factors and cognitive, motor (gait) and mood consequences of functional and structural brain changes among 503 independently living, non-demented elderly, aged between 50 and 85 years, with cerebral SVD (**chapter 3**).

A conventional MRI approach

In **part II** we describe the associations between conventional MRI SVD parameters and cognitive performance. WML were manually segmented and volumes were calculated and the number of lacunar infarcts and MB were rated. All subjects had some degree of WML and lacunar infarcts were present in 31%. Presence, number and location of MB were rated in the lobar (frontal, parietal, temporal, occipital), deep (basal ganglia and thalamus, the white matter of the corpus callosum, internal, external and extreme capsule), and infratentorial (brainstem and cerebellum) regions. Ten percent of the subjects had one or more MB. Cognitive performance was assessed with an extensive neuropsychological test battery covering global cognitive function, verbal and visuospatial memory performance, executive function (e.g. psychomotor speed, verbal fluency and concept shifting) and attention. The mean of the Mini Mental State Examination (MMSE) of our study population was 28.1 (SD 1.6). In addition, subjective cognitive complaints were assessed with a semi-structured interview, 91% of the participants reported complaints in some degree.

MB have been related to cognitive impairment in elderly individuals, however this relation has predominantly been investigated in memory clinic patients, usually without taking location into account and without adjustment of coexisting SVD (i.e. quantitative WML).

volume and number of lacunar infarcts) We found that the presence, number and location of MB correlated with cognitive performance, independent of other manifestations of SVD (**chapter 4**) Furthermore MB were related to subjective cognitive, memory and executive complaints and were associated with progression of these failures over the past 5 years, especially in individuals with good objective cognitive function (**chapter 5**) Especially MB located in the frontal and temporal lobe were related to subjective cognitive complaints and cognitive performance In addition, lower hippocampal volumes have been related to subjective cognitive complaints however without taking into account the degree of SVD We described that lower hippocampal volume is related to subjective cognitive complaints independent of SVD and that this relation was strongest in those without objective cognitive impairment (**chapter 6**)

A diffusion tensor imaging approach

In **part III** we report on the associations between the microstructural integrity of the white matter and cognitive performance, assessed by DTI In **chapter 7** we investigated the relation between the mean diffusivity (MD) and fractional anisotropy (FA) within the WML and the normal appearing white matter (NAWM) and cognitive performance We demonstrated that these DTI parameters in both the WML and NAWM correlate with cognitive performance, independent of SVD visible on conventional MRI These relations were most pronounced for subjects with severe WML As the hippocampus and cingulum bundle are important structures in verbal memory performance, we investigated the relation between hippocampal and cingulum microstructural integrity and verbal memory performance In **chapter 8** we report on the relation we found between the microstructural integrity of the hippocampus and verbal memory performance, independent of SVD, these relations were most outspoken for subjects with a normal hippocampal volume In **chapter 9** we used a region of interest analysis to assess the microstructural integrity in six regions along the cingulum bundle The microstructural integrity of the mid and posterior cingulum was associated with verbal memory performance independent of hippocampal volume and integrity and SVD

In **chapter 10** we use Tract-Based Spatial Statistics (TBSS), a method restricted to the white matter voxels that constitute the skeleton of the brain's connectonal architecture, and demonstrated that the tracts associated with cognitive performance are scattered throughout the white matter In addition, we showed that the strongest spatially localizing associations were found in the corpus callosum, predominantly the genu and splenium, the cingulum bundle for verbal memory performance and the frontal white matter for psychomotor speed The microstructural integrity of the corpus callosum is important in cognitive performance in all cognitive domains

Conventional MRI compared to diffusion tensor imaging

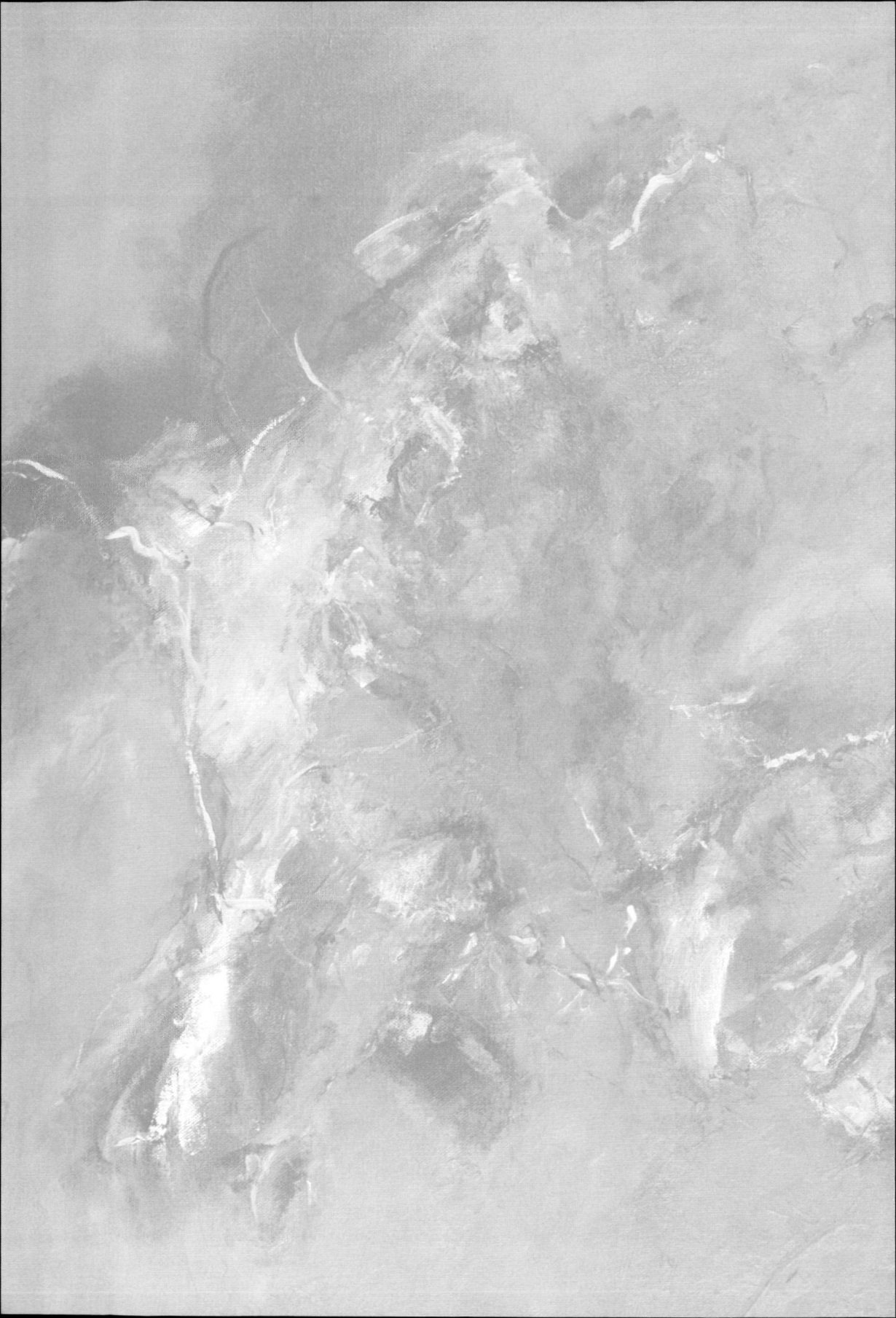
In **part IV** of this thesis we investigated the additional variance in cognitive performance explained by DTI measures to that of SVD related lesions visible on conventional MRI. Our results described in part III of the thesis suggested that microstructural damage in the WML but also in the NAWM plays an important role in cognitive impairment. In **chapter 11** we describe that the structural integrity of the WML, NAWM and total WM accounted for very little additional explained variance (1%) in cognitive performance on top of co-existing conventional MRI markers of SVD, e.g. WML and lacunar infarcts. Of note, the total explained variance of all cerebral MRI markers, apart from age, sex and education (which explained 36% of the variance in global cognitive performance), was about 4.2%, in which the MD in the (NA)WM played an important role.

In conclusion, SVD related brain changes are associated with cognitive performance, although the cross-sectional relation is rather weak and microstructural integrity of the WM accounted for very little additional explained variance. However, considering the whole range of SVD features may better predict the clinical consequences.

Conclusion

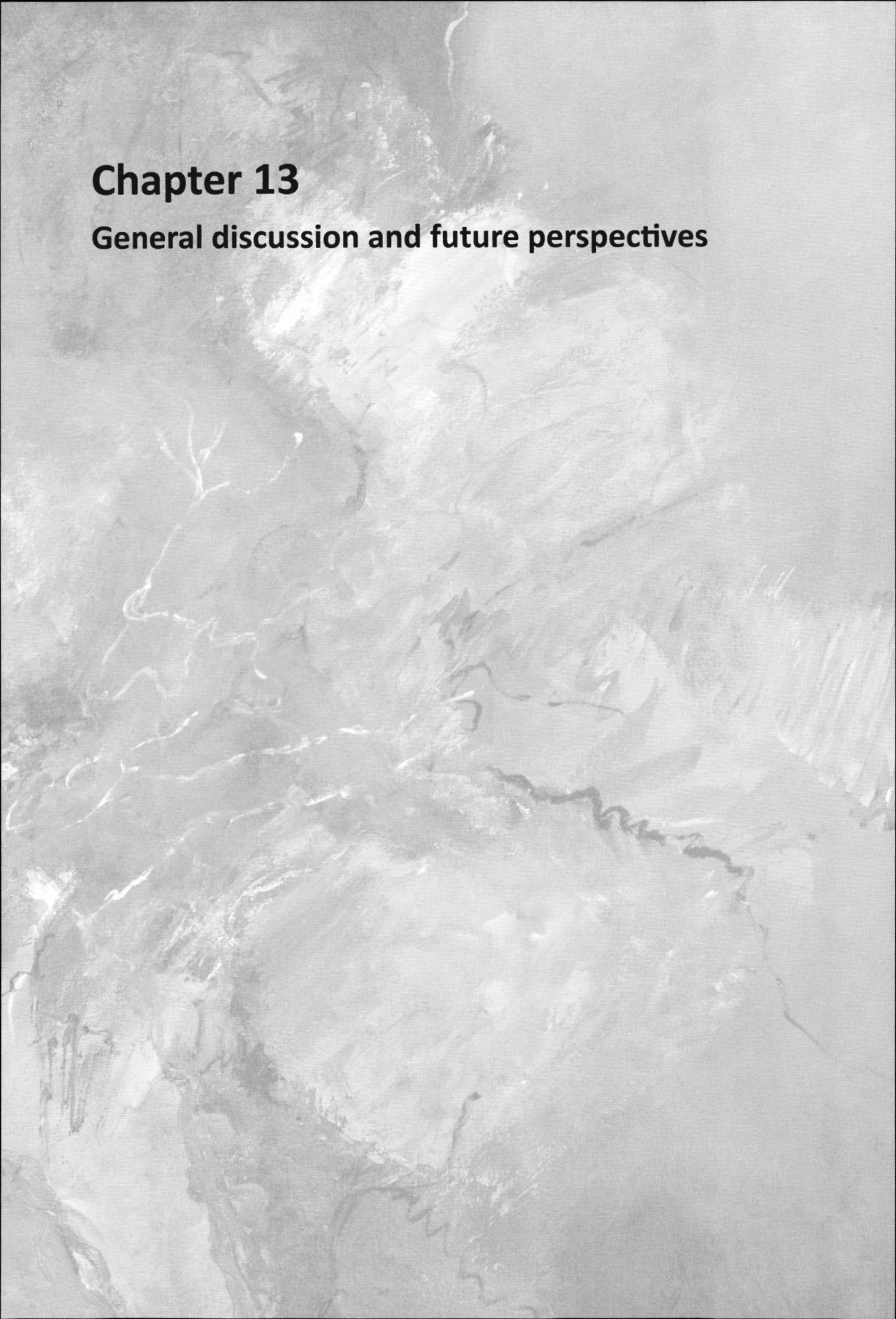
The studies described in this thesis identified MRI markers that can serve as the basis for future research and help us to gain more insight in the pathophysiological processes underlying cognitive disturbances in cerebral SVD.

Longitudinal examination is needed to assess whether these imaging markers indeed indicate early disease. The true impact of identifying those at risk will depend on the availability and effectiveness of preventive treatment for the disease.



Chapter 13

General discussion and future perspectives



The overall objective of this thesis was to gain more insight in the role of brain changes due to cerebral small vessel disease (SVD) in cognitive impairment with the use of conventional MRI and diffusion tensor imaging (DTI). The studies described in this thesis are based on data from the RUN DMC study, a prospective cohort study on the risk factors and cognitive, motor (gait) and mood consequences of functional and structural brain changes among 503 independently living, non-demented elderly with cerebral SVD, aged between 50 and 85 years. In this chapter methodological considerations will be addressed, main findings and potential clinical implications will be discussed, and finally suggestions for future research will be made.

Methodological considerations

Selection of the study population

Studies described in this thesis aimed at investigating the role of SVD in cognitive performance. As the onset of cerebral SVD is often insidious, clinically heterogeneous and typically with mild symptoms, patients with SVD are difficult to diagnose on the basis of clinical symptoms alone, making it rather inefficient to include them in studies only on clinical grounds as many individuals without SVD may have similar symptoms. Consequently, fairly large numbers of patients would then be needed in order to be able to include the number required. It has therefore been suggested that the selection of subjects with cerebral SVD should be based on the more consistent, generalizable features namely neuroimaging.¹ We invited subjects to participate in the RUN DMC study based on SVD on neuroimaging. The selected age range of 50-85 years was chosen because prevalence of both determinant (SVD) and the outcome (cognitive performance) is high in this age range. The most important exclusion criteria were baseline dementia and psychiatric disease interfering with cognitive testing.

Internal validity

Validity is the most important issue when a study is judged on its methodological merits. Validity of results involves restricting systematic error or bias. Three general types of bias can be identified: selection bias, information bias and confounding.

Selection bias results from a different association between the determinant (MRI and DTI parameters for SVD) and the outcome (cognitive performance) between participants and non-participants, who were potentially eligible for the study. The RUN DMC study had a high response rate of 71.3%. Non-participants compared to participants were older (response rate in subjects between 50 and 60 years was 82% compared to 56.5% in subjects aged between 70 and 85 years) and had more severe WML than participants, as assessed by the Age Related White Matter Changes scale.² Since age and SVD predict cognitive performance and decline, older people with severe SVD and severe cognitive disturbances may have

refused to participate more often than those without or less cognitive disturbances. We obviously could not collect data on cognitive function in these non-responders, however it is likely that we missed those with the highest degrees of SVD and highest degrees of cognitive disturbances. This would have resulted in a less precise estimation of the strength of the relation between the most severe SVD and the most severe cognitive dysfunction.

Information bias results from measurement error of the determinant (different SVD parameters) that depends on the outcome (cognitive performance) or when assessment of the outcome is influenced by (the severity of) the determinant. In our study the assessment of the determinants (WML, lacunar infarcts, microbleeds (MB) and DTI parameters) was performed independently and blinded for clinical information. In addition, assessment of outcome (subjective cognitive complaints and objective cognitive function) was performed in the days before MRI scanning and therefore independent from the knowledge of the determinant, minimizing any observer bias. Therefore it is very unlikely that misclassification would have been differential and hence would only have resulted in a random error. Some measurement error may have occurred in the rating of the MB, as novel MRI techniques have improved the detection of MB detecting more MB in more patients.³ This may have resulted in an underestimation of the actual number of MB in our population. However, a recent study did not find stronger relations (or more variance explained) between cognition and MB detected with novel MRI techniques.⁴ In addition, it is not clear whether this improved detection is linearly present across the whole range of MB, or if this predominantly plays a role in those with multiple MB, which probably would have resulted in a differential measurement error.

Confounding results from confusion of the determinant under study with that of an extraneous factor. A confounder is associated with both the determinant and the outcome and is not an intermediate factor in the causal chain leading to the outcome variable under study. In our studies we dealt with confounding by adjusting for potential confounders in multivariate regression models. Adjusting for an intermediate factor would result in an underestimation of the effect of that factor.

The most important confounder in the relation between SVD and cognitive function is age, and potentially, gender, level of education, the presence of depressive symptoms and other neuroimaging characteristics, such as territorial infarcts, brain- and hippocampal volume. Because of the large number of subjects included in our study, we were able to control for these confounders. We intentionally did not adjust for cardiovascular risk factors such as hypertension as they were considered to cause SVD and in that way they are an earlier part of the causal chain between SVD and cognitive performance.

The question is whether or not to adjust for SVD markers, visible on conventional MRI, when investigating the association between the microstructural integrity of both the WML

and NAWM and cognitive performance. When we stratified these analyses on severity of WML, we found that especially in those with severe WML, damage to the microstructural integrity in the NAWM is related to impairment in cognitive function. This suggests effect modification between the DTI parameters in the NAWM and WML volume in relation with cognitive function. The question remains whether WML volume is also a confounder in this relation, a factor in the causal relationship or both. It could be, that in almost none of the individuals with very mild WML the integrity of the NAWM is affected, and therefore the microstructural integrity of the NAWM in these individuals is not associated with cognitive performance. In individuals with the highest severity of WML, the observed association between the microstructural integrity of the NAWM and cognitive performance could reflect, 1) direct additional damage to NAWM in some by the same risk factors that are involved in the development of severe WML, and 2) indirect damage to the NAWM by distant effects of functionally the most severe WML. Both are biologically more plausible effects than WML inducing a spurious association between NAWM integrity and cognitive performance. Therefore adjustment for WML volume in these analyses would probably result in over-adjustment.

Precision

Precision reflects the amount of random error (non-differential misclassification) in a given measurement and thereby affects the reproducibility of that measurement. We tried to increase overall precision in the RUN DMC study by having a large sample size ($n=503$). In the assessment of the determinant (different SVD parameters), all imaging data were collected with a single scanner, without adjustment in hardware or software during the study period, and most of the determinants were assessed quantitatively. WML and hippocampal volumes were manually segmented and lacunar and territorial infarcts and MB were rated visually by two trained raters. Both raters independently assessed 10 percent of all manual and visual assessments with high inter- and intra-rater agreements. Brain tissue segmentation and the analysis of the DTI data were fully automated, to prevent systematic error (such as co-registration errors) these data were visually checked.

Furthermore, the precision in measurement of the outcome (cognitive performance) was greatly enhanced by performing multiple neuropsychological tests, chosen because of their robustness in detecting age-related impairment and sensitivity to subcortical dysfunction^{5,7}. Tests were assessed in the same order, on the same time of the day, by the same two investigators. Compound scores were constructed for eight cognitive domains taking the mean of several tests that were individually standardized to z-scores. The tests within one compound score assess the same cognitive domain and act therefore as multiple measurement of the same variable, thereby reducing the intra-individual variability and giving a better estimate of the true value per domain.

External validity

External validity involves the extent to which the results of the study can be generalized beyond the study population. We included a large group of consecutive patients aged between 50 and 85 years with SVD who were referred to the Department of Neurology and who underwent neuroimaging. We initially selected patients based on the neuroimaging characteristics of SVD and subsequently asked for acute or subacute clinical symptoms of SVD in order to fulfill established criteria for SVD.¹ We think that our results can be generalized to patients with SVD in general hospitals and to community-dwelling population for several reasons. First, although our participants were seen in an university hospital our results can be generalized to the general SVD population, as most of them were seen as a first opinion. In addition, SVD is a common disorder in the elderly with a prevalence over 90% in the general population over 60 years of age.⁸ We included, independently living, non-demented subjects with a mean Mini Mental State Examination of 28.1 (SD 1.6), which corresponds with the estimates in the general population,^{5,9,10} a cognitively rather healthy population, all with some degree of SVD on neuroimaging. Furthermore, the prevalence of vascular risk factors for SVD was very similar compared to community-dwelling cohorts with SVD. We therefore think that our study has a reasonably high external validity for the general population aged between 50 and 85 years of age with SVD on neuroimaging likely to be related to arterio(lo)sclerosis and vascular risk factors. We do not think our results can be extended to individuals with other causes of SVD or to individuals with SVD at a much younger age.

Causal inference

A precisely measured association between a factor and an outcome that is free of bias, does not imply that the observed association is a causal one. There has been much debate on the criteria for causal inference, which has elaborated in a widely recognized set of inductively oriented causal criteria useful as a basis for inferring causality.¹¹ One of the most important criteria for establishing a causal relation is temporality. Temporality refers to the necessity that the cause precedes the effect in time. Our studies were based on cross-sectional data, limiting our abilities to establish a temporal relationship, leaving the probability that the proposed determinant is in fact the cause (inverse causality). This would be the case if cognitive decline induces lifestyle changes, which result in the development of SVD. In addition, it could be possible that change in cognitive function is caused by another factor that also caused SVD, in that way SVD is an epiphenomenon and not a cause of cognitive impairment. However based on prior data and our results, the last two hypotheses are much less likely than that SVD causes cognitive impairment. The RUN DMC study has a longitudinal design, the 5 year follow-up of the study population has already started to evaluate the parenchymal changes over time, changes in cognitive performance, development of cognitive decline and incident dementia. This prospective

nature with repeated measurements of both the determinant and the outcome will reduce the possibility of inverse relations

General discussion of the main findings

A conventional MRI approach (part II)

We found that microbleeds (MB), which are another manifestation of SVD on conventional MRI, were related to cognitive performance and subjective cognitive complaints, independent of co-existing quantitatively assessed WML volume and lacunar infarcts and that location played a role in the association. Our findings suggest that MB have a direct effect on cognitive performance rather than simply reflecting the presence of other markers of SVD. First, this might be explained by the presence of MB representing subjects with the highest severity of SVD, as all our subjects some degree of WML, 31% of these subjects had lacunar infarcts and almost all subjects with MB (92%) also had lacunar infarcts. Second, a dose-effect relation is suggested as in both studies there was a stronger relation between the number of MB, than the mere presence of MB with cognitive performance. A third explanation might be the location of MB. We demonstrated a relation between frontal and temporal located MB and subjective cognitive complaints and objective cognitive performance. These are both important locations involved in executive function and memory performance. Although the temporal lobe is not a predilection site for WML, we found a relation with temporal located MB and cognitive performance.¹² In addition, a recent study found lobar MB significantly more often in the temporal lobe.¹³ Fourth, the underlying mechanisms of the association between MB and cognitive function are unknown. Histopathologic studies have shown that the MB indicate widespread damage of arterioles by hypertension or by amyloid deposition, but also of damage to the white matter due to surrounding gliosis and frank necrosis or infarction, resulting in microstructural damage.^{14,15} Taken together, MB may represent additional disruption of white matter tracts important for cognitive function on top of the damage caused by WML and lacunar infarcts. However, the imaging techniques used in these studies could not identify this microstructural damage in relation to the presence of MB.

Furthermore, we investigated the relation between the location of MB and cognitive performance. It is suggested that the distribution of MB may reflect the underlying etiology of MB. It might be that this location of MB also differentiates in the different underlying pathologies of concomitant WML and lacunar infarcts and in that way partly influence their relation with cognition, lobar MB are presumably attributable to cerebral amyloid angiopathy (CAA), whereas MB in the deep/infratentorial regions are considered to represent hypertension related arteriosclerotic angiopathy.^{16,17} Together with the fact that

the temporal lobe is known to be more affected by CAA, the relation observed between the frontal and temporal located MB and lower cognitive performance is in agreement with the notion that CAA may be the underlying pathology. This finding could be of importance as CAA is highly frequent among persons with Alzheimer's disease.¹⁸ Follow-up study may help to elucidate the potential relation between MB, CAA and the risk of Alzheimer's disease.

A diffusion tensor imaging approach (part III)

The microstructural integrity of the normal appearing white matter (NAWM) and WML was related to cognitive performance on several cognitive domains. In our study population WML volume highly correlated with DTI parameters in the NAWM, which is in line with other studies in which larger WML volumes correlated with a higher mean diffusivity (MD) and lower fractional anisotropy (FA) in the NAWM.¹⁹ WML on conventional MRI merely reflect increased water content, and conventional MRI does not differentiate between mildly and more severely damaged areas, while the latter results in (more outspoken) disruption of white matter tracts. DTI reveals changes in the white matter that are imperceptible with conventional MRI. These changes may precede lesions visible on conventional MRI and in that way DTI may be a sensitive tool to assess early white matter damage. However, we found the strongest relations between microstructural integrity of the NAWM and cognitive performance in those with the highest degree of WML. The used cognitive tests may not be sensitive enough to detect differences in cognitive performance associated with loss of microstructural integrity in those with mild WML. On the other hand it might be that those with mild WML the integrity of the NAWM is affected and that changes in the NAWM in fact reflect late damage due to the presence of severe WML. Severe WML may lead to axonal loss in areas (i.e. NAWM) connected by axons that travel through these WML by anterograde (Wallerian) or retrograde neural degeneration, resulting in disconnection and cognitive dysfunction. In addition it could be that SVD on conventional MRI and microstructural changes of the NAWM assessed by DTI share similar risk factors for SVD, such as hypertension.²⁰ Furthermore, it may be that the relation between the microstructural integrity of the NAWM and cognitive performance is, at least in part, explained by the coexisting WML, and not so much by the loss of microstructural integrity of the NAWM. Although the relation found for the NAWM was relatively weak, the observed relations between the microstructural integrity of the corpus callosum, cingulum bundle and hippocampus with cognitive performance, all regions usually without WML on conventional MRI, do show the importance of loss of microstructural integrity in this part of the white matter in relation to cognitive disturbances in SVD.

In conclusion, all our findings suggest that SVD is a widespread disease throughout the whole brain, exceeding WML, lacunar infarct and MB visible on conventional MRI with loss of microstructural integrity in the NAWM. These findings together with those from functional and pathological studies suggest a disruption of cortical-cortical and cortical-

subcortical connections, and a subsequent 'disconnection-syndrome' accounting for the cognitive disturbances in SVD patients. Although DTI does not add a lot to conventional MRI in explaining the variance of cognitive impairment in individuals with SVD, DTI is a promising tool in gaining more insight in the pathophysiological processes underlying cognitive disturbances in cerebral SVD assessing cerebral connectivity *in vivo*.

Cognitive function

In patients with SVD, cognitive impairment is usually of a characteristic 'subcortical' pattern and include psychomotor slowing due to impaired executive function, deficits of attention, planning and set-shifting²¹. In our studies an extensive neuropsychological test battery was used to assess objective cognitive function. This encompasses items from other large scale epidemiological studies that cover virtually all cognitive domains and were chosen because of their robustness in detecting age-related impairment and sensitivity to subcortical dysfunction^{5,7}. We constructed compound scores which consisted of the mean of several tests that were individually standardized to z-scores⁵. As measures of global cognitive function we used a combination of all tests^{5,6} as well as the widely used Mini Mental State Examination (MMSE)²². For both memory compound scores, verbal memory and visuospatial memory, we used the free recall capabilities, which are affected by cortical as well as subcortical dysfunction²³. We intentionally used only the most basic elements of the test parameters for the compound score of psychomotor function, as this enabled us to strictly assess the psychomotor speed that was intended to be measured. The Stroop test consists of three subtasks of which we used the simplest, the reading task, in most analyses²⁴. The interference task, the more complex subtasks of the Stroop test, was used for the domain concept shifting, as it measures psychomotor speed, but also sensorimotor processes as stimulus encoding and response selection²⁴.

There was no relation between the MMSE, a widely used bedside test, and the number of MB, whereas there was only a weak association with microstructural integrity measures of the white matter. This might be because the MMSE is not sensitive to 'subcortical' (dys)function and merely reflects 'cortical' (dys)function. In addition, in our rather healthy population (mean MMSE of 28.1 (SD 1.6, 24-30)), the MMSE score might be a too robust measure to detect subtle cognitive changes, or the variance in the outcome measure (MMSE) might be too small, to detect correlations with MB of loss of microstructural integrity.

Explained variance by cerebral small vessel disease

We found an association between SVD markers on conventional MRI (i.e. MB) and cognitive function, as well as a relation between loss of microstructural integrity, assessed by DTI, and cognitive function. Although, only a small part of the variance in cognitive function was accounted for by SVD. There may be several explanations for this. First, in the elderly, neurodegenerative disease and cerebrovascular disease are both common and both entities often coexist. Evidence is accumulating that the joint occurrence of these diseases is based not merely on chance, they share many identical vascular risk factors²⁵ and brains of persons diagnosed with late-onset dementia often show a mixture of ischemic and typical Alzheimer's disease pathology at autopsy²⁶. Although, we tried to adjust for neurodegenerative disease by normalized total brain or hippocampal volume, neurodegenerative disease could play a role in the relation with cognitive function in our study population. In addition, lobar MB are associated with CAA,^{16,17} and we found a relation between lobar MB and cognitive function. Part of this relation with cognitive function could be explained by underlying CAA.

Second, age (sex and educational level) were considered to be confounding factors in all our analyses. It might be that the strengths of the associations is influenced by age, as the presence and severity of SVD increases with age, and 90% of the general population over the age of 60 years has some degree of SVD⁸, in that way SVD may to some extent be regarded to as 'normal aging'. Higher age is related to longer exposure to risk factors and their possible interactions. 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. In addition there could be a distortion of the effect of SVD on cognitive performance due to several reasons. First, selective survival may play a role. SVD is associated both with cognitive performance and mortality. It may therefore be that only those who are not or differently vulnerable to the effect of SVD survive, and that the effect of SVD on cognition is different in these survivors. Another potential source of bias is selection of participants, especially in the oldest category, in which response rate was lowest, as discussed earlier. Non-responders were older and had more severe SVD and may have had more severe cognitive disturbances. Finally, due to end-stage of disease effect, in patients with SVD, low blood pressure might be a risk for poor cerebral perfusion and in that way lead to cognitive impairment. By adjusting for age in our analyses the role of SVD in relation to cognitive impairment could have been underestimated, due to decreased susceptibility to the effects of SVD and selective non-participation of survivors with severe SVD and severe cognitive impairment. Third, it might be that the neuropsychological tests used are not sensitive enough to detect subtle changes in cognitive function that relate to SVD related lesions assessed with conventional MRI and DTI. Greater variance in cognitive function during follow-up examination could result in a more outspoken relation between cognitive (dys)function and MRI and DTI parameters.

Finally, another explanation could be the concept of brain and cognitive reserve, which postulates that the brain must undergo a certain amount of pathological change before the attendant clinical symptoms emerge. The idea of the cognitive reserve theory posits that individuals with higher reserve are more able to cope with brain pathology, through some form of compensatory strategy, i.e. by functioning on a previously higher cognitive level.²⁷ Consequently, they may experience a longer subclinical cognitive deterioration, despite the presence of SVD. Another explanation could be the presence of compensatory networks that may compensate for SVD based disruption of pre-existing networks. This may obscure the relation between SVD burden and cognitive performance. In younger individuals the presence of cognitive reserve could have underestimated the effect of SVD on cognitive performance, in older age adaptive and compensating mechanisms may fail and increase the susceptibility for disease.

Clinical relevance

People with severe WML and silent lacunar brain infarcts on MRI have an increased risk of dementia, depression, stroke and death.²⁸⁻³² By the time people are diagnosed with cognitive impairment and dementia, they already have irreversible brain damage. Therefore the focus in brain research is gradually shifting to early detection of pathologic changes and possible prevention. Studies like ours can identify early markers of disease which may help us to modify the risk of development or prevent progression of disease.

Our results suggest that subjective cognitive complaints reported by subjects with SVD should not be ignored as they are related to cerebral MB and hippocampal volume, even in those without objective cognitive impairment, and these complaints have shown to be related to the development of dementia years later.³³⁻³⁴

In addition to the identification of WML and lacunar infarcts on conventional T1 and T2 imaging, we showed that additional T2* imaging could be of interest in gaining more insight in the role of SVD in cognitive impairment as our results suggest that MB have a direct effect on cognitive performance rather than simply reflecting the presence of other markers of SVD. Even more, because cerebral MB have been identified as potential imaging biomarkers of arteriosclerotic microangiopathy or CAA, and therefore might reflect different etiologies of both MB and WML.

In the clinical setting, additional DTI sequences seem not necessary, as it does not seem to be of added value to conventional MRI parameters, in explaining the variance of cognitive impairment in subjects with SVD. However, we showed that loss of microstructural white matter integrity of the NAWM and WML, and also of the corpus callosum, cingulum bundle and hippocampus has an impact on the cognitive performance in SVD. This indicates that DTI promising tool in gaining more insight in the pathophysiological processes underlying

cognitive disturbances in cerebral SVD. In addition DTI may provide surrogate markers to monitor treatment effects, as DTI parameters have shown to change over a short period of time, compared to conventional SVD parameters and were related to change in cognitive performance³⁵

Identification of individuals at risk for cognitive decline and dementia becomes more and more important. The imaging markers described in this thesis will not directly identify those at risk for disease. However, the studies described in this thesis identified MRI markers that can serve as the basis for future research and help us to gain more insight in the pathophysiological processes underlying cognitive disturbances in cerebral SVD and in that way might open a way for intervention. Longitudinal examination is needed to assess whether these imaging markers indeed indicate early disease. Until now no randomized trial has specifically addressed the treatment of people with SVD to prevent dementia in late life. The true impact of identifying those at risk will depend on the availability and effectiveness of preventive treatment for the disease.

Recommendations for future research

Study design - prospective

Dementia poses a major burden on individuals, caregivers and society. Within our aging society research on possible modifiable risk factors for the development of dementia is therefore very important. Cardiovascular risk factors may be one of these modifiable risk factors. In this thesis we report on the mediating role of SVD in the relation between cardiovascular risk factors and cognitive function and showed that, next to WML and lacunar infarcts, MB and white matters' microstructural integrity are potential relevant biomarkers in the relation with cognitive function.

However, our findings need to be replicated in other cross-sectional studies, as the observed associations between MB and microstructural integrity of the white matter with cognitive function have been shown by others, although not or only sporadically in SVD populations. Findings from our cross-sectional studies can be verified by relating progression changes to cognitive decline and the development of dementia in the follow-up of the RUN DMC participants. If, using serial neuroimaging assessment, change SVD severity can be related to change in cognitive performance and incident dementia it would greatly enhance the evidence for a causal relation. The longitudinal part the RUN DMC study could also clarify whether subjects who report progression of subjective cognitive complaints are a high risk group for the development of cognitive dysfunction and dementia and more importantly whether this coincides with an increase of MB (and/or other manifestations of SVD) and decrease in hippocampal volume. Of note, longitudinal studies on DTI in aging are rare, however a few longitudinal studies showed that change in DTI parameters could be detected

over a short period of time, and DTI parameters correlated with change in cognitive performance.^{35,36} In contrast there were no correlations with change in WML volume and cognition. Therefore it will be of great interest to analyze serial DTI scans for developing SVD pathology. In addition, it might be of special interest to investigate whether change in the microstructural integrity of the NAWM predicts/ is related to cognitive decline and the development of incident dementia in those with the highest degree of WML severity. Furthermore, follow-up of the RUN DMC participants over time for the occurrence of new MB, cognitive decline and incident dementia may help to elucidate the potential link between lobar MB, the supposed underlying pathology CAA and the risk of Alzheimer's disease.

Advances in MRI data analysis

Visualization of white matter tracts and performing tract-based spatial statistics are promising methods investigating the neural pathways disrupted in cognitive disturbances. DTI tractography can also help to explore structural connectivity between brain regions, increasing our understanding of how SVD pathology in one region may affect the structural integrity within the other, connected, region and results in cognitive impairment.^{37,38} With these techniques it might be able to gain evidence for the reserve theory and visualize compensatory networks.

Study design - intervention

Hypertension is recognized as one of the major risk factors for SVD, but evidence for the benefit of antihypertensive therapy on WML progression is still scarce.³⁹ Ultimately, future studies should unravel whether adequate treatment of vascular risk factors in subjects with SVD may reduce the course of brain lesions, and subsequently reduce the number of patients who develop cognitive decline and dementia. Of note, a Cochrane review showed that blood pressure lowering in late life does not prevent the development of cognitive decline and dementia.⁴⁰ However, one of the possible explanations for this unequivocal role of (treatment of) hypertension could be that the deleterious effects of vascular risk factors in general and hypertension specific already occurs decades before either cardiovascular or cognitive symptoms occur. By the time the risk factors result into disease, the damage is not remediable anymore.

Future studies should try to include people early in mid-life (30-40 years), as this might be an effective strategy to detect people at risk for SVD and possibly cognitive decline relatively early in life, thus opening a way for prevention or reduction of severity. A major drawback of this option is that it will take a long time before the outcome under study occurs.

A major question to be answered is whether and which proportion of subjects with SVD will eventually progress to dementia. As the rate of cognitive decline is slow, requiring large

samples and/or long lasting studies, neuropsychological testing is time consuming and learning effects reduce sensitivity, our study and other studies suggest that MRI markers of SVD could serve as surrogate markers for cognitive disturbances in future studies and therapeutic trials. However, future studies are needed to evaluate which imaging parameter, WML volume, number of lacunar infarcts, increase in number of MB, change in DTI markers, or a combination thereof will be the best predictor of cognitive decline and incident dementia. Probably it will not be one imaging marker alone, but a combination of this neuroimaging with genetic risk factors and biochemical biomarkers.

In conclusion, SVD related brain changes, assessed by conventional MRI and DTI, are associated with cognitive performance, although the cross-sectional relation is rather weak and microstructural integrity of the NAWM accounted for very little additional explained variance, within this cohort of cognitive healthy elderly individuals. However, considering the whole range of SVD features they may better predict the clinical consequences. The studies described in this thesis identified MRI markers that can serve as the basis for future research and help us to gain more insight in the pathophysiological processes underlying cognitive disturbances in cerebral SVD.

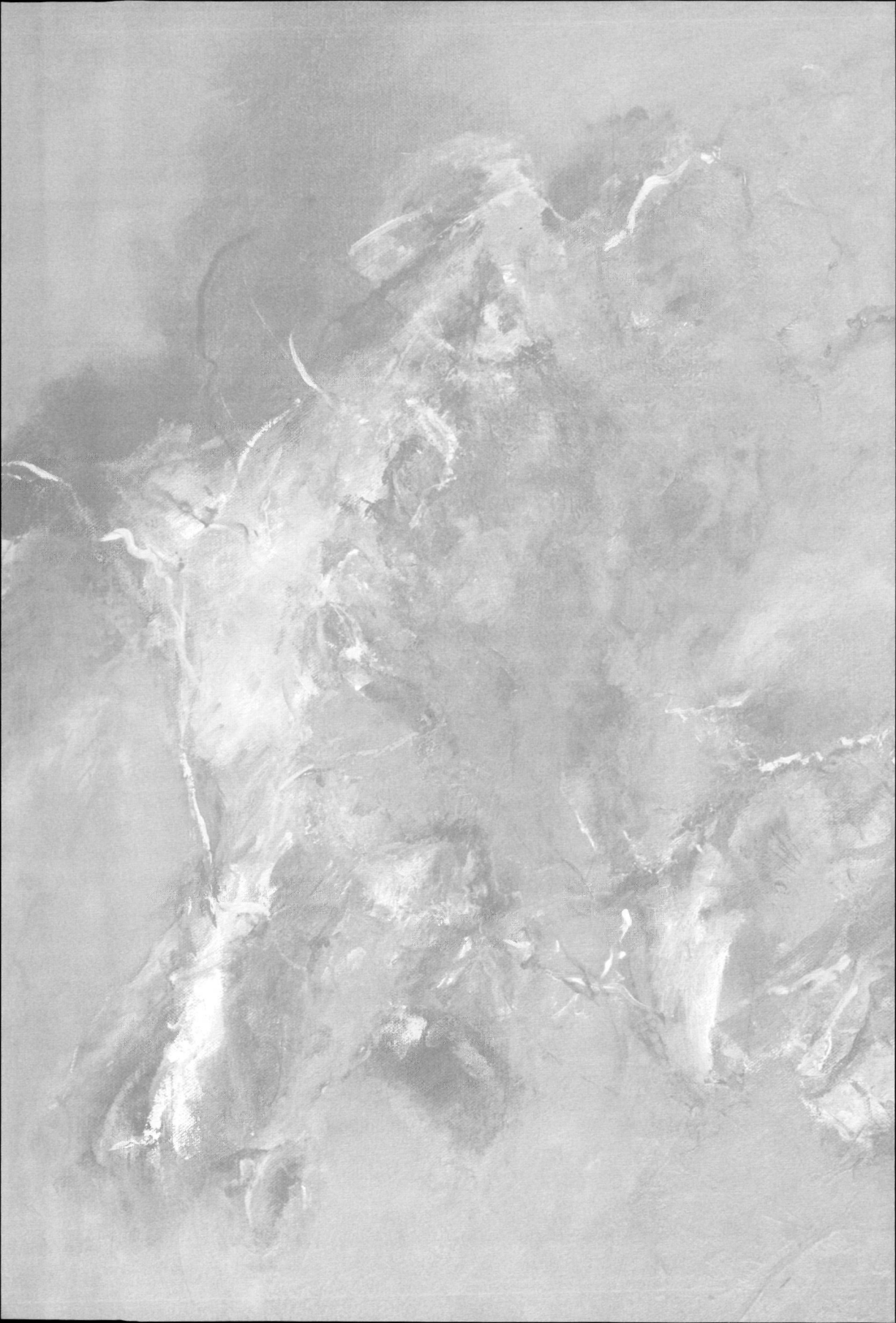
Longitudinal examination is needed to assess whether these imaging markers indeed indicate early disease. The true impact of identifying those at risk will depend on the availability and effectiveness of preventive treatment for the disease.

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Chapter 14

Nederlandse samenvatting

Summary in Dutch

Cognitief functioneren bij oudere mensen met cerebrale microangiopathie: een MRI-studie

Beschadiging van kleine bloedvaten in de hersenen, ook wel cerebrale microangiopathie genoemd, wordt in het Engels 'cerebral small vessel disease (SVD)' genoemd. Op een MRI (magnetic resonance imaging) scan van de hersenen wordt SVD gekenmerkt door witte stofafwijkingen, lacunaire herseninfarcten en microbloedingen. SVD is geassocieerd met vasculaire risicofactoren, zoals hypertensie, en komt veel voor bij ouderen. Meer dan 90 procent van de ouderen heeft enige mate van SVD op de MRI-scan en de prevalentie neemt toe met het voortschrijden van de leeftijd. Door die beschadigingen van de kleine bloedvaten kunnen stoornissen in de doorbloeding van de hersenen ontstaan, met als gevolg het beschadigen van witte stofbanen waardoor verbindingen tussen belangrijke hersengebieden verbroken worden. Als gevolg van onderbrekingen kunnen cognitieve stoornissen, cognitieve achteruitgang en dementie ontstaan. Echter, mensen met dezelfde mate van SVD op conventionele MRI-beelden, (T1 en T2 gewogen), presteren zeer verschillend op cognitieve taken, variërend van normaal cognitief functioneren tot dementie. Naast afwijkingen op een conventionele MRI die passen bij SVD, zijn er mogelijk andere factoren, naast de reeds bekende aan Alzheimer gerelateerde factoren, die bepalen waarom mensen met identieke afwijkingen op conventionele MRI-beelden een grote variatie in cognitief functioneren vertonen. Uit pathologisch onderzoek komt naar voren dat alhoewel witte stofafwijkingen er op een conventionele MRI hetzelfde uitzien, deze toch behoorlijk kunnen verschillen in mate van beschadiging (microstructurele integriteit) van de witte stof. Het zou kunnen zijn dat de microstructurele integriteit van de witte stofafwijkingen juist bepaalt welke personen stoornissen krijgen in het cognitief functioneren. Daarnaast zou de microstructurele integriteit van de witte stof die er normaal uitziet op conventionele MRI kunnen bijdragen aan de aanwezigheid van cognitieve stoornissen. De microstructurele integriteit van de witte stof kan gemeten worden met een nieuwe MRI-techniek genaamd 'diffusion tensor imaging' (DTI).

In dit proefschrift beschrijven we de associaties tussen veranderingen in de hersenen gerelateerd aan SVD en cognitief functioneren, in beeld gebracht met conventionele MRI- en DTI-scans.

In **deel I** van het proefschrift beschrijven we de gedachte achter en de opzet van de RUN DMC (Radboud University Nijmegen Diffusion tensor and Magnetic resonance imaging Cohort) studie; een prospectieve cohortstudie die de risicofactoren voor functionele en structurele veranderingen van de hersenen onderzoekt en de gevolgen daarvan op cognitie, lopen en stemming. Dit is onderzocht bij 503 onafhankelijk levende, niet dementerende ouderen, tussen de 50 en 85 jaar, met tekenen van SVD op de MRI-scan van de hersenen. Tijdens het eerste cross-sectionele onderzoek in 2006, waar dit proefschrift op is gebaseerd, had niemand dementie of parkinsonisme (**hoofdstuk 3**)

De relatie tussen ‘small vessel disease’ en cognitief functioneren: een conventionele MRI benadering

In **deel II** van dit proefschrift beschrijven we de associaties tussen parameters van SVD zoals te zien op conventionele MRI-beelden en cognitief functioneren. Wittestofafwijkingen werden handmatig gesegmenteerd en de volumes werden berekend, daarnaast werd het aantal lacunaire infarcten en microbloedingen geteld. Alle deelnemers aan het onderzoek hadden enige mate van wittestofafwijkingen en 31 procent van de deelnemers had lacunaire infarcten. De aanwezigheid, het aantal en de locatie van de microbloedingen werden geteld per kwab (frontaal, parietaal, temporaal en occipitaal), in diepe (basale kernen, thalamus en de wittestof van het corpus callosum en de capsula interna, externa en extrema), en infratentoriele (hersenstam en cerebellum) regio's. Tien procent van de deelnemers had een of meer microbloedingen. Het cognitief functioneren werd getest met behulp van een uitgebreid neuropsychologisch testprotocol, waarbij het globaal cognitief functioneren, het verbale en visuospatiele geheugen, het executief functioneren, met onder andere de psychomotorische snelheid, en de aandacht werd getest. De gemiddelde MMSE (Mini Mental State Examination) van onze studie populatie was 28,1 (SD 1,6). Daarnaast werden subjectieve geheugenklachten vastgelegd met behulp van een semigestructureerd interview, 91 procent van de deelnemers meldde enige mate van subjectieve geheugenklachten. Microbloedingen werden in eerder onderzoek reeds gerelateerd aan cognitief falen bij ouderen, echter deze relatie werd met name onderzocht bij patiënten die geheugenpoliklinieken bezochten, waarbij niet werd gekeken naar de locatie van de microbloedingen en zonder correctie voor de aanwezigheid van andere kenmerken van SVD, zoals kwantitatieve volumes van de wittestofafwijkingen en het aantal lacunaire infarcten. Wij stelden vast dat de aanwezigheid, het aantal en de locatie van microbloedingen zijn gerelateerd aan cognitief functioneren, onafhankelijk van andere manifestaties van SVD (**hoofdstuk 4**). Tevens zijn microbloedingen gerelateerd aan subjectieve cognitieve klachten en zijn zij gerelateerd aan toename van deze klachten over de afgelopen vijf jaar. Deze relatie was het meest duidelijk bij mensen met een goed objectief cognitief functioneren (**hoofdstuk 5**). Met name microbloedingen in de frontale en temporale kwabben zijn gerelateerd aan subjectieve cognitieve klachten en cognitief functioneren. Een kleiner hippocampusvolume werd reeds gerelateerd aan subjectieve cognitieve klachten, echter zonder dat werd bekeken in hoeverre SVD hieraan mogelijk bijdraagt. Wij stelden vast dat een kleiner hippocampusvolume is gerelateerd aan subjectieve cognitieve klachten, onafhankelijk van de aanwezigheid van SVD. Deze relatie was het sterkst bij de deelnemers zonder objectief cognitief falen (**hoofdstuk 6**).

De relatie tussen ‘small vessel disease’ en cognitief functioneren: een DTI benadering

In **deel III** van het proefschrift beschrijven we de relaties tussen de microstructurele integriteit van de witte stof en het cognitief functioneren, gemeten met DTI. In **hoofdstuk 7** hebben we de relatie onderzocht tussen de gemiddelde diffusiviteit (MD) en de fractionele anisotropie (FA) in de witte stofafwijkingen en de normaal ogende witte stof en cognitief functioneren. Uit de resultaten blijkt dat deze DTI-parameters in zowel de witte stofafwijkingen als de normaal ogende witte stof correleerden met cognitief functioneren, onafhankelijk van beschadiging van de kleine bloedvaten zichtbaar op conventionele MRI. Deze relaties waren het meest sterk in de groep mensen met de meest ernstige beschadigingen van de kleine bloedvaten. Aangezien de hippocampus en het cingulum belangrijke hersenstructuren zijn voor verbaal geheugen, hebben we deze structuren specifiek bekeken. In **hoofdstuk 8** beschrijven we de relatie tussen de microstructurele integriteit van de hippocampus en het verbaal geheugen, onafhankelijk van parameters voor SVD op conventionele MRI. Deze relaties waren het meest sterk bij personen met een normaal hippocampusvolume. In **hoofdstuk 9** hebben we een ‘region of interest’-analyse gebruikt om de microstructurele integriteit te bepalen in zes regio's van het cingulum. De microstructurele integriteit van de mid- en het posterieure cingulum was geassocieerd met verbaal geheugen onafhankelijk van het hippocampus volume en de hippocampus integriteit en onafhankelijk van SVD.

In **hoofdstuk 10** hebben we gebruik gemaakt van ‘Tract-Based Spatial Statistics’. Dit is een methode die zich beperkt tot de witte stof-voxels die het skelet vormen van de verbindingenarchitectuur van de hersenen. Uit onze analyses blijkt dat de banen die geassocieerd zijn met cognitief functioneren verspreid zijn door de gehele witte stof van de hersenen. De sterkste relaties voor verbaal geheugen werden gevonden in het corpus callosum, met name de genu en het splenium, en het cingulum en voor psychomotorische snelheid in de frontale witte stof. De microstructurele integriteit van het corpus callosum is belangrijk voor het functioneren op alle cognitieve domeinen.

Conventionele MRI in vergelijking met DTI

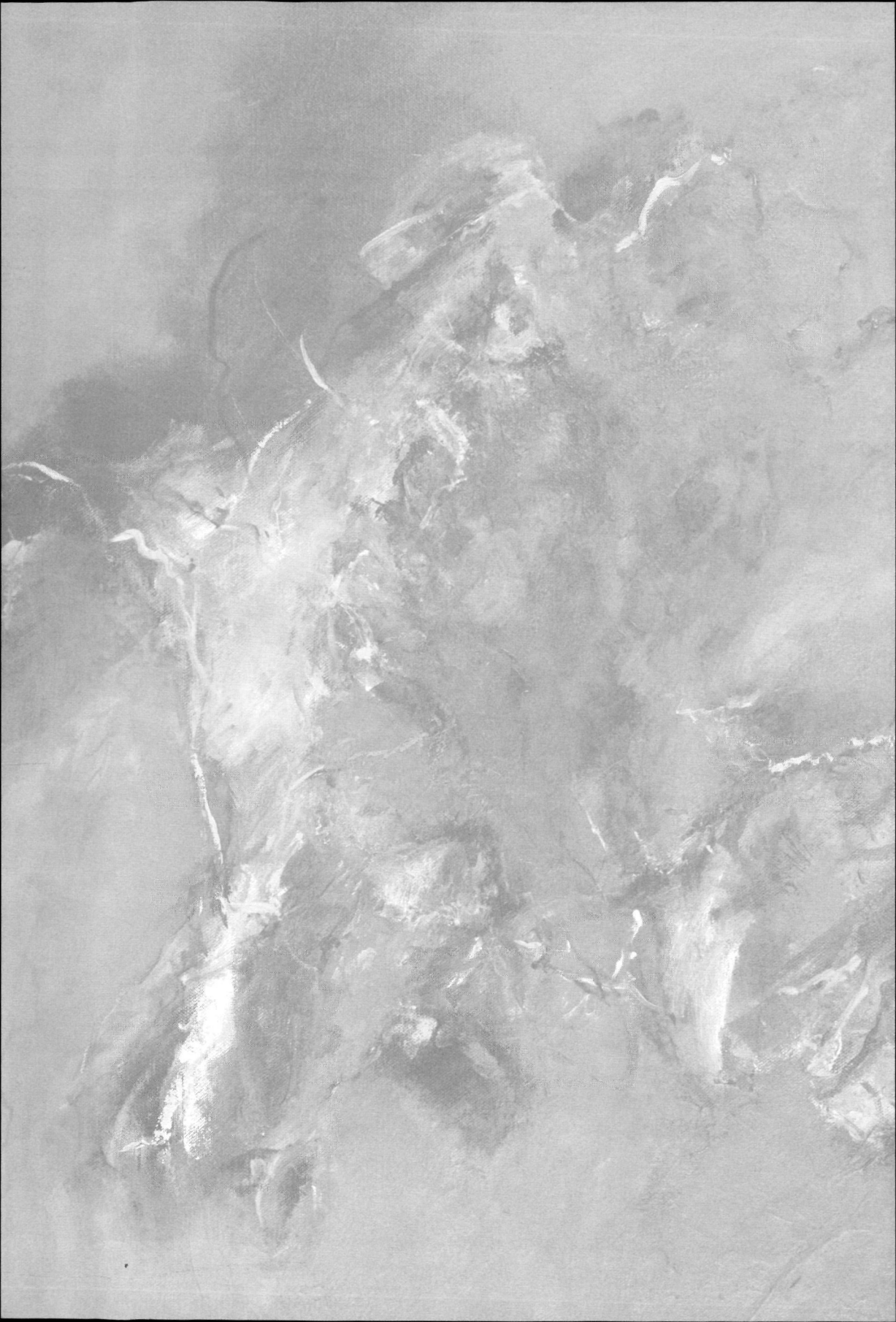
In **deel IV** van het proefschrift beschrijven we of het maken van een DTI-scan naast een conventionele MRI-scan nuttig is om als clinicus beter in te kunnen schatten in hoeverre cognitieve stoornissen gerelateerd zijn aan eventuele beschadigingen in de hersenen. De resultaten beschreven in deel III van dit proefschrift suggereren dat de microstructurele schade in de witte stofafwijkingen, maar ook in de normaal ogende witte stof een rol speelt in het cognitief falen. In **hoofdstuk 11** beschrijven we dat de microstructurele integriteit van de witte stofafwijkingen, de normaal ogende witte stof en de totale witte stof verantwoordelijk

zijn voor maar een zeer klein deel van de variantie in cognitief functioneren bovenop dat wat verklaard werd door de conventionele MRI-markers voor SVD, zoals wittestofafwijkingen, lacunaire infarcten en microbloedingen. Daarnaast wilden we weten of een DTI-scan wetenschappelijk van aanvullende waarde kan zijn in bijvoorbeeld trials bij mensen met SVD. Om dit te onderzoeken hebben we bekeken of de gemiddelde diffusiviteit van de totale wittestof een betere voorspeller is voor cognitief functioneren dan de afwijkingen op conventionele MRI-scans. Leeftijd, geslacht en opleiding verklaarden tot 36 procent van het cognitief functioneren. De gemiddelde diffusiviteit van de totale wittestof bij een aantal cognitieve taken verklaarde meer dan elk van de conventionele MRI-parameters apart, echter dit was maar maximaal 1 procent. Bovendien werd slechts 4,2 procent van de variantie in cognitief functioneren verklaard door de conventionele MRI-parameters (wittestofafwijkingen, lacunaire infarcten, microbloedingen, grijze- en wittestof volume). Concluderend kunnen we zeggen dat veranderingen van de hersenen gerelateerd aan SVD zijn geassocieerd met cognitief functioneren. Echter de cross-sectionele relatie is vrij zwak en de microstructurele integriteit van de wittestof is verantwoordelijk voor maar een klein deel van de variantie in cognitief functioneren. DTI-parameters lijken geen toegevoegde waarde te hebben bovenop conventionele MRI-parameters in relatie tot cognitie in de klinische praktijk. Desalniettemin, de microstructurele integriteit van de totale wittestof is verantwoordelijk voor een kleine variantie in cognitie. Mogelijk zijn veranderingen in DTI-parameters eerder en beter detecteerbaar dan veranderingen in conventionele MRI-maten. DTI zou derhalve een goede marker voor verandering in tijd kunnen zijn en zou kunnen fungeren als een surrogaat uitkomstmaat in therapeutische trials bij mensen met SVD.


Conclusie

In de studies beschreven in dit proefschrift hebben we MRI-markers gevonden die kunnen fungeren als basis voor toekomstig onderzoek en die ons kunnen helpen om meer inzicht te krijgen in de onderliggende pathofysiologische processen die achteruitgang in cognitie veroorzaken bij mensen met SVD.

Longitudinaal onderzoek is nodig om vast te stellen of deze markers op beeldvorming inderdaad tekenen zijn van een vroeg of laat stadium van de ziekte. Het daadwerkelijke belang van het identificeren van mensen met een risico op het ontwikkelen van cognitief falen als gevolg van SVD wordt bepaald door de beschikbaarheid en effectiviteit van een preventieve behandeling.

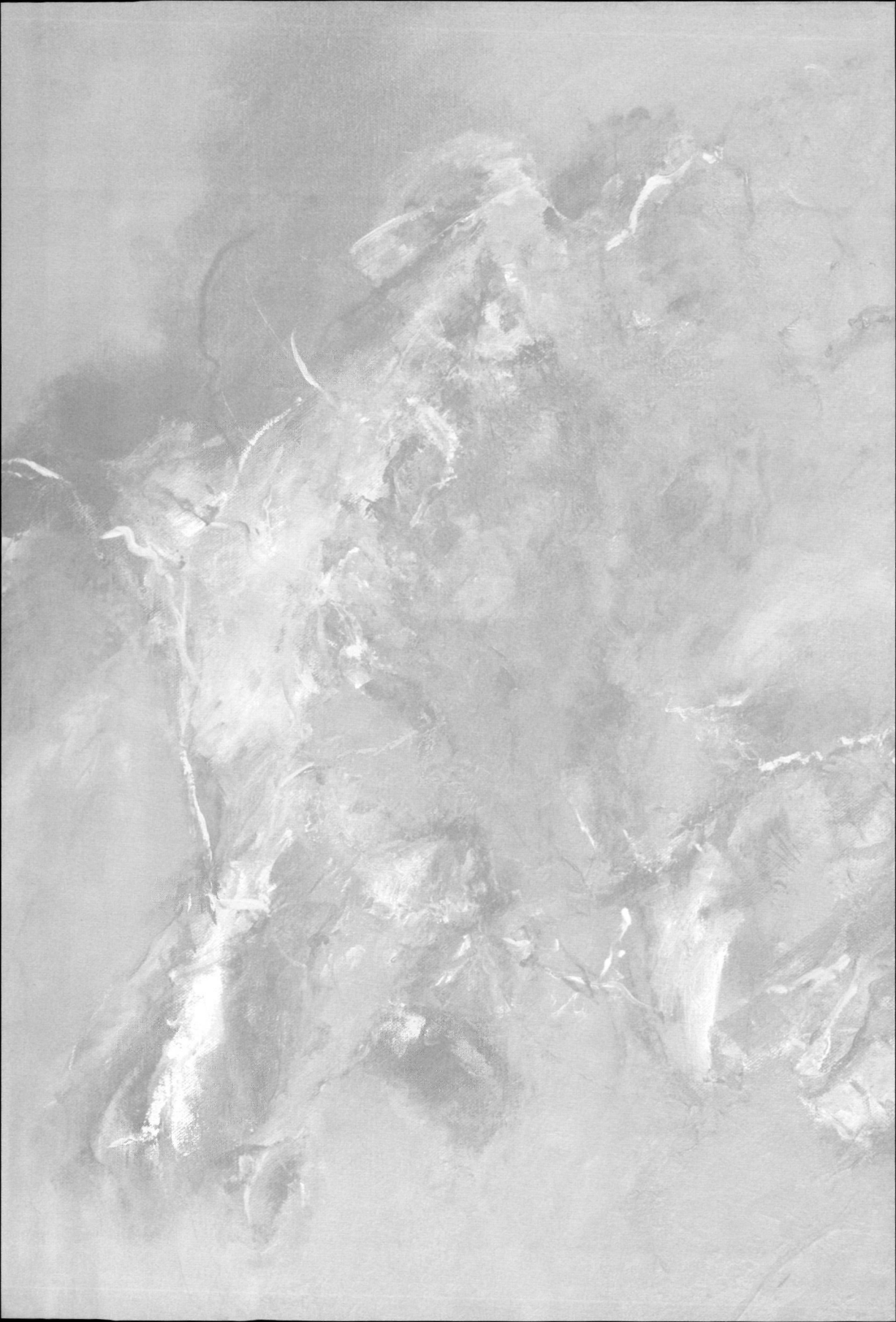


List of abbreviations



List of abbreviations

AD	Alzheimer's Disease
ApoE	Apolipoprotein E
APP	Amyloid Precursor Protein
CAA	Cerebral Amyloid Angiopathy
CES-D	Center of Epidemiologic Studies on Depression Scale
CSF	Cerebrospinal Fluid
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, 4 th Edition
DTI	Diffusion Tensor Imaging
FA	Fractional Anisotropy
GM	Gray Matter
MB	Microbleeds
MD	Mean Diffusivity
MMSE	Mini Mental State Examination
MRI	Magnetic Resonance Imaging
MTA	Medial Temporal Lobe Atrophy
NAWM	Normal Appearing White Matter
PET	Positron Emission Tomography
PIB	Pittsburg Imaging Compound B
RAVLT	Rey Auditory Verbal Learning Test
RCFT	Reys Complex Figure Test
RUN DMC	Radboud University Nijmegen Diffusion Tensor and Magnetic Resonance Imaging Cohort
SCF	Subjective Cognitive Failures
SD	Standard Deviation
SVD	Small Vessel Disease
VSAT	Verbal Series Attention Test
WM	White Matter
WML	White Matter Lesions



List of publications



List of publications

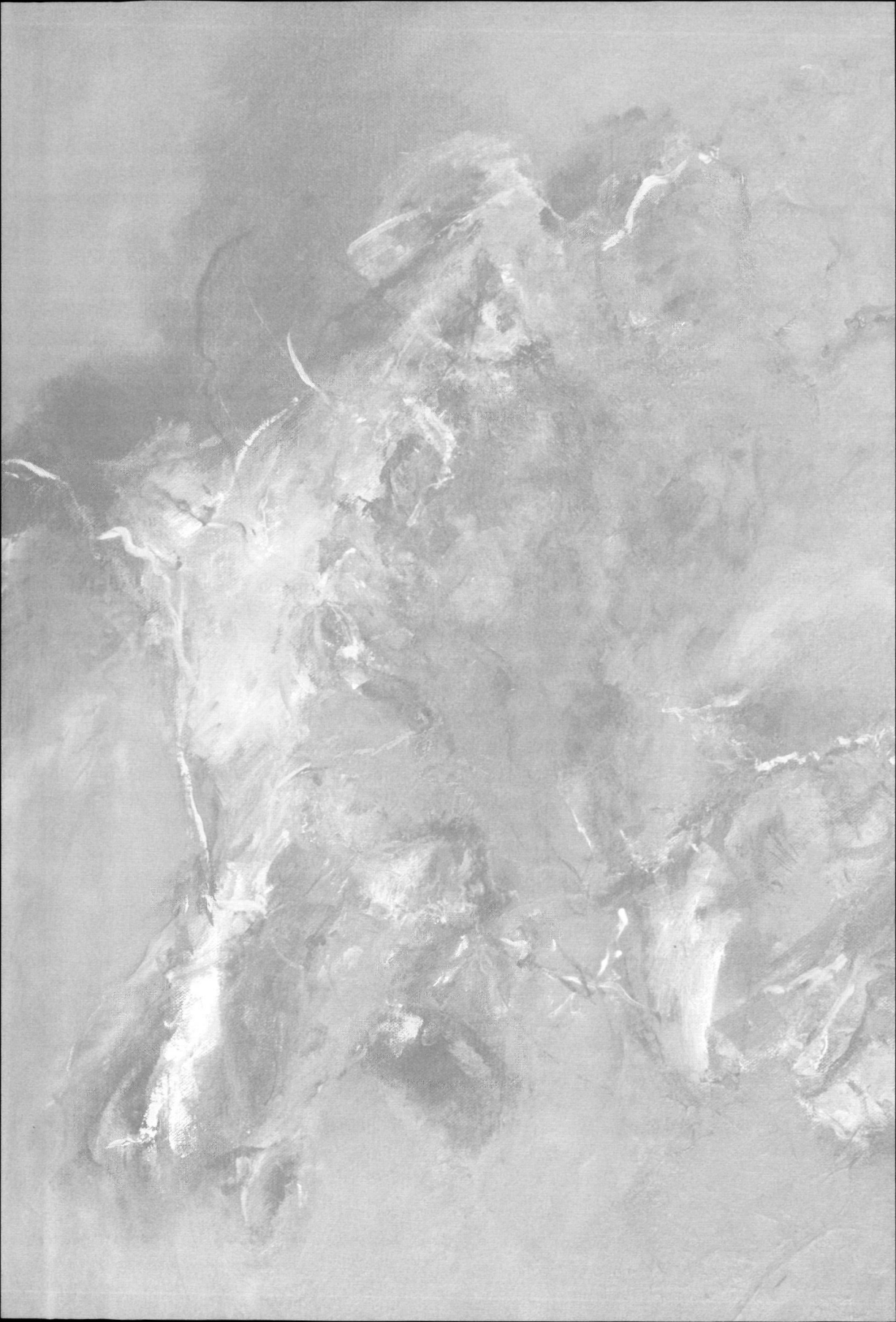
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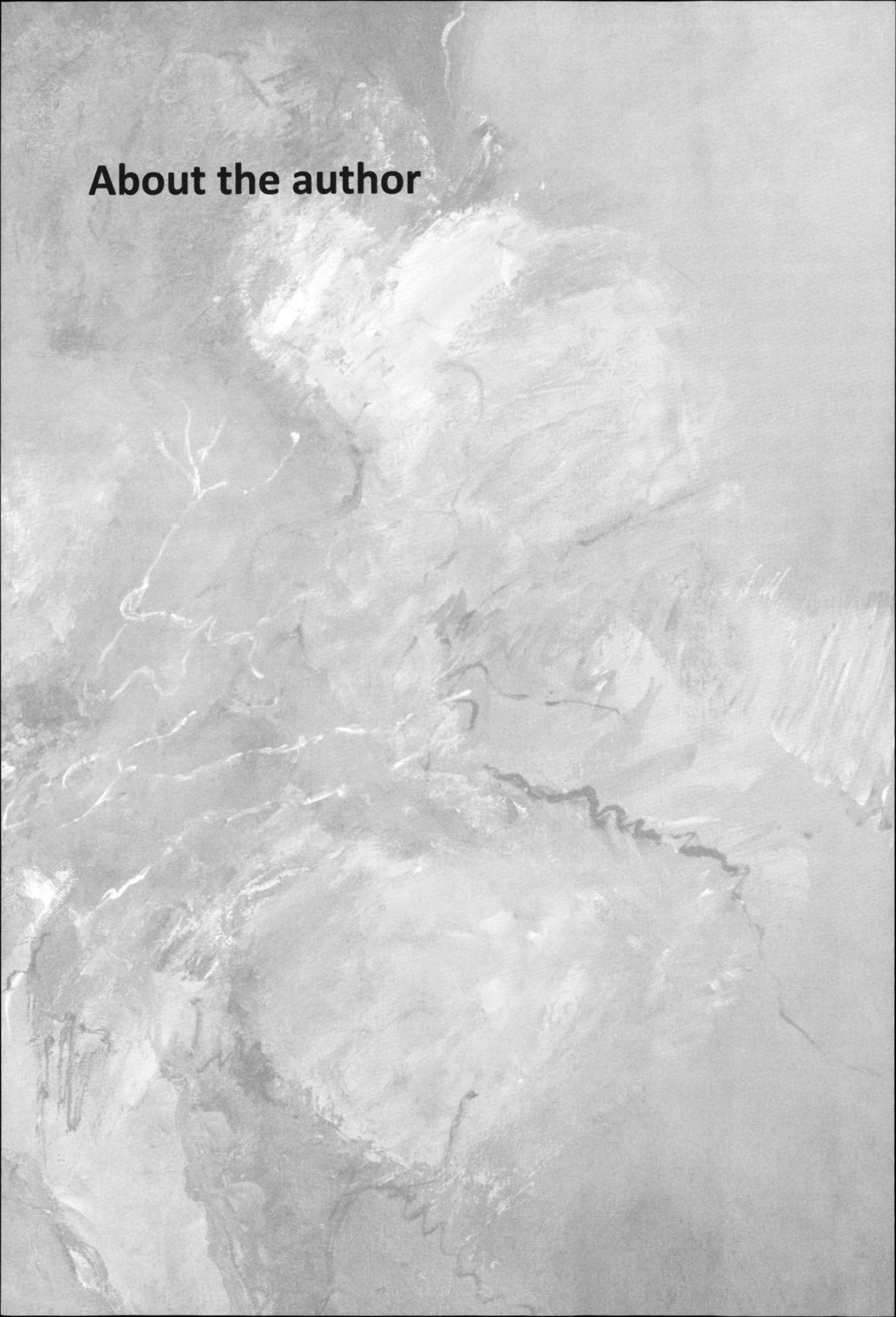
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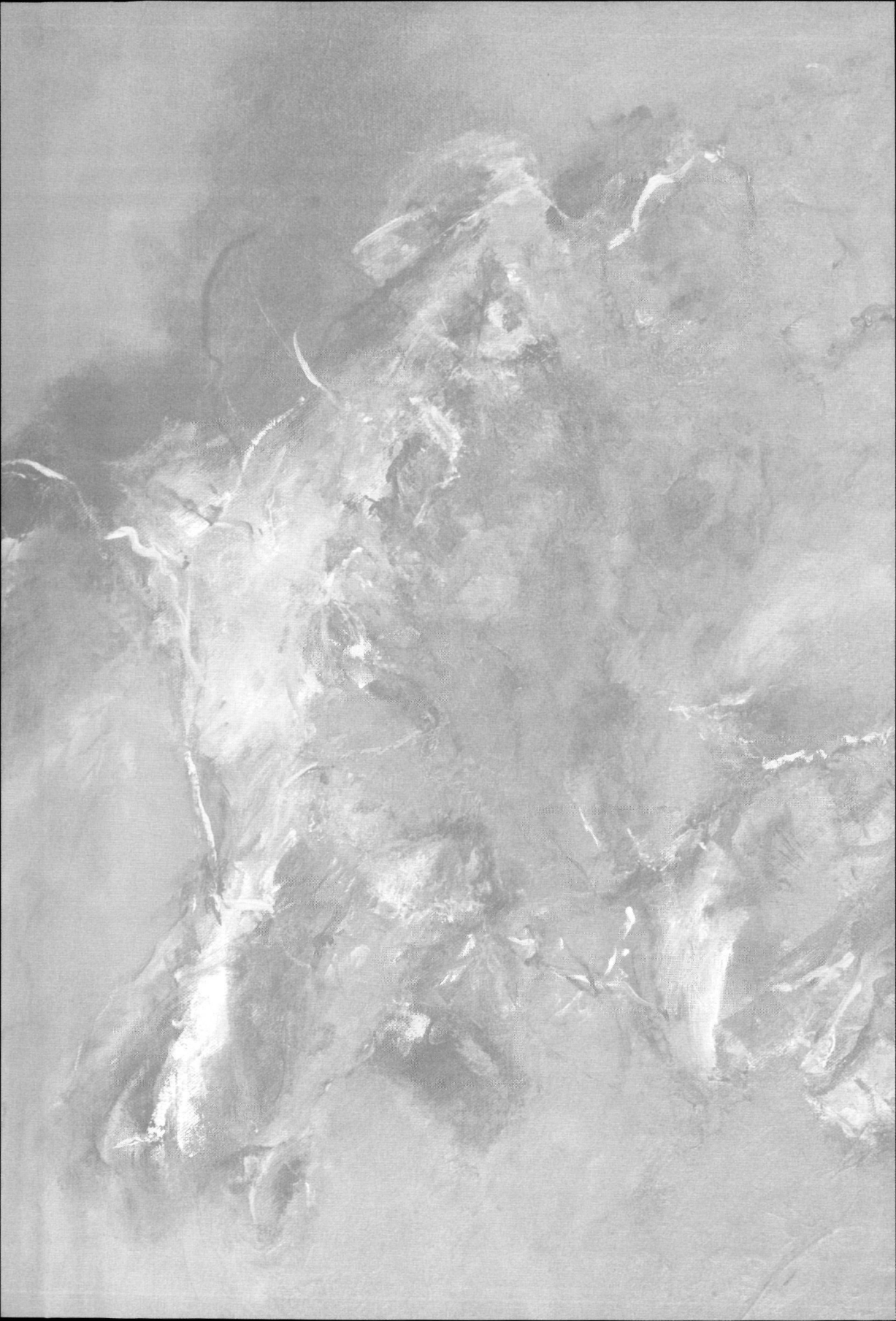


About the author



About the author

Anouk van Norden was born on May 6th, 1978 in Sittard, The Netherlands. She attended secondary school at the Bisschoppelijk College in Sittard and graduated in 1996. That year she started medical school at the University of Utrecht. During this period she performed a research internship on subarachnoid hemorrhages, at the department of Neurology, University Medical Centre Utrecht, Prof dr GJE Rinkel and Dr GW van Dijk. In September 2003 she started working as a resident at the department of Neurology, Radboud University Nijmegen Medical Centre, Prof dr GWAM Padberg, and in December 2003 she started her specialization. She started her PhD project, which resulted in this thesis, in 2005. In this period she had the opportunity to visit the Imaging of Dementia and Aging laboratory, at the University of California Davis in the United States of America during a research fellowship in 2008, supervised by Prof C DeCarli. From 2005 until 2010 she was a member of the board of directors of the Dutch society of neurologists in training and a member of the Neurology Consilium. Since 2009 she is a member of the editorial board of the Dutch Journal of Neurology and Neurosurgery. In 2007 she won an investigators award on the Scientific Conference of Dutch Neurologists and in 2010 she won the young investigators award of the European Stroke Conference, Barcelona, Spain. In March 2012 she will finish her specialization after which she will leave for a fellowship in Stroke Medicine at the Toronto Western Hospital in Toronto, Canada.



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Dankwoord

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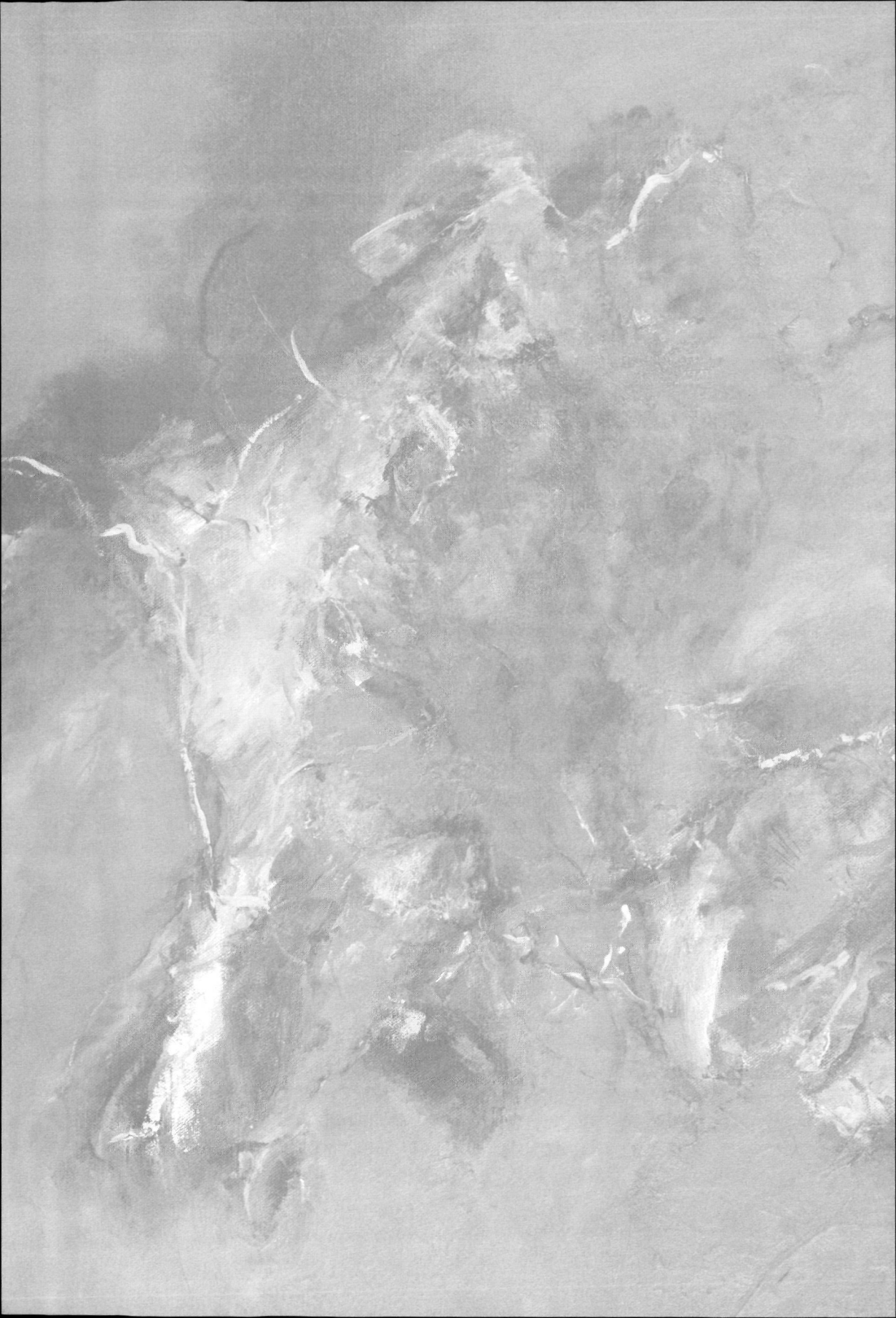
Ester, mijn Sittard-vriendinnetje, een van de weinige 'niet'-dokter vriendinnetjes, maar altijd bijzonder geïnteresseerd en betrokken, ook tijdens de afronding van dit promotie traject Je gezelligheid en vriendschap zijn voor mij onmisbaar Ik hoop dat ook wij weer snel samen met onze gezinnen op vakantie gaan

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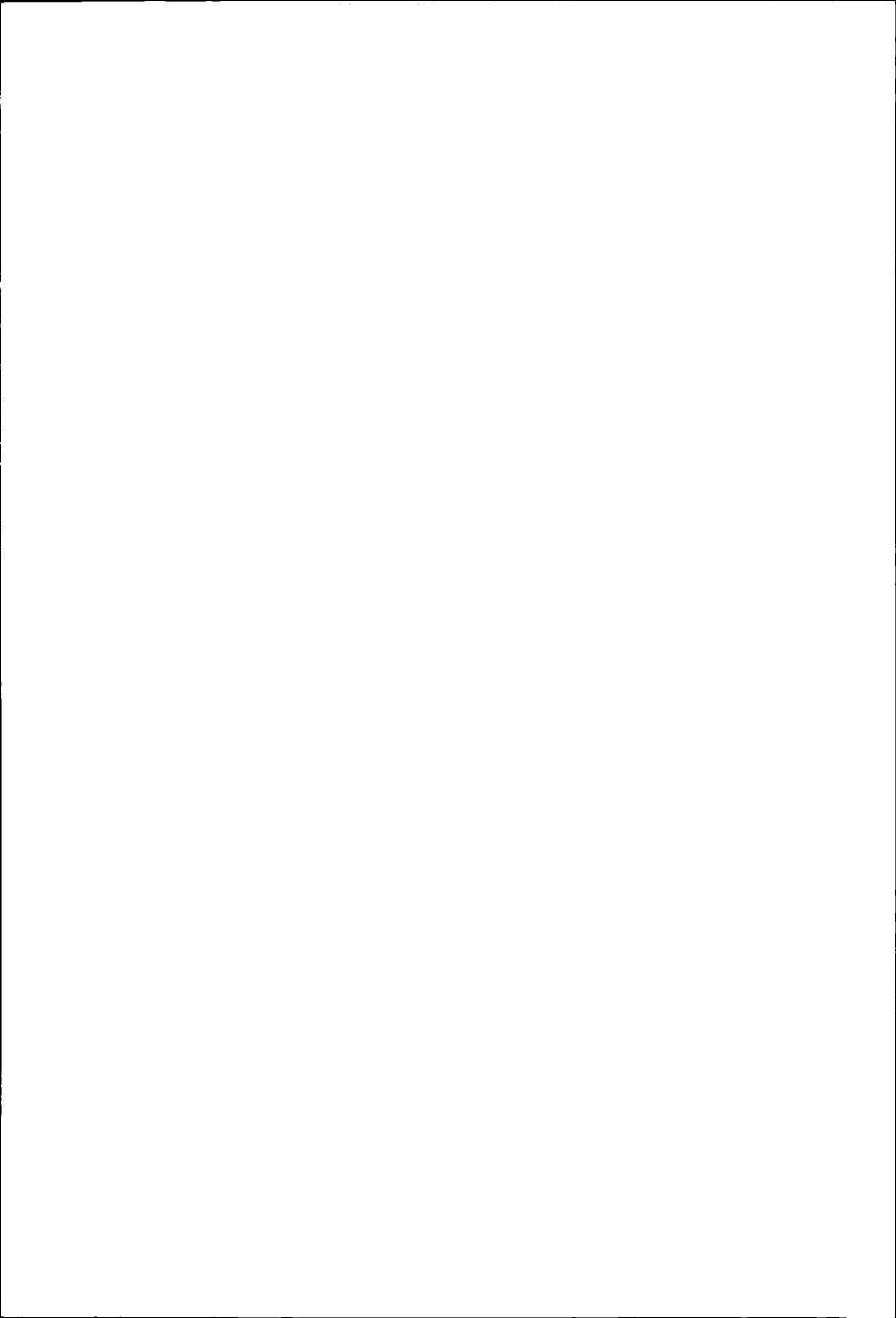
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Stellingen

behorend bij het proefschrift
'Cognitive function in elderly individuals with cerebral small vessel disease: An MRI study'

Anouk G W van Norden, 30 november 2011

- 1 'Cerebral small vessel disease' kan variabele stoornissen veroorzaken binnen het volledige spectrum aan cognitieve functies (dit proefschrift)
- 2 Subjectieve geheugenklachten hebben een radiologisch detecteerbaar pathologisch-anatomisch substraat (dit proefschrift)
- 3 Longitudinaal onderzoek naar de relatie tussen microbloedingen en cognitieve stoornissen is nodig om een onderling oorzakelijk verband te onderscheiden van een gemeenschappelijke oorzakelijke factor (dit proefschrift)
- 4 'Cerebral small vessel disease' is een uitgebreide ziekte waarbij ook de microstructurele integriteit van de normaal ogende witte stof betrokken is bij het cognitief functioneren (dit proefschrift)
- 5 Imaging van de microstructurele integriteit van de witte stof heeft voor de klinische praktijk van patienten met cognitieve stoornissen geen toegevoegde waarde bovenop conventionele MRI (dit proefschrift)
- 6 De microstructurele integriteit van de witte stof geeft meer inzicht in de onderliggende pathofysiologische processen van cognitieve achteruitgang (dit proefschrift)
- 7 De verklaarde variantie in cognitief functioneren door 'cerebral small vessel disease' wordt deels bepaald door de homogeniteit van een studiepopulatie (dit proefschrift)
- 8 Dementie op oudere leeftijd wordt veroorzaakt door meerdere elkaar versterkende factoren
- 9 De uitdaging in de moderne geneeskunst is om medische ontwikkelingen tijdig plaats te laten maken voor een natuurlijk ziektebeloop
- 10 Het liberale rookbeleid van de huidige minister van Volksgezondheid geeft de vrijheid om gevangene te blijven van je eigen verslaving en anderen daar onvrijwillig in te betrekken
- 11 Het competentiegericht opleiden vergt een grote investering van zowel opleider als AIOS, maar brengt completer opgeleide medisch specialisten voort
- 12 Het bijhouden van een opleidingsportfolio is uitsluitend een middel maar niet een doel op zich
- 13 Werkende moeders worden nogal eens als ontaard bestempeld terwijl ze zich niet anders gedragen dan werkende vaders
- 14 Een kraamhoofd (cerebrale sinus trombose tijdens het kraambed) is voor zowel neuroloog als gynaecoloog een bloedstollende ziekte



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