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Unraveling the heterogeneity of cerebral small vessel disease

From local to remote effects

Esther M.C. van Leijsen
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Unraveling the heterogeneity of cerebral small vessel disease
From local to remote effects

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volgens besluit van het college van decanen
in het openbaar te verdedigen
op maandag 19 november 2018
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door

Esther Maria Catharina van Leijsen
geboren op 13 november 1989
te Tilburg
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Chapter 1
General introduction, aims and outline of the thesis
Cognitive impairment and dementia

Dementia is a progressive and irreversible disease in which there is deterioration of cognitive function, characterized by memory loss and difficulties with thinking and problem-solving, severe enough to interfere with daily functioning. The prevalence of dementia is high and as people live longer, the burden of cognitive impairment in society becomes increasingly important. In the Netherlands, over 270,000 people are currently diagnosed with dementia and this number is expected to be doubled within twenty years [1]. Worldwide, around 50 million people have dementia and this number is projected to reach over 150 million in 2050 [2]. Thus, dementia presents society with a major health problem, and therefore, a better understanding of underlying mechanisms is of utmost importance and may eventually lead to personalized treatment of cognitive impairment and dementia.

Cerebral small vessel disease

Cerebral small vessel disease (SVD) has been recognized as the most important vascular contributor to cognitive decline and the development of dementia [3, 4], and will be the topic of this thesis. SVD refers to a variety of pathological processes affecting the smallest cerebral blood vessels. Since in vivo assessment of these small vessels is not yet possible with conventional neuroimaging techniques, SVD is usually operationalized as a spectrum of radiological manifestations that are thought to result from SVD. These neuroimaging markers include white matter hyperintensities (WMH), lacunes and cerebral microbleeds (Box 1) [5], and are present in virtually every individual over 60 years of age, although at a highly variable degree [6, 7].

Temporal dynamics of cerebral small vessel disease

We can only start to understand the complexity of underlying mechanisms and consequences of SVD if we have proper understanding of the time course of the disease. Progression of SVD has been extensively studied over the years, however, studies investigating SVD progression have usually operationalized the change in SVD markers over time with the aid of neuroimaging at two time points. Consequently, SVD progression is usually considered a continuous process that linearly progresses over time, without actual proof of this assumption. In this thesis, I will elaborate on the dynamics of SVD progression over time using repeated neuroimaging assessments at three time points, further challenging the assumption of a continuous progressive nature of SVD markers.
Thus far, studies on SVD have mainly used conventional MRI, thereby visualizing only the tip of the “SVD” iceberg, since pathological changes of the white matter are not only limited to SVD visible on conventional MRI but may also occur in the normal appearing white matter (NAWM). More advanced neuroimaging techniques such as diffusion tensor imaging (DTI) can possibly provide additional information on the underlying mechanisms of SVD – i.e. before they become visible on conventional imaging – by the assessment of the microstructural organization of the white matter (Box 2) [27-30]. Previous studies have shown associations between changes in baseline DTI parameters – i.e. increased mean diffusivity (MD) and decreased fractional anisotropy (FA) – and WMH progression [31, 32]. However, the temporal course of DTI changes in NAWM regions later converting into WMH remains to be elucidated.

The etiology of cerebral small vessel disease

The most common causes of SVD are hypertension and cerebral amyloid angiopathy (CAA) [8]. Hypertension causes arteriopathy that predominantly affects small perforating end-arteries in deep brain areas, whereas CAA is characterized by the deposition of amyloid beta (Aβ) within cortical arteries [9-13]. In parallel with this distribution of the underlying pathology, hypertensive arteriopathy is commonly associated with microbleeds in deep brain regions (e.g. basal ganglia, thalamus, and brainstem), whereas CAA is characterized by (micro)bleeds in a lobar distribution.

Apart from hypertension, other major risk factors for SVD include aging and the presence of vascular risk factors. The presence of vascular risk factors has been associated with cognitive decline and dementia as well [14, 15]. Hypertension [16, 17], diabetes mellitus [18], hypercholesterolemia [19, 20], obesity [21, 22], smoking [23], and lack of physical exercise [24-26] have all been related to an increased risk of cognitive impairment and dementia. Yet, the underlying mechanisms of SVD and its progression remain poorly understood. In this thesis, I will use more advanced neuroimaging techniques and biomarkers in blood to further elaborate on the underlying mechanisms of SVD.
In addition to neuroimaging markers, perhaps biomarkers in blood, that reflect the SVD pathology, can also reveal insights on the etiology of SVD. Along with traditional vascular risk factors such as hypertension, \( A\beta \) has emerged as a contributor to SVD. \( A\beta \) has previously been associated with AD and CAA, albeit in different forms. In Alzheimer’s Disease (AD), \( A\beta \) accumulates in the brain as senile plaques, predominantly composed of \( A\beta42 \) peptides. This is in contrast to CAA in which \( A\beta \) accumulates in the blood vessels, mostly composed of \( A\beta40 \) peptides. Several cross-sectional studies have suggested a role for circulating \( A\beta \) in SVD, reporting elevated plasma \( A\beta \) levels in participants with SVD [33-36]. However, since cross-sectional studies are limited in elaborating on causality, prospective studies are required to provide more insights on temporal precedence in the associations between plasma \( A\beta \) levels and SVD. In addition, neurofilament light chain (NfL) might provide additional information on the pathophysiological mechanisms of SVD, since NfL is an emerging blood marker for neuroaxonal damage. A role of serum NfL has been established in multiple neurological diseases affecting the elderly population, such as multiple sclerosis [37] and neurodegenerative disorders [38], including AD [39] and frontotemporal dementia [40]. However, a detailed account of the relationship between NfL in the blood and SVD burden and is lacking.

**Cognitive consequences of cerebral small vessel disease**

SVD has been associated with the development of cognitive impairment and dementia [3, 4, 41], as well as with gait impairment and an increased risk of parkinsonism [42], stroke [43], admission to a nursing home and even with an increased risk of mortality [44]. However, clinical symptoms in patients with a virtually identical SVD burden are often remarkably heterogeneous, both in nature and severity. Where SVD goes clinically unnoticed in the majority of patients, it leads to major cognitive deficits or dementia in others. In addition, the spectrum of cognitive symptoms attributable to SVD is more diverse than previously thought. SVD has previously mainly been associated with deficits in psychomotor speed and executive function [3, 43], but nowadays deficits in memory, attention, language and visuospatial abilities are also considered cognitive consequences of SVD [45, 46]. However, episodic memory deficits are not well understood by the location of SVD, since episodic memory performance is mainly supported by structures in the medial temporal lobes and especially the hippocampus [47, 48], which are typically unaffected by SVD [49]. Consequently, better understanding of the mechanisms by which SVD leads to cognitive deficits is needed.
Additional insights into the mechanisms by which SVD leads to cognitive deficits might be gained by taking into account remote effects of SVD, the interaction of SVD markers with hallmarks of AD and disruptions in network connectivity. The temporal interactions between SVD markers and hippocampal atrophy—either as a remote effect of SVD or a hallmark of AD—and their effect on cognitive decline, can be investigated using advanced statistical models. In addition, structural networks, constructed with the use of DTI (Box 3) [50-52], provide a measure of whole brain connectivity which might drive the associations between SVD and cognitive performance [53, 54].

Especially a set of centrally located and highly interconnected regions—also called rich clubs—are thought to play a pivotal role in maintaining global network function [55, 56]. In other words, due to their central role in brain networks and high connectivity with other nodes, damage to rich clubs or rich club connections might have a more widespread effect on network function compared with damage to peripheral nodes or peripheral connections in a network (Box 3, Figure F). Earlier cross-sectional studies in SVD have shown that SVD affects preferentially these rich club connections, thereby leading to cognitive impairment [57, 58]. The question remains how SVD affects progression of brain network impairments over time and whether this affects the rate of cognitive decline. Therefore, prospective studies are required to assess the temporal dynamics and the directionality of events.

**Aim of this thesis and study design**

The aim of this thesis is to gain insights into the time course, the etiology and the cognitive consequences of SVD. The studies presented in this thesis are based on the RUN DMC study, a prospective cohort study on the risk factors and clinical consequences of SVD in elderly with SVD using three neuroimaging and cognitive assessments over nine years (Box 4).
Outline of this thesis

In Part II of this thesis, I will start with describing how SVD changes over time. In Chapter 2, I will review evidence on progression and regression of SVD markers from the existing literature and in Chapter 3 I will describe the temporal dynamics of SVD within the RUN DMC study.

In Part III, I will use the insights on the time course of SVD to elaborate on the underlying mechanisms of SVD and its progression. I will start with describing changes in microstructural integrity preceding progression of white matter hyperintensities (Chapter 4). Thereafter, I will examine how amyloid beta levels in plasma are associated with severity and progression of SVD markers (Chapter 5). Additionally, I will assess the associations between neurofilament light chain levels in serum with severity (Chapter 6) and progression (Chapter 7) of SVD markers.

In part IV, I will elaborate on cognitive consequences of SVD. I will first provide a framework of mechanisms by which SVD can lead to clinical symptoms (Chapter 8). In Chapter 9, I will describe the cognitive consequences of regression of SVD markers over time. Thereafter, in Chapter 10, I will elaborate on the interaction of white matter hyperintensities with hippocampal atrophy and how this explains memory decline in elderly with SVD. In Chapter 11, I will investigate whether changes in rich club organization over time are associated with cognitive decline.

Finally, in Part V, I will summarize the main results of this thesis (Chapter 12) and will provide a discussion (Chapter 13) with general interpretation and clinical implications of these findings, together with some methodological considerations and future perspectives.
Part II
Temporal dynamics of cerebral small vessel disease
Chapter 2
Disease progression and regression in cerebral small vessel disease

Published as:
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Disease progression and regression in sporadic small vessel disease - insights from neuroimaging
Clinical Science. 2017;131(12):1191-1206
Abstract

Cerebral small vessel disease (SVD) is considered the most important vascular contributor to the development of dementia. Comprehensive characterization of the time course of disease progression will result in better understanding of etiology and clinical consequences of SVD. SVD progression has been studied extensively over the years, usually describing change in SVD markers over time using neuroimaging at two time points. As a consequence, SVD is usually seen as a rather linear, continuously progressive process. This assumption of continuous progression of SVD markers was recently challenged by several studies that showed regression of SVD markers. Here we provide a review on disease progression in sporadic SVD, thereby taking into account both progression and regression of SVD markers with emphasis on white matter hyperintensities, lacunes and microbleeds. We will elaborate on temporal dynamics of SVD progression and discuss the view of SVD progression as a dynamic process, rather than the traditional view of SVD as a continuous progressive process, that might better fit evidence from longitudinal neuroimaging studies. We will discuss possible mechanisms and clinical implications of a dynamic time course of SVD, with both progression and regression of SVD markers.

Introduction

Markers of cerebral small vessel disease (SVD) include, amongst others, white matter hyperintensities (WMH), lacunes of presumed vascular origin and microbleeds [5] and are present on neuroimaging in virtually every individual over 60 years of age [6], although in highly variable degree. SVD is considered the most important vascular contributor to the development of cognitive impairment and dementia [3, 4, 41], and is associated with an increased risk of stroke [43], admission to a nursing home and even with an increased risk of mortality [44].

Progression of SVD has been studied extensively over the years, usually by operationalizing the change in SVD markers over time with the aid of neuroimaging at two time points. Consequently, SVD progression is usually expressed as volume change or incidence per year, assuming rather linear, continuous progression over time. More recently also a decrease in WMH volume [28, 59-62], number of lacunes [63, 64] and microbleeds over time [65, 66] has been reported, challenging the assumption of a continuous progressive nature of SVD markers.

Comprehensive characterization of the time course of disease progression will result in better understanding of underlying mechanisms and clinical consequences of SVD. This might be particularly valuable in clinical trials that use SVD markers as outcome measure and as such may in time lead to personalized treatment approaches. It remains to be established whether progression is interrupted by regression or whether progression and regression occur in identifiable phases or simultaneously in different brain regions.

In this opinion review, we will summarize evidence on the progression and regression of SVD markers based on data from longitudinal neuroimaging studies, with an emphasis on WMH, lacunes and microbleeds. By reviewing studies on SVD progression we combine evidence on the time course of progression of various SVD markers over multiple time points in different cohorts. We will elaborate on the time course of SVD and discuss possible mechanisms and clinical implications of a dynamic time course of SVD.

Literature search

We identified articles by searching PubMed using the search terms as described in the Appendix to cover three main SVD markers: WMH, lacunes and microbleeds. We limited the search to full-text manuscripts published in English from January 1st 1990 to October 10th 2016. We included serial magnetic resonance imaging studies (i.e. studies with at least two time points) on sporadic SVD in participants.
above the age of 50 years examining change of these markers over time. We also searched reference lists of identified papers for further relevant articles.

The search strategy yielded 204 articles for WMH, 194 articles for lacunes and 167 articles for microbleeds. After screening the titles and abstracts and adding relevant literature from reference lists 74 articles (not mutually exclusive) fulfilled the inclusion criteria: 49 articles for WMH, 16 for lacunes and 22 for microbleeds (Flowchart in the Supplementary material).

Progression of SVD markers

Data on progression of SVD markers over time come from 41 hospital-based and 30 population-based studies. We will discuss the findings from longitudinal neuro-imaging studies describing progression of WMH, lacunes and microbleeds over time.

WMH progression

Both population-based and hospital-based studies have shown that WMH volume increases over time, although the range of WMH progression varies considerably across the studies (Table 1). Average increase of WMH volume varied over 20-fold, ranging between 0.1 and 2.2 ml/yr, depending on the study population [32, 59-62, 67-110]. The range of WMH progression did not differ between population-based and hospital-based cohorts (Table 1), probably due to heterogeneity within these cohorts.

Predictors of WMH progression were age, baseline WMH severity, hypertension and current smoking [3, 60, 62, 94, 97, 111-115]. For example, the Rotterdam Study reported more WMH progression in the strata of higher age [60] and participants with uncontrolled untreated hypertension showed more WMH progression than people with uncontrolled, but treated hypertension [114]. Moreover, the Austrian Stroke Prevention Study reported an annual increase in WMH volume of 1.3 ml per year in those with confluent lesions and almost no progression in participants with punctuate lesions [97]. In the RUN DMC study, participants with moderate or severe WMH at baseline had a high likelihood of progression of their WMH, whereas participants with only mild WMH did not show progression, not even over a period of nine years [110]. Finally, the WMH penumbra - a region surrounding the WMH composed of normal appearing white matter but with lower structural integrity - has been reported to be at increased risk of becoming WMH over time [116, 117].

Incidence of lacunes

The incidence of lacunes varied notably between different hospital- and population-based studies. The proportion of participants with incident lacunes varied almost 25-fold across studies between 0.4 and 9.5 percent per year [60, 64, 67, 68, 71, 73, 81, 83, 101, 102, 110, 118-122], with higher incidence in hospital-based cohorts (Table 2). The Age, Gene/Environment Susceptibility-Reykjavik Study, a large population-based study, reported a yearly incidence of lacunes of 0.8% [102]. The Rotterdam Scan Study [60] and the Cardiovascular Health Study [87] reported higher incidence (3.3% and 2.5% per year, respectively). Incidence of lacunes was higher in the hospital-based LADIS and SCANS studies, reporting incidence of 5.8% of 9.5% per year respectively [68, 119], probably due to the high proportion of participants with lacunes at baseline. In the RUN DMC study, 20.3% of the participants had incident lacunes over the course of nine years (2.3% per year)[110].

Predictors for incident lacunes were severity of WMH and presence of lacunes at baseline [119], history of stroke, atrial fibrillation and carotid atherosclerosis and presence of vascular risk factors such as hypertension and hypercholesterolemia [60, 81, 119, 121]. Incident lacunes were predominantly located in brain regions with contact or partial overlap with pre-existing WMH, suggesting that especially tissue adjacent to WMH is susceptible to further ischemia [123].

Incidence of microbleeds

The yearly incidence of microbleeds ranged between 2.9 and 3.5 percent in population-based studies and between 2.2 and 31.5 percent in hospital-based studies [65-67, 78, 85, 102, 110, 124-134] (Table 3). In participants with intracerebral hemorrhages or CAA the incidence was up to 41.8% per year [72, 135-137]. In the RUN DMC study, yearly incidence of microbleeds was 2.2% per year [110].

Major predictors for microbleeds incidence were age and presence of SVD at baseline: in the Rotterdam Study incidence was 7.6% in subjects aged 60 to 69 years, 15.6% in those between 70 and 79 years, and 18.6% in subjects older than 80 years [132]. Subjects with microbleeds at baseline had higher risk for incident microbleeds as compared with participants without microbleeds at baseline [65]. Besides the number of microbleeds at baseline, presence of lacunes and baseline WMH severity also predicted incident microbleeds [78]. In addition, APOE genotype and vascular risk factors (i.e. smoking and blood pressure) were predictors of incident microbleeds [78]. Predictors of microbleeds differed across brain regions, suggesting differences in etiology for deep versus lobar microbleeds [9, 138]. Deep microbleeds were associated with cardiovascular risk factors such as hypertension and smoking [9] and therefore considered to be due to hypertensive arteriopathy. In contrast, lobar microbleeds are considered to be due to cerebral amyloid angiopathy (CAA), because of their association with known risk factors for CAA including APOE ε4 genotype [9, 139].
Table 1  Characteristics of longitudinal changes of WMH by study population

<table>
<thead>
<tr>
<th>Ref</th>
<th>Population</th>
<th>n (%)</th>
<th>Age</th>
<th>FU time</th>
<th>Nr of scans</th>
<th>Field strength</th>
<th>Identical MRI B &amp; FU?</th>
<th>WMH assessment</th>
<th>Progression of WMH*</th>
<th>Regression of WMH**</th>
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<tbody>
<tr>
<td></td>
<td>Hospital-based studies</td>
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<td></td>
<td>SVD</td>
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</tr>
<tr>
<td>[110]</td>
<td>SVD (RUN DMC) (multicentre)</td>
<td>276 (54.9%)</td>
<td>62.5 ± 7.7</td>
<td>8.7</td>
<td>3</td>
<td>1.5T</td>
<td>No; FLAIR voxel size adjusted</td>
<td>Semiautomated volumetrics - FLAIR/T1; FU FLAIR sequence resliced to FLAIR</td>
<td>+0.54 ml/y</td>
<td>9.4 %; -0.1 ml/y</td>
</tr>
<tr>
<td>[81]</td>
<td>SVD (LADIS) (multicentre)</td>
<td>396 (62.0%)</td>
<td>73.6 ± 5.0</td>
<td>3.1</td>
<td>2</td>
<td>1.5T</td>
<td>No; new MRI in 3 centers</td>
<td>Modified Rotterdam Progression scale - FLAIR</td>
<td>VS</td>
<td>-</td>
</tr>
<tr>
<td>[96]</td>
<td>SVD (LADIS) (multicentre)</td>
<td>394 (61.7%)</td>
<td>73.1 ± 5.0</td>
<td>3.2</td>
<td>2</td>
<td>0.5T</td>
<td>No; Modified Rotterdam Progression scale - FLAIR</td>
<td>VS</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>[82]</td>
<td>Vascular disease (PROSPER)</td>
<td>554 (85.8%)</td>
<td>75.0 ± 3.2</td>
<td>2.8</td>
<td>2</td>
<td>1.5T</td>
<td>Yes</td>
<td>Semiautomated volumetrics - FLAIR</td>
<td>+2.2 ml/y</td>
<td>US</td>
</tr>
<tr>
<td>[79]</td>
<td>SVD</td>
<td>88 (72.7%)</td>
<td>52 ± 8.5</td>
<td>2.2</td>
<td>2</td>
<td>1.5T</td>
<td>Yes</td>
<td>Semiautomated volumetrics - FLAIR</td>
<td>+0.3 ml/y</td>
<td>-</td>
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<tr>
<td></td>
<td>Stroke</td>
<td></td>
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<tr>
<td>[68]</td>
<td>Lacunar stroke (SCANS)</td>
<td>99 (81.8%)</td>
<td>70 ± 9.8</td>
<td>3</td>
<td>2-4</td>
<td>1.5T</td>
<td>Yes</td>
<td>Automated volumetrics - FLAIR</td>
<td>+0.8 %/y</td>
<td>-</td>
</tr>
<tr>
<td>[59]</td>
<td>Ischemic stroke</td>
<td>100 (100%)</td>
<td>67.5 ± 11.8</td>
<td>2.3</td>
<td>2</td>
<td>-</td>
<td>Yes</td>
<td>Semiautomated volumetrics - FLAIR or T2</td>
<td>-27%; +1.4 ml/y; -0.3 ml/y</td>
<td></td>
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<tr>
<td></td>
<td>Stroke or TIA (PROGRESS)</td>
<td></td>
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<tr>
<td>[75]</td>
<td>Stroke or TIA (VITATOPS)</td>
<td>359 (76.2%)</td>
<td>64.3 ± 12.7</td>
<td>2.1</td>
<td>2</td>
<td>1.5T</td>
<td>Yes</td>
<td>Semiautomated volumetrics - FLAIR/T2</td>
<td>+0.05 ml/y</td>
<td>-</td>
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<tr>
<td></td>
<td>Stroke patients (CASSP)</td>
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<td>Stroke patients (CATCH)</td>
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<tr>
<td>[32]</td>
<td>Memory clinic (ADNI) (multicentre)</td>
<td>56</td>
<td>67.9 ± 8.0</td>
<td>1.8</td>
<td>-</td>
<td>-</td>
<td>Yes</td>
<td>Automated volumetrics - FLAIR/T1/2/PD</td>
<td>- +/MCI: +0.4 ml/y Controls: +0.4 ml/y</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Memory clinic (Sunnybrook Dementia)</td>
<td>57</td>
<td>74.3 ± 8.3</td>
<td>1.7</td>
<td>2</td>
<td>1.5T</td>
<td>Yes</td>
<td>Semiautomated volumetrics - T1/PD/T2</td>
<td>AD +SVD: +1.0 ml/y</td>
<td></td>
</tr>
<tr>
<td>[86]</td>
<td>Stroke patients (CATCH)</td>
<td>193</td>
<td>74.6 ± 7.5</td>
<td>1.8</td>
<td>2</td>
<td>1.5T</td>
<td>Yes</td>
<td>Automated volumetrics - T1/T2/PD</td>
<td>AD: +7.4 ml MCI: +7.6 ml NC: +4.9 ml</td>
<td></td>
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<tr>
<td>[89]</td>
<td>Stroke patients (CATCH)</td>
<td>47</td>
<td>75.7 ± 6.8</td>
<td>3.5</td>
<td>-</td>
<td>-</td>
<td>Yes</td>
<td>Semiautomated volumetrics - FLAIR/T1</td>
<td>+1.6 ml/y MCI: +1.7 ml Controls: +0.7 ml</td>
<td></td>
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<tr>
<td>[32]</td>
<td>Memory clinic (ADNI) (multicentre)</td>
<td>44</td>
<td>69.4 ± 7.0</td>
<td>2.0</td>
<td>-</td>
<td>-</td>
<td>Yes</td>
<td>Automated volumetrics - FLAIR/T1</td>
<td>- +/MCI: +0.5 ml/y Controls: +0.4 ml/y</td>
<td></td>
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<tr>
<td></td>
<td>Memory clinic (Sunnybrook Dementia)</td>
<td>56</td>
<td>67.9 ± 8.0</td>
<td>1.8</td>
<td>-</td>
<td>-</td>
<td>Yes</td>
<td>Automated volumetrics - FLAIR/T1/2/PD</td>
<td>- +/MCI: +0.4 ml/y Controls: +0.4 ml/y</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Memory clinic (Sunnybrook Dementia)</td>
<td>57</td>
<td>74.3 ± 8.3</td>
<td>1.7</td>
<td>2</td>
<td>1.5T</td>
<td>Yes</td>
<td>Semiautomated volumetrics - T1/PD/T2</td>
<td>AD +SVD: +1.0 ml/y</td>
<td></td>
</tr>
<tr>
<td>[83]</td>
<td>Atherosclerosis (SMART-MR)</td>
<td>565 (43.2%)</td>
<td>57 ± 9.0</td>
<td>3.9</td>
<td>2</td>
<td>1.5T</td>
<td>Yes</td>
<td>Automated volumetrics - FLAIR/T1</td>
<td>+0.6 ml/y</td>
<td>-</td>
</tr>
<tr>
<td>[92]</td>
<td>Artery stenosis (ROCAS)</td>
<td>208 (91.6%)</td>
<td>61 ± 9.0</td>
<td>2.2</td>
<td>2</td>
<td>1.5T</td>
<td>Yes</td>
<td>Semiautomated volumetrics - T1/PD/T2</td>
<td>+0.2 ml/y</td>
<td>-</td>
</tr>
<tr>
<td>[101]</td>
<td>Diabetes type 2</td>
<td>190 (86.8%)</td>
<td>62.7 ± 8.1</td>
<td>6</td>
<td>3</td>
<td>1.5T</td>
<td>Yes</td>
<td>Modified Rotterdam Progression scale - FLAIR</td>
<td>VS</td>
<td>-</td>
</tr>
<tr>
<td>[72]</td>
<td>CAA patients</td>
<td>26 (36.6%)</td>
<td>69.1 ± 6.5</td>
<td>1.1T</td>
<td>2</td>
<td>1.5T</td>
<td>Yes</td>
<td>Semiautomated volumetrics - FLAIR or T2</td>
<td>+0.5 ml/y</td>
<td>-</td>
</tr>
<tr>
<td>[76]</td>
<td>Migraine patients</td>
<td>17</td>
<td>470 ± 11.2</td>
<td>3</td>
<td>2</td>
<td>0.5T</td>
<td>Yes</td>
<td>3D slicer - FLAIR/T2/1T</td>
<td>+0.1 ml/y</td>
<td>US</td>
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<tr>
<td>[95]</td>
<td>Cirrhosis patients</td>
<td>19 (63.3%)</td>
<td>60 ± 9.0</td>
<td>0.8</td>
<td>2</td>
<td>1.5T</td>
<td>Yes</td>
<td>Semiautomated volumetrics - T2/FLAIR/T1</td>
<td>-0.6 ml/y</td>
<td>-</td>
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### Table 1 Continued

<table>
<thead>
<tr>
<th>Ref</th>
<th>Population</th>
<th>n (%)</th>
<th>Age</th>
<th>FU time</th>
<th>Nr of scans</th>
<th>Field strength</th>
<th>Identical MRI B &amp; FU?</th>
<th>WMH assessment</th>
<th>Progression of WMH*</th>
<th>Regression of WMH**</th>
</tr>
</thead>
<tbody>
<tr>
<td>[67]</td>
<td>Population-based (Rotterdam Scan)</td>
<td>803 (75.6%)</td>
<td>68.3 ± 6.2</td>
<td>3.4</td>
<td>2</td>
<td>1.5T</td>
<td>Yes</td>
<td>Automated volumetrics - T2/PD</td>
<td>+0.18 ml/y ‡</td>
<td>-</td>
</tr>
<tr>
<td>[60]</td>
<td>Population-based (Rotterdam Scan)</td>
<td>668 (62.0%)</td>
<td>71 ± 7</td>
<td>3.4</td>
<td>2</td>
<td>Yes</td>
<td>Rotterdam Progression scale - T2/PD</td>
<td>VS</td>
<td>US</td>
<td></td>
</tr>
<tr>
<td>[94]</td>
<td>Population-based (ASPS)</td>
<td>243 (47.7%)</td>
<td>60.2 ± 6.3</td>
<td>6.0</td>
<td>3</td>
<td>1.5T</td>
<td>Yes</td>
<td>Semiautomated volumetrics - PD</td>
<td>+0.2 ml/y</td>
<td>US</td>
</tr>
<tr>
<td>[87]</td>
<td>Population-based (CHS) (multicentre)</td>
<td>1919 (32.6%)</td>
<td>74.0</td>
<td>5</td>
<td>2</td>
<td>1.5 / 0.35T</td>
<td>Yes</td>
<td>CHS scoring - PD</td>
<td>VS</td>
<td>-</td>
</tr>
<tr>
<td>[102]</td>
<td>Population-based (AGES-Reykjavik)</td>
<td>1949 (33.3%)</td>
<td>74.6 ± 4.6</td>
<td>5.2</td>
<td>2</td>
<td>1.5T</td>
<td>Yes</td>
<td>Automated volumetrics - PD/T2/FLAIR/T1</td>
<td>+1.2 ml/y</td>
<td>-</td>
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<tr>
<td>[107]</td>
<td>Population-based (Sydney Stroke)</td>
<td>1949  (33.3%)</td>
<td>81.7 ± 3.9</td>
<td>4.0</td>
<td>2</td>
<td>3.0T</td>
<td>Yes</td>
<td>Semiautomated volumetrics - FLAIR/T1</td>
<td>+0.2 %/y †</td>
<td>-</td>
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<tr>
<td>[73]</td>
<td>Population-based (ARIC) (multicentre)</td>
<td>983 (50.4%)</td>
<td>61 ± 4</td>
<td>11</td>
<td>2</td>
<td>1.5T</td>
<td>Yes</td>
<td>Semiautomated volumetrics - FLAIR</td>
<td>+0.5 ml/y</td>
<td>-</td>
</tr>
<tr>
<td>[74]</td>
<td>Population-based (Sydney Stroke)</td>
<td>1118 (62.1%)</td>
<td>72.0 ± 4.0</td>
<td>3.6</td>
<td>2</td>
<td>1.5T</td>
<td>Yes</td>
<td>Automated volumetrics - T2</td>
<td>+0.25 ml/y</td>
<td>US</td>
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<tr>
<td>[62]</td>
<td>Population-based (ASPI)</td>
<td>51 (63.8%)</td>
<td>71.0 ± 5.9</td>
<td>3</td>
<td>2</td>
<td>1.5T</td>
<td>Yes</td>
<td>Semiautomated volumetrics - FLAIR</td>
<td>+2.2 ml/y</td>
<td>US</td>
</tr>
<tr>
<td>[106]</td>
<td>Population-based (Framingham Heart)</td>
<td>70 (50.7%)</td>
<td>79</td>
<td>4</td>
<td>2</td>
<td>1.5T</td>
<td>Yes</td>
<td>Manual grid volumetrics - T2</td>
<td>+0.27 ml/y</td>
<td>23 %</td>
</tr>
<tr>
<td>[91]</td>
<td>Population-based (NCODE)</td>
<td>110 (79.9%)</td>
<td>70.7 ± 5.6</td>
<td>2.0</td>
<td>2</td>
<td>1.5T</td>
<td>Yes</td>
<td>Semiautomated volumetrics - T2/PD</td>
<td>+0.6 ml/y</td>
<td>-</td>
</tr>
<tr>
<td>[98]</td>
<td>Population-based (OBAS)</td>
<td>104</td>
<td>85.1 ± 5.6</td>
<td>4.6</td>
<td>3</td>
<td>1.5T</td>
<td>Yes</td>
<td>Semiautomated volumetrics - PD/T2</td>
<td>+1.0 ml/y</td>
<td>-</td>
</tr>
<tr>
<td>[99]</td>
<td>Population-based (FRS)</td>
<td>117</td>
<td>69.1 ± 6.2</td>
<td>2.1</td>
<td>2</td>
<td>1.5T</td>
<td>Yes</td>
<td>Semiautomated volumetrics - PD</td>
<td>+0.7 ml/y</td>
<td>-</td>
</tr>
<tr>
<td>[93]</td>
<td>Population-based (WHICAP)</td>
<td>250 (85.0%)</td>
<td>84.4 ± 2.5</td>
<td>4</td>
<td>2</td>
<td>3.0T</td>
<td>Yes</td>
<td>Automated volumetrics - T1</td>
<td>+1.0 ml/y</td>
<td>-</td>
</tr>
<tr>
<td>[70]</td>
<td>Population-based (WHICAP)</td>
<td>303 (39.4%)</td>
<td>79.2 ± 5.3</td>
<td>4.6</td>
<td>2</td>
<td>1.5T</td>
<td>Yes</td>
<td>Semiautomated volumetrics - FLAIR</td>
<td>+0.2 ml/y</td>
<td>-</td>
</tr>
<tr>
<td>[108]</td>
<td>Population-based (WHICAP)</td>
<td>210 (71.9%)</td>
<td>70.9 ± 0.9</td>
<td>4</td>
<td>2</td>
<td>0.5T</td>
<td>Yes</td>
<td>Visual rating scale - FLAIR</td>
<td>VS</td>
<td>-</td>
</tr>
<tr>
<td>[84]</td>
<td>Population-based (WHICAP)</td>
<td>56</td>
<td>73.9 ± 6.6</td>
<td>3.7</td>
<td>2</td>
<td>1.5T</td>
<td>Yes</td>
<td>Semiautomated volumetrics - T2</td>
<td>+0.6 ml/y</td>
<td>-</td>
</tr>
<tr>
<td>[77]</td>
<td>Population-based (1914 cohort)</td>
<td>30 (3.7%)</td>
<td>80.7 ± 0.4</td>
<td>3.6</td>
<td>2</td>
<td>1.5T</td>
<td>Yes</td>
<td>Automated volumetrics - PD/T2</td>
<td>+1.3 ml/y</td>
<td>-</td>
</tr>
<tr>
<td>[103]</td>
<td>Population-based (1914 cohort)</td>
<td>14 (23.7%)</td>
<td>76 ± 5</td>
<td>2</td>
<td>2</td>
<td>0.6T</td>
<td>Yes</td>
<td>Scheltens scale - T2</td>
<td>VS</td>
<td>14 %</td>
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<tr>
<td>[104]</td>
<td>Population-based (1914 cohort)</td>
<td>13 (56.5%)</td>
<td>79</td>
<td>2</td>
<td>2</td>
<td>0.02/ 0.5T</td>
<td>No</td>
<td>0.02 to 0.5T</td>
<td>Scheltens scale - T2</td>
<td>-</td>
</tr>
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</table>

Studies describing change in WMH volume over time, subdivided into hospital-based, population-based and case studies. *WMH progression is presented as the annual unadjusted change and expressed as mean volume change in ml/year. **If studies mentioned regression of WMH volume, it is reported in this column. When available, the percentage of the study population with WMH regression and volume of WMH decrease in ml/year are also reported. FU: mean follow-up duration in years; N: number of participants with available imaging data during the entire follow-up; Age: mean age at baseline in years; VS: Visual scale score: no quantitative WMH volume assessments available; US: unspecified; ‡ Median follow-up duration reported or median WMH volume change reported in ml/year; † WMH volume change is expressed as %TBV/year; †† 10^-3 log-transformed volume/month; ° WMH volume change is expressed in ml and is not extrapolated to ml/year.
Vanishing SVD
Many studies have described overall net progression of SVD markers over time, but some studies also reported regression or vanishing of SVD markers over time. All longitudinal neuroimaging studies reporting regression of SVD markers are summarized in Table 1 for WMH, Table 2 for lacunes and Table 3 for microbleeds. We will discuss vanishing SVD for WMH, lacunes and microbleeds separately.

WMH regression
Some longitudinal population studies [28, 59-62, 82, 90, 94, 95, 97, 106, 109] reported regression of WMH in some participants, but reported negative volume changes without further comment [82, 94], attributed the reduction in WMH volume to measurement error or variability [62, 90, 97, 106, 109], or classified it as “no progression” without further explanation [60]. A recent longitudinal imaging study performed in a memory clinic population described progression, regression and stable WMH simultaneously in different brain regions in healthy elderly and Alzheimer's Dementia patients [61]. Two other studies reported WMH regression in stroke populations: Wardlaw and colleagues noted reductions in WMH volume a year after stroke in a third of 200 patients with minor stroke [28] and a recent serial MRI study performed on ischemic stroke patients demonstrated both progression and regression, with WMH regression observed in 21.5% of stroke patients [59], mainly in per ventricular white matter, posterior horn, frontal sub cortical or parietal sub cortical areas. In the RUN DMC study decline in WMH volume was observed in 9.4% of the participants with symptomatic SVD [110]. Maillard and colleagues reported decrease in FLAIR signal intensity over time in areas of stagnant WMH [117], providing further evidence for regression of WMH. Significant WMH regression has also been demonstrated in case-studies reporting regression of WMH volume one year after cerebral infarction [140] or lacunar stroke [141].

The observed reductions in WMH volume may have several explanations: methodological, radiological or biological. First, WMH regression could be missed when using two neuroimaging assessments with a long interval: WMH decline within a certain time window can be compensated by WMH progression thereafter (or vice versa) in a cohort that on average showed progression. Second, it might be that changes in WMH volume could in part be explained by partial volume effects, leading to less accurate WMH volume estimations. Third, since the signal change on T2 or FLAIR is not just due to permanent myelin loss or axonal damage but may also be due to (reversible) shifts in water content [28], WMH can reduce or disappear on follow-up imaging. Recently developed WMH might also include areas of tissue edema and reduction in tissue edema at a later stage could then lead to reduced WMH volume [28], which might be the reason that WMH regression is more often observed in stroke patients. This hypothesis is confirmed by Yao and colleagues who found that in patients with CADASIL new WMH were associated with subtle increased brain volume [142]. Fourth, factors influencing the blood-brain barrier might play a role in reducing WMH volume [28, 39]. After acute infarction the blood-brain barrier might be disturbed, causing leakage of cerebral fluid into the white matter, which might recover afterward [140, 141].

Vanishing lacunes
Only two studies reported a decrease in number of lacunes [64, 110]. On follow-up imaging in lacunar stroke patients, 94% of the lacunes visible at baseline imaging were completely or incompletely cavitated, most had reduction in diameter, and 5 lacunes (6%) were not visible anymore. In the RUN DMC study cohort, 15 lacunes disappeared in 10 participants (3.6% of the total population that underwent follow-up imaging) over the course of nine years [110].

There might be several explanations for vanishing lacunes. It is possible that the brain tissue recovered after an acute lacunar infarction, without the formation of a lacune. Besides, it can be that lacunes are either collapsed into small lacunes that can be missed by brain imaging [64] or became incorporated into the ventricles. Also, since some primary studies were performed before the STRIVE criteria [5] were developed and reported, it is also possible that some lacunes were confused with enlarged perivascular spaces. Further, it is also possible that due to partial volume effects lacunes can be rated at baseline imaging but are not visible at follow-up imaging anymore.

Vanishing microbleeds
Although numerous studies have reported incident microbleeds over time, there are also some reports of a decrease in the number of microbleeds [65, 66, 78, 129, 130]. However, most of these studies classified participants with vanishing microbleeds as “no incident microbleeds” [65, 78, 130] or mentioned less microbleeds at follow-up without further comment [129]. A study in patients with stroke or transient ischemic attack mentioned a decrease in number of microbleeds in 14.5% of the patients and reported a dynamic temporal change of microbleeds during follow-up [66]. In the RUN DMC study, 42 microbleeds vanished in 16 participants (6.1%) over the time course of nine years [110].
### Table 2: Characteristics of longitudinal changes of lacunes by study population

<table>
<thead>
<tr>
<th>Ref</th>
<th>Population</th>
<th>n (%)</th>
<th>Age</th>
<th>FU time</th>
<th>Nr of scans</th>
<th>Field strength</th>
<th>Identical MRI B &amp; FU?</th>
<th>Assessment of lacunes</th>
<th>Incident lacunes*</th>
<th>Vanishing lacunes**</th>
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<tr>
<td><strong>Hospital-based studies</strong></td>
<td></td>
<td></td>
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<td></td>
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<td>SVD</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[110] SVD (RUN DMC)</td>
<td>276 (54.9%)</td>
<td>62.5 ± 7.7</td>
<td>8.7</td>
<td>3</td>
<td>1.5T</td>
<td>No; FLAIR voxel size adjusted</td>
<td>STRIVE – Manually rated on FLAIR/T1; FU FLAIR sequence resliced to B FLAIR</td>
<td>2.3 %/y</td>
<td>0.4 %/y</td>
<td></td>
</tr>
<tr>
<td>[81] SVD (LADIS) (multicentre)</td>
<td>396 (62.0%)</td>
<td>73.6 ± 5.0</td>
<td>3.1</td>
<td>2</td>
<td>1.5</td>
<td>No: new MRI in 3 centers</td>
<td>Manually rated on FLAIR/T1/T2</td>
<td>6.1 %/y</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>[120] SVD (LADIS) (multicentre)</td>
<td>358 (56.0%)</td>
<td>74 ± 5</td>
<td>3</td>
<td>2</td>
<td></td>
<td></td>
<td>Manually rated</td>
<td>5.8 %/y</td>
<td>-</td>
<td></td>
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<tr>
<td>[120] SVD (LADIS) (multicentre)</td>
<td>387 (60.6%)</td>
<td>73.1 ± 5</td>
<td>3</td>
<td>2</td>
<td></td>
<td></td>
<td>Manually rated on FLAIR/T2</td>
<td>6.2 %/y</td>
<td>-</td>
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<tr>
<td><strong>Stroke</strong></td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>[68] Lacunar stroke (SCANS)</td>
<td>70 (57.9%)</td>
<td>70 ± 9.8</td>
<td>3</td>
<td>2-4</td>
<td>1.5T</td>
<td>Yes</td>
<td>STRIVE - Manually rated on FLAIR/T1/T2</td>
<td>9.5 %/y</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>[71] Stroke or TIA (VITATOPS) (multicentre)</td>
<td>359 (76.2%)</td>
<td>64.3 ± 12.7</td>
<td>2.1</td>
<td>2</td>
<td>1.5T</td>
<td>Yes</td>
<td>Manually rated</td>
<td>3.3 %/y</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>[64] Lacunar stroke</td>
<td>82 (59.4%)</td>
<td>63 ± 11</td>
<td>2.1</td>
<td>2</td>
<td>1.5</td>
<td>Yes</td>
<td>Manually rated on FLAIR/T2</td>
<td>6.1 %/y</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>Other</strong></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[83] Atherosclerotic disease (SMART-MR)</td>
<td>565 (43.2%)</td>
<td>57 ± 9</td>
<td>3.9</td>
<td>2</td>
<td>1.5T</td>
<td>Yes</td>
<td>STRIVE – Manually rated on FLAIR/T1</td>
<td>2.2 %/y</td>
<td>-</td>
<td></td>
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<tr>
<td>[101] Diabetes type 2</td>
<td>190 (86.8%)</td>
<td>62.7 ± 8.1</td>
<td>6</td>
<td>3</td>
<td>1.5T</td>
<td>Yes</td>
<td>Manually rated on T2/T1/FLAIR</td>
<td>3.7 %/y</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>Population-based studies</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[67] Population-based (Rotterdam Scan)</td>
<td>803 (75.6%)</td>
<td>68.3 ± 6.2</td>
<td>3.4</td>
<td>2</td>
<td>1.5T</td>
<td>Yes</td>
<td>Manually rated on FLAIR/PD/T1</td>
<td>0.7 %/y</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>[122] Population-based (Rotterdam Scan)</td>
<td>668 (62.0%)</td>
<td>71 ± 7</td>
<td>3.4</td>
<td>2</td>
<td></td>
<td></td>
<td>Manually rated on FLAIR/PD/T1</td>
<td>3.6 %/y</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>[60] Population-based (Rotterdam Scan)</td>
<td>668 (62.0%)</td>
<td>71 ± 7</td>
<td>3.4</td>
<td>2</td>
<td></td>
<td></td>
<td>Manually rated on FLAIR/PD/T1</td>
<td>3.5 %/y</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>[121] Population-based (CHS) (multicentre)</td>
<td>1433 (24.3%)</td>
<td>74</td>
<td>5</td>
<td>2</td>
<td>1.5</td>
<td>Yes</td>
<td>Manually rated on T2/T1</td>
<td>2.9 %/y</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>[73] Population-based (ARGC) (multicentre)</td>
<td>810 (42.2%)</td>
<td>61.6 ± 4.2</td>
<td>10</td>
<td>2</td>
<td>1.5T</td>
<td>No</td>
<td>Manually rated on T1/T2/PD; FU scan best matched to B</td>
<td>1.6 %/y</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>[102] Population-based (AGES-Reykjavik)</td>
<td>1949 (33.3%)</td>
<td>74.6 ± 4.6</td>
<td>5.2</td>
<td>2</td>
<td>1.5T</td>
<td>Yes</td>
<td>Manually rated on FLAIR/T2/PD</td>
<td>0.8 %/y</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>[118] Population-based (PATH TLS)</td>
<td>375 (78.6%)</td>
<td>62.6 ± 1.5</td>
<td>4.0</td>
<td>2</td>
<td>1.5T</td>
<td>No; new scanner</td>
<td>Manually rated on FLAIR/T1</td>
<td>0.4 %/y</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Studies describing change in number of lacunes over time, divided into hospital-based and population-based studies. "Incidence of lacunes is expressed as percentage of participants with incident lacunes in %/year. *If studies mentioned vanishing lacunes it is reported in this column. When available, the proportion of the study population with vanishing lacunes is reported in %/year. FU: mean follow-up duration in years; N: number of participants with available imaging data during the entire follow-up; Age: mean age at baseline in years; US: unspecified; ‡ Median follow-up duration reported."
### Table 3  Characteristics of longitudinal changes of microbleeds by study population

<table>
<thead>
<tr>
<th>Ref</th>
<th>Population</th>
<th>n (%)</th>
<th>Age</th>
<th>FU time</th>
<th>Nr of scans</th>
<th>Field strength</th>
<th>Identical MRI B &amp; FU?</th>
<th>Assessment of microbleeds</th>
<th>Incident microbleeds*</th>
<th>Vanishing microbleeds**</th>
</tr>
</thead>
<tbody>
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<td></td>
<td>Hospital-based studies</td>
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<td></td>
<td><strong>SVD</strong></td>
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<tr>
<td>[110]</td>
<td>SVD (RUN DMC)</td>
<td>264 (52.5%)</td>
<td>62.5 ± 7.7</td>
<td>8.7</td>
<td>3</td>
<td>1.5T</td>
<td>No; MRI update identical T2*</td>
<td>STRIVE – Manually rated on T2*</td>
<td>2.2 %/y</td>
<td>0.7 %/y</td>
</tr>
<tr>
<td></td>
<td><strong>Stroke</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[130]</td>
<td>Lacunar stroke</td>
<td>96</td>
<td>64.5 ± 11.1</td>
<td>2.1</td>
<td>2</td>
<td>1.5</td>
<td>3.0T</td>
<td>Yes</td>
<td>STRIVE – Manually rated on T2*</td>
<td>8.6 %/y</td>
</tr>
<tr>
<td>[85]</td>
<td>Stroke patients (CASISP) (multicentre)</td>
<td>500 (69.4%)</td>
<td>59.7 ± 9.8</td>
<td>1.2‡</td>
<td>2</td>
<td>1.5T</td>
<td>Yes</td>
<td>MARS - Manually rated on T2*</td>
<td>11 %/y</td>
<td>-</td>
</tr>
<tr>
<td>[131]</td>
<td>Stroke patients</td>
<td>204 (73.6%)</td>
<td>68</td>
<td>2</td>
<td>2</td>
<td>1.5T</td>
<td>Yes</td>
<td>Manually rated on T2*</td>
<td>5 %/y</td>
<td>-</td>
</tr>
<tr>
<td>[66]</td>
<td>Stroke or TIA</td>
<td>224 (37.9%)</td>
<td>64.6 ± 11.3</td>
<td>2.3</td>
<td>2</td>
<td>1.5T</td>
<td>Yes</td>
<td>Greenberg – Manually rated on T2*</td>
<td>8.0 %/y</td>
<td>6.5 %/y</td>
</tr>
<tr>
<td>[127]</td>
<td>Stroke patients</td>
<td>508 (46.4%)</td>
<td>68.9 ± 11.5</td>
<td>3.7</td>
<td>2</td>
<td>1.5T</td>
<td>Yes</td>
<td>MARS - Manually rated on T2*</td>
<td>7.4 %/y</td>
<td>4.1 %/y</td>
</tr>
<tr>
<td>[126]</td>
<td>Ischemic stroke</td>
<td>21 (43.8%)</td>
<td>65 ‡</td>
<td>5.6</td>
<td>2</td>
<td>1.5T</td>
<td>Yes</td>
<td>MARS – Manually rated on T2*</td>
<td>4.1 %/y</td>
<td>US</td>
</tr>
<tr>
<td>[128]</td>
<td>Acute stroke</td>
<td>237 (13.6%)</td>
<td>64.0 ± 12.8</td>
<td>4 d</td>
<td>2</td>
<td>1.5T</td>
<td>Yes</td>
<td>Manually rated on T2*</td>
<td>12.7 %</td>
<td>3 %</td>
</tr>
<tr>
<td>[136]</td>
<td>Primary ICH</td>
<td>61 (28.6%)</td>
<td>58.3</td>
<td>1.9‡</td>
<td>2</td>
<td>1.5T</td>
<td>Yes</td>
<td>Manually rated on T2*</td>
<td>15.9 %/y †</td>
<td>-</td>
</tr>
<tr>
<td>[137]</td>
<td>ICH patients (DECIPHER)</td>
<td>84 (42.0%)</td>
<td>58.0 ± 13.6</td>
<td>1</td>
<td>3</td>
<td>1.5</td>
<td>3.0T</td>
<td>No; some on other scanner</td>
<td>Manually rated on T2*</td>
<td>33.3 %/y</td>
</tr>
<tr>
<td>[135]</td>
<td>Elderly with lobar ICH</td>
<td>34 (36.2%)</td>
<td>71.0</td>
<td>1.3</td>
<td>2</td>
<td>1.5T</td>
<td>Yes</td>
<td>Greenberg – Manually rated on T2*</td>
<td>38.5 %/y</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td><strong>Memory clinic</strong></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>[78]</td>
<td>Memory clinic</td>
<td>254</td>
<td>66 ± 10</td>
<td>1.9</td>
<td>2</td>
<td>1.0T</td>
<td>Yes</td>
<td>Manually rated on T2*</td>
<td>6.3 %/y</td>
<td>1.1 %/y</td>
</tr>
<tr>
<td>[129]</td>
<td>Memory clinic</td>
<td>26</td>
<td>78.9</td>
<td>1.1</td>
<td>2</td>
<td>1.5T</td>
<td>Yes</td>
<td>Manually rated on SWI</td>
<td>MCI / D; 31.5 %/y</td>
<td>20 %/y</td>
</tr>
<tr>
<td></td>
<td></td>
<td>23</td>
<td>75.5</td>
<td>1.1</td>
<td>-</td>
<td>-</td>
<td>Yes</td>
<td>MARS – Manually rated on T2*</td>
<td>MCI 0 %/y</td>
<td>Controls: 0 %/y</td>
</tr>
<tr>
<td>[134]</td>
<td>AD, MCI &amp; controls (AIBL)</td>
<td>123 (70.7%)</td>
<td>75</td>
<td>3</td>
<td>3</td>
<td>3.0T</td>
<td>Yes</td>
<td>Manually rated on SWI</td>
<td>9.7 %/y</td>
<td>-</td>
</tr>
<tr>
<td>[124]</td>
<td>MCI &amp; controls</td>
<td>103</td>
<td>73</td>
<td>4</td>
<td>2-4</td>
<td>1.5T</td>
<td>Yes</td>
<td>Manually rated on SWI</td>
<td>US</td>
<td>-</td>
</tr>
<tr>
<td>[133]</td>
<td>Elderly with AF &amp; controls</td>
<td>77 (31.7%)</td>
<td>69.2 ± 9.3</td>
<td>2.6</td>
<td>3-5</td>
<td>1.5T</td>
<td>No; 3 scanners</td>
<td>Greenberg – Manually rated on T2*</td>
<td>5.5 %/y</td>
<td>-</td>
</tr>
<tr>
<td>[72]</td>
<td>CAA patients</td>
<td>26 (36.6%)</td>
<td>69.1 ± 6.5</td>
<td>1.1‡</td>
<td>2</td>
<td>1.5T</td>
<td>Yes</td>
<td>Manually rated on T2*</td>
<td>41.8 %/y †</td>
<td>-</td>
</tr>
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<td></td>
<td><strong>Population-based studies</strong></td>
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<tr>
<td>[67]</td>
<td>Population-based (Rotterdam Scan)</td>
<td>803 (75.6%)</td>
<td>68.3 ± 6.2</td>
<td>3.4</td>
<td>2</td>
<td>1.5T</td>
<td>Yes</td>
<td>Manually rated on T2*</td>
<td>2.9 %/y</td>
<td>-</td>
</tr>
<tr>
<td>[65, 132]</td>
<td>Population-based (AGES – Reykjavik)</td>
<td>831</td>
<td>68.5 ± 6.3</td>
<td>3.4</td>
<td>2</td>
<td>-</td>
<td>Yes</td>
<td>Manually rated on T2*</td>
<td>3.0 %/y</td>
<td>0.2 %/y</td>
</tr>
<tr>
<td>[102]</td>
<td>Population-based (AGES – Reykjavik)</td>
<td>1949 (33.3%)</td>
<td>74.6 ± 4.6</td>
<td>5.2</td>
<td>2</td>
<td>1.5T</td>
<td>Yes</td>
<td>Manually rated on T2*</td>
<td>3.4 %/y</td>
<td>-</td>
</tr>
<tr>
<td>[125]</td>
<td></td>
<td>2635</td>
<td>74.6</td>
<td>5.2</td>
<td>2</td>
<td>-</td>
<td>Yes</td>
<td>Manually rated on T2*</td>
<td>3.5 %/y</td>
<td>-</td>
</tr>
</tbody>
</table>

Studies describing change in number of microbleeds over time, subdivided into hospital-based and population-based studies. *Incidence of microbleeds is expressed as percentage of participants with incident microbleeds in %/year. **If studies mentioned vanishing microbleeds it is reported in this column. When available, the proportion of the study population with vanishing microbleeds is reported in %/year.

FU: mean follow-up duration in years; N: number of participants with available imaging data during the entire follow-up; Age: mean age at baseline in years; MB: microbleeds; US: unspecified; d: days; ‡ Median follow-up duration reported; ° Incidence is expressed in % and is not extrapolated to %/y.
Vanishing microbleeds may depend on methodological issues or imaging artefacts [78, 130, 143]. It may be the result of a biological process, including physiological resorption [78, 129, 130, 143]. The disappearance of microbleeds may also be explained by clearance of hemosiderin-containing macrophages, if we consider the pathology of microbleeds as hemosiderin pigment accumulations in macrophages adjacent to ruptured atherosclerotic microvessels [66, 144].

Clinical implications
SVD is associated with poor clinical outcome. The association between SVD severity and progression with cognitive decline and dementia [3, 4, 41]. Incident stroke [43], gait dysfunction [98] and mortality [44] is well established. If, and how, regression of SVD markers might affect clinical outcome remains to be established. There are two hypotheses how SVD regression might affect clinical outcome. First, WMH regression might co-occur with atrophy of the white matter, leading to impaired clinical outcome. Second, WMH regression might reflect resolution of transient white matter damage (i.e. before permanent axonal injury or demyelination has occurred), and might therefore account for recovery of clinical symptoms [28]. So far, studies on SVD dynamics could not find any significant associations between SVD regression and clinical outcome [59, 61]. Further studies are required to further explore the clinical significance of vanishing SVD.

Understanding the dynamic nature of SVD may be particularly valuable in clinical trials that use change of SVD markers as surrogate endpoints. Future therapeutic strategies that target vascular contributions to dementia, such as the use of anti-hypertensive treatments, may be particularly interested in dynamics and regression of SVD.

Limitations
Several methodological considerations need to be addressed. First, the studies included in this review are heterogeneous, as we included all longitudinal neuroimaging studies assessing SVD markers over time, irrespective of inclusion criteria or neuroimaging methodologies. Note that MRI technique varies largely across studies, with magnetic field strengths ranging from 0.02T in early studies to 3.0T in more recent studies, and varying voxel sizes and imaging parameters. Additionally, different methods for SVD assessment were used including visual scales and fully automatic quantitative volume measurements. Second, the reproducibility of SVD markers is a major concern in cross-sectional and longitudinal observational studies, especially across centers [145]. For example, quantification of SVD markers depends on the choice of MR sequence; microbleeds are more likely to be detected using SWI compared to T2* images [145, 146]. Also, smaller voxel sizes and slice gaps improve detection of WMH, lacunes and microbleeds, due to a reduction of partial volume effects [145]. Therefore, there is a need for studies to directly assess the reproducibility of any longitudinal measurement of WMH, lacunes or microbleeds. Third, primary studies might be susceptible to bias. Dropout rates vary between 30 and 80%, as displayed in Tables 1 to 3. Although this is inevitable in longitudinal cohort studies, this attrition bias presumably leads to an underestimation of progression rates, since those without follow-up were often older with more severe SVD at baseline. Misclassification might also have occurred since regression of SVD was previously seen as measurement error. Many studies added participants with regression to the groups without progression and did not report SVD regression. The regression of SVD markers over time in this review might therefore be an underestimation of the true regression. Furthermore, the variability in data acquisition of SVD markers may confound the detection of MRI changes, thereby limiting the power to detect and follow the progression of imaging markers of SVD over time [145].

Due to these limitations, interpretation of results from the primary studies can be difficult and caution is warranted when results from the primary studies are combined. As most studies used identical MRI protocols at baseline and follow-up – or made adequate adjustments for longitudinal assessments – we feel that within studies the extent of progression and regression of SVD over time can be reliably reported.

Concluding remarks
Although SVD progression was traditionally seen as a continuous progressive process, it should rather be seen as a dynamic and highly variable process, sometimes with regression of SVD. Where previously regression of SVD markers was often attributed to measurement error or classified as ‘no progression’, SVD regression might instead be a true phenomenon with clinical implications. Further understanding of temporal dynamics of SVD can be obtained by performing serial imaging at both more and shorter time intervals. Studies using multiple (i.e. three or more) imaging assessments will allow disentangling of episodes with regression from those with progression and hence are necessary to elaborate on the course of SVD progression. Future studies are required to examine the associated factors and clinical significance of dynamical time course of SVD.
Supplementary material

Literature search methods and terms

We identified articles by searching PubMed using the search terms below to cover three main SVD markers: WMH, lacunes and microbleeds. The MeSH terms “cerebral small vessel disease” and “MRI” were used to ensure potentially relevant articles, and the MeSH terms “lacunar stroke” and “leukoaraiosis” were used where relevant. All terms except for “cerebral small vessel disease” were exploded. Inclusion criteria were: 1) longitudinal MRI on at least two time points; 2) sporadic SVD; 3) elderly above age of 50 years.

The search strategy yielded 204 articles for WMH, 194 articles for lacunes and 167 articles for microbleeds. After screening the titles and abstracts and adding relevant literature from reference lists 71 articles (not mutually exclusive) fulfilled the inclusion criteria: 49 articles for WMH, 16 for lacunes and 22 for microbleeds.

The final search terms were as follows:

**WMH**

((White matter hyperintens* [tiab]) OR (white matter lesion* [tiab]) OR (white matter disease* [tiab]) OR (white matter change* [tiab])) AND ((cerebral small vessel disease [mh:noexp]) OR (small vessel disease* [tiab]) OR (leukoaraiosis [mh]) OR (leukoaraiosis* [tiab]) AND (MRI [mh]) AND ((longitudinal studies [mh]) OR (longitudinal* [tiab]) OR (follow-up studies [mh]) OR (incidence [mh]) OR (incidence* [tiab]) OR (progression [mh]) OR (progress* [tiab])) AND (1990/01/01 : 2016/10/10 [dp]))

⇒ 204 articles

⇒ After screening of titles and abstracts, 36 articles fulfilled the inclusion criteria

**Lacunes**

((Lacun* [tiab]) OR (deep infarct* [tiab]) OR (subcortical infarct* [tiab]) OR (deep stroke* [tiab]) OR (subcortical stroke* [tiab]) OR (silent stroke* [tiab]) OR (silent brain infarct* [tiab]) OR (small vessel infarct* [tiab]) OR (small vessel stroke* [tiab]) OR (lacunar stroke* [tiab]) OR (microinfarct* [tiab]) OR (microscopic infarct* [tiab])) AND ((cerebral small vessel disease [mh:noexp]) OR (small vessel disease* [tiab]) OR (lacun* [ti]) AND (MRI [mh]) AND (longitudinal studies [mh]) OR (longitudinal* [tiab]) OR (follow-up studies [mh]) OR (incidence [mh]) OR (incidence* [tiab]) OR (progression [mh]) OR (progress* [tiab])) AND (1990/01/01 : 2016/10/10 [dp]))

⇒ 194 articles

⇒ After screening of titles and abstracts, 24 articles fulfilled the inclusion criteria

**Microbleeds**

((microbleed* [tiab]) OR (microhemorrhag* [tiab]) OR (microhaemorrhag* [tiab]) OR ("dot-like" [tiab] AND (suscept* [tiab] OR hemosid* [tiab])) AND ((cerebral small vessel disease [mh:noexp]) OR (small vessel disease* [tiab]) OR (cerebral hemorrhage [mh]) OR (microbleed* [ti]) AND (MRI [mh]) AND (longitudinal studies [mh]) OR (longitudinal* [tiab]) OR (follow-up studies [mh]) OR (incidence [mh]) OR (inciden* [tiab]) OR (disease progression [mh]) OR (progress* [tiab])) AND (1990/01/01 : 2016/10/10 [dp]))

⇒ 167 articles

⇒ After screening of titles and abstracts, 24 articles fulfilled the inclusion criteria
Supplementary Figure  Flowchart of the literature search process

Records identified through PubMed search for WMH (n=204)

Records identified through PubMed search for lacunes (n=134)

Records identified through PubMed search for microbleeds (n=167)

Records after duplicates removed (n=429)

Records screened (n=429)

Records excluded (no serial MRI and/or other than sporadic SVD) (n=368)

Articles after screening (n=61)

Additional articles identified through other sources (n=35)

Articles assessed for eligibility (n=96)

Articles excluded (no serial MRI and/or other than sporadic SVD) (n=25)

Studies included in this review (Tables 1 to 3) (sporadic SVD; elderly over 50 years; longitudinal MRI) (n=71)

Studies included in WMH summary (Table 1) (n=49)

Studies included in lacunes summary (Table 2) (n=15)

Studies included in microbleeds summary (Table 3) (n=22)
Chapter 3

Nonlinear temporal dynamics of cerebral small vessel disease

Published as:

Nonlinear temporal dynamics of cerebral small vessel disease: The RUN DMC study
Neurology. 2017;89:1569-1577

* Both authors contributed equally
CHAPTER 3 NONLINEAR TEMPORAL DYNAMICS OF CEREBRAL SMALL VESSEL DISEASE

Abstract

Objective: To investigate the temporal dynamics of cerebral small vessel disease (SVD) by three consecutive assessments over a period of nine years, distinguishing progression from regression.

Methods: Changes in SVD markers of 276 participants of the RUN DMC cohort were assessed at three time points over 9-years. We assessed white matter hyper-intensities (WMH) volume by semi-automatic segmentation, and rated lacunes and microbleeds manually. We categorized baseline WMH severity as mild, moderate or severe according to the modified Fazekas scale. We performed mixed-effects regression analysis including a quadratic term for increasing age.

Results: Mean WMH progression over nine years was 4.7 ml (0.54 ml/yr; IQR 0.95–5.5 ml), 20.3% of patients had incident lacunes (2.3%/yr) and 18.9% incident microbleeds (2.2%/yr). WMH volume declined in 9.4% of the participants during the first follow-up interval, but only for 1 participant (0.4%) throughout the whole follow-up. Lacunes disappeared in 3.6% and microbleeds in 5.7% of the participants. WMH progression accelerated over time: including a quadratic term for increasing age during follow-up significantly improved the model (p<0.001). SVD progression was predominantly seen in participants with moderate to severe WMH at baseline compared to those with mild WMH (OR 35.5, 95% CI 15.8-80.0; p<0.001 for WMH progression; OR 5.7, 95% CI 2.8-11.2; p<0.001 for incident lacunes and OR 2.9, 95% CI 1.4-5.9; p=0.003 for incident microbleeds).

Conclusions: SVD progression is non-linear, accelerating over time, and is a highly dynamic process, with progression interrupted by reduction in some, in a population that on average shows progression.

Introduction

Markers of cerebral small vessel disease (SVD) are present on neuroimaging in virtually every individual over 60 years. They include white matter hyperintensities (WMH), lacunes and cerebral microbleeds [5]. SVD, and its progression, has been recognized as the most important vascular contributor to dementia [3, 4]. Therefore understanding of the time course of SVD progression will result in better understanding of both etiology and consequences of SVD.

Current knowledge regarding temporal dynamics of SVD is limited due to lack of studies with more than one follow-up assessment. Consequently, these studies could only report the average, presumably linear change in SVD severity [67, 68, 82, 90, 97, 125, 130]. Previous studies, however, have suggested that SVD progression may be a non-linear process accelerating over time [60, 61]. Recent studies suggest that SVD might exert its clinical effects by affecting remote brain structure and function [49, 147]. The temporal relation between changes in SVD and the subsequent atrophy of these remote brain structures is thus far unknown.

Recently, a decrease of WMH volume [28, 59-62] as well as a decrease in number of lacunes [63, 64] and microbleeds [65, 66] have been reported, further challenging the assumption of linear progression of SVD markers. Neither the time course, nor the magnitude of this “disappearing SVD” has been investigated.

In this study we investigated the temporal dynamics of SVD by three consecutive neuroimaging assessments over a period of nine years in participants with SVD, distinguishing progression from regression. As secondary analysis we investigated the temporal dynamics related to atrophy of remote brain structures.

Methods

Study population
This study is part of the RUN DMC study that prospectively investigates risk factors and clinical consequences of SVD. The detailed study protocol has been published previously [148]. Of 503 baseline participants, 281 underwent repeated MRI assessment at three time points. Five participants were excluded because of insufficient scan quality, yielding a final sample of 276 participants for the present study (Supplementary Figure 1).
**Figure 1** Temporal dynamics of WMH progression

A. Acceleration of WMH volume change over two follow-up periods (mL/ year) by age at individual level. B. Change in WMH volume (mL) over three time points by age at individual level. C. Baseline WMH severity was classified as mild (Fazekas 0–1; n=211), moderate (Fazekas 2; n=33), or severe (Fazekas 3; n=20). Smoothed curves using least smoothing express average WMH change with increasing age.

**MRI protocol**
Images were acquired at three time points on 1.5-Tesla MRI (2006: Siemens, Magnetom Sonata; 2011 and 2015: Siemens, Magnetom Avanto) and included the following whole brain scans: 3D T1 MPRAGE imaging (voxel size 1.0×1.0×1.0 mm); FLAIR pulse sequences (baseline: voxel size 1.2×1.0×5.0 mm, interslice gap 1.0 mm; follow-up: voxel size 1.2×1.0×2.5 mm; interslice gap 0.5 mm) and a transversal T2-weighted gradient echo sequence (voxel size 1.3×1.0×5.0 mm, interslice gap 1.0 mm). Full acquisition details have been described previously [148]. The same head coil was used at all three time points. To minimize effects of changes in FLAIR sequence we resliced follow-up FLAIR images to match slice thickness of baseline images using linear interpolation.

**Brain volumetry**
Grey matter (GM), white matter (WM) and CSF probability maps were computed using SPM12 [http://www.fil.ion.ucl.ac.uk/spm/] unified segmentation routines on the T1 MPRAGE images. Additionally, we used the WMH masks to correct the segmentation images, since several brain regions with WMH damage were initially misclassified. All WMH voxels were given mean WM intensity and these corrected T1 images were segmented using SPM12. All images were visually checked for co-registration and segmentation artefacts. GM, WM and CSF volumes (GMV, WMV and CSFV) were computed by summing all voxels belonging to that tissue class multiplied by voxel volume in mL. Intracranial volume (ICV) was determined by summing GMV, WMV and CSFV and total brain volume (TBV) by summing GMV and WMV. To account for inter-scan effects we corrected for differences in ICV between baseline and follow-up. We normalized all volumes to baseline ICV to account for head size [149].

**Small vessel disease**
SVD was rated according to the STRIVE criteria [5]. WMH volumes were calculated by a semi-automated WMH segmentation method, which has been described in detail elsewhere [150]. Segmentation errors by one trained rater, blinded for clinical data. WMH volumes were corrected for inter-scan differences in ICV and then normalized to baseline ICV. Besides, we calculated WMH volumes for odd and even slices separately to determine the effects of change in slice thickness of the FLAIR sequence. We used the modified Fazekas scale to categorize WMH severity at baseline (mild: Fazekas 0–1; moderate: Fazekas 2; and severe: Fazekas 3) [151].

**Standard Protocol Approvals, Registrations, and Patient Consents**
The Medical Review Ethics Committee region Arnhem-Nijmegen approved the study and all participants gave written informed consent.
Both number and location of lacunes and microbleeds were rated manually on FLAIR/T1-weighted and T2*-weighted MRI scans according to the STRIVE criteria [5] by two trained raters blinded for clinical data. Follow-up FLAIR images were resliced to match the baseline scans to prevent differences in partial volume effects between baseline and follow-up scans. Inter and intrarater reliabilities were excellent [152]. Incidence was expressed as number of participants with new lacunes or microbleeds. We identified whether lacunes or microbleeds were truly incident or disappeared and in which time-period. To minimize risk of misclassification due to co-registrations, we visually inspected all WMH segmentations and corrected lacune occurrence maps based on manual ratings.

### Vascular risk factors

We assessed presence of hypertension, smoking, alcohol use, body mass index, diabetes and hypercholesterolemia, by standardized questionnaires, as described previously [148].

### Statistical analysis

We calculated differences in baseline characteristics between participants and those without follow-up using univariate analyses. Differences in MMSE score between individuals with mild versus moderate or severe WMH at baseline and with or without WMH progression were examined using nonparametric tests.

We created WMH probability maps and distribution maps of lacunes. WMH decline was defined as more than 0.25 ml volume decline, as this was shown to be smallest change that could be confirmed visually [59]. We plotted change of WMH volumes by age at individual level using R package ggplot2 (version 2.1.0) [153]. R package lme4 was used to perform linear mixed-effects regression analysis to analyze WMH change as function of baseline age and time (version 1.1-12) [154]. We used a random intercept and random slope model, which permits the estimation of an average slope across the whole cohort while allowing for inter-individual variability. By smoothed curves using loess smoothing we explored average WMH change with increasing age. To evaluate a possible quadratic relationship, indicating non-linear progression of SVD, we compared model fit between the full model and the full model with a quadratic term for increasing age during follow-up included, using a likelihood ratio test and we evaluated change in Akaike information criterion (AIC).

To determine remote effects of SVD progression, we analyzed the relation between WMH progression in the first follow-up interval, and subsequent brain atrophy, by means of linear regression analysis. Multicollinearity between different SVD markers was investigated using regression analysis.

To identify differences in vascular risk factors in individuals with regression of SVD markers, we compared vascular risk factors for participants with and without regression of SVD markers and with those who remained relatively stable, by ANOVA followed by Bonferroni correction.

We created WMH probability maps stratified by baseline age and by baseline WMH severity. We repeated mixed-effects regression analysis stratified by baseline WMH severity to explore change in WMH within these groups separately. We calculated odds of SVD progression according to baseline Fazekas 0-1 versus Fazekas 2-3 by logistic regression analysis, adjusted for age and sex.

Statistical analyses were performed using SPSS Statistics version 20 and R Programming Language version 3.2.1.

### Results

Baseline characteristics are presented in Table 1. Mean age at baseline was 62.5 ± 7.7 years and 59.1% was male. Mean follow-up duration was 5.4 ± 0.2 years until first and 8.7 ± 0.2 years until second follow-up. Participants with moderate to severe WMH at baseline had lower MMSE scores (27.7±1.7 vs. 28.4±1.5; p<0.001) compared to participants with mild WMH. Steeper decline in MMSE score was seen in participants with WMH progression compared to participants whose WMH remained relatively stable (-0.95±2.5 vs. -0.32±1.8; p=0.031). Those lost to follow-up were significantly older and had more severe baseline SVD characteristics compared to participants (Supplementary Table 1).

Temporal dynamics of SVD

WMH probability maps are shown in Supplementary Figure 2 and Supplementary Video 1. Mean WMH progression was 0.54 ml/yr (median 0.24; IQR 0.11-0.64 ml/yr). Progression of WMH increased with baseline age (Figure 1A-B, Supplementary Video 2). In mixed-effects regression analysis, each year increase of age at baseline resulted in an increase in WMH as percentage of WM of 0.10% (95%CI: 0.07-0.13%). Including a quadratic term for increasing age during follow-up significantly improved the model (AIC base model 2995.2 versus AIC extended model 2932.9, likelihood ratio test,  χ² =64.3, df=1, p<0.001). Severity and progression of WMH were comparable for men and women.
Fifty-six participants (20.3%) developed new lacunes over nine years (2.3%/yr; Table 2). The distribution of lacunes is shown in Supplementary Figure 2 and Supplementary Video 3. Incidence of lacunes was higher for the second follow-up period (3.5%/yr) than for the first follow-up period (2.7%/yr).

Fifty participants (18.9%) developed new microbleeds over nine years (2.2%/yr) (Table 2). Incidence of microbleeds in the second follow-up period (4.2%/yr) was higher than in the first follow-up period (1.7%/yr). WMH progression in the first follow-up period was associated with brain atrophy in the second follow-up period ($\beta=0.124; p=0.040$) as well as with WM atrophy ($\beta=0.149; p=0.013$) but not with GM atrophy ($\beta=0.045; p=0.461$). Multicollinearity between SVD markers is shown in Supplementary Table 2.

Regression of SVD markers

We also observed regression of SVD markers. We observed decline in WMH volume in 26 participants (9.4%; median decline -0.5 ml; IQR: -0.9 to -0.3 ml) during the first follow-up period and in 5 participants (1.8%; median -0.5 ml; IQR: -0.9 to -0.4 ml) during the second follow-up period. In one participant WMH volume declined over the course of nine years (0.4%; -0.4 ml). In 10 participants (3.6%) 14 lacunes could not be found at follow-up imaging (Table 2). In 15 participants (5.7%) 37 microbleeds were no longer detectable after nine years of follow-up (Table 2). Examples of lacunes and microbleeds that were no longer visible on follow-up imaging are shown in Figure 2. There were no differences for any of the vascular risk factors between participants with and without regression of SVD markers, also compared with those who remained relatively stable over the nine year course (data not shown).

Heterogeneity in temporal dynamics of SVD

Mean WMH progression over nine years was 2.4 ml for participants with mild WMH at baseline, 12.0 ml for those with moderate WMH and 15.2 ml for those with severe WMH (Figure 1C, Figure 3, and Supplementary Video 4). From participants with mild WMH at baseline, six percent showed WMH progression beyond measurement error, compared with 75% of participants with moderate or severe WMH. Participants with moderate to severe WMH at baseline had 36 times higher risk of WMH progression compared to participants with mild WMH (OR 35.5; 95% CI 15.8-80.0; p<0.001). Participants with moderate to severe WMH also more often developed incident lacunes (OR 5.7; 95% CI 2.8-11.2; p<0.001) and microbleeds (OR 2.9; 95% CI 1.4-5.9; p=0.003) compared to participants with mild WMH at baseline.

<table>
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</tr>
</thead>
<tbody>
<tr>
<td>Age, years, mean (SD)</td>
<td>62.5 (7.7)</td>
<td>71.2 (7.8)</td>
<td>67.8 (7.8)</td>
<td>5.3 (0.2)</td>
<td>9.1 (0.2)</td>
<td>14.4 (0.2)</td>
</tr>
<tr>
<td>Sex, male, n (%)</td>
<td>213 (59.1)</td>
<td>215 (44.5)</td>
<td>201 (49.6)</td>
<td>-18 (0.1)</td>
<td>14 (0.2)</td>
<td>32 (0.1)</td>
</tr>
<tr>
<td>Education &gt;primary school, n (%)</td>
<td>259 (93.8)</td>
<td>257 (79.4)</td>
<td>259 (93.8)</td>
<td>-2 (0.1)</td>
<td>2 (0.2)</td>
<td>4 (0.2)</td>
</tr>
<tr>
<td>MMSE score, mean (SD)</td>
<td>28.6 (1.3)</td>
<td>28.4 (1.8)</td>
<td>28.2 (2.0)</td>
<td>-0.2 (0.2)</td>
<td>-0.1 (0.1)</td>
<td>-0.3 (0.2)</td>
</tr>
<tr>
<td>WMH volume ml, median (IQR)</td>
<td>2.1 (0.8-6.1)</td>
<td>2.8 (1.2-7.7)</td>
<td>3.5 (1.6-8.2)</td>
<td>0.7 (1.5)</td>
<td>0.7 (1.2)</td>
<td>1.4 (1.8)</td>
</tr>
<tr>
<td>WMH volume ml, mean (SD)</td>
<td>5.8 (9.3)</td>
<td>7.4 (11.3)</td>
<td>9.5 (14.4)</td>
<td>1.7 (3.6)</td>
<td>2.1 (3.6)</td>
<td>3.8 (3.6)</td>
</tr>
<tr>
<td>% WMH of WM, mean (SD)</td>
<td>1.7 (1.4)</td>
<td>1.7 (1.4)</td>
<td>1.9 (1.6)</td>
<td>0.02 (0.1)</td>
<td>0.02 (0.1)</td>
<td>0.04 (0.2)</td>
</tr>
<tr>
<td>Participants with any lacunes, n (%)</td>
<td>117 (40.3)</td>
<td>165 (55.2)</td>
<td>157 (61.8)</td>
<td>48 (15.9)</td>
<td>286 (22.0)</td>
<td>403 (17.3)</td>
</tr>
<tr>
<td>Total number of lacunes</td>
<td>117 (40.3)</td>
<td>165 (55.2)</td>
<td>157 (61.8)</td>
<td>48 (15.9)</td>
<td>286 (22.0)</td>
<td>403 (17.3)</td>
</tr>
<tr>
<td>Participants with any microbleeds, n (%)</td>
<td>117 (40.3)</td>
<td>165 (55.2)</td>
<td>157 (61.8)</td>
<td>48 (15.9)</td>
<td>286 (22.0)</td>
<td>403 (17.3)</td>
</tr>
<tr>
<td>Total number of microbleeds</td>
<td>117 (40.3)</td>
<td>165 (55.2)</td>
<td>157 (61.8)</td>
<td>48 (15.9)</td>
<td>286 (22.0)</td>
<td>403 (17.3)</td>
</tr>
<tr>
<td>Brain volumes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White matter volume, ml (SD)</td>
<td>465.6 (38.9)</td>
<td>465.4 (38.9)</td>
<td>465.4 (38.9)</td>
<td>0.2 (0.3)</td>
<td>0.2 (0.3)</td>
<td>0.4 (0.4)</td>
</tr>
<tr>
<td>Grey matter volume, ml (SD)</td>
<td>620.7 (48.9)</td>
<td>620.3 (48.9)</td>
<td>620.3 (48.9)</td>
<td>0.4 (0.4)</td>
<td>0.4 (0.4)</td>
<td>0.8 (0.8)</td>
</tr>
</tbody>
</table>

Data shown are unadjusted values and represent numbers (%), mean (SD) or median (IQR). Change represents the number of participants without lacunes or microbleeds at baseline who developed lacunes or microbleeds during follow-up. For rating of microbleeds, 12 participants were additionally excluded based on missing T2* or scanartefacts at any time point.
### Table 2: Lacunes and microbleeds per brain location

<table>
<thead>
<tr>
<th></th>
<th>Lacunes</th>
<th>Microbleeds</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>22 (8.0)</td>
<td>27 (9.8)</td>
<td>31 (11.2)</td>
<td>18 (6.5)</td>
</tr>
<tr>
<td></td>
<td>9 (3.3)</td>
<td>10 (3.6)</td>
<td>10 (3.6)</td>
<td>3 (1.1)</td>
</tr>
<tr>
<td></td>
<td>1 (0.4)</td>
<td>1 (0.4)</td>
<td>1 (0.4)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td></td>
<td>9 (3.3)</td>
<td>9 (3.3)</td>
<td>11 (4.0)</td>
<td>3 (1.1)</td>
</tr>
<tr>
<td>Incident Disappearing</td>
<td>31 (11.2)</td>
<td>34 (12.3)</td>
<td>40 (14.5)</td>
<td>21 (7.6)</td>
</tr>
<tr>
<td></td>
<td>25 (9.1)</td>
<td>39 (14.1)</td>
<td>46 (16.7)</td>
<td>35 (12.7)</td>
</tr>
<tr>
<td>Deep</td>
<td>4 (1.4)</td>
<td>7 (2.5)</td>
<td>10 (3.6)</td>
<td>6 (2.2)</td>
</tr>
<tr>
<td>Basal Ganglia*</td>
<td>4 (1.4)</td>
<td>5 (1.8)</td>
<td>7 (2.5)</td>
<td>3 (1.1)</td>
</tr>
<tr>
<td>Thalamus, n (%)</td>
<td>29 (10.5)</td>
<td>44 (15.9)</td>
<td>52 (18.8)</td>
<td>41 (14.9)</td>
</tr>
<tr>
<td>Internal Capsule, n (%)</td>
<td>13 (4.7)</td>
<td>20 (7.2)</td>
<td>21 (7.6)</td>
<td>10 (3.6)</td>
</tr>
<tr>
<td>Any deep, n (%)</td>
<td>55 (19.9)</td>
<td>70 (25.4)</td>
<td>77 (27.9)</td>
<td>56 (20.3)</td>
</tr>
<tr>
<td>Any infratentorial, n (%)</td>
<td>13 (4.7)</td>
<td>20 (7.2)</td>
<td>21 (7.6)</td>
<td>10 (3.6)</td>
</tr>
<tr>
<td>Any, n (%)</td>
<td>55 (19.9)</td>
<td>70 (25.4)</td>
<td>77 (27.9)</td>
<td>56 (20.3)</td>
</tr>
</tbody>
</table>

Data represent number of participants (%) with lacunes/microbleeds per brain location. *Basal ganglia include Globus Pallidus, Putamen and Caudate Nucleus. Any infratentorial includes pons, mesencephalon, medulla oblongata and cerebellum.

**Figure 2:** Lacunes and microbleeds no longer visible on follow-up imaging.

A. Lacunes

B. Microbleeds

Examples of lacunes that are no longer detectable following imaging in A, which appear to be assimilated by the ventricle. Microbleeds in B appear to have faded away over time.
Discussion

In this study we showed the temporal dynamics of SVD, revealing both SVD progression and regression, using three imaging assessments over a period of nine years. We demonstrated that progression of all SVD markers occurred in a non-linear fashion, accelerating over time consistent with a quadratic course. In addition, we showed that participants with moderate or severe WMH had a high likelihood of progression of their SVD, whereas participants with mild baseline SVD showed mild progression over a period of nine years.

Our study demonstrates that SVD progression is not linear but accelerates with increasing age. While the average progression in our study is comparable with other studies [67, 68, 82, 90, 97, 119, 125, 130], the use of three imaging assessments allowed us to show that SVD progression accelerated over time, providing evidence for a non-linear process [60, 61]. Moreover, our results suggest that a quadratic course of SVD progression over time is plausible, since including a quadratic term improved the model. Although we would need more than three time points to further study exponential functions, our study indicates non-linear temporal dynamics of SVD progression. Our findings do not support the hypothesized ceiling effect in which WMH progression reaches a certain threshold at high age and high lesion volume [3], as we also saw WMH progression in those at high age and with high SVD lesion load.

The relation between WMH progression and subsequent WM atrophy and TBV atrophy, suggests that SVD affects adjacent brain structures. WM atrophy might be the result of disconnected white matter tracts due to SVD, leading to axonal loss by anterograde or retrograde degeneration, and subsequently the loss of brain volume [4, 155]. The clinical observation that patients with similar SVD burden show heterogeneity in clinical symptoms might be explained by disconnection of WM tracts.

Imaging assessments at three time points also enabled us to identify regression of SVD markers followed by progression, in a cohort that on average showed progression. This observation provides further evidence that SVD does not gradually evolve but is a dynamic process, with progression interrupted by regression in some. Thus far only few other studies have reported a decline in WMH volume [28, 59-61], possibly because WMH decline within a certain time window was compensated by WMH progression thereafter (or vice versa). Two imaging assessments do not allow disentangling of episodes with regression from those with progression.
The observed decline in WMH may have several explanations. First, WMH decline in the first follow-up period could be explained in part by partial volume effects caused by slight adjustments in FLAIR sequences between baseline and first follow-up. However, we think this is unlikely because WMH volumes calculated from even and odd slices were identical and because we also found WMH decline between the second and third MRI assessment. Second, different orientation of participants in the scanner might also partly explain disappearing SVD, especially for smaller lesions. In order to prevent this, we classified WMH regression as more than 0.25 ml volume decline. Third, recently developed WMH might represent areas of tissue edema. Reduction in tissue edema at a later stage could lead to reduced WMH volume [28]. Fourth, improved control of vascular risk factors or factors influencing the blood-brain-barrier might play a role by reducing WMH volume [28, 59]. Disappearance of lacunes could be due to partial volume effects, due to “collapsing” lacunes or to incorporation of the lacune into the ventricle (Figure 2) [63, 64]. Disappearing microbleeds may be explained by partial volume effects as well as by clearance of hemosiderin-containing macrophages [66]. Our findings are in line with the latter hypothesis. In most cases microbleeds seemed to “fade away” between 2006 and 2011 and were no longer visible in 2015.

All SVD markers at baseline were important predictors for SVD progression, in a non-linear way and independent of age. Additional analyses on progression of SVD markers by distribution of microbleeds did not reveal significantly different progression for participants with strictly lobar compared to participants with deep microbleeds (data not shown), although this analysis might have been underpowered. Although we would require an even longer follow-up to exclude the possibility that all participants with mild baseline WMH will ultimately progress to severe WMH, our data show that even the oldest participants with mild baseline WMH rarely show progression over a time course of nine years. This suggests different progression curves for participants with mild versus severe baseline WMH, implying heterogeneity in etiology of mild versus severe SVD. Small WMH volumes, representing punctuate WMH or small periventricular caps, probably consist of enlarged perivascular spaces and subependymal gliosis [97]. On the contrary, confluent WMH represent a continuum of ischemic tissue damage, ranging from mild fiber loss to complete infarction and may have a more malignant course in terms of cognitive deterioration. These different etiologies call upon a different diagnostic and therapeutic approach. The correlation between WMH severity and progression and MMSE score underlines the clinical relevance of our findings on inter-individual variability in SVD progression.

Strengths of this study include the large cohort of participants with SVD and the long follow-up duration. Furthermore, imaging assessments at three time points allowed us to characterize change in SVD over time, including SVD regression. SVD was rated according to standardized procedures [5], minimizing risk of misclassification. Moreover, semi-automatic WMH quantification reduced risk of information bias [3]. Furthermore, brain volumes were determined with the newest segmentation routines of SPM12 and corrected for segmentation errors using WMH masks. Finally, our study has high external validity for patients with SVD in a general neurology clinic.

A limitation of our study is change of MRI scanner between baseline and first follow-up. However, by taking into account the third MRI assessment we are able to capture most of this possible bias. A slight adjustment in FLAIR sequence between baseline and first follow-up may have caused an overestimation of incident lacunes. However, we limited the possible negative effects by reclassifying follow-up to baseline FLAIR images before rating lacunes. Besides, changes in signal characteristics of normal brain tissue and WMH might have lead to artefactually higher rates of lesion development. However we considered this unlikely, since we also observed regression of SVD markers from the second to third time-period in a considerable proportion of participants. Further, due to low-resolution T2* sequences, we might have missed smaller microbleeds. However, since similar GRE sequences are applied for all time points, risk of misclassification will result in comparable systematic error for all time points. Inevitably, attrition bias may have occurred due to the very long-term follow-up, probably leading to an underestimation of progression of SVD, since those who dropped-out were older, and had more severe SVD.

Our study demonstrates that SVD progression is a non-linear, dynamic and highly variable process, predominantly seen in participants with moderate or severe WMH at baseline. And, equally important, those with mild WMH rarely show progression over a nine year course. Since SVD progression has been linked to cognitive decline and development of dementia, our findings on inter-individual variability in SVD progression might be a major step forward in developing personalized treatment approaches. The findings that progression of SVD is sometimes interrupted by regression and that SVD progression occurs in a quadratic way and hence is not gradually, linearly progressive as was previously thought, suggest a paradigm shift on how SVD processes should be considered. Future studies should elaborate on the clinical consequences of this non-linear dynamic time course of SVD progression.
Supplementary material

Supplementary Figure 1 Flowchart

Baseline (2006) participants with MRI
n=503

49 participants deceased

Participants eligible for follow-up 1 (2011)
n=454

- Lost to follow-up n=2
- Unable to visit research centre n=54
- MRI contra-indications n=37

Note: These 93 participants were again contacted for second follow-up assessment in 2015.

Follow-up 1 (2011) participants with MRI
n=361

43 participants deceased

Participants eligible for follow-up 2 (2015)
n=411

115 participants without MRI in 2015
- Unable to visit research centre n=65
- MRI contra-indications n=50

Follow-up 2 (2015) participants with MRI
n=296

20 participants excluded in the present study
- MRI assessment in 2015 but not 2011 n=15
- Insufficient scan quality n=5

Participants with MRI in 2006 & 2011 & 2015
n=276

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

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

<table>
<thead>
<tr>
<th></th>
<th>Participants</th>
<th>Lost to follow-up</th>
<th>Significance</th>
</tr>
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<tbody>
<tr>
<td>n=276</td>
<td></td>
<td>n=227</td>
<td></td>
</tr>
<tr>
<td>Demographics</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Age (years), mean (SD)</td>
<td>62.5 (7.7)</td>
<td>69.5 (8.5)</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Sex, male, n (%)</td>
<td>163 (59.1)</td>
<td>121 (53.3)</td>
<td>p=0.207</td>
</tr>
<tr>
<td>Education &gt; primary school, n (%)</td>
<td>259 (93.8)</td>
<td>32 (14.1)</td>
<td>p=0.004</td>
</tr>
<tr>
<td>MMSE score, mean (SD)</td>
<td>28.6 (1.3)</td>
<td>276 (1.8)</td>
<td>p=0.001</td>
</tr>
<tr>
<td>SVD Characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WMH volume, ml, median (IQR)</td>
<td>2.3 (0.8-6.1)</td>
<td>7.7 (2.6-16.2)</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>WMH volume, ml, Mean (SD)</td>
<td>5.8 (9.5)</td>
<td>11.9 (13.6)</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>% WMH of WM, mean (SD)</td>
<td>1.3 (2.3)</td>
<td>2.9 (4.4)</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Participants with any lacunes, n (%)</td>
<td>55 (19.9)</td>
<td>77 (33.9)</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Total number of lacunes</td>
<td>117</td>
<td>135</td>
<td>p=0.038</td>
</tr>
<tr>
<td>Participants with any microbleeds, n (%)</td>
<td>36 (13.1)</td>
<td>47 (20.9)</td>
<td>p=0.022</td>
</tr>
<tr>
<td>Total number of microbleeds</td>
<td>140</td>
<td>159</td>
<td>p=0.496</td>
</tr>
<tr>
<td>Territorial Infarcts, n (%)</td>
<td>23 (8.3)</td>
<td>34 (15.0)</td>
<td>p=0.023</td>
</tr>
<tr>
<td>Modified Fazekas score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild WMH (0-1), n (%)</td>
<td>218 (79.0)</td>
<td>114 (50.2)</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Moderate WMH (2), n (%)</td>
<td>38 (13.8)</td>
<td>70 (30.8)</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Severe WMH (3), n (%)</td>
<td>20 (7.2)</td>
<td>43 (18.9)</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Brain volumes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White matter volume, ml (SD)</td>
<td>465.6 (38.9)</td>
<td>441.5 (50.2)</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Grey matter volume, ml (SD)</td>
<td>620.7 (48.9)</td>
<td>588.7 (51.6)</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Vascular risk factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking, ever, n (%)</td>
<td>196 (71.0)</td>
<td>157 (69.2)</td>
<td>p=0.696</td>
</tr>
<tr>
<td>Alcohol, glasses/week, mean (SD)</td>
<td>8.3 (9.0)</td>
<td>7.5 (9.7)</td>
<td>p=0.367</td>
</tr>
<tr>
<td>Glucose lowering drugs, n (%)</td>
<td>23 (8.3)</td>
<td>43 (18.9)</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>190 (68.8)</td>
<td>179 (78.9)</td>
<td>p=0.015</td>
</tr>
<tr>
<td>BMI, mean (SD)</td>
<td>27.1 (4.1)</td>
<td>27.2 (4.2)</td>
<td>p=0.778</td>
</tr>
<tr>
<td>Lipid/lowering drugs, n (%)</td>
<td>118 (42.8)</td>
<td>119 (52.4)</td>
<td>p=0.032</td>
</tr>
</tbody>
</table>

Data are represented as numbers (%), mean (SD) or median (IQR). Comparisons between participants and those lost to follow-up were performed by t-test, Chi-square or Mann-Whitney-U test. *For ratings of microbleeds 4 participants were additional excluded based on missing T2* or scan artefacts at baseline.
Supplementary Figure 2. Probability maps of white matter hyperintensities and lacunes.

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

Supplementary Table 2. Correlation matrix for baseline SVD characteristics

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>Sex</th>
<th>Baseline WMH volume</th>
<th>Baseline lacunes</th>
<th>Baseline microbleeds</th>
<th>Baseline WM volume</th>
<th>Baseline GM volume</th>
<th>Change WMH volume</th>
<th>Incident lacunes</th>
<th>Incident microbleeds</th>
<th>Change WM volume</th>
<th>Change GM volume</th>
</tr>
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<tbody>
<tr>
<td>Age</td>
<td>-</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>Sex</td>
<td>0.010</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Baseline WMH volume</td>
<td>0.315***</td>
<td>0.088</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Baseline lacunes</td>
<td>0.189**</td>
<td>-0.102</td>
<td>0.310***</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Baseline microbleeds</td>
<td>0.127*</td>
<td>0.006</td>
<td>0.222***</td>
<td>0.237***</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Baseline GM volume</td>
<td>-0.460***</td>
<td>0.001</td>
<td>-0.244***</td>
<td>-0.205**</td>
<td>-0.132*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Change WMH volume</td>
<td>-0.531***</td>
<td>0.034***</td>
<td>-0.258***</td>
<td>-0.265***</td>
<td>-0.161***</td>
<td>0.283***</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Change GM volume</td>
<td>-0.299***</td>
<td>0.101</td>
<td>0.577***</td>
<td>0.229***</td>
<td>0.110</td>
<td>-0.182**</td>
<td>-0.281***</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Incident lacunes</td>
<td>-0.379***</td>
<td>0.164***</td>
<td>-0.168**</td>
<td>-0.166**</td>
<td>-0.131*</td>
<td>0.202**</td>
<td>0.266***</td>
<td>-0.295***</td>
<td>0.085</td>
<td>-0.172**</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Incident microbleeds</td>
<td>-0.131*</td>
<td>0.038</td>
<td>-0.036</td>
<td>0.109</td>
<td>0.026</td>
<td>0.046</td>
<td>-0.093</td>
<td>-0.051</td>
<td>0.022**</td>
<td>0.161***</td>
<td>0.189***</td>
<td>-</td>
</tr>
</tbody>
</table>

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

All Supplementary Videos are available at Neurology.org

**Supplementary Video 1** WMH probability maps
Probability maps of WMH through the whole brain, color-coded in percentage from 5 to 75%. This movie shows the probability of presence of WMH at three different time points (2006-2011-2015).

**Supplementary Video 2** WMH probability maps stratified by baseline age
Probability maps of WMH progression stratified by baseline age through the whole brain, color-coded in percentage from 5 to 75%. This movie shows the WMH increase after 9 years of follow-up for participants aged <60 years, between 60 and 70 years and over 70 years.

**Supplementary Video 3** Distribution maps of lacunes
This movie shows the distribution map of presence of lacunes in three different time points (2006-2011-2015) in green, with incident lacunes in red.

**Supplementary Video 4** WMH probability maps stratified by baseline WMH severity
Probability maps of WMH progression stratified by baseline WMH severity through the whole brain, color-coded in percentage from 5 to 75%. This movie shows the probability of WMH increase over 9 years of follow-up for participants with mild (Fazekas 0-1; n=211), moderate (Fazekas 2; n=33) and severe (Fazekas 3; n=20) WMH at baseline.
Part III

The etiology of cerebral small vessel disease
Chapter 4

Progression of white matter hyperintensities preceded by heterogeneous decline of microstructural integrity

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van Leijsen EMC, Bergkamp MI, van Uden IWM, Ghafoorian M, van der Holst HM, Norris DG, Platel B, Tuladhar AM, de Leeuw FE

Progression of white matter hyperintensities preceded by heterogeneous decline of microstructural integrity

Stroke. 2018;49:1386-1393
CHAPTER 4 WHAT PRECEDED WHITE MATTER HYPERINTENSITIES?

Abstract

Background and Purpose: White matter hyperintensities (WMH) are frequently seen on neuroimaging of elderly and are associated with cognitive decline and the development of dementia. Yet, the temporal dynamics of conversion of normal appearing white matter (NAWM) into WMH remains unknown. We examined whether and when progression of WMH was preceded by changes in FLAIR and diffusion tensor imaging values, thereby taking into account differences between participants with mild versus severe baseline WMH.

Methods: From 266 participants of the RUN DMC study, we semi-automatically segmented WMH at 3 time points for 9 years. Images were registered to standard-space through a subject-template. We analyzed differences in baseline FLAIR, fractional anisotropy (FA) and mean diffusivity (MD) values and changes in MD values over time between 4 regions: (1) remaining NAWM; (2) NAWM converting into WMH in the second follow-up period; (3) NAWM converting into WMH in the first follow-up period; and (4) WMH.

Results: NAWM converting into WMH in the first or second time-interval showed higher FLAIR and MD values than remaining NAWM. MD values in NAWM converting into WMH in the first time-interval were similar to MD values in WMH. When stratified by baseline WMH severity, participants with severe WMH had higher FLAIR and MD, and lower fractional anisotropy values than participants with mild WMH, in all areas including the NAWM. MD values in WMH and in NAWM that converted into WMH continuously increased over time.

Conclusions: Impaired microstructural integrity preceded conversion into WMH and continuously declined over time, suggesting a continuous disease process of white matter integrity loss that can be detected using diffusion tensor imaging even years before WMH become visible on conventional neuroimaging. Differences in microstructural integrity between participants with mild versus severe WMH suggest heterogeneity of both NAWM and WMH, which might explain the clinical variability observed in patients with similar small vessel disease severity.

Introduction

White matter hyperintensities (WMH) are part of the spectrum of cerebral small vessel disease (SVD) markers and are frequently observed on magnetic resonance imaging (MRI) scans in individuals >60 years of age [5-7]. WMH are associated with cognitive decline and the development of dementia [3, 43]. The pathophysiology of WMH remains poorly understood, in part because studies on WMH and its progression have mainly used conventional MRI, thereby unable to assess its earlier stages that perhaps were not yet visible. Imaging techniques such as diffusion tensor imaging (DTI) can possibly provide additional information on these earlier stages by the assessment of the microstructural organization of the white matter (WM) [27-30]. Previous longitudinal studies have shown changes in baseline DTI parameters, that is, decreased fractional anisotropy (FA) and increased mean diffusivity (MD) that predicted incident WMH at follow-up [31, 32]. However, the temporal course of DTI changes in normal appearing WM (NAWM) preceding conversion to WMH remains to be elucidated. This is especially important because there is increasing evidence that WMH progression accelerates over time [110]. Besides, progression was found most pronounced in participants with severe WMH [97, 110], suggesting differences in pathogenesis for mild versus severe WMH. More knowledge of the sequence of events that precedes the conversion of NAWM toward WMH might result in better identification of patients at risk for further WMH progression and the attendant clinical symptoms. This would especially be useful to identify in which individuals and at what moment preventive therapies could be beneficial.

In the present study we therefore examined whether and when progression of WMH was preceded by changes in FLAIR and DTI values, using neuroimaging assessments at 3 time points for 9 years in 266 participants with SVD. We also studied differences between those with mild versus severe WMH. Finally, we analyzed the degree of WMH progression during follow-up according to baseline FLAIR and DTI measures.

Materials and methods

Study population
The RUN DMC study is a prospective cohort study of elderly with SVD that investigates risk factors and clinical consequences of SVD. The detailed study protocol has been published previously [148]. Of 503 baseline participants, 281
Spatial normalization

The spatial normalization process is described in the online-only Data Supplement and a schematic overview of the spatial normalization process is displayed in Figure I in the online-only Data Supplement.

Normalization of FLAIR values

To account for inter-individual differences in FLAIR intensities, we normalized the FLAIR values [32]. We calculated the mean (μFLAIR) and SD (σFLAIR) of FLAIR values in the NAWM for each participant and defined a z-score per voxel (\(z\)). The same procedure was applied to follow-up FLAIR images.

Definition of regions

We created 4 masks for each participant: (1) remaining NAWM through all three MRI assessments; (2) NAWM converting into WMH in the second follow-up period (i.e. between 2011 and 2015; in other words converting into WMH after at least 5 years); (3) NAWM converting into WMH in the first follow-up period (i.e. between 2006 and 2011; in other words converting into WMH within 5 years); and (4) WMH at baseline (Fig. 1). For mask (1), we created a binary NAWM mask by subtracting the baseline and incident WMH masks from the baseline WM mask. Mask (2) was created by subtracting the mask with incident WMH voxels in the first time-period from the mask with incident WMH voxels over the entire follow-up. Mask (3) was created by subtracting the baseline WMH map from the first follow-up WMH map. For mask (4) we used the baseline WMH mask.

Statistical analysis

We used t-tests to compare mean baseline FLAIR, FA and MD values in (1) remaining NAWM with values in (2) NAWM voxels converting into WMH in the second follow-up period, (3) NAWM converting into WMH in the first follow-up period, and (4) WMH. We additionally compared baseline FLAIR, MD and FA values in NAWM converting into WMH and in WMH with values in remaining NAWM for all time points separately (i.e. 2006-2011; 2011-2015; and overall 2006-2015) in order to validate the results. Further, we stratified by the modified Fazekas scale (mild: Fazekas 0-1; and severe: Fazekas 2-3 [151]) to evaluate whether changes in microstructural integrity preceding WMH progression differed between participants with mild versus severe baseline WMH. We used one-way ANOVA to investigate whether baseline FLAIR and DTI values in the previously mentioned four areas differed between participants with mild versus severe WMH. Additionally, we used repeated-measures ANOVA to investigate changes in MD values over time in the four areas. To investigate associations between baseline MD and FLAIR values and severity of WMH progression, we calculated quintiles of
CHAPTER 4 WHAT PRECEDED WHITE MATTER HYPERINTENSITIES?

Baseline MD and FLAIR values and analyzed WMH progression according to these strata, by one-way ANOVA adjusted for age and sex, followed by a Bonferroni correction, and tested continuous linear trend per stratum. Statistical analyses were performed using Matlab version 2014b and SPSS Statistics version 20.

Results

Baseline characteristics of the study population are presented in Table 1. Mean age at baseline was 62.5 (SD 7.8) years. Mean follow-up duration until first follow-up assessment was 5.4 (SD 0.2) years and 8.7 (SD 0.2) years until second follow-up assessment. Median WMH volume progressed from 2.2 ml (IQR 0.8–6.1 ml) at baseline to 2.8 ml (IQR 1.2–7.5 ml) at first follow-up and to 4.7 ml (IQR 2.0–11.5 ml) at second follow-up. We observed lacunes in 52 participants (19.0%) at baseline and incident lacunes in 20.3% of participants. Presence of lacunes was more frequent in participants with severe WMH compared with participants with mild WMH (49.1% versus 31.8%; p<0.001). Participants who had not completed follow-up assessment were significantly older at baseline, had more vascular risk factors, and more severe SVD characteristics (Supplementary Table 1).

Baseline FLAIR and DTI parameters in areas converting into WMH

In Figure 2A, differences in baseline FLAIR, MD and FA values are shown between the 4 areas. Compared with remaining NAWM areas through all 3 time points, NAWM areas converting into WMH in the second time-interval had higher normalized FLAIR intensity and higher MD, but similar FA values. NAWM areas converting into WMH in the first time-interval had higher normalized FLAIR intensity, higher MD, and lower FA values compared with remaining NAWM. In contrast, when compared with WMH, these NAWM areas converting into WMH in the first time-period had lower normalized FLAIR intensity and higher FA, but MD values were similar between areas of WMH and areas converting into WMH in the first 5 years. Comparable differences in baseline FLAIR and DTI values were observed between the 4 areas when the follow-up periods were investigated separately (Supplementary Table 2).

Baseline FLAIR and DTI parameters by WMH severity

Figure 2B illustrates baseline FLAIR and DTI measures in the 4 areas stratified by baseline WMH severity. Participants with Fazekas 2-3 had significantly higher normalized FLAIR and MD and lower FA values than those with Fazekas 0-1, in all WM areas, including the NAWM (p<0.005 for all comparisons). MD values in NAWM converting into WMH in the second time-period in participants with

<table>
<thead>
<tr>
<th>Table 1 Characteristics of the study population</th>
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<tbody>
<tr>
<td>Study population (n=266)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Demographics</td>
</tr>
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</tr>
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</tr>
<tr>
<td>MMSE score</td>
</tr>
<tr>
<td>Education, years</td>
</tr>
<tr>
<td>Vascular risk factors</td>
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<tr>
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<tr>
<td>Diabetes, no</td>
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<tr>
<td>Hypercholesterolemia, no</td>
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<td>Smoking, ever, no</td>
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<td>Alcohol, glasses/week</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
</tr>
<tr>
<td>Imaging characteristics</td>
</tr>
<tr>
<td>Total brain volume, ml</td>
</tr>
<tr>
<td>Grey matter volume, ml</td>
</tr>
<tr>
<td>White matter volume, ml</td>
</tr>
<tr>
<td>WMH volume, ml</td>
</tr>
<tr>
<td>Lacunes, no</td>
</tr>
<tr>
<td>Microbleeds, no</td>
</tr>
<tr>
<td>NAWM MD, 10⁻³ mm²/s</td>
</tr>
<tr>
<td>NAWM FA</td>
</tr>
</tbody>
</table>

Data represent mean ± SD, number of participants (%), or median (IQR). Comparisons between participants with Fazekas 0-1 and Fazekas 2-3 were performed by t-test, Chi square or Mann-Whitney-U test. No: number of participants; MMSE: Mini-Mental State Examination; WMH: white matter hyperintensities; NAWM: normal appearing white matter; MD: mean diffusivity; FA: fractional anisotropy.

Fazekas score 2-3 were similar to MD values in NAWM converting into WMH in the first time-period and to WMH in participants with Fazekas 0-1.

Changes in MD values over time

In Figure 3, changes in MD over time are shown for the 4 areas. The MD value in areas of remaining NAWM remained constant over time (Figure 3, diamonds). MD values in persisting WMH during the 9-year course continued to increase over time (circles in Figure 3; p<0.001 for all time-periods). Interestingly, MD values in
NAWM converting into WMH within the first 5 years were similar to MD values in persisting WMH at baseline and continued to increase over time (Figure 3, triangles; p<0.005 for all repeated measures ANOVAs). MD values in NAWM converting into WMH between 5 and 10 years were slightly less elevated at baseline compared to WMH, but continuously increased over time (p<0.005 for all time-periods), reaching the level of MD in WMH areas at first follow-up in 2011 (Figure 3, squares).

**WMH progression according to baseline FLAIR and DTI parameters**

We evaluated the progression of WMH at different levels of baseline FLAIR and DTI parameters by dividing the groups into quintiles. Cut-off values of quintiles were 0.816; 0.833; 0.848 and 0.874 *10^{-3} \text{mm}^2/\text{s} for MD values and 315; 322; 329 and 336 for FLAIR values. The degree of WMH progression according to baseline MD and FLAIR strata in NAWM is presented in Figure 4. Higher baseline MD values in NAWM were associated with increased WMH progression over nine years (mean difference highest versus lowest quintile: 0.73ml/y; 95%CI 0.26-1.19; p<0.001

Intensities of FLAIR, FA and MD values at baseline in a) remaining NAWM; b) NAWM converting into WMH in the second follow-up period; c) NAWM converting into WMH in the first follow-up period; d) WMH. Figure A: Overall baseline FLAIR intensities and FA and MD values (mean with 95%CI); statistical differences between the four areas are calculated by t-tests. Figure B: Baseline FLAIR intensities, FA and MD values (mean with 95%CI) stratified by baseline Fazekas scores (Fazekas 0-1: squares; Fazekas 2-3: diamonds); statistical differences between participants with Fazekas 0-1 and Fazekas 2-3 are calculated for the four areas separately, by one-way ANOVA. *p<0.05; **p<0.01; ***p<0.001.

![Figure 1 Progression of white matter hyperintensities and definition of regions](image-url)

Left: WMH progression during follow-up in a single representative patient: WMH at baseline (yellow), at first follow-up (green), and at second follow-up (blue). Right: Overview of the four masks included in analyses: a) remaining NAWM; b) NAWM converting into WMH in the second follow-up period; c) NAWM converting into WMH in the first follow-up period; d) WMH.

![Figure 2 Baseline FLAIR and DTI parameters in regions preceding WMH](image-url)
these findings by adding an additional time point with a total follow-up of 9 years, which enabled us to distinguish between NAWM converting into WMH within the first and the second time-period. We observed that baseline MD values were higher in NAWM areas converting into WMH within 5 years than in NAWM areas that converted into WMH between 5 and 9 years. Besides, MD values continued to increase over time. The continuously ongoing decline of microstructural integrity within the WM underlines that WMH progression visible on conventional FLAIR imaging is only the “tip of the iceberg” with underlying loss of microstructural integrity that can only be visualized using more advanced neuroimaging techniques.

Discussion

In this longitudinal study with 3 imaging assessments during 9 years, we observed that impaired microstructural integrity in the NAWM preceded conversion into WMH and that WM microstructural integrity declined over time. Participants with severe baseline WMH showed more loss of structural integrity compared with participants with mild WMH in all areas of the WM, including the remaining NAWM. These results suggest that WMH progression is an ongoing process characterized by loss of WM microstructural integrity occurring years before WMH can be detected on conventional MRI.

These results are in line with 2 longitudinal studies showing changes in baseline DTI and FLAIR signal intensities that were related to incident WMH at follow-up, with mean follow-up durations of 3.5 and 3.7 years [31, 32]. Here, we extended...
Participants with severe baseline WMH showed higher normalized FLAIR and MD values and lower FA values than participants with mild baseline WMH in all areas, including the remaining NAWM. Differences between participants with mild versus severe baseline WMH have been reported before with respect to progression of their WMH, suggesting heterogeneity in pathogenesis of mild versus severe WMH [60, 97, 110]. Our observed differences in microstructural integrity between participants with mild and severe baseline WMH in both remaining NAWM and WMH confirm and extend this hypothesis. The observation of impaired microstructural integrity in remaining NAWM in participants with baseline Fazekas 2-3 indicates that NAWM is not as normal one would expect from conventional MRI, and underlines the hypothesis of a continuous, ongoing disease of the WM in SVD. Moreover, it might be an explanation for the observation that WMH progression was most pronounced in participants with severe WMH at baseline [110].

The findings that the microstructural integrity within WMH was more impaired in participants with severe baseline WMH than in participants with mild baseline WMH and also continuously declined over time within WMH in both groups, suggest that not only NAWM, but also WMH is heterogeneous. This heterogeneity of microstructural integrity in WMH might explain clinical variances observed in subjects with similar SVD severity. For example, participants with impaired WMH microstructural integrity might have more severe clinical symptoms compared with participants with the same degree of WMH, but with higher microstructural integrity in their WMH. This hypothesis is supported by findings in patients with SVD, showing that patients with severe loss of microstructural integrity within their WMH showed decreased cognitive performance and had higher risk of parkinsonian signs, independent of WMH volume [158, 159].

Our findings can be clinically relevant because differences in microstructural integrity between remaining NAWM and incident WMH suggest that it would be possible to predict which NAWM voxels will convert into WMH, based on baseline FLAIR and DTI values. Our observation that impaired microstructural integrity at baseline was associated with more extensive WMH progression during 9 years implies that measures of microstructural integrity can possibly be used as biomarker for WMH progression as well as for personalized treatment approaches. Increasing evidence suggests that endothelial dysfunction might be an important factor in the pathogenesis of SVD because loss of WM microstructure has been associated with reduced endothelial function [160]. Although speculative, potential treatment strategies might target the brains’ microvascular endothelium with endothelin antagonists or nitric oxide donors [161], although randomized controlled trials are required. For now, we would argue a stringent control of vascular risk factors according to current guidelines, until further data from randomized controlled trials are available.

Major strengths of this study include the longitudinal design of our study with 3 neuroimaging assessments during 9 years, which enabled us to elaborate on the temporal dynamics of WMH progression. Furthermore, the availability of these 3 time points enabled us to validate the findings in the other follow-up periods.

Several methodological issues and limitations deserve consideration. First, differences in MRI scanner and FLAIR sequences between baseline and first follow-up might be a potential source of bias because this might have led to differences in co-registrations of the FLAIR images and WMH masks to MNI space between baseline and first and second follow-up images. However, we consider this unlikely because we performed several analyses to estimate and minimize the effects of possible interscan effects. Co-registrations of all images to MNI space have been performed with great caution using an intermediate subject-template and the most robust and accurate registration routines [162]. Besides, in additional analyses we only used those WMH voxels that were classified as WMH both at baseline and at follow-up imaging, reducing the risk of bias because of possible overestimation of WMH at baseline. These analyses revealed similar results (data not shown). Moreover, we validated our findings by repeating analyses for all time-periods separately (Supplementary Table 1). The results for the first follow-up and the overall time-interval were comparable with the results for the second follow-up period in which scanner and sequence protocols remained identical. Hence it is unlikely that change in scanner between baseline and first follow-up biased the results. Second, so-called partial volume effects might have introduced a potential bias, that is, NAWM voxels around the border of existing WMH can contain a fraction of WMH signal intensity that affects measurements in that voxel. It is possible that this has led to an overestimation of intensities in the masks consisting of NAWM voxels that converted into WMH during follow-up when WMH progression occurred adjacent to already existing WMH. However, it cannot explain all differences, especially because also NAWM voxels remote from preexisting WMH were included in the masks. Third, because of the long-term follow-up of our study a proportion of the participants was unable to complete the entire follow-up. This attrition bias might have led to an underestimation of the effects because those lost to follow-up had more severe SVD already at baseline (Supplementary Table 1), and severity of WMH at baseline is associated with progression of WMH [60, 97, 110]. A fourth limitation might be that we would need even more advanced neuroimaging protocols to visualize the underlying mechanisms of final conversion of NAWM into WMH because MD values in NAWM
that converted into WMH within 5 years were similar to MD values in WMH. Otherwise, it might be that other processes such as secondary neurodegeneration, hypoperfusion, inflammation or small acute infarctions play a role in the final conversion into WMH. Studies with more follow-up ascertainment on shorter time-intervals may shed more light on the underlying processes of the final conversion into WMH. Because there is increasing awareness that SVD exerts its clinical effects by affecting remote brain structures [49, 147, 163, 164], future studies should also address the role of GM atrophy and how it is affected by WMH progression. In addition, it would be of interest to study changes in microstructural integrity preceding incident lacunes, since the presence of lacunes plays an important role in the development of cognitive deficits as well [165, 166].

In conclusion, WMH progression visible on conventional FLAIR imaging is only the “tip of the iceberg” with underlying loss of WM microstructural integrity that continuously declines over time. These findings indicate that microstructural measures derived from DTI can predict the development of WMH years before they are visible on conventional neuroimaging. Future studies should elaborate on the possibility to use these measures of microstructural integrity for personalized treatment approaches.

Supplementary material

Supplementary methods
Spatial normalization
A schematic overview of the spatial normalization process is displayed in Suppl. Fig. 1. We created a T1-weighted subject-template from the T1-weighted scans of all three time points(1) using ‘buildtemplateparallel’, part of Advanced Neuroimaging Tools (ANTs) (http://stnava.github.io/ANTs/) [167], and registered the subject-template to atlas space (2) using ‘antsRegistrationSyN’ [167, 168]. All T1-weighted images were co-registered to the subject-template and subsequently to atlas-space (3) by ‘ApplyTransforms’ using the affine matrix and warp image from step (1) and (2), resulting in three T1-weighted images in atlas-space.

The B0-images, along with the MD and FA maps, were registered to the T1-weighted images (4) using ‘epi_reg’, part of FMRIB’s Linear Image Registration Tool (FLIRT), FSL v5.0.9 (http://www.fmrib.ox.ac.uk/fsl/) [169, 170]. The FLAIR images, together with the binary WMH masks, were registered to the T1-weighted images using ‘antsRegistrationSyN’ (5). All intermediate images were co-registered to the subject-template and subsequently to atlas-space, using ‘ApplyTransforms’.
CHAPTER 4 WHAT PRECEDED WHITE MATTER HYPERINTENSITIES?

Supplementary Figure 1 Schematic overview of the normalization process

Supplementary Table 1 Baseline characteristics of the study population versus those lost to follow-up

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Study population (n=266)</th>
<th>Lost to follow-up (n=237)</th>
<th>Significance, p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>62.5 ± 7.8</td>
<td>69.2 ± 8.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male sex, number of participants</td>
<td>157 (59.0)</td>
<td>127 (53.6)</td>
<td>0.177</td>
</tr>
<tr>
<td>MMSE score</td>
<td>28.6 ± 1.3</td>
<td>27.6 ± 1.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Education, years</td>
<td>10.1 ± 1.5</td>
<td>9.5 ± 2.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vascular risk factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension, number of participants</td>
<td>181 (68.0)</td>
<td>188 (79.3)</td>
<td>0.005</td>
</tr>
<tr>
<td>Diabetes, number of participants</td>
<td>28 (10.5)</td>
<td>47 (19.8)</td>
<td>0.002</td>
</tr>
<tr>
<td>Hypercholesterolemia, number of participants</td>
<td>112 (42.1)</td>
<td>125 (52.7)</td>
<td>0.020</td>
</tr>
<tr>
<td>Smoking, ever, number of participants</td>
<td>191 (71.8)</td>
<td>162 (68.4)</td>
<td>0.496</td>
</tr>
<tr>
<td>Alcohol, glasses/week</td>
<td>8.3 ± 9.1</td>
<td>7.5 ± 9.6</td>
<td>0.346</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>27.1 ± 4.1</td>
<td>27.2 ± 4.2</td>
<td>0.664</td>
</tr>
<tr>
<td>Imaging characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total brain volume, ml</td>
<td>1087.1 ± 69.8</td>
<td>1031.6 ± 81.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Grey matter volume, ml</td>
<td>621.2 ± 48.6</td>
<td>589.4 ± 51.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>White matter volume, ml</td>
<td>465.8 ± 38.6</td>
<td>442.2 ± 50.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>WMH volume, ml</td>
<td>2.2 (0.8 – 6.1)</td>
<td>7.3 (2.6 – 15.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lacunes, number of participants</td>
<td>52 (19.5)</td>
<td>80 (33.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Microbleeds, number of participants</td>
<td>34 (12.8)</td>
<td>49 (20.7)</td>
<td>0.012</td>
</tr>
</tbody>
</table>

Data represent mean ± SD, nr of participants (%) or median (IQR). Comparisons between participants and those lost to follow-up were performed by t-test, Chi-square or Mann-Whitney-U test.
Supplementary Table 2 FLAIR and DTI parameters in areas preceding WMH for all time-periods

<table>
<thead>
<tr>
<th></th>
<th>NAWM</th>
<th>NAWM converting into WMH</th>
<th>WMH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intensity</td>
<td>p-value</td>
<td>Intensity</td>
</tr>
<tr>
<td>2006 - 2015</td>
<td>FLAIR, $z$-score</td>
<td>-0.01 ± 0.02</td>
<td>Ref</td>
</tr>
<tr>
<td></td>
<td>MD, $10^{-3}$ mm$^2$/s</td>
<td>0.85 ± 0.04</td>
<td>Ref</td>
</tr>
<tr>
<td></td>
<td>FA</td>
<td>0.35 ± 0.02</td>
<td>Ref</td>
</tr>
<tr>
<td>2006 - 2011</td>
<td>FLAIR, $z$-score</td>
<td>-0.01 ± 0.02</td>
<td>Ref</td>
</tr>
<tr>
<td></td>
<td>MD, $10^{-3}$ mm$^2$/s</td>
<td>0.85 ± 0.04</td>
<td>Ref</td>
</tr>
<tr>
<td></td>
<td>FA</td>
<td>0.35 ± 0.02</td>
<td>Ref</td>
</tr>
<tr>
<td>2011 - 2015</td>
<td>FLAIR, $z$-score</td>
<td>-0.02 ± 0.02</td>
<td>Ref</td>
</tr>
<tr>
<td></td>
<td>MD, $10^{-3}$ mm$^2$/s</td>
<td>0.84 ± 0.04</td>
<td>Ref</td>
</tr>
<tr>
<td></td>
<td>FA</td>
<td>0.38 ± 0.02</td>
<td>Ref</td>
</tr>
</tbody>
</table>

Intensities of FLAIR and FA and MD values in areas preceding WMH. Values represent mean ± SD. Statistical differences are calculated by t tests, comparing mean FLAIR, MD, and FA values of NAWM converting into WMH versus NAWM, and of WMH versus NAWM. FLAIR values are normalized to the mean NAWM FLAIR intensity and presented as $z$-scores.
Chapter 5

Plasma amyloid beta levels and severity and progression of cerebral small vessel disease

Published as:

Plasma Aβ (amyloid-beta) levels and severity and progression of small vessel disease

Stroke. 2018;49:884-890
CHAPTER 5 PLASMA AMYLOID BETA AND CEREBRAL SMALL VESSEL DISEASE

Abstract

Background and purpose: Cerebral small vessel disease (SVD) is a frequent pathology in aging and contributor to the development of dementia. Plasma amyloid beta (Aβ) levels may be useful as early biomarker, but the role of plasma Aβ in SVD remains to be elucidated. We investigated the association of plasma Aβ levels with severity and progression of SVD markers.

Methods: We studied 487 participants from the RUN DMC study of whom 258 participants underwent three MRI assessments over nine years. We determined baseline plasma Aβ38, Aβ40 and Aβ42 levels using ELISAs. We longitudinally assessed volume of white matter hyperintensities (WMH) semi-automatically, and manually rated lacunes and microbleeds. We analyzed associations between plasma Aβ and SVD markers by ANCOVA adjusted for age, sex and hypertension.

Results: Cross-sectionally, plasma Aβ40 levels were elevated in participants with microbleeds (mean 205.4 vs. 186.4 pg/ml; p<0.01) and lacunes (194.8 vs. 181.2 pg/ml; p<0.05). Both Aβ38 and Aβ40 were elevated in participants with severe WMH (Aβ38 25.3 vs. 22.7 pg/ml; p<0.01; Aβ40 201.8 vs. 183.3 pg/ml; p<0.05). Longitudinally, plasma Aβ40 levels were elevated in participants with WMH progression (mean 194.6 vs. 182.9 pg/ml; p<0.05). Both Aβ38 and Aβ40 were elevated in participants with incident lacunes (Aβ38 24.5 vs. 22.5 pg/ml; p<0.05; Aβ40 194.9 vs. 181.2 pg/ml; p<0.01), and Aβ42 in participants with incident microbleeds (62.8 vs. 60.4 pg/ml; p<0.05).

Conclusions: Plasma Aβ levels are associated with both presence and progression of SVD markers, suggesting that Aβ pathology might contribute to the development and progression of SVD. Plasma Aβ levels might thereby serve as inexpensive and non-invasive measure for identifying individuals with increased risk for progression of SVD.

Introduction

Cerebral small vessel disease (SVD) is frequently seen on neuroimaging of elderly as white matter hyperintensities (WMH), lacunes and microbleeds [5], and is recognized as the most important vascular contributor to the development of dementia [3, 4]. Hypertensive arteriopathy and cerebral amyloid angiopathy (CAA) are the most common causes of SVD [8], which are distinguished based on both the localization of the lesions and the distribution of neuroimaging characteristics, i.e. whereas CAA predominantly affects cortical arteries and hence is characterized by lobar microbleeds, hypertensive arteriopathy typically affects small perforating end arteries in deep brain areas and is characterized by deep microbleeds [9-11, 13].

Along with traditional vascular risk factors such as hypertension, amyloid β (Aβ) has emerged as contributor to SVD. Aβ has previously been associated with Alzheimer’s Disease (AD) and CAA, albeit in different forms. In AD, Aβ accumulates in the brain to form amyloid plaques, predominantly composed of Aβ42 peptides. This is in contrast to CAA in which Aβ accumulates in the blood vessels, mostly composed of Aβ40 peptides. Several cross-sectional studies have suggested a role for circulating Aβ in SVD, reporting elevated plasma Aβ levels in participants with SVD [33-36]. Prospective studies would help describe the associations between Aβ levels and SVD over time. One longitudinal study reported associations between plasma Aβ levels and WMH progression over time, although they did not find any cross-sectional associations [171]. Recent studies reported associations between plasma Aβ and hypertension [34, 36, 172, 173], suggesting that these etiologies might interact. It remains to be investigated whether plasma Aβ levels are differentially associated with CAA or hypertensive SVD.

In this study we investigated the association of baseline levels of plasma Aβ38, Aβ40 and Aβ42 peptides with both severity and progression of SVD markers, independent of hypertension. To assess whether plasma Aβ should be considered either a general SVD marker or specific for CAA, we examined Aβ levels by distribution of microbleeds.

Materials and Methods

Study population
The Radboud University Nijmegen Diffusion tensor and Magnetic resonance imaging Cohort (RUN DMC) study is a prospective cohort study of 503 non-demented elderly with SVD, that investigates risk factors and clinical consequences of SVD.
The detailed study protocol including the inclusion and exclusion criteria has been published previously [148]. In short, all consecutive patients referred to the Neurology department who underwent diagnostic neuroimaging were selected for participation. Inclusion criteria were age between 50 and 85 years and SVD on neuroimaging. SVD was defined as the presence of any WMH or lacunes of presumed vascular origin on brain imaging [174].

Of 503 participants, twelve were excluded based on quality of plasma analyses: five participants because their Aβ levels for at least one out of three Aβ peptides were below detection limits and seven participants because the coefficients of variation for Aβ measures were higher than 10%. Four participants were additionally excluded because of insufficient scan quality, yielding a final sample of 487 participants for the present study. Of these, 258 participants underwent repeated MRI assessment at three time points (baseline in 2006, first follow-up in 2011 and second follow-up in 2015) and could be included for longitudinal analyses. The Medical Review Ethics Committee region Arnhem-Nijmegen approved the study and all participants gave written informed consent. The data that support the findings of this study are available from the corresponding author upon request.

Plasma analyses
We collected blood samples from all participants at baseline in 2006. EDTA plasma was separated from whole-blood samples (10 ml blood with EDTA acting as anti-coagulant) by routine methods. These samples were stored at -80°C and were only thawed immediately before Aβ quantification. We determined plasma levels of Aβ1-38, Aβ1-40 and Aβ1-42 isoforms (hereafter called Aβ38, Aβ40 and Aβ42) by enzyme-linked colorimetric immunosorbent assay (ELISA; EUROIMMUN AG, Lübeck, Germany). No samples needed further dilution. Details on the assay procedure have been described earlier [175]. The operators were trained with a familiarization panel immunosorbent assay (ELISA; EUROIMMUN AG, Lübeck, Germany) to get acquainted with the assay protocol and sample manipulation.

Neuroimaging protocols
Images were acquired at three time points on 1.5-Tesla MRI (2006: Siemens, Magnetom Sonata; 2011 and 2015: Siemens, Magnetom Avanto) and included the following whole brain scans: T1-weighted 3D MPRAGE, FLAIR and T2*-weighted MRI scans according to the STRIVE criteria [5] by two trained raters blinded for clinical data. Intra and inter-rater agreement was good (weighted kappa 0.87 and 0.95 respectively for WMH and 0.85 and 0.86 for microbleeds) [152]. We also assessed the distribution of microbleeds, distinguishing lobar from deep microbleeds, since lobar microbleeds are considered a hallmark of CAA and deep or infratentorial microbleeds are hypothesized to result from hypertensive or atherosclerotic microangiopathy [9-11]. Incidence was expressed as number of participants with any new microbleeds or lacunes at nine-year follow-up. Baseline and follow-up scans were checked side-by-side to verify incidence of microbleeds or lacunes.

Vascular risk factors
We assessed presence of hypertension, smoking, alcohol use, diabetes and hypercholesterolemia at baseline by standardized questionnaires, as described previously [148]. We defined hypertension as the use of antihypertensive agents and/or systolic blood pressure greater than or equal to 140 mm Hg and/or diastolic blood pressure greater than or equal to 90 mm Hg [148].

Statistical analysis
We assessed associations between plasma Aβ38, Aβ40 and Aβ42 levels and SVD markers by one-way ANOVA, followed by a Bonferroni correction in order to correct for multiple comparisons. In cross-sectional analyses we examined differences in plasma Aβ levels for participants with or without microbleeds or lacunes at baseline. In longitudinal analyses we assessed differences in plasma Aβ levels for participants with or without incident microbleeds or lacunes during follow-up.
We used linear regression analyses to examine associations between plasma \( A\beta \) levels and both WMH severity at baseline and WMH progression over time.

We used four models to investigate the associations between plasma \( A\beta \) levels and SVD markers: Model 1: unadjusted; Model 2: adjusted for age and sex. In Model 3 we additionally adjusted for hypertension to examine whether the associations between plasma \( A\beta \) levels and SVD markers were independent of hypertension status. In Model 4 we additionally adjusted for total brain volume or atrophy to examine whether the associations were independent of neurodegeneration. In analyses on longitudinal data we also adjusted for follow-up duration and baseline severity of SVD in Models 2, 3 and 4.

We additionally analyzed the associations between the ratios of plasma \( A\beta_{38}/A\beta_{40}, A\beta_{38}/A\beta_{42} \) and \( A\beta_{42}/A\beta_{40} \) and SVD markers, as ratios might enhance our understanding of underlying mechanisms. To further investigate the role of hypertension, we investigated whether plasma \( A\beta \) levels were associated with hypertension status, by one-way ANOVA followed by Bonferroni correction.

To quantify the strength of the associations between plasma \( A\beta \) levels and SVD markers, we analyzed \( A\beta \) levels in quintiles of their distribution and tested continuous linear trend per stratum. We analyzed odds ratios (OR) of presence of microbleeds and lacunes by quintiles of \( A\beta \) using logistic regression analyses, adjusted for age and sex. We calculated WMH volumes by quintiles of \( A\beta \) levels and displayed median, 25th, and 75th percentile values as box, and lower and upper adjacent values as whiskers.

To assess whether plasma \( A\beta \) should be considered either a general SVD marker or a specific marker for CAA, we also analyzed the relation between plasma \( A\beta \) levels and microbleeds, stratified by their location (i.e. lobar vs. deep or infratentorial) by one-way ANOVA, followed by a Bonferroni correction.

Statistical analyses were performed using SPSS Statistics version 20.

### Results

Demographics, imaging characteristics and plasma \( A\beta \) levels of the study population are shown in Table 1. At baseline, 81 participants (16.6%) had microbleeds, 132 (27.1%) lacunes and 163 (33.5%) had moderate to severe WMH. We observed incident microbleeds in 49 participants (19.0%), incident lacunes in 62 (24.0%) and WMH progression in 34 participants (13.2%) over the follow-up of almost nine years.

#### Table 1 Characteristics of the study population

<table>
<thead>
<tr>
<th></th>
<th>Baseline (n=487)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>65.7 ± 8.7</td>
</tr>
<tr>
<td>Male sex, nr of participants</td>
<td>278 (57.1)</td>
</tr>
<tr>
<td>MMSE score</td>
<td>28.1 ± 1.7</td>
</tr>
<tr>
<td>Education &gt; primary school, nr of participants</td>
<td>438 (89.9)</td>
</tr>
<tr>
<td><strong>Imaging characteristics</strong></td>
<td></td>
</tr>
<tr>
<td>Microbleeds, nr of participants</td>
<td>81 (16.6)</td>
</tr>
<tr>
<td>Lacunes, nr of participants</td>
<td>132 (27.1)</td>
</tr>
<tr>
<td>WMH volume, ml</td>
<td>3.5 (1.2 – 10.8)</td>
</tr>
<tr>
<td>Total brain volume, ml</td>
<td>1061.6 ± 80.4</td>
</tr>
<tr>
<td>White matter volume, ml</td>
<td>455.0 ± 46.3</td>
</tr>
<tr>
<td>Grey matter volume, ml</td>
<td>606.7 ± 52.6</td>
</tr>
<tr>
<td><strong>Plasma A\beta levels</strong></td>
<td></td>
</tr>
<tr>
<td>A\beta_{38}, pg/ml</td>
<td>23.5 ± 5.6</td>
</tr>
<tr>
<td>A\beta_{40}, pg/ml</td>
<td>189.5 ± 38.4</td>
</tr>
<tr>
<td>A\beta_{42}, pg/ml</td>
<td>60.9 ± 6.1</td>
</tr>
<tr>
<td>A\beta_{38}/A\beta_{40} ratio</td>
<td>0.13 ± 0.02</td>
</tr>
<tr>
<td>A\beta_{38}/A\beta_{42} ratio</td>
<td>0.39 ± 0.09</td>
</tr>
<tr>
<td>A\beta_{42}/A\beta_{40} ratio</td>
<td>0.34 ± 0.13</td>
</tr>
<tr>
<td><strong>Vascular risk factors</strong></td>
<td></td>
</tr>
<tr>
<td>Hypertension, nr of participants</td>
<td>356 (73.1)</td>
</tr>
<tr>
<td>Smoking, ever, nr of participants</td>
<td>343 (70.4)</td>
</tr>
<tr>
<td>Alcohol, glasses/week</td>
<td>7.9 ± 9.4</td>
</tr>
<tr>
<td>Body mass index</td>
<td>27.1 ± 4.1</td>
</tr>
<tr>
<td>Glucose-lowering drugs, nr of participants</td>
<td>64 (13.1)</td>
</tr>
<tr>
<td>Lipid-lowering drugs, nr of participants</td>
<td>231 (47.4)</td>
</tr>
</tbody>
</table>

Data represent mean ± SD, nr of participants (%) or median (IQR). Hypertension is defined as usage of antihypertensive agents and/or blood pressure equal or higher than 140 systolic or 90 diastolic.
Compared to participants who completed follow-up (n=258), those lost to follow-up were older, more often had hypertension or diabetes, and had more severe SVD characteristics at baseline (Supplementary Table 1).

Plasma Aβ38 and Aβ40 levels, but not Aβ42 levels, were elevated in participants with microbleeds or lacunes, although only the associations with Aβ40 remained significant after adjustments for age, sex, hypertension and total brain volume (Table 2). Additional analyses separating participants with 1 versus >1 microbleeds or lacunes revealed similar results (data not shown). A similar pattern was observed for WMH severity: plasma Aβ38 and Aβ40 levels were positively associated with WMH volume (Table 2). Additional adjustments did not change these associations. Higher Aβ38/Aβ42 ratios and lower Aβ42/Aβ40 ratios were observed in participants with presence of SVD markers, but, except for the association between Aβ38/Aβ42 ratios and WMH, the significance of these associations was lost after adjustment for age, sex and hypertension (Supplementary Table 2). Plasma Aβ38, Aβ40 and Aβ42 levels were elevated in participants with hypertension (Supplementary Table 3).

Figure 1 illustrates a linear increase in the prevalence of microbleeds, lacunes and WMH volume with higher plasma Aβ40 quintiles (p-trend ≤0.001), but not with Aβ38 or Aβ42 quintiles. Participants with highest quintile of plasma Aβ40 levels had both higher risk of microbleeds (OR 3.3, 95%CI 1.5-7.3, p=0.003 unadjusted; OR 2.2, 95%CI 0.95-5.0, p=0.066 age and sex adjusted) and higher risk of lacunes (OR 3.5, 95%CI 1.7-6.9, p=0.001 unadjusted; OR 2.2, 95%CI 1.1-4.6, p=0.029 age and sex adjusted). WMH volume was also higher in highest quintile of plasma Aβ40 (median: 7.2 ml) compared to the lowest quintile (median: 1.9 ml).

Longitudinally, plasma Aβ40 levels were elevated in participants with incident lacunes and were positively associated with WMH progression, although these associations were no longer significant after additional adjustments (Table 2). Plasma Aβ38 levels were elevated in participants with incident lacunes during follow-up and plasma Aβ42 levels were elevated in participants with incident microbleeds (Table 2). These associations remained significant after additional adjustments for age, sex, follow-up duration, hypertension, total brain atrophy and presence of SVD at baseline.

Elevated plasma Aβ40 levels were associated with higher odds of incident lacunes (p-trend=0.002; highest quintile OR 3.0, 95%CI 1.2-7.5, p=0.016 unadjusted; OR 2.5, 95%CI 0.97-6.3, p=0.058 adjusted) and WMH progression (highest quintile median 2.3 ml; lowest quintile median 1.3 ml; p=0.006) over the follow-up (Figure 2). Elevated plasma Aβ38 levels were associated with higher odds of incident lacunes.

Figure 1 Associations between baseline plasma Aβ levels and presence of SVD markers
## Table 2: Plasma Aβ levels by presence and progression of SVD markers

<table>
<thead>
<tr>
<th></th>
<th>Cross-sectional</th>
<th>Longitudinal</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No microbleeds</td>
<td>≥1 microbleeds</td>
<td>No lacunes</td>
<td>≥1 lacunes</td>
</tr>
<tr>
<td></td>
<td>mean ± SD (n=405)</td>
<td>mean ± SD (n=81)</td>
<td>mean ± SD (n=355)</td>
<td>mean ± SD (n=132)</td>
</tr>
<tr>
<td>Aβ levels</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma Aβ38 (pg/ml)</td>
<td>23.2 ± 5.5</td>
<td>25.2 ± 6.1 b</td>
<td>23.1 ± 5.5</td>
<td>247 ± 5.9 b</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.192 (0.104; 0.280) b</td>
</tr>
<tr>
<td>Plasma Aβ40 (pg/ml)</td>
<td>186.4 ± 36.1</td>
<td>215.4 ± 45.3 c, d, f, h</td>
<td>185.6 ± 35.8</td>
<td>200.0 ± 43.2 a, s, e</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.255 (0.168; 0.341) a, e</td>
</tr>
<tr>
<td>Plasma Aβ42 (pg/ml)</td>
<td>60.8 ± 5.9</td>
<td>61.3 ± 6.9</td>
<td>60.9 ± 5.9</td>
<td>60.9 ± 6.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.002 (0.0.87; 0.091)</td>
</tr>
</tbody>
</table>

### Associations between plasma Aβ levels and presence and progression of SVD markers

Plasma Aβ levels (mean ± SD in pg/ml) for participants without vs. with microbleeds or lacunes at baseline (top; n=487) and for participants without vs. with incident microbleeds or lacunes over the course of nine years (bottom; n=258).

Statistical differences are analyzed by one-way ANOVA, followed by a Bonferroni correction. Associations between plasma Aβ levels and WMH are analyzed by linear regression analyses; displayed as unadjusted standardized betas with 95% confidence intervals. *p<0.001, b p<0.01, c p<0.05 unadjusted; d p<0.01, e p<0.05 adjusted for age and sex and additionally for follow-up duration and SVD severity at baseline for analyses on SVD progression; f p<0.01, g p<0.05 additionally adjusted for hypertension; h p<0.01, i p<0.05 additionally adjusted for total brain volume or atrophy.
CHAPTER 5 PLASMA AMYLOID BETA AND CEREBRAL SMALL VESSEL DISEASE

(p-trend=0.045; highest quintile OR 2.9, 95%CI 1.2-7.3, p=0.022 unadjusted; OR 2.5, 95%CI 0.98-6.4, p=0.054 adjusted) and elevated plasma Aβ42 levels with higher odds of incident microbleeds (p-trend=0.041; highest quintile OR 2.2, 95%CI 0.78-5.9, p=0.140 unadjusted; OR 2.2, 95%CI 0.76-6.2, p=0.146 adjusted).

Additional analyses on plasma Aβ levels by spatial distribution of microbleeds (Table 3) revealed significantly elevated plasma Aβ40 levels in participants with both lobar and deep microbleeds (n=17). This was not found for plasma Aβ38 and Aβ42 levels.

<table>
<thead>
<tr>
<th>Table 3 Plasma Aβ levels by distribution of microbleeds</th>
</tr>
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<tbody>
<tr>
<td></td>
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<tr>
<td>------------------------</td>
</tr>
<tr>
<td>Plasma Aβ levels</td>
</tr>
<tr>
<td>Aβ38, pg/ml</td>
</tr>
<tr>
<td>Aβ40, pg/ml</td>
</tr>
<tr>
<td>Aβ42, pg/ml</td>
</tr>
</tbody>
</table>

Plasma Aβ levels are expressed as mean ± SD (in pg/ml) and stratified by distribution of microbleeds. Statistical differences in plasma Aβ levels vs. group without microbleeds, analyzed by one-way ANOVA followed by Bonferroni correction. p<0.05 unadjusted.

Discussion

Our study shows that elevated plasma Aβ levels were associated with neuroimaging markers of SVD, including microbleeds, lacunes and WMH cross-sectionally, and with progression of SVD longitudinally over a follow-up of almost nine years. These findings suggest a relationship between amyloid β pathology and the development and progression of SVD.

Our cross-sectional findings that plasma Aβ levels are elevated in participants with SVD are in line with results from previous cross-sectional studies. The Swedish BioFINDER study reported associations between elevated plasma Aβ40 and Aβ42 levels and WMH and microbleeds [34] and the ADNI study reported elevated Aβ40 levels in participants with lacunar infarctions [36]. Another study in elderly with CAA, Alzheimer’s Disease (AD) and mild cognitive impairment (MCI) reported associations between elevated plasma Aβ40 levels and increased WMH and lacunes in all groups [33]. In the population-based Rotterdam Scan Study elevated plasma Aβ40 and Aβ42 levels were associated with the extent of WMH and the presence of lacunes in APOE 4 carriers only [35]. Unfortunately, we do not have data on APOE genotype for our participants. More recently, associations between elevated plasma Aβ38, Aβ40 and Aβ42 levels and markers of SVD have been reported in two subsamples of the same population-based study [176]. Our results showing elevated plasma Aβ levels in participants with SVD strengthen the evidence for an association between plasma Aβ and SVD cross-sectionally.

The longitudinal design of our study enabled us to elaborate on the directionality of the associations between plasma Aβ levels and cerebrovascular pathology. In our study, progression of WMH and incident microbleeds and lacunes were associated with elevated plasma Aβ levels of one or more of the peptides. In contrast, the Three-City Dijon Study showed that decreased plasma Aβ40 and Aβ42 levels were associated with WMH progression over time, but did not find cross-sectional associations of plasma Aβ with SVD markers [171]. Discrepancies in these results might be explained by the different study populations with variation in SVD burden, different assay designs, or how the analysis of the samples was done. Especially, the multicenter design might have complicated longitudinal SVD assessment [145]. Interestingly, in our study associations were different for different Aβ peptides: elevated Aβ42 levels were associated with incident microbleeds whereas elevated Aβ38 and Aβ40 levels were associated with WMH progression and incident lacunes. This might be explained by the characteristics of the different Aβ peptides as the Aβ42 peptide is known to have more tendency to aggregate compared to the Aβ38 and Aβ40 peptides [177, 178]. Enhanced accumulation of Aβ42 in the media of arterioles [179] might weaken the vessel walls and ultimately lead to hemorrhages [180], although experimental studies would be required to investigate the underlying mechanisms. Altogether, our findings that elevated plasma Aβ levels were not only associated with severity of SVD cross-sectionally but also predicted the progression of SVD over time, suggest that amyloid β pathology - either neurodegeneration or CAA - might contribute to the development and progression of SVD.

Several mechanisms could explain the associations between plasma Aβ levels and SVD. First, plasma Aβ might enhance endothelium-dependent vasoconstriction, leading to cerebral hypoperfusion which in turn might result in WMH and lacunes [181-183]. Second, an inverse relation could also explain our findings, i.e. that cerebral hypoperfusion – or reduced cerebral blood flow – promotes overproduction of Aβ and its secretion into the circulation [184]. Our findings showing associations between plasma Aβ levels and progression of SVD markers...
over time support the first mechanism. Third, a relative overproduction of Aβ might lead to enhanced Aβ accumulation in the media of arterioles [179], weakening the vessel walls and leading to microbleeds. Fourth, SVD might affect plasma amyloid levels through impairment in perivascular clearance [185], leading to vascular amyloid deposits and decreased plasma Aβ levels. Since we found elevated plasma Aβ levels in participants with SVD we consider the latter hypothesis less likely. Finally, the reported associations between plasma Aβ and SVD might also be explained by mixed age-related pathologies, i.e. SVD might interact with Alzheimer’s type neurodegeneration. This hypothesis has been supported by previous studies reporting presence of WMH in AD patients [186, 187], though our findings that associations between plasma Aβ levels and SVD markers were independent of markers for neurodegeneration attenuates this hypothesis.

Our findings indicate that plasma Aβ levels are elevated in participants with hypertension (Supplementary Table 3), which, in turn, is associated with SVD markers (data not shown). This association between hypertension and plasma Aβ is also reported in previous studies [34, 36, 172, 173]. In our analyses we adjusted for hypertension to confirm that the associations between plasma Aβ levels and SVD markers were independent of hypertension. In future it would be interesting to study the interactions between blood pressure, plasma Aβ levels and cerebrovascular pathology in more detail.

Additional analyses on plasma Aβ levels by distribution of microbleeds showed no clear distinction in plasma Aβ levels between deep versus lobar microbleeds, although analyses might have been underpowered. Together with the associations found between plasma Aβ levels and WMH and lacunes, these results suggest that plasma Aβ might be a general cerebrovascular disease marker rather than specific for CAA.

Major strengths of this study include the large number of elderly participants with SVD in whom both plasma Aβ levels and neuroimaging were assessed, and the longitudinal design of the study with a long follow-up duration. The long follow-up duration enabled us to study progression of SVD makers including WMH, lacunes and microbleeds over the course of almost a decade. Furthermore, assessments of SVD neuroimaging markers and Aβ levels were performed blinded to clinical data, limiting the risk of bias. Finally, our study has high external validity for patients with SVD in a general neurology clinic.

Several limitations deserve consideration. First, since we only collected plasma samples at baseline we were not able to perform repeated plasma Aβ measurements. As a consequence, we could not assess the influence of changes in plasma Aβ levels over time on progression of SVD markers. Second, we do not have information on APOE genotype, which precluded us from studying the interaction between APOE status, plasma Aβ levels and SVD markers. Third, attrition bias might have led to an underestimation of the effects. Due to the long-term follow-up of our study a proportion of the participants was unable to complete the entire follow-up which may have reduced power in analyses on progression of SVD markers over time, since those lost to follow-up had more severe SVD already at baseline (Supplementary Table 1).

Summary

In summary, we report associations between plasma Aβ levels and neuroimaging markers of SVD, both cross-sectional on severity of SVD at baseline and longitudinal on progression of SVD over time. These findings suggest a relationship between Aβ and vascular disease. Plasma Aβ levels might thereby serve as measure for identifying individuals with increased risk for progression of SVD.
### Supplementary Table 1 Baseline characteristics of participants who completed follow-up compared to those lost to follow-up

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Completed follow-up (n=258)</th>
<th>Lost to follow-up (n=245)</th>
<th>Significance, p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>62.4 ± 7.7</td>
<td>69.1 ± 8.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male sex, nr of participants</td>
<td>153 (59.3)</td>
<td>131 (53.5)</td>
<td>0.208</td>
</tr>
<tr>
<td>MMSE score</td>
<td>28.6 ± 1.3</td>
<td>27.6 ± 1.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Education &gt; primary school, nr of participants</td>
<td>243 (94.2)</td>
<td>211 (86.1)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Imaging characteristics</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Microbleeds, nr of participants</td>
<td>36 (14.0)</td>
<td>47 (19.2)</td>
<td>0.118</td>
</tr>
<tr>
<td>Lacunes, nr of participants</td>
<td>53 (20.5)</td>
<td>85 (34.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>WMH volume, ml</td>
<td>2.2 ± (0.8 – 5.7)</td>
<td>7.3 ± (2.6 – 16.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total brain volume, ml</td>
<td>1087.2 ± 70.9</td>
<td>1032.2 ± 80.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>White matter volume, ml</td>
<td>465.8 ± 39.5</td>
<td>443.0 ± 49.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Grey matter volume, ml</td>
<td>621.4 ± 49.0</td>
<td>590.2 ± 51.5</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Plasma Aβ levels</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Aβ38, pg/ml</td>
<td>23.0 ± 5.1</td>
<td>23.9 ± 7.3</td>
<td>0.108</td>
</tr>
<tr>
<td>Aβ40, pg/ml</td>
<td>184.5 ± 30.8</td>
<td>193.1 ± 54.9</td>
<td>0.029</td>
</tr>
<tr>
<td>Aβ42, pg/ml</td>
<td>60.9 ± 6.2</td>
<td>61.6 ± 18.7</td>
<td>0.527</td>
</tr>
<tr>
<td>Aβ38/Aβ40 ratio</td>
<td>0.13 ± 0.02</td>
<td>0.12 ± 0.02</td>
<td>0.916</td>
</tr>
<tr>
<td>Aβ38/Aβ42 ratio</td>
<td>0.38 ± 0.09</td>
<td>0.39 ± 0.10</td>
<td>0.264</td>
</tr>
<tr>
<td>Aβ42/Aβ40 ratio</td>
<td>0.35 ± 0.15</td>
<td>0.34 ± 0.15</td>
<td>0.472</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vascular risk factors</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension, nr of participants</td>
<td>178 (69.0)</td>
<td>191 (78.0)</td>
<td>0.026</td>
</tr>
<tr>
<td>Smoking, ever, nr of participants</td>
<td>182 (70.5)</td>
<td>171 (69.8)</td>
<td>0.922</td>
</tr>
<tr>
<td>Alcohol, glasses/week</td>
<td>8.2 ± 9.1</td>
<td>7.7 ± 9.6</td>
<td>0.550</td>
</tr>
<tr>
<td>Body mass index</td>
<td>27.2 ± 4.1</td>
<td>27.1 ± 4.1</td>
<td>0.733</td>
</tr>
<tr>
<td>Glucose-lowering drugs, nr of participants</td>
<td>20 (7.8)</td>
<td>46 (18.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lipid-lowering drugs, nr of participants</td>
<td>112 (43.4)</td>
<td>125 (51.0)</td>
<td>0.090</td>
</tr>
</tbody>
</table>

Data represent mean ± SD, nr of participants (%) or median (IQR). Hypertension is defined as usage of antihypertensive agents and/or blood pressure equal or higher than 140 systolic or 90 diastolic.

### Supplementary Table 2 Plasma Aβ ratios by presence and progression of SVD markers

<table>
<thead>
<tr>
<th></th>
<th>Microbleeds, mean ± SD</th>
<th>Lacunes, mean ± SD</th>
<th>WMH progression, β (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross-sectional</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No microbleeds</td>
<td>0.13 ± 0.02</td>
<td>0.12 ± 0.02</td>
<td>-0.26 (-0.14; 0.09)</td>
</tr>
<tr>
<td>≥1 microbleeds</td>
<td>0.12 ± 0.02</td>
<td>0.12 ± 0.02</td>
<td>0.044 (0.02; 0.06)</td>
</tr>
<tr>
<td>No lacunes</td>
<td>0.13 ± 0.02</td>
<td>0.12 ± 0.02</td>
<td>-0.26 (-0.14; 0.09)</td>
</tr>
<tr>
<td>≥1 lacunes</td>
<td>0.12 ± 0.02</td>
<td>0.12 ± 0.02</td>
<td>0.044 (0.02; 0.06)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Longitudinal</th>
<th>No incident microbleeds, mean ± SD</th>
<th>No incident lacunes, mean ± SD</th>
<th>Incident microbleeds, mean ± SD</th>
<th>Incident lacunes, mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Aβ38/Aβ40 ratio</td>
<td>0.13 ± 0.02</td>
<td>0.12 ± 0.02</td>
<td>-0.018 (-0.13; 0.10)</td>
<td>-0.13 (-0.02; 0.09)</td>
</tr>
<tr>
<td>No Aβ38/Aβ42 ratio</td>
<td>0.38 ± 0.09</td>
<td>0.37 ± 0.09</td>
<td>0.129 (0.00; 0.24)</td>
<td>-0.13 (-0.02; 0.09)</td>
</tr>
<tr>
<td>No Aβ42/Aβ40 ratio</td>
<td>0.34 ± 0.14</td>
<td>0.35 ± 0.15</td>
<td>0.13 ± 0.02</td>
<td>-0.13 (-0.02; 0.09)</td>
</tr>
</tbody>
</table>

Association between plasma Aβ ratios and presence and progression of SVD markers. Plasma Aβ ratios (mean ± SD) for participants without vs. with microbleeds or lacunes at baseline (top; n=487) and for participants without vs. with incident microbleeds or lacunes over the course of nine years (bottom; n=258). Statistical differences are analyzed by one-way ANOVA, followed by a Bonferroni correction. Association between plasma Aβ2/Aβ40 ratio and WMH progression is analyzed by linear regression analyses; displayed as unadjusted standardized betas with 95% confidence intervals. a p<0.001, b p<0.01, c p<0.05.
### Supplementary Table 3  Plasma Aβ levels stratified by hypertension status

<table>
<thead>
<tr>
<th>Plasma Aβ levels</th>
<th>No hypertension (n=131)</th>
<th>Hypertension (n=356)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aβ38, pg/ml</td>
<td>22.0 ± 5.0</td>
<td>24.1 ± 5.7 (^a, c)</td>
</tr>
<tr>
<td>Aβ40, pg/ml</td>
<td>179.3 ± 30.2</td>
<td>193.3 ± 40.5 (^a)</td>
</tr>
<tr>
<td>Aβ42, pg/ml</td>
<td>60.0 ± 6.0</td>
<td>61.3 ± 6.1 (^b, c)</td>
</tr>
</tbody>
</table>

Plasma Aβ levels (in pg/ml) stratified by hypertension status at baseline. Statistical differences are analyzed by one-way ANOVA, followed by a Bonferroni correction. \(^a\) p<0.001, \(^b\) p<0.05 unadjusted; \(^c\) p<0.05 adjusted for age and sex.
Chapter 6

Serum neurofilament light chain levels are related to cerebral small vessel disease burden

Published as:

Serum neurofilament light chain levels are related to small vessel disease burden

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CHAPTER 6

Introduction

Neurofilament Light Chain (NfL) is an emerging blood marker for neuroaxonal damage. A role of serum NfL has been established in multiple neurological diseases affecting the elderly, such as motor neuron disease [188], Alzheimer’s disease [39], and frontotemporal dementia [40]. However, the factors influencing serum NfL levels in the elderly are incompletely understood. Cerebral small vessel disease (SVD) is a highly prevalent condition and a major cause of stroke, vascular cognitive impairment and eventually loss of independence [8]. Recent studies suggest that cerebrovascular pathology has an effect on serum NfL. Elevated serum levels were observed in patients with a recent small subcortical infarct [189] and in patients with stroke caused by cervical artery dissection [190]. Also, NfL levels in CSF correlated significantly with white matter hyperintensity (WMH) load, one MRI marker of SVD burden [191]. However, a detailed account of the relationship between SVD burden and NfL in blood is lacking.

The aim of the current study was to assess the association between serum NfL and SVD burden as assessed by MRI and clinical status. We first analyzed patients with the genetically defined SVD CADASIL (Cerebral Autosomal-Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy). Because these patients show severe SVD already at young age, confounding by other age-related pathologies impacting on serum NfL levels, such as neurodegenerative pathology, can be largely excluded [123]. To validate our findings and to assess their generalizability towards the more common, sporadic form of SVD, we further analyzed a large, independent sample of patients with sporadic SVD.

We hypothesized that serum NfL levels are associated with MRI markers for SVD, i.e. white WMH volume, lacune volume, brain volume, microbleed count and mean diffusivity obtained from diffusion tensor imaging. Clinical characterization included neuropsychological testing in both SVD samples. CADASIL patients were further characterized for focal neurological deficits (NIH stroke scale, NIHSS) and disability (modified Rankin scale, mRS).

Methods

Study participants

CADASIL patients were recruited into the ongoing, prospective ‘VASCAMY’ study. The diagnosis was confirmed by either molecular genetic testing or ultrastructural analysis of skin biopsies. Exclusion criteria were the presence or history for i) diabetes mellitus (because of its pronounced effect on brain structure), ii) other...
known neurological or psychiatric diseases, and iii) clinically apparent stroke within the last three months. Fifty-four patients gave consent for the biobanking procedure and were therefore included in this analysis. All examinations (clinical assessment, neuropsychological testing, blood draw, MRI) were performed on the same day or within 2 consecutive days. One subject was excluded because of missing MRI data (scanner malfunction). The final sample comprised 53 CADASIL patients.

Data for sporadic SVD patients was obtained from the Radboud University Nijmegen Diffusion tensor and Magnetic resonance Cohort (RUN DMC). This prospective study recruited 563 non-demented elderly (age 50-85) with SVD, defined as the presence of lacunes and/or WMH on neuroimaging. Patients were recruited in a hospital-based setting. Clinical examination and blood draw were performed on the same day, and the majority of patients received the MRI scan within 2 weeks. More details can be found in the previously published study protocol [148]. Sixty-four subjects were excluded because of the presence of an old, large vessel territorial infarct (n=55), insufficient MRI quality (n=4) or missing clinical/neuropsychological data (n=5). The final sporadic SVD sample comprised 439 patients.

In addition, healthy control subjects (n=93) were drawn from our biobank repository, where we collect samples from healthy subjects across multiple studies. These subjects were relatives of outpatient clinic patients or volunteers recruited through advertisements. The absence of cerebrovascular events or other neurological symptoms/diagnoses was established through a clinical interview and a neurological examination by a board-certified neurologist.

All measurements were performed blinded from each other. The two SVD samples were subjected to independent statistical analyses. The studies were approved by the ethics committee of the respective institution. Written and informed consent was obtained from all subjects after receiving a complete description of the study.

Clinical characterization
All SVD patients were characterized according to standardized procedures. Clinical severity was assessed using established scales for activities of daily living (Barthel scale), focal neurological deficits (NIH stroke scale [NIHSS]), and disability (modified Rankin scale [mRS]) [192]. The latter two were only available in the CADASIL sample.

Neuropsychological testing
Patients underwent comprehensive neuropsychological testing. In the current study, we focused on processing speed performance, the main cognitive deficit in SVD patients [193, 194]. For CADASIL patients, trail making tests (matrix A and B) were available. For sporadic SVD patients, the 1-letter subtask of the Paper-Pencil Memory Scanning Test and the Letter-Digit Substitution Task were available. For each test, age and education corrected z-scores were first calculated in reference to a healthy control population from the literature [195-197]. The mean of both z-scores was then used as compound processing speed z-score.

Serum biobanking
Blood samples were collected through a standardized procedure. Blood was drawn into serum containers with clotting activator and subsequently allowed to clot for at least 30 minutes at room temperature. Separation of serum was achieved by centrifugation at 2,000g for 10 minutes. Samples were aliquoted in polypropylene screw cap vials and stored deep-frozen until analysis.

Neurofilament light chain (NfL) assay
All samples were analyzed on the same single molecule array instrument (Simoa HD-1, Quanterix, Lexington, MA, USA) in Basel. We used the capture monoclonal antibody (mAB) 47:3, and the biotinylated detector mAB 2:1 (UmanDiagnostics, Umeå, Sweden) [198], transferred onto the Simoa platform. Bovine lyophilized NfL was obtained from UmanDiagnostics. Calibrators ranged from 0 to 2,000 pg/ml. Intra- and inter-assay variability of the assay were below 20%, respectively. The analytical sensitivity was 0.32 pg/ml. All samples produced signals above the analytical sensitivity of the assay.

MRI scanning and analysis
Within a study, all SVD patients were examined on a single MRI scanner (CADASIL: 3 Tesla Siemens Magnetom Verio; sporadic SVD: 1.5 Tesla Siemens Magnetom Sonata, Siemens Healthcare, Erlangen, Germany) with a standardized protocol including 3D-T1, FLAIR, T2, and DTI sequences. Sequence parameters have already been published [199]. We calculated white matter hyperintensity (WMH) volume, lacune volume and brain volume and counted cerebral microbleeds as previously described [199, 200]. For normalization, all volumes were divided by the volume of the intracranial cavity.

Mean diffusivity is an established measure for SVD burden calculated from DTI. It captures the microstructural integrity of white matter through diffusivity of water in the tissue. Diffusion images were first pre-processed to correct for eddy
current induced distortions and motion as previously described [199]. The diffusion tensor was estimated using ‘dtifit’, part of the Functional Magnetic Resonance Imaging of the Brain (FMRIB) Software Library (FSL) [201, 202]. To avoid contamination by CSF partial volume, the analysis of mean diffusivity was restricted to the main fiber tracts. For this purpose, diffusion data were skeletonized as implemented in the tract-based spatial statistics pipeline (TBSS) of FSL [203]. In brief, all subjects’ fractional anisotropy data were aligned into a common space using nonlinear registration and the standard FA template provided within FSL. Each subject’s fractional anisotropy data was then projected onto the tract skeleton in standard space. Finally, mean diffusivity images were projected onto the skeleton using the fractional anisotropy derived projection parameters. The final mean diffusivity skeletons were masked with the standard skeleton thresholded at a value of 0.3 to focus the analysis on main fiber tracts. Furthermore, regions of the skeleton directly adjacent to the ventricles were removed by a custom-made mask to further minimize the contamination of the skeleton by CSF partial volume. Finally, the mean over the entire mean diffusivity skeleton was used for subsequent analyses.

Statistical analyses
Statistical analyses were performed in ‘R’, version 3.1.2 [204]. For group comparisons, we used the non-parametric Wilcoxon rank sum test (with Bonferroni correction for multiple comparisons). The ability of serum NFL to discriminate between SVD patients and healthy controls was assessed using receiver operating characteristic analysis as implemented in the R package ‘ROCR’ (version 1.0-7) [205].

We applied linear regression analysis to assess associations with serum NFL levels as well as processing speed compound scores as dependent variables. All R² values reported are ‘adjusted R²’. Variables were power transformed when necessary to ensure the appropriateness of linear models as indicated by the distribution of residuals. To ensure that regression results were robust and not driven by outliers, we conducted a statistical regression outlier test as implemented in the ‘car’ package of ‘R’ [206]. As a result, two CADASIL patients and two sporadic SVD patients had to be excluded from regression analyses. In order to determine associations with clinical scores (NIHSS and mRS as dependent variables), we used ordinal logistic regression as implemented in the ‘R’ package ‘ordinal’ [207]. For all analyses, correction for multiple testing was performed via the Bonferroni method.

For multiple regression, we first applied lasso (least absolute shrinkage and selection operator) regression for variable selection as implemented in the R package ‘glmnet’ (for linear response) or ‘glmnetcr’ (for ordinal response) with standard parameters [208]. Lasso performs both variable selection and regularization in order to enhance the prediction accuracy and interpretability of regression models. Serum NFL, all MRI markers, age and sex were included as independent variables in lasso regression and variables with non-zero coefficients after cross-validation were carried forward to the final multiple regression model.

Results
Characteristics of SVD patients and healthy controls are presented in Table 1. Patients in both SVD samples, CADASIL and sporadic SVD, were relatively mildly affected as indicated by unimpaired activities of daily living (Barthel scale). Healthy controls covered the entire age range of both SVD samples (30 – 85 years). In both SVD samples, a substantial number of patients had suffered from a prior stroke or transient ischemic attack. Serum NFL levels in these patients were not higher than in patients without history for a cerebrovascular event (CADASIL: p=0.144, sporadic SVD: p=0.190).

Serum NFL is increased in SVD
In comparison with healthy controls, we found increased serum NFL levels in both SVD samples (CADASIL: p=4.1e-06, sporadic SVD: p<1e-15, Wilcoxon rank sum tests with Bonferroni correction) (Figure 1A). These differences were also significant after correction for age (Supplementary Figure, CADASIL: p=4.2e-12, sporadic SVD: p<1e-15). The ability of serum NFL to differentiate between diagnostic groups as assessed by receiver operating characteristics is shown in Figure 1B.

In all groups, serum NFL was positively correlated with age (Table 2) but there was a significant interaction with diagnosis: Patients with sporadic SVD showed a steeper increase of serum NFL levels with age than healthy controls (p=0.011 for interaction).

Serum NFL is associated with MRI markers for SVD
We next assessed associations between serum NFL levels and established MRI markers for SVD as well as age and sex. Simple linear regression analyses (Table 2) showed significant associations between serum NFL and all imaging markers as well as age, both in the CADASIL sample and in the sporadic SVD sample. Regarding SVD imaging markers, the strongest effect was found for mean diffusivity in both samples. Most associations remained significant after controlling for age (Table 2).
CHAPTER 6 SERUM NEUROFILAMENT LIGHT CHAIN AND CEREBRAL SMALL VESSEL DISEASES

Explaining more processing speed variance than serum NfL levels. The associations between the other imaging markers and processing speed were weaker than for serum NfL.

In the CADASIL sample, the lasso regression model including serum NfL, all imaging markers, age and sex as independent variables selected mean diffusivity, lacune volume and microbleed count for the final model. In the sporadic SVD sample, lasso regression selected mean diffusivity and serum NfL (Table 3).

Serum NfL levels are independently related to symptom severity and disability

Using the CADASIL sample, we were also able to assess the association between serum NfL levels and clinical scores capturing focal neurological deficits (NIHSS) and disability (mRS).

**Table 1 Characteristics of the SVD samples**

<table>
<thead>
<tr>
<th></th>
<th>CADASIL (Munich) n=53</th>
<th>Sporadic SVD (RUN DMC) n=439</th>
<th>Healthy controls n=93</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, median (IQR) [years]</td>
<td>56.0 (11.2)</td>
<td>64.3 (15.2)</td>
<td>59 (12.6)</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>36 (67.9)</td>
<td>199 (45.3)</td>
<td>58 (62.4)</td>
</tr>
<tr>
<td>Prior stroke/TIA, n (%)</td>
<td>28 (52.8)</td>
<td>162 (36.9)</td>
<td>0</td>
</tr>
<tr>
<td>Last stroke/TIA, median (IQR) [years]</td>
<td>2.9 (8.9)</td>
<td>0.7 (1.7)</td>
<td>n/a</td>
</tr>
<tr>
<td><strong>Vascular risk factors, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>11 (20.8)</td>
<td>316 (72)</td>
<td>16 (17.2)</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>24 (45.3)</td>
<td>193 (44)</td>
<td>12 (12.9)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0 (0)</td>
<td>59 (13.4)</td>
<td>5 (5.4)</td>
</tr>
<tr>
<td>Current or past smoking</td>
<td>34 (64.2)</td>
<td>304 (69.2)</td>
<td>n/a</td>
</tr>
<tr>
<td><strong>Clinical scores, median (IQR)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barthel scale score</td>
<td>100 (0)</td>
<td>100 (0)</td>
<td>n/a</td>
</tr>
<tr>
<td>Processing speed z-score</td>
<td>1.62 (2.21)</td>
<td>-0.31 (1.58)</td>
<td>n/a</td>
</tr>
<tr>
<td>NIHSS score</td>
<td>0 (1)</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>mRS score</td>
<td>0 (1)</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td><strong>SVD markers, median (IQR)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum NfL [pg/ml]</td>
<td>41.7 (46.9)</td>
<td>50.8 (38.9)</td>
<td>26.0 (14.7)</td>
</tr>
<tr>
<td>WMH volume [%]³</td>
<td>7.52 (7.39)</td>
<td>0.59 (1.23)</td>
<td>n/a</td>
</tr>
<tr>
<td>Lacune volume [%]³</td>
<td>0.024 (0.064)</td>
<td>0 (0)</td>
<td>n/a</td>
</tr>
<tr>
<td>Microbleed count</td>
<td>0 (3)</td>
<td>0 (0)</td>
<td>n/a</td>
</tr>
<tr>
<td>Brain volume [%]³</td>
<td>78.4 (6.82)</td>
<td>65.5 (7.69)</td>
<td>n/a</td>
</tr>
<tr>
<td>Mean diffusivity [10⁻⁴ mm²/s]</td>
<td>9.86 (1.83)</td>
<td>8.02 (0.55)</td>
<td>n/a</td>
</tr>
</tbody>
</table>
CHAPTER 6 SERUM NEUROFILAMENT LIGHT CHAIN AND CEREBRAL SMALL VESSEL DISEASES

For NIHSS, simple ordinal logistic regression showed the strongest association with serum NfL levels (Figure 2B, Table 4). Significant, albeit weaker associations were also found between imaging markers (except for microbleeds) and NIHSS scores. After ordinal lasso regression including serum NfL levels, all imaging markers, age and sex as independent variables, the final model comprised mean diffusivity, brain volume and serum NfL (Table 4).

For disability, simple ordinal logistic regression showed the most significant association with serum NfL levels (Figure 2C, Table 4). Significant, albeit weaker associations were also found between imaging markers, age and sex as independent variables, the final model comprised mean diffusivity, brain volume and serum NfL (Table 4).

| Table 2 Linear regression models with serum NfL levels as dependent variable |
|---|---|---|---|
| **Beta**<sup>†</sup> | **p** | **p|age**<sup>‡</sup> | **R²** |
| **CADASIL** | Mean diffusivity | 0.730 | 1.2e-09<sup>∗</sup> | 1.2e-05<sup>∗</sup> | 0.52 |
| | Age | 0.629 | 7.8e-07<sup>∗</sup> | – | 0.38 |
| | Brain volume<sup>§</sup> | -0.595 | 4.1e-06<sup>∗</sup> | 0.005<sup>∗</sup> | 0.34 |
| | WMH volume<sup>§</sup> | 0.575 | 1.0e-05<sup>∗</sup> | 0.023 | 0.32 |
| | Lacune volume<sup>§</sup> | 0.558 | 2.1e-05<sup>∗</sup> | 6.41e-04<sup>∗</sup> | 0.30 |
| | Microbleed count | 0.366 | 0.008 | 0.021 | 0.11 |
| | Sex | 0.117 | 0.418 | 0.825 | 0 |
| **Sporadic SVD** | Age | 0.558 | <1e-15<sup>∗</sup> | – | 0.31 |
| | Mean diffusivity | 0.461 | <1e-15<sup>∗</sup> | 7.31e-05<sup>∗</sup> | 0.21 |
| | WMH volume<sup>§</sup> | 0.382 | <1e-15<sup>∗</sup> | 1.08e-05<sup>∗</sup> | 0.14 |
| | Brain volume<sup>§</sup> | -0.376 | <1e-15<sup>∗</sup> | 0.248 | 0.14 |
| | Lacune volume<sup>§</sup> | 0.264 | 2.2e-08<sup>∗</sup> | 2.18e-06<sup>∗</sup> | 0.07 |
| | Microbleed count | 0.149 | 0.0019<sup>∗</sup> | 0.007 | 0.02 |
| | Sex | -0.003 | 0.947 | 0.993 | 0 |

NfL, neurofilament light chain; WMH, white matter hyperintensity  
<sup>†</sup>standardized beta,  
<sup>‡</sup>controlled for age,  
<sup>§</sup>normalized to intracranial volume  
<sup>∗</sup>significant after correction for multiple testing (Bonferroni)

For NIHSS, simple ordinal logistic regression showed the strongest association with serum NfL levels (Figure 2B, Table 4). Significant, albeit weaker associations were also found between imaging markers (except for microbleeds) and NIHSS scores. After ordinal lasso regression including serum NfL levels, all imaging markers, age and sex as independent variables, the final model comprised mean diffusivity, brain volume and serum NfL (Table 4).

For disability, simple ordinal logistic regression showed the most significant association between serum NfL levels and mRS scores (Figure 2C, Table 4), and associations between all imaging markers and mRS scores were weaker. The final model after lasso regression comprised serum NfL, brain volume, and (at trend level) microbleed count and mean diffusivity (Table 4).

| Table 3 Linear regression models with processing speed as dependent variable |
|---|---|---|
| **Beta**<sup>†</sup> | **p** | **R²** |
| **CADASIL: Simple linear regression** | Mean diffusivity | -0.626 | 9.0e-07<sup>∗</sup> | 0.38 |
| | Serum NfL | -0.526 | 7.6e-05<sup>∗</sup> | 0.27 |
| | Lacune volume<sup>‡</sup> | -0.521 | 9.0e-05<sup>∗</sup> | 0.26 |
| | Microbleed count | -0.486 | 3.0e-04<sup>∗</sup> | 0.22 |
| | WMH volume<sup>‡</sup> | -0.474 | 4.5e-04<sup>∗</sup> | 0.21 |
| | Brain volume<sup>‡</sup> | 0.374 | 0.007 | 0.12 |
| | Age | -0.240 | 0.091 | 0.04 |
| | Sex | 0.019 | 0.895 | 0 |
| **CADASIL: Multiple linear regression (lasso): p=6.6e-07, R<sup>2</sup>=0.45** | Mean diffusivity | -0.267 | 1.5e-08<sup>∗</sup> | 0.07 |
| | Serum NfL | -0.257 | 4.8e-08<sup>∗</sup> | 0.06 |
| | WMH volume<sup>‡</sup> | -0.207 | 1.3e-05<sup>∗</sup> | 0.04 |
| | Lacune volume<sup>‡</sup> | -0.174 | 2.6e-04<sup>∗</sup> | 0.03 |
| | Brain volume<sup>‡</sup> | 0.173 | 2.7e-04<sup>∗</sup> | 0.03 |
| | Age | -0.149 | 0.0018<sup>∗</sup> | 0.02 |
| | Microbleed count | -0.140 | 0.0035<sup>∗</sup> | 0.02 |
| | Sex | 0.105 | 0.0279 | 0.01 |
| **Sporadic SVD: Simple linear regression** | Mean diffusivity | -0.188 | 2.92e-04 | - |
| | Serum NfL | -0.171 | 9.97e-04 | - |

NfL, neurofilament light chain; WMH, white matter hyperintensity  
<sup>†</sup>standardized beta,  
<sup>‡</sup>normalized to intracranial volume  
<sup>∗</sup>simple regression significant after correction for multiple testing (Bonferroni)
Figure 2  Serum NfL levels are associated with clinical deficits

A) Simple linear regression analysis between serum NfL levels and processing speed in the CADASIL (left panel) and sporadic SVD (right panel) sample. Dashed lines depict the 95% confidence interval for the linear regression.

B, C) In the CADASIL sample, logistic regression analysis showed an independent association between serum NfL levels and NIH stroke scale (NIHSS) scores (B) as well as modified Rankin scale (mRS) scores (C). Associations are visualized using violin plots. Vertical lines depict the median serum NfL levels in each score category. For both scores, values of 3 and 4 were grouped together for visualization because of the low number of patients in these categories. NfL, neurofilament light chain.

Table 4  Logistic regression models with NIHSS and mRS scores as dependent variables in the CADASIL sample

<table>
<thead>
<tr>
<th></th>
<th>OR†</th>
<th>OR 95%CI‡</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NIHSS score: Simple ordinal logistic regression</strong></td>
<td></td>
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<tr>
<td>Serum NfL</td>
<td>4.11</td>
<td>2.16–8.47</td>
<td>4.2e-05*</td>
</tr>
<tr>
<td>Mean diffusivity</td>
<td>3.67</td>
<td>1.93–7.67</td>
<td>1.8e-04*</td>
</tr>
<tr>
<td>Lacune volume§</td>
<td>2.99</td>
<td>1.68–5.80</td>
<td>4.3e-04*</td>
</tr>
<tr>
<td>Brain volume§</td>
<td>0.26</td>
<td>0.11–0.53</td>
<td>6.6e-04*</td>
</tr>
<tr>
<td>WMH volume§</td>
<td>2.53</td>
<td>1.38–4.96</td>
<td>0.004*</td>
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<tr>
<td>Age</td>
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<td>0.99–4.01</td>
<td>0.069</td>
</tr>
<tr>
<td>Microbleed count</td>
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<td>0.89–2.36</td>
<td>0.121</td>
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<tr>
<td>Sex</td>
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<td>0.79–5.43</td>
<td>0.245</td>
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<tr>
<td><strong>NIHSS score: Multiple ordinal logistic regression (lasso)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean diffusivity</td>
<td>3.27</td>
<td>1.23–9.53</td>
<td>0.021</td>
</tr>
<tr>
<td>Brain volumec</td>
<td>0.33</td>
<td>0.11–0.86</td>
<td>0.030</td>
</tr>
<tr>
<td>Serum NfL</td>
<td>2.51</td>
<td>1.10–6.08</td>
<td>0.032</td>
</tr>
<tr>
<td><strong>mRS score: Simple ordinal logistic regression</strong></td>
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<td></td>
</tr>
<tr>
<td>Serum NfL</td>
<td>5.52</td>
<td>2.83–12.0</td>
<td>3.0e-06*</td>
</tr>
<tr>
<td>Mean diffusivity</td>
<td>5.97</td>
<td>2.84–14.4</td>
<td>1.2e-05*</td>
</tr>
<tr>
<td>Brain volume§</td>
<td>0.20</td>
<td>0.08–0.41</td>
<td>7.0e-05*</td>
</tr>
<tr>
<td>WMH volume§</td>
<td>3.47</td>
<td>1.78–7.40</td>
<td>5.5e-04*</td>
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<tr>
<td>Lacune volume§</td>
<td>2.71</td>
<td>1.57–5.10</td>
<td>7.7e-04*</td>
</tr>
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<td>Microbleed count</td>
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<td>2.17–35.9</td>
<td>0.008</td>
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<tr>
<td>Age</td>
<td>2.52</td>
<td>1.33–5.45</td>
<td>0.009</td>
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<tr>
<td>Sex</td>
<td>1.57</td>
<td>0.92–2.72</td>
<td>0.097</td>
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<td><strong>mRS score: Multiple ordinal logistic regression (lasso)</strong></td>
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<tr>
<td>Serum NfL</td>
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<td>1.13–6.63</td>
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<tr>
<td>Brain volumec</td>
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<td>0.029</td>
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<tr>
<td>Microbleed count</td>
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<td>0.99–9.29</td>
<td>0.103</td>
</tr>
<tr>
<td>Mean diffusivity</td>
<td>2.14</td>
<td>0.75–6.21</td>
<td>0.151</td>
</tr>
</tbody>
</table>

NfL, Neurofilament light chain; NIHSS, NIH stroke scale; mRS, modified Rankin scale; WMH, white matter hyperintensity; †cumulative odds ratio, ‡95% confidence interval for the cumulative odds ratio, §normalized to intracranial volume.

*simple regression significant after correction for multiple testing (Bonferroni)
Discussion

Analyzing two independent SVD samples, we found elevated serum NfL levels compared with healthy controls. Serum NfL levels were associated with all MRI markers of SVD burden, in particular mean diffusivity. Regarding clinical symptoms, serum NfL levels were strongly associated with impaired processing speed performance, the cognitive domain most prominently affected in SVD. These findings were consistent across young patients with genetically defined SVD (CADASIL) and patients with sporadic SVD. In CADASIL patients, serum NfL levels were also strongly and independently associated with measures of focal neurological deficits and disability.

Elevated serum NfL is not specific for a particular pathology, as it is released upon neuroaxonal damage of any cause. Recent findings indicate a role of serum NfL in multiple sclerosis [37], motor neuron disease [188] and – particularly important in the elderly – neurodegenerative disorders [38], i.e. Alzheimer’s disease [39] and frontotemporal dementia [40]. Our findings demonstrate that SVD burden is a relevant contributor to elevated serum NfL levels. The increase of serum NfL in SVD compared with healthy controls (approx. 2-fold on average) is equal if not higher than the increase seen for patients with Alzheimer’s disease [38, 39]. The association between SVD burden and serum NfL together with the high prevalence of SVD suggests that SVD needs to be considered when interpreting serum NfL levels in elderly patients.

Our results potentially extend the utility of serum NfL as a marker for SVD burden. To our knowledge, our analysis of serum NfL is the first study to identify a blood based marker associated with both imaging as well as clinical features of SVD. For processing speed performance in the sporadic SVD sample, there was an added value of serum NfL levels beyond MRI markers. Moreover, in both samples, only mean diffusivity explained more variance in processing speed than serum NfL. All other imaging markers of SVD – i.e. WMH, lacune or brain volume – showed a weaker association with processing speed, suggesting that serum NfL might be of equal or even greater utility than these conventional MRI markers.

The strong relationship between DTI measures and vascular cognitive impairment is well established, both in cross-sectional and longitudinal studies [68, 199, 209]. DTI quantifies alterations in the microstructural integrity of brain tissue and this seems to be a major substrate underlying cognitive impairment in SVD [210]. Interestingly, among imaging markers serum NfL levels showed the strongest association with mean diffusivity, suggesting that the two parameters capture a similar disease process: NfL is regarded as a marker for neuroaxonal damage, and damage to axons is also a potential explanation for mean diffusivity changes in the white matter. The high inter-correlation might also explain why the association between serum NfL and processing was not independent from mean diffusivity in the CADASIL sample.

A recent study showed that serum NfL is sensitive to active SVD, i.e. recent subcortical infarcts, and that serum NfL levels remain elevated at least 3 months after the acute infarct [189]. We did not find a difference in serum NfL levels between patients with and without history for stroke or TIA. However, by design, all of the events in CADASIL patients occurred more than 3 months ago. In the same study, serum NfL levels were especially high in patients with incident SVD lesions on follow-up scans. Adding to that study, our results suggest that serum NfL levels also reflect the chronic SVD burden, as e.g. captured by mean diffusivity, as well as the clinical severity.

MRI will remain the gold standard for assessment of SVD burden. Imaging markers have already been refined to serve as surrogate treatment response markers for clinical trials in SVD [68, 199, 211]. However, there are relevant limitations of MRI in the context of clinical routine and trials: First, some patients cannot be assessed because of contraindications (e.g. metal implants) or claustrophobia. Second, data quality can be affected by disease severity, therefore leading to bias. For example, cognitively impaired patients are more likely to cause motion artifacts [212]. Third, MR imaging is relatively expensive and time-consuming. This applies to both the examination itself as well as the mandatory post-processing for assessing SVD burden [5]. Finally, even after protocol harmonization, MRI is prone to center effects in multicenter studies, introduced by inevitable differences in MRI scanner hardware and software [145]. In contrast to MRI, blood draws are less costly and less time consuming, can be readily repeated at multiple time points in longitudinal studies and are less prone to bias. Furthermore, for multicenter-studies, blood biomarkers can be centrally analyzed at a single, experienced (reference) laboratory. Serum NfL might therefore complement MRI measures in assessing disease burden in SVD patients.

Several other blood biomarkers have been studied in SVD, such as markers for inflammation, endothelial dysfunction, subclinical cardiac injury and coagulation. Results on these markers have so far been ambiguous: while in some studies associations with imaging parameters, such as WMH, have been reported [213-217], this was not the case in others [218, 219]. Most importantly, in contrast to our present findings for NfL, no other blood marker has been reported to have robust associations with both imaging as well as clinical features of SVD. Although NfL is not specific for SVD, our results suggest that serum NfL may be used to stage disease...
Supplementary material

Supplementary Figure  Age-adjusted comparisons

A) To account for age differences between the study samples, we calculated age-adjusted serum NfL levels (residuals after regressing out the effect of age). Compared with healthy controls (HC), age adjusted serum NfL levels were increased in CADASIL (CAD) and sporadic SVD (sSVD) patients.

B) Comparison between serum NfL levels after matching healthy control (HC) subgroups for age (using similar median and IQR) to either the CADASIL (young HC) or the sporadic SVD (old HC) sample. Please note the logarithmic scale in both panels. ***p < 0.001 (Wilcoxon rank sum test with Bonferroni correction).

Conclusions

SVD burden is reflected in serum NfL levels and needs to be considered when interpreting elevated NfL levels in the elderly. Serum NfL is associated with imaging as well as clinical features in hereditary and sporadic SVD and might therefore complement established MRI markers in assessing SVD burden. Since neurodegenerative pathology and SVD often co-occur in elderly patients, serum NfL might offer the possibility to assess the combined effect of these pathologies on brain integrity. Serum NfL might also be a promising candidate marker for prognostication and treatment response monitoring, which needs to be addressed in longitudinal studies.
Chapter 7

Increased levels of serum neurofilament light chain are associated with incident white matter damage and cognitive decline in cerebral small vessel disease

Submitted as:

*Both authors contributed equally
Abstract

Objective: Serum neurofilament light chain (NfL) is a circulating marker for axonal injury and has been shown to be associated with severity of cerebral small vessel disease (SVD). Here we explored the predictive value of serum NfL for incident SVD and cognitive impairment.

Methods: From 503 subjects with sporadic SVD from the RUN DMC-study, baseline and follow-up MR-imaging was available for 264 (mean age 62.4 ± 7.7 years, 59.3% male) participants with a follow-up period of 8.7 ± 0.2 years. Baseline serum NfL was measured by an ultrasensitive single-molecule array assay. MRI markers of SVD including white matter hyperintensity (WMH) volume, lacunes, microbleeds, brain atrophy and mean diffusivity (MD) values were assessed both at baseline and at follow-up. Baseline and follow-up cognitive testing could be performed in 335 participants, including the 264 that had also undergone imaging. Cognitive testing included performance of global cognition, memory, processing speed and executive function. Associations of serum NfL with MRI-markers and cognition were assessed using linear regression analyses and ANCOVA.

Results: Serum NfL levels were associated with baseline WMH volume and MD values, as well as with presence of lacunes and microbleeds, after correction for age. Furthermore, serum NfL levels were associated with future MRI markers of SVD (WMH: β=0.173; 95%CI [0.062, 0.327]; p=0.004; MD: β=0.165; 95%CI [0.048, 0.334]; p=0.009). In addition, NfL levels were associated with occurrence of incident lacunes during the follow-up period. Clinically, NfL levels were associated with future cognitive impairment, including processing speed at follow-up (β=0.135; 95%CI [-0.226, -0.039]; p=0.005), the cognitive domain most affected in SVD. Risk of developing dementia increased with higher NfL levels (HR: 5.0; 95%CI 2.6-9.4; p=0.001), but significance was lost after adjusting for age (HR: 1.6; 95%CI 0.6-4.1; p=0.312).

Conclusions: Serum NfL is associated with incident SVD, as well as with cognitive decline. Serum NfL may thus potentially serve as a marker for disease monitoring and outcome in SVD and capture both vascular as well as neurodegenerative processes in the elderly.

Introduction

Neurofilament light chain (NfL) is an emerging blood marker for axonal damage in various neurological diseases affecting the elderly, including neurodegenerative as well as cerebral small vessel disease (SVD) [39, 40, 188, 189, 223, 224]. SVD is a frequent condition with increasing prevalence with age and the most important vascular contributor to dementia as well as an important cause of stroke [8]. The diagnosis of SVD is based on clinical findings as well as the presence of markers on magnetic resonance imaging (MRI), including white matter hyperintensities (WMH), lacunes and microbleeds [5]. Cross-sectionally, MRI markers have been shown to be associated with clinical symptoms attributed to SVD. However, there are various potential limitations of MR-imaging, such as contraindications interfering with performing MRI, limited availability of MRI, poor image quality due to motion artefacts in demented subjects as well as costs and time needed for performing imaging. Thus, other more easily accessible markers being less prone to the aforementioned limitations, such as blood based markers reflecting the severity underlying condition, are warranted.

In a recent cross-sectional study we observed elevated serum NfL levels in individuals with SVD compared to healthy controls and found a relation between serum NfL levels and the severity of MRI markers of SVD, especially with the white matters' microstructural tissue damage as operationalized by mean diffusivity (MD), assessed by diffusion-tensor-imaging (DTI) [223]. Also, serum NfL levels were strongly associated with cognitive impairment of processing speed performance, the cognitive domain most affected in SVD patients [193, 194]. Given these significant associations, serum NfL may potentially serve as a marker of disease progression in SVD, but prospective studies to validate this are currently lacking.

In the present study we therefore wanted to assess the association of serum NfL levels with the burden of future MRI markers of SVD as well as with incident cognitive decline and dementia in a large cohort of patients with sporadic SVD. We hypothesized that baseline serum NfL levels are related to future severity of MRI markers of SVD and cognitive performance.
Methods

Study population
The Radboud University Nijmegen Diffusion tensor and Magnetic resonance imaging Cohort (RUN DMC) study is a prospective cohort study of 503 non-demented elderly with SVD, that investigates risk factors and clinical consequences of SVD. The detailed study protocol including the inclusion and exclusion criteria has been published previously [148]. Information on dementia status at follow-up was available of all 503 participants. Participants underwent repeated neuroimaging and cognitive assessments; baseline assessment has been performed in 2006 and follow-up assessment in 2015 (mean follow-up duration was 8.7 ± 0.2 years). Of 503 participants, 335 participants underwent repeated cognitive assessments and could be included in longitudinal analyses on cognitive performance. Of these, 276 participants underwent repeated MRI assessment [110]. Twelve participants were excluded due to missing T2* sequences or scan artefacts, yielding a sample of 264 participants for longitudinal neuroimaging analyses. The Medical Review Ethics Committee region Arnhem-Nijmegen approved the study and all participants gave written informed consent.

Neurofilament light chain (NFL) assay
All samples were analyzed on the same single molecule array instrument (Simoa HD-1, Quanterix, Lexington, MA, USA) in Basel. We used the capture monoclonal antibody (mAB) 47:3, and the biotinylated detector mAB 2:1 (UmanDiagnostics, Umeå, Sweden) [198], transferred onto the Simoa platform. Bovine lyophilized NFL was obtained from UmanDiagnostics. Calibrators ranged from 0 to 2,000 pg/ml. Intra- and inter-assay variability of the assay were below 20%, respectively. The analytical sensitivity was 0.32 pg/ml. All samples produced signals above the analytical sensitivity of the assay.

Neuroimaging protocol
MR images were acquired at three time points on 1.5-Tesla MRI, though only MRI assessments from the baseline and follow up imaging were used in the present study (2006: Siemens, Magnetom Sonata; 2015: Siemens, Magnetom Avanto). The neuroimaging protocol included the following whole brain scans: a T1-weighted 3D MPRAGE sequence (isotropic voxel size 1.0 mm³), a FLAIR sequence (2006: voxel size 0.5x0.5x0.5 mm, interslice gap 1.0 mm; 2015: voxel size 0.5x0.5x2.5 mm; interslice gap 0.5 mm) and a DTI sequence (2006: isotropic voxel size 2.5 mm³, 4 unweighted scans, 30 diffusion weighted scans at b=900 s/mm²; 2015: isotropic voxel size 2.5 mm³, 8 unweighted scans, 60 diffusion weighted scans at b=900 s/mm²). Full acquisition details have been described previously [110, 148].

Small vessel disease and brain volumetry
We rated SVD markers according to the STRIVE criteria [5]. WMH volumes were calculated semi-automatically at all three time-points using FLAIR and T1 sequences [150]. Segmentations were visually checked for segmentation errors by one trained rater, blinded for clinical data. Lacunes and microbleeds were rated manually on FLAIR/T1-weighted scans and T2*-weighted MRI scans by two trained raters blinded for clinical data. Inter and intra-rater reliability were excellent [152].

We calculated grey matter (GM), white matter (WM) and CSF volumes using SPM12 (http://www.fil.ion.ucl.ac.uk/spm/ ) unified segmentation routines on T1 images corrected for WMH [110]. All images were visually checked for co-registration and segmentation artefacts. All volumes were calculated in ml, corrected for interscan differences in ICV and normalized to baseline ICV.

DTI processing
All diffusion weighted images were denoised using a Local Principal Component Analyses filter [156], and corrected for cardiac, head motion, and eddy current artefacts simultaneously using the 'PATCH' algorithm [157], as described previously [152]. Diffusion tensor and scalar parameters, including mean diffusivity (MD) and fractional anisotropy (FA), were calculated using DTIFIT from FSL’s FDT toolbox. We calculated mean MD of the normal appearing white matter (NAWM), calculated by subtracting the WMH from the WM masks.

Dementia diagnosis
Dementia case finding was extensively described previously [225]. In short, dementia diagnosis was made after examination at the Radboud Alzheimer Center or by a consensus diagnosis by a panel consisting of a neurologist, clinical neuropsychologist and a geriatrician with expertise in dementia reviewing all available cognitive assessments and medical records. All available neuropsychological and neuroimaging information was reviewed; this included (i) the difference in neuropsychological performance between baseline and follow-up, (ii) outcome of the Mini International Neuropsychiatric Interview MINI [226], (iii) the follow-up MRI scan, or if not available, the baseline MRI scan for classification, (iv) Age and level of education were taken into account for the interpretation of the data, next to interference with daily living, confirmed by family or caregivers. In case participants were not available for follow-up assessment, we reviewed all available medical records. In addition, we contacted their general practitioners and medical specialists for information on their cognitive status. The diagnosis of dementia was based on the DSM-IV criteria [227]. In total, 65 out of 503 participants were diagnosed with dementia at the nine-year follow-up.
CHAPTER 7  SERUM NEUROFILAMENT LIGHT CHAIN AND FUTURE CEREBRAL SMALL VESSEL DISEASE

Cognitive function

Cognitive performance was measured using an extensive neuropsychological test battery, as has been described previously [228]. In short, the test battery included the Mini-Mental State Examination (MMSE), Rey Auditory Verbal Learning Test (RAVLT), Rey Complex Figure Task (RCFT), verbal fluency, Paper-Pencil Memory Scanning Task (PPMST), Stroop Color Word Test (short form), Symbol Digit Substitution Task (SDST), and Verbal Series Attention Test (VSAT). To account for possible learning effects, parallel versions of the RAVLT, RCFT and verbal fluency task were used for the follow-up assessment. Raw scores of all time-points were transformed into z-scores based on the mean and standard deviation of the overall study population at baseline. We calculated Speed–Accuracy Trade-Off (SAT) scores where appropriate. Cognitive decline over time was calculated for each subject individually, by subtracting baseline scores from the follow-up scores.

We calculated compound scores for global cognitive function, memory, processing speed and executive function. We calculated the Cognitive Index as a compound score for global cognitive function, using the mean of the z-scores of all tests from the neuropsychological test battery. Memory was measured using the 2-letter and 3-letter subtasks of the PPMST and the immediate and delayed recall of the RAVLT and the RCFT. Processing speed was calculated as the mean of the z-scores of the 1-letter subtask of the PPMST, the reading and color naming tasks of the Stroop Test and the SDST. Executive function was measured using the verbal fluency task, the interference score of the Stroop Test, which was calculated by dividing the color-word task by the mean of the reading and color naming tasks of the Stroop Test, and the VSAT.

Vascular risk factors

We assessed presence of hypertension, smoking, alcohol use, diabetes and hypercholesterolemia at baseline, by standardized questionnaires, as described previously [148]. We defined hypertension as the use of antihypertensive agents and/or systolic blood pressure greater than or equal to 140 mm Hg and/or diastolic blood pressure greater than or equal to 90 mm Hg. We defined diabetes as treatment with antidiabetic medication and hypercholesterolemia as the use of lipid-lowering drugs [148].

Statistical analyses

Statistical analyses were performed using SPSS Statistics version 20. Serum NFL levels and WMH volumes were log-transformed because of skewedness. Associations between baseline serum NFL levels and neuroimaging markers (TBV, GMV, WMV, WMH volume, MD, FA) both at baseline and follow-up were analyzed by linear regression analyses, adjusted for age and sex. Associations between NFL levels and (incident) lacunes and microbleeds were calculated by ANCOVA, adjusted for age and sex. We performed linear regression analyses, separately for cognitive index, memory, processing speed and executive function, adjusted for age and sex. The association between NFL levels and development of dementia was analyzed by cox proportional hazard analyses, adjusted for age and sex.

Results

Baseline characteristics are presented in Table 1. Participants for whom follow-up MR-imaging and/or cognitive testing was available were slightly younger and had less severe MRI markers of SVD than for those without this information available (Table 1).

<table>
<thead>
<tr>
<th>Table 1 Baseline characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Demographics</td>
</tr>
<tr>
<td>Age, years</td>
</tr>
<tr>
<td>Male sex, nr of participants (%)</td>
</tr>
<tr>
<td>MMSE score</td>
</tr>
<tr>
<td>Education, years</td>
</tr>
<tr>
<td>Vascular risk factors</td>
</tr>
<tr>
<td>Hypertension, nr of participants (%)</td>
</tr>
<tr>
<td>Diabetes, nr of participants (%)</td>
</tr>
<tr>
<td>Hypercholesterolemia, nr of participants (%)</td>
</tr>
<tr>
<td>Smoking, ever, nr of participants (%)</td>
</tr>
<tr>
<td>Alcohol, glasses/week</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
</tr>
<tr>
<td>MRI markers</td>
</tr>
<tr>
<td>Total brain volume, ml</td>
</tr>
<tr>
<td>Grey matter volume, ml</td>
</tr>
<tr>
<td>White matter volume, ml</td>
</tr>
<tr>
<td>WMH volume, ml</td>
</tr>
<tr>
<td>Lacunes, nr of participants (%)</td>
</tr>
<tr>
<td>Microbleeds, nr of participants (%)</td>
</tr>
<tr>
<td>Serum NFL, pg/ml</td>
</tr>
</tbody>
</table>

Data represent mean ± SD, nr of participants (%) or median (IQR).
Median level of serum NfL was 44.4 (IQR: 33.0 - 62.0) pg/ml in the 264 participants with longitudinal neuroimaging assessments and 47.2 (IQR: 35.0 – 68.8) pg/ml in the 335 participants with baseline and follow-up cognitive testing. Changes in MRI markers of SVD and cognitive performance over time are shown in Supplementary Table 1: while there was no change of MD in NAWM over time, all other markers showed progression over the follow-up period (p<0.001). This was also the case for cognitive performance.

**Serum NfL levels are associated with SVD markers at baseline and follow-up**

For both – baseline and follow-up – NfL levels were associated with WMH volume, lacunes, as well as MD (Table 2 and 3, Figure 1), but not with microbleeds. Furthermore, NfL levels were associated with incident lacunes (Table 3). These associations remained present after adjustment for age.

**Associations of serum NfL levels with cognitive performance and dementia**

In the overall cohort, serum NfL levels were higher in the subgroup of participants who developed dementia during the follow-up period (n=65; NfL 74.8 pg/ml) versus those who did not develop dementia (n=438; NfL 50.1 pg/ml; p<0.001). However, this difference disappeared when adjusting for age. Cox proportional hazard analyses revealed increased risk of developing dementia with higher NfL levels (HR: 5.0; 95%CI 2.6-9.4; p<0.001), but again significance was lost after adjusting for age (HR: 1.6; 95%CI 0.6-4.1; p=0.312).

Next, we analyzed the associations of serum NfL levels with cognitive performance, both at baseline and follow-up (Table 4). For both baseline and follow-up, NfL levels were associated with cognitive index and processing speed, which remained after correction for age. Figure 2 displays the association of NfL with future processing speed. In addition, NfL was also associated with memory impairment at follow-up, again independent of age (Table 4).

---

**Table 2** Associations between NfL levels and brain volumes and MRI markers of SVD

<table>
<thead>
<tr>
<th>MRI markers</th>
<th>Baseline MRI markers</th>
<th>Future MRI markers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β [95%CI]</td>
<td>P-value</td>
</tr>
<tr>
<td>Total brain volume</td>
<td>-0.086 [-0.207; 0.014]</td>
<td>0.087</td>
</tr>
<tr>
<td>Gray matter volume</td>
<td>-0.061 [-0.184; 0.047]</td>
<td>0.241</td>
</tr>
<tr>
<td>White matter volume</td>
<td>-0.079 [-0.221; 0.043]</td>
<td>0.187</td>
</tr>
<tr>
<td>WMH volume</td>
<td>0.212 [0.106; 0.370]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean Diffusivity</td>
<td>0.142 [0.045; 0.284]</td>
<td>0.007</td>
</tr>
<tr>
<td>Fractional Anisotropy</td>
<td>-0.084 [-0.236; 0.048]</td>
<td>0.195</td>
</tr>
</tbody>
</table>

Associations are presented as standardized betas with 95% confidence intervals, analyzed by linear regression analyses adjusted for age and sex. NfL levels and WMH volumes were log-transformed.

**Table 3** Serum NfL levels in SVD patients with or without lacunes and microbleeds

<table>
<thead>
<tr>
<th>Lacunes</th>
<th>Absent</th>
<th>Present</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>42.7 [31.9-58.5]</td>
<td>55.7 [43.1-85.5]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Follow-up</td>
<td>41.2 [31.6-55.4]</td>
<td>55.3 [42.4-78.5]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Incidence</td>
<td>41.3 [31.8-55.9]</td>
<td>57.2 [42.9-82.4]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Microbleeds</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>44.0 [32.6-58.9]</td>
<td>56.9 [41.2-82.2]</td>
<td>0.002</td>
</tr>
<tr>
<td>Follow-up</td>
<td>43.1 [32.3-59.3]</td>
<td>50.1 [40.1-73.5]</td>
<td>0.549</td>
</tr>
<tr>
<td>Incidence</td>
<td>43.5 [32.6-60.2]</td>
<td>50.7 [41.2-71.5]</td>
<td>0.921</td>
</tr>
</tbody>
</table>

Data represent NfL levels in pg/ml (median with interquartile range) for participants with and without (incident) lacunes or microbleeds. Differences are calculated by ANCOVA with Bonferroni correction, adjusted for age and sex.
Serum neurofilament light chain (NfL) levels were associated with future MRI markers. Simple linear regression analysis between serum NfL levels and WMH volume and mean diffusivity (MD) at follow-up, adjusted for age and sex. Dashed lines depict the 95% confidence interval for the linear regression. NfL levels and WMH volumes were log-transformed.

### Table 4: Associations between baseline NfL levels and cognitive performance during baseline and follow-up

<table>
<thead>
<tr>
<th>Cognitive performance</th>
<th>Baseline cognition</th>
<th>Future cognition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β [95%CI]</td>
<td>P-value</td>
</tr>
<tr>
<td>Cognitive index</td>
<td>-0.131 [-0.173; -0.017]</td>
<td>0.017</td>
</tr>
<tr>
<td>Memory</td>
<td>-0.109 [-0.158; 0.001]</td>
<td>0.053</td>
</tr>
<tr>
<td>Processing speed</td>
<td>-0.180 [-0.251; -0.064]</td>
<td>0.001</td>
</tr>
<tr>
<td>Executive function</td>
<td>-0.045 [-0.120; 0.050]</td>
<td>0.419</td>
</tr>
</tbody>
</table>

Associations between NfL levels and cognitive performance for all participants who completed repeated cognitive assessments (n=335), presented as standardized betas with 95% confidence intervals, analyzed by linear regression analyses. NfL levels were log-transformed. P-values, adjusted for age and sex.
Discussion

In the present longitudinal study, we found serum NfL levels to be related to future and incident MRI markers of SVD after a follow-up period of approximately nine years. These included MD, WMH and lacunes. Furthermore, NfL was associated with future cognitive impairment comprising processing speed, the cognitive domain mostly affected in SVD [232], but also other cognitive deficits, including memory function.

Our findings extend the results from our recent cross-sectional study of the value of NfL as a marker of SVD burden and cognitive performance [223], where we identified serum NfL as the first blood-based marker in SVD, reflecting both SVD-related lesion burden on MR-imaging as well as its related cognitive impairment. Adding to this, our present study showed an association of serum NfL levels and markers of SVD after an average a follow-up period of nine years. This was true for WMH volume, the most established MRI-marker of SVD in clinical routine, as well as for microstructural tissue damage as assessed by MD on DTI. In SVD, MD indicates microstructural tissue damage in normal appearing white matter (NAWM) that later may convert into WMH [229]. The here observed strong association of serum NfL with MD and WMH – both cross-sectional as well as longitudinally – suggest a pathological process of tissue damage in SVD with axonal damage as a common denominator. As for MD, we observed an association of serum NfL with WMH volume at follow-up. Although we also observed progression of WMH volume over time, this was not associated with baseline NfL, most likely due to the strong correlation of both WMH progression and NfL with age [223]. In contrast to WMH volume, we did not observe an association with overall white matter volume, indicating that NfL may reflect earlier, more active white matter damage, rather than later stages of secondary atrophy.

Of note, NfL was not only related to the presence of lacunes at follow-up, but also to the occurrence of incident lacunes during the follow-up period. Besides WMH, lacunes are a hallmark of SVD and the occurrence of lacunes is known to be of clinical relevance in SVD [230, 231]. Lacunes have been shown to occur in a tempo-spatial relation to WMH, appearing later than WMH and often being localised at the edge of WMH [123]. Our finding of serum NfL as a sensitive marker of neuroaxonal damage being associated with white matter damage and predicting the later occurrence of incident lacunes is in line with this course of tissue damage in SVD. In fact, a recent study showed serum NfL to be sensitive to active SVD [8]. Furthermore, NfL has recently been shown to predict lacunar cavitation in small subcortical infarcts [232]. Our findings thus support this notion of serum NfL being an early marker of more progressive subsequent white matter damage related to SVD.

Besides association with MRI markers of SVD, we found NfL to be associated with processing speed, the cognitive domain mostly affected in SVD [193, 194]. The association of serum NfL with processing speed was observed independent of age at baseline and at follow-up and was observed also after adjustment for MD. DTI measures such as MD have been recognized as an important underlying substrate of vascular cognitive impairment in SVD [68, 199, 210]. Our findings indicate that serum NfL also seems to be a sensitive marker of vascular neuroaxonal damage, reflecting the disease process underlying cognitive impairment in SVD. However, the associations of NfL levels with both global cognitive index and memory performance at follow-up and the association with age suggest that NfL may not only reflect vascular, but also neurodegenerative pathologies associated with aging.

Some methodological considerations need to be addressed: we did not have serum NfL values at follow-up, thus not allowing to study the temporal course of NfL levels over the follow-up period. Besides, there were differences in MRI scanner and FLAIR and DTI sequences between baseline and follow-up, which might have led to segmentation differences between baseline and follow-up images. However, we consider this unlikely because we previously validated our findings on the associations between MD and WMH progression by repeating analyses for all time-periods within the RUN DMC-cohort separately: the results for the first follow-up and the overall time-interval were comparable to the results for the second follow-up period in which scanner and sequence protocols remained identical [229]. Finally, due to the long-term follow-up of our study a proportion of the participants was unable to complete the entire follow-up. However, this attrition bias most likely will lead to an underestimation of the observed findings, since more affected subjects not included in the imaging analyses are likely to have more severe lesion burden. The strengths of our study are the large cohort of SVD patients and the longitudinal design over a period of approximately nine years and the comprehensive and standardized clinical and imaging work-up that was performed at both time points. Furthermore, measurements of serum NfL were performed at a single, experienced centre using a very sensitive state-of-the-art Simoa assay [222].

Further research should investigate whether NfL levels predict progression of MRI markers of SVD better than its baseline MRI markers. In addition, it needs to be investigated whether NfL levels are able to disentangle SVD progression from for
example age related reductions in brain volumes. When proven, the next step should include the investigation of NfL as a biomarker of SVD progression, that can for example be used in a clinical trial.

In conclusion, serum NfL is associated with future white matter tissue damage as well as cognitive performance in SVD. This included association with newly occurring lacunar lesions, indicating a potential value of serum NfL as a marker of active and progressive SVD. Serum NfL may thus potentially serve as a marker for disease monitoring and outcome in SVD. Furthermore, given the association with a broader spectrum of future cognitive impairment, NfL may reflect both vascular as well as neurodegenerative processes in the elderly.

Supplementary material

Supplementary Table 1 MRI markers and cognitive performance over time

<table>
<thead>
<tr>
<th>MRI markers</th>
<th>Baseline (n=264)</th>
<th>Follow-up (n=264)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total brain volume, ml</td>
<td>1086.4 ± 71.1</td>
<td>1043.6 ± 80.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Grey matter volume, ml</td>
<td>620.8 ± 49.1</td>
<td>599.2 ± 51.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>White matter volume, ml</td>
<td>465.6 ± 39.3</td>
<td>444.4 ± 46.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>WMH volume, ml</td>
<td>2.2 (0.8 – 5.8)</td>
<td>4.6 (2.0 – 11.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lacunes, nr of participants (%)</td>
<td>54 (20.5)</td>
<td>83 (31.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Microbleeds, nr of participants (%)</td>
<td>36 (13.6)</td>
<td>66 (25.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NAWM mean diffusivity, 10⁻³ mm²/s</td>
<td>0.85 ± 0.04</td>
<td>0.85± 0.07</td>
<td>0.999</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cognitive performance</th>
<th>Baseline (n=335)</th>
<th>Follow-up (n=355)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cognitive index</td>
<td>0.19 ± 0.68</td>
<td>-0.15 ± 0.85</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Memory</td>
<td>0.17 ± 0.67</td>
<td>-0.07 ± 0.89</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Processing speed</td>
<td>0.21 ± 0.82</td>
<td>-0.27 ± 0.92</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Executive function</td>
<td>0.16 ± 0.73</td>
<td>-0.14 ± 0.87</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data represent mean ± SD, nr of participants (%) or median (IQR). For cognitive performance z-scores based on the mean and standard deviation of the overall study population at baseline were used. Significant differences were calculated by repeated measures ANOVA for normally distributed variables and nonparametric tests. NAWM: normal appearing white matter.
Part IV

Cognitive consequences of cerebral small vessel disease
Chapter 8
Cerebral small vessel disease: from focal to global perspective

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ter Telgte A, van Leijsen EMC, Wiegerijtjes K, Klijn CJM, Tuladhar AM, de Leeuw FE

Cerebral small vessel disease: from focal to global perspective

Abstract

Cerebral small vessel disease (SVD) is commonly observed on neuroimaging among elderly individuals and is recognized as a major vascular contributor to dementia, cognitive decline, gait impairment, mood disturbance and stroke. However, clinical symptoms are often highly inconsistent in nature and severity among patients with similar degrees of SVD on brain imaging. Here, we provide a new framework based on new advances in structural and functional neuroimaging that aims to explain the remarkable clinical variation in SVD. First, we discuss the heterogeneous pathology present in SVD lesions despite an identical appearance on imaging, and the perilisional and remote effects of these lesions. We review effects of SVD on structural and functional connectivity in the brain, and discuss how network disruption by SVD can lead to clinical deficits. We address reserve and compensatory mechanisms in SVD and discuss the part played by other age-related pathologies. Finally, we conclude that SVD should be considered a global rather than a focal disease, as the classically recognized focal lesions affect remote brain structures, and structural and functional network connections. The large variability in clinical symptoms among patients with SVD can probably be understood by taking into account the heterogeneity of SVD lesions, the effects of SVD beyond the focal lesions, and the interaction with reserve mechanisms, compensatory mechanisms and the contribution of neurodegenerative pathologies other than SVD.

Key points

• Cerebral small vessel disease (SVD) is associated with a remarkable degree of variation in clinical symptoms — both in nature and in severity — which cannot be explained fully by conventional markers of SVD.

• Conventional MRI does not capture the heterogeneity present in SVD lesions with a similar appearance, and only reveals the tip of the iceberg of the total SVD-related brain damage.

• SVD affects brain tissue beyond the commonly recognized focal lesions by inducing a cascade of events that spread from the initial lesion to remote brain areas, which probably contributes to clinical outcome.

• SVD disturbs structural and functional network connectivity and thereby disrupts efficient communication in brain networks, which is necessary for functional performance.

• Brain resilience protects against clinical deterioration caused by SVD via reserve and compensatory mechanisms, which explains the clinical variation observed in patients with apparently equal SVD lesion burden.

• The clinical notion that SVD mostly constitutes a subcortical disease of focal lesions requires reconsideration.

Introduction

Life expectancy is higher than ever before and is predicted to increase continuously in the future in industrialized countries [233]. As a result, age-related diseases will increasingly pose challenges to societies and healthcare systems. Globally, over 40 million people currently have dementia and this number is predicted to almost double every 20 years [234]. Cerebral small vessel disease (SVD), which was once thought to be innocuous, is now recognized to be the most important vascular contributor to dementia [239]. Furthermore, this condition causes ~20% of all ischemic strokes and is associated with gait impairment and mood disturbances [5, 43, 236, 237]. Consequently, a proper understanding of how SVD exerts its action on the ageing brain and leads to clinical symptoms is urgently needed.

SVD is present to some extent in virtually every individual aged 60 years or older [6]. It affects the smallest cerebral blood vessels, including the perforating arterioles, capillaries and venules [8]. Although in vivo assessment of the smallest blood vessels is not yet possible with conventional imaging techniques, a spectrum of radiological manifestations can be detected that are thought to result from SVD. Common radiological markers of SVD include white matter hyperintensities (WMH), lacunes, enlarged perivascular spaces, microbleeds, recent small subcortical infarcts and brain atrophy, and the detection of these features is now complemented by the examination of losses in microstructural integrity of the white matter and the presence of cortical microinfarcts (FIG. 1) [5].

SVD historically has been perceived as a slowly progressing disease that affects frontal subcortical networks, leading to corresponding frontal symptoms [238]. These symptoms include loss of mental processing speed and executive function, and affect cognitive function, motor performance and mood regulation. Compared with the healthy ageing population, individuals with SVD are at an increased risk of cognitive decline and, ultimately, dementia [43, 239]. In addition, slowing of gait is a frequent observation in people with SVD and can result in parkinsonism [42, 236, 240, 241]. Finally, apathy (defined as a lack of motivation expressed by reduced initiative, diminished interest and lowered emotional responsiveness to stimuli [242, 243]), and depressive symptoms are common in individuals with SVD [237, 244, 245].

At least two reasons support re-evaluation of this classic concept of SVD. First, despite loss of executive control and speed in behavioral performance, the spectrum of cognitive symptoms attributable to SVD is more diverse than previously thought and can also include deficits in language, memory, attention and visuospatial
The focus of this Review is on the most frequently occurring, sporadic form of SVD—namely, age-related and vascular risk-factor-related SVD [8] with predominantly ischemic manifestations (Box 1). Illustrative examples of mechanisms of action that have been demonstrated in other types of SVD, such as cerebral amyloid angiopathy (CAA) or inherited SVD are sometimes used as a model.

**Focal and remote effects of SVD**

**Heterogeneity of SVD lesions with an identical appearance on imaging**

MRI-based markers of SVD have a homogeneous appearance on conventional imaging. However, post-mortem histopathology studies have shown that these markers are in fact heterogeneous with regard to disease severity and etiology [255-258].

Pathological examination of WMH has shown different degrees of demyelination, gliosis and loss of fibers and oligodendrocytes in different lesions, with more-extensive abnormalities in more-severe and confluent lesions [255]. Periventricular and deep WMH are characterized by vessel wall thickening, enlarged perivascular spaces, decreased vascular density, increased vessel tortuosity and the presence of

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Cerebral small vessel disease (SVD) is associated with a wide range of tissue alterations detectable with MRI, which are reported here according to the STRIVE (Standards for Reporting Vascular Changes on Neuroimaging) criteria [5]. CSF, cerebrospinal fluid; FLAIR, fluid-attenuated inversion recovery.
Thickening of the walls of venules due to collagen deposition could contribute to changes of the venules in the origin of WMH, especially venous collagenosis [261]. In addition, evidence increasingly suggests a role for pathological and failure of the glymphatic system, responsible for clearance of neurotoxic waste of oligodendrocyte precursor cells, which are involved in the formation of myelin, however, new hypotheses on the pathophysiology of WMH include the dysfunction of endothelial cells within capillaries, especially in deep WMH, and immunologically activated microglial cells, in particular in periventricular WMH, have been observed [255]. On the basis of these different pathological observations, ischemia and blood–brain barrier breakdown have been suggested as mechanisms that contribute to the origin of WMH [255].

Although the pathological processes that underlie MRI markers of cerebral small vessel disease (SVD) are incompletely understood, changes that result in disorganization of the vessel structure and function of the intraparenchymal and leptomeningeal blood vessel walls are key [8]. A previous report described six types of SVD [6]. The most prevalent type of SVD comprises a set of pathological changes under the influence of age and vascular risk factors, especially hypertension, that mainly affect the perforating arterioles [8, 341, 342]. This type is characterized by arteriolar wall thickening (mainly due to deposition of collagen, plasma proteins and inflammatory cells in the vessel wall), loss of smooth muscle cells involved in the regulation of arterial pressure and blood flow [343], and leakage of plasma proteins into the perivascular tissue [8, 341, 342]. Arteriosclerosis represents an early stage of the disease, whereas lipohyalinosis and fibroid necrosis are observed at later stages — although these early and late stages can occur simultaneously in one vessel [8, 341, 342]. The second most common type of SVD is cerebral amyloid angiopathy (CAA), which is characterized by the deposition of amyloid-β in the walls of small arteries, arterioles and, infrequently, capillaries and venules, predominantly in the cerebral cortex and leptomeninges [8, 341]. In addition, vasculopathic changes observed in CAA include vessel wall thickening, loss of smooth muscle cells, fibrinoid necrosis and exudation of blood breakdown products into perivascular tissue [13, 341]. In both of these types of SVD, the described vessel wall alterations are associated with enlarged perivascular spaces [341]. Furthermore, occlusion or rupture of the blood vessel can occur, which leads to infarction or hemorrhage, although CAA is typically associated with lobar hemorrhages [13, 341]. Sometimes, microatheroma and microaneurysms are formed, which can also cause an infarct or hemorrhage [8, 341]. The remaining four types of SVD result from rare causes, and consist of hereditary forms of SVD (such as CADASIL), inflammatory and immunologically mediated SVD, venous collagenosis and finally a category of other causes of SVD (such as post-radiation angiopathy) [8].

**Box 1** Different types of cerebral small vessel disease

Although the pathological processes that underlie MRI markers of cerebral small vessel disease (SVD) are incompletely understood, changes that result in disorganization of the vessel structure and function of the intraparenchymal and leptomeningeal blood vessel walls are key [8]. A previous report described six types of SVD [6]. The most prevalent type of SVD comprises a set of pathological changes under the influence of age and vascular risk factors, especially hypertension, that mainly affect the perforating arterioles [8, 341, 342]. This type is characterized by arteriolar wall thickening (mainly due to deposition of collagen, plasma proteins and inflammatory cells in the vessel wall), loss of smooth muscle cells involved in the regulation of arterial pressure and blood flow [343], and leakage of plasma proteins into the perivascular tissue [8, 341, 342]. Arteriosclerosis represents an early stage of the disease, whereas lipohyalinosis and fibroid necrosis are observed at later stages — although these early and late stages can occur simultaneously in one vessel [8, 341, 342]. The second most common type of SVD is cerebral amyloid angiopathy (CAA), which is characterized by the deposition of amyloid-β in the walls of small arteries, arterioles and, infrequently, capillaries and venules, predominantly in the cerebral cortex and leptomeninges [8, 341]. In addition, vasculopathic changes observed in CAA include vessel wall thickening, loss of smooth muscle cells, fibrinoid necrosis and exudation of blood breakdown products into perivascular tissue [13, 341]. In both of these types of SVD, the described vessel wall alterations are associated with enlarged perivascular spaces [341]. Furthermore, occlusion or rupture of the blood vessel can occur, which leads to infarction or hemorrhage, although CAA is typically associated with lobar hemorrhages [13, 341]. Sometimes, microatheroma and microaneurysms are formed, which can also cause an infarct or hemorrhage [8, 341]. The remaining four types of SVD result from rare causes, and consist of hereditary forms of SVD (such as CADASIL), inflammatory and immunologically mediated SVD, venous collagenosis and finally a category of other causes of SVD (such as post-radiation angiopathy) [8].

On histopathological examination, lacunes are irregularly shaped fluid-filled cavities that are surrounded by some degree of myelin and axonal loss and gliosis [255, 263]. Different pathological findings have been described with respect to the age of the lacune. In contrast to more recently formed lacunes, older lacunes are characterized by a decreased concentration of macrophages and necrotic waste, and an increased density of gliosis [255, 263]. In addition, a distinct type of lacunar infarcts has been proposed; these so-called incomplete infarcts are either not cavitated or are only minimally cavitated but show evidence of myelin loss and neuronal loss and a variable extent of gliosis [256].

MRI-defined microbleeds have also been associated with various degrees of gliosis and tissue loss and different pathological correlates have been identified. A pathology study that examined patients with CAA distinguished between acute and old microbleeds by the presence of intact red blood cells in acute lesions and of hemosiderin-laden macrophages in older microbleeds [258]. Histopathological evidence suggests that the majority of microbleeds reflect true microhemorrhages caused by vessel wall disruption [255, 257, 258]. However, some microbleed mimics have been identified reflecting a vasculopathy rather than a parenchymal hemorrhage, including vessel wall dissection, microaneurysms or vessel wall thickening due to accumulation of fibrin and red blood cells in the vessel wall [255, 257, 258]. Finally, markers of an underlying ischemic process have been observed in some microbleeds, suggesting hemorrhagic transformation of a previous infarct [255, 258].

These pathological findings indicate heterogeneity of radiological similar appearing SVD lesions, which is also increasingly being recognized in vivo on MRI [117, 264-266]. Conventional MRI techniques, such as fluid-attenuated inversion recovery (FLAIR), coarsely dichotomize tissue into abnormal and normal tissue and do not, for example, reliably reflect the degree of demyelination [267], whereas quantitative imaging techniques, including magnetization transfer imaging and diffusion tensor imaging (DTI), can provide a detailed evaluation of the underlying tissue alterations at the voxel level [28]. DTI is a technique that applies a tensor-based model to acquired diffusion-weighted imaging (DWI) scans, a MRI sequence sensitive to the diffusion of water molecules, and thereby provides information on the microstructural organization of white matter [29]. In a study that investigated DTI changes within WMH that had not changed on FLAIR imaging over a 3-year period, diffusion metrics showed a significant change over time in these WMH [117]. Similarly, within acute or subacute incidental DWI+...
lesions, suggestive of being infarcts, diffusion metrics were significantly changed on follow-up imaging 7 months later, compared to the pre-lesional scan [264]. Interestingly, this ongoing change in diffusion parameters occurred irrespective of whether or not an infarct remained visible on FLAIR or T1-weighted MRI [264].

Besides showing heterogeneity with regard to disease severity, MRI is increasingly able to contribute to our understanding of the various mechanisms implicated in the origin of SVD, including perfusion deficits, blood-brain barrier breakdown and venous pathology [268-270]. Finally, imaging evidence has converged on a shared origin of different types of SVD lesions. Acute DWI-positive lesions have now been shown to evolve either into a WMH, lacune, microbleed or normal-appearing tissue [264, 271, 272].

In summary, the histopathological characteristics of lesions with similar appearances on MRI can vary. Consequently, the effects that these lesions have on the cerebral tissue can also vary (Figure 2A,B).

Perilesional effects of SVD

In addition to overlooking the heterogeneous pathology present in similar appearing SVD lesions, conventional MRI manifestations of SVD are well known to represent only the tip of the iceberg with regards to SVD-related brain damage. In fact, DTI studies have shown that SVD lesions, such as WMH, are surrounded by areas with altered diffusion metrics that otherwise look normal on conventional MRI, the so-called SVD penumbra (Figure 2B) [273, 274]. Diffusion abnormalities in penumbral areas are associated with the overall WMH load and depend on the distance from the WMH, with more severe abnormalities in regions proximal to WMH than in distant regions [273, 274].

A cross-sectional study demonstrated that a gradient of diffusion abnormalities also surrounds lacunes and extends up to centimeters from the lacune into the white matter tract containing that lacune (Figure 2B) [275]. In a longitudinal study on the effect of acute and subacute DWI+ lesions on the DTI characteristics of the perilesional tissue, water diffusion in perilesional tissue was slightly less anisotropic at the post-lesional scan compared to the pre-lesional scan — albeit not to a significant extent, perhaps due to the small number of six patients demonstrating a lesion [264]. An autopsy study in six patients with a lacunar infarct confirmed previous imaging findings suggesting the presence of a penumbra surrounding the lacunar infarct. The investigators revealed axonal damage in the penumbral area of the lacunar infarct at a distance 1.5 times the diameter of the lacune [276]. Changes included loss of nodes of Ranvier and increased length between each node.

**Figure 2** What you see is not what you get

**a. Conventional neuroimaging markers of SVD**

**b. Heterogeneity, perilesional and remote effects of SVD**

**c. Structural and functional connectivity in SVD**

**d. Brain reserve and compensatory mechanisms in SVD**

A: MRI markers of cerebral small vessel disease (SVD), including white matter hyperintensities (WMH), lacunes, enlarged perivascular spaces, microbleeds and cortical microinfarcts. B: Pathology studies show heterogeneity with regard to underlying disease severity and nature in SVD lesions with a similar appearance on MRI (shown for WMH). A penumbra (lighter areas) can surround SVD lesions. When penumbra of cortical microinfarcts overlap, these microinfarcts can coalesce with a resultant larger cortical infarct. Besides having perilesional effects, SVD is also associated with remote effects, such as cortical thinning (purple) caused by lacunes and possibly WMH via the disconnection of white matter tracts (green). C: SVD lesions can impair structural (left) and functional (right) connectivity by affecting the nodes of a network or the connections between them. Strategic infarcts affect rich clubs (red circles) or rich club connections (red lines). D: A larger total brain volume, increased network efficiency and compensatory (increased) functional connectivity can make the brain resilient to a certain degree of damage. ACC, anterior cingulate cortex; dlPFC, dorsolateral prefrontal cortex; PPC, posterior parietal cortex.
Although the axon and myelin sheath were preserved, the investigators argued that the injured axons could be susceptible to future axonal degeneration [276].

In addition to WMH and lacunes, a penumbra can also surround cerebral microbleeds and perivascular spaces, although this phenomenon requires further investigation. One case report observed a temporary perilesional edema surrounding an acute microbleed, which was thought to cause the transient clinical symptoms observed in this individual [277]. The dilation of perivascular spaces could affect the integrity of surrounding grey or white matter tissue, although this remains to be proven.

Interestingly, in contrast to conventional markers of SVD such as WMH or lacunar volume, which have consistently shown only weak relations with clinical symptoms, diffusion metrics generally yield robust relations with cognitive, motor and mood symptoms associated with SVD, which remain after adjustment for the typical SVD imaging markers in statistical analyses [116, 278, 279]. A DTI study demonstrated an association between abnormal diffusion metrics in the penumbra surrounding lacunes and deficits on tests of executive functioning and information processing speed, independent of the size and side (that is, left or right hemisphere) of the lacune and independent of total WMH load [278]. This finding indicates that cognitive performance is determined not only by SVD lesions that are visible on conventional MRI, but also by the extent to which the penumbra is affected. In the past few years, two global brain DTI derived metrics — the peak width of skeletonized mean diffusivity (which is based on skeletonization of white matter tracts and histogram analysis [199]) and segmentation of DTI images [280] — have been shown to be strongly related with processing speed [199, 280] and executive functioning [280] in patients with SVD, well beyond the associations with classic SVD makers. These findings illustrate that SVD truly exerts its action outside the visible lesion, which contributes to clinical outcome.

A penumbra seems to surround not only conventional SVD markers, but also cortical microinfarcts (Figure 2B) [281-283]. Cortical microinfarcts are frequently observed at brain autopsy in elderly people, are associated with cognitive decline and dementia [284] and, within the past few years, have also been recognized on MRI [285]. Two studies in rodents demonstrated that the occlusion of a single penetrating arteriole to induce a cortical microinfarct led to structural, functional and hemodynamic changes that were measureable millimeters away from the infarct core [282, 283]. In fact, decreased neuronal activity was estimated to extend over a cortical region at least 12 times larger than the volume of the microinfarct core itself, whereas MRI could only visualize the core [283]. Remarkably, when perilesional areas of multiple, isolated microinfarcts overlapped, a coalescence of infarcts was observed, resulting in a larger cortical infarct (Figure 2B) [282]. An ex vivo study in human brain tissue showed that, similar to what was observed in the penumbra surrounding lacunar infarcts, cortical microinfarcts caused disruption in the organization of adjacent axons, which was characterized predominantly by a loss of axon initial segments and increased length between nodes of Ranvier [281]. The investigators suggested that these changes could ultimately reduce the capability of the axons to conduct action potentials [281]. The debilitating effect of cortical microinfarcts on axonal communication might be yet another mechanism by which the accumulation of cortical microinfarcts can induce clinical deficits (Figure 2B) [281].

Remote effects of SVD
Previous studies have demonstrated suppression of brain function in areas far from subcortical infarcts and WMH, as indicated by reduced perfusion and glucose metabolism on PET, suggesting the presence of sequelae of SVD remote from subcortical MRI markers [247, 251, 286]. In addition to these functional changes, a growing body of evidence suggests that SVD is accompanied by structural changes remote from the initial SVD MRI markers. Several cross-sectional studies have shown negative associations between WMH and cortical thickness [287-289]. More specifically, one study demonstrated a particular pattern of frontal–parietal–occipital grey matter atrophy related to WMH progression, which was thought to be responsible for the observed decline in total brain volume [49]. However, among patients less-severely affected with SVD, a longitudinal association between progression of WMH and reduction of cortical thickness could not be confirmed [287].

A study of patients with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), a genetic form of SVD, demonstrated that incident lacunar infarcts cause thinning in cortical tissue connected to the infarct area, providing the first direct proof for SVD-induced secondary degeneration in remote cortex [147]. Cortical thinning was most pronounced in areas that had a high probability of connectivity via the lacune-affected white matter tract (Figure 2B). Indeed, in a subsequent study this group demonstrated that infarcts are accompanied by DTI changes in white matter tracts connecting to the infarct area, with larger changes of white matter microstructure associated with more cortical thinning [290]. Interestingly, cortical thinning was independent of the final infarct fate — that is, whether the infarct was cavitating (complete or partial tissue loss) or non-cavitating (no visible tissue loss) — suggesting that less-severe infarcts can also induce long-term remote effects [290].
Brain atrophy is an important predictor of cognitive decline and has been shown to mediate the relationship between presence of WMH and cognitive decline in individuals [109, 291]. Similar to lacunar infarcts, WMH can affect the cortex via disruption of white matter tracts (Figure 2B) and consequently can lead to cognitive symptoms, although this hypothesis remains to be proven in longitudinal studies. We previously showed that decreased cortical thickness played an intermediate role in the associations between WMH volume and deficits in various cognitive domains (namely global cognition, verbal memory, psychomotor speed, and attention and executive functioning) [289]. In line with our observations, others have identified an intermediate role for cortical thickness of the left medial frontal lobe in the relationship between lacunar infarct volume within the anterior thalamic radiation and reductions in processing speed, whereas anterior thalamic radiation lacunar volume itself was not related to processing speed [292]. The role of cortical thickness in SVD is probably not limited to its effects on cognitive performance: another study showed that periventricular WMH affected gait impairment via disruption of white matter tracts and cortical thinning [293].

**Structural and functional connectivity**

Functional performance results from the complex interplay between brain regions connected with each other through the white matter tracts, together forming brain networks. Advances in human connectomics have led to better understanding of how SVD might give rise to clinical symptoms through its effects on structural or functional connected brain networks (Box 2).

**Structural connectivity in SVD**

The white matter tracts are crucial for information transfer between brain regions. These tracts can be reconstructed using DTI tractography techniques and represent the connections in structural brain networks. Subsequently, the organizational properties of a network, such as its global efficiency of information transfer, a measure of how well-connected the brain regions are, can be quantified using principles from graph theory (Box 2). Several studies demonstrated structural network changes in patients with SVD through the application of this approach [53, 54, 57, 294]. These network changes were characterized by a reduction in the number of connections, reduced strength of connectivity and decreased local and global efficiency. The degree of brain network disruption was associated with the extent of MRI markers of SVD, including WMH volume, number of lacunes and number of microbleeds, and with brain volume and microstructural integrity.

With respect to the specific connections being disrupted in patients with severe SVD, a study revealed a subnetwork comprising the most impaired connections, which included predominantly interhemispheric and prefrontal connections [53]. Whereas the distribution of SVD near the ventricles and within the centrum semiovale could explain the reduced connectivity of interhemispheric tracts, the disruption of frontal connections was interpreted as remote effects of SVD. Additional insights into the extent and location of structural network disruption in SVD have been provided by rich-club analyses (Box 2): rich-club organization of a network refers to the presence of nodes (brain regions) that are rich in connections that are more densely connected to each other than to other nodes [56]. The connections between the rich-club regions are centrally located in the network and are thereby the most important connections for integration of information [55]. Rich-club nodes identified in SVD comprise the precuneus, putamen, thalamus, superior occipital gyrus and regions in the superior frontal gyrus [57]. Reduced connectivity was predominantly observed for connections between the rich-club nodes rather than a generalized, random reduction of the white matter connectivity (Figure 2C) [57]. This observation might be explained in part by the location of SVD damage, which often overlaps with the location of rich clubs.

Several studies in patients with SVD have demonstrated that the degree of brain network disruption, reflected by decreased global efficiency, was related to increased cognitive impairment [53, 54, 295] and depressive symptoms [296]. Furthermore, decreased global efficiency was linked to an increased risk of all-cause dementia over a 5-year period [297]. In addition, associations between the presence of conventional MRI markers of SVD and decreased cognitive functions were, at least in part, mediated through network disruption [53, 54, 289]. In particular, rich-club connectivity strength mediated the association of WMH with processing speed and executive functioning, in that higher rich-club connectivity strength was associated with better cognitive performance [57]. These findings were corroborated by others, who found that only microstructural changes in central network connections, as opposed to non-central connections, mediated the association between WMH volume and executive functioning [58]. Due to their central role in brain networks and high connectivity with other nodes, damage to rich clubs or central connections might have a more widespread effect on network functioning compared with damage to peripheral nodes or peripheral connections in a network. Consequently, strategic infarctions, even when small in size, could result in a highly heterogeneous clinical phenotype, deviant from the typical ‘subcortical’ clinical picture when they are located near rich clubs or central connections (Figure 2C) [298].
**Box 2 Brain networks and graph theory**

**a. Constructing brain networks**

Brain networks can be explored using graph theory, which conceptualizes the brain as a network (i.e., a graph) with connections that mediate the interaction between brain regions. A network consists of nodes (brain regions) linked by edges (the connections between nodes), in which nodes are defined by a parcellation scheme (arbitrary scheme shown in figure). Brain networks can be constructed using structural and functional neuroimaging data. In structural imaging, connectivity between nodes A and B is retrieved from diffusion tensor tractography and thereby represents their anatomical connections. In functional imaging, connectivity between nodes A and B is based on the correlation between their functional MRI blood-oxygen-level-dependent time series. Functionally connected regions might or might not have a structural underpinning. Regions with correlated activity are likely to form a network and several such brain networks have been identified, including the default mode network, dorsal attention network and frontoparietal control network [300, 344]. Once brain networks are constructed, the topological organization and properties of these networks can be explored [50, 51], of which some are discussed here. Path length is defined as the shortest distance or minimum number of connections between two nodes (orange connections). Network efficiency is inversely related to path length, whereby global efficiency of the network is reflected by the average efficiency for all node pairs. Node degree refers to the number of connections that link a node to the rest of the network (orange connections). The human brain consists of a few central connected regions, known as hubs. Several hubs are also highly interconnected with each other and with the rest of the network, and represent rich clubs. Connections between the rich club nodes are referred to as rich club connections and play an important part in the integration of information by creating short paths [55, 56].

**b. Graph theoretical measures**

![Graph theoretical measures](image)
Nevertheless, these clinical deficits cannot entirely be explained by SVD alone as discussed the mechanisms by which SVD might lead to these clinical deficits. In the previous sections we described the clinical consequences of SVD and brain resilience and DAN (Figure 2C) [301].

Networks responsible for cognitive control or attention, such as the DMN, FPCN, and long association fibers that in turn impair communication between crucial neural networks. Furthermore, brain regions within the DMN normally show highly correlated brain activity during rest and reduced activity during attentionally demanding tasks, which is considered to be important for the maintenance of task-related goals. However, patients with SVD showed hyperactivation or impaired DMN deactivation during attentionally demanding tasks [311-314]. For example, WMH severity was associated with hyperactivation of the anterior cingulated cortex, a key structure of the DMN [311].

These alterations to functional networks provide novel insights into the mechanisms of cognitive decline in SVD. Several studies have reported that reduced functional connectivity within the FPCN, DAN and DMN was related to an increased degree of cognitive impairment [302, 303, 307-310]. Evidence suggests that cognitive impairments due to SVD result from disruption of frontal–subcortical circuits and long association fibers that in turn impair communication between crucial neural networks responsible for cognitive control or attention, such as the DMN, FPCN, and DAN (Figure 2C) [301].

Brain resilience
In the previous sections we described the clinical consequences of SVD and discussed the mechanisms by which SVD might lead to these clinical deficits. Nevertheless, these clinical deficits cannot entirely be explained by SVD alone as many individuals remain functionally independent despite a considerable burden of SVD. An alternative approach might be to look not only at the sum of SVD burden and brain damage, but also to explicitly take into account brain resilience, which is the capacity for patients to tolerate a certain degree of brain damage before clinical symptoms become manifest. Brain resilience can be separated into reserve mechanisms including brain reserve (referring to structural or functional metrics of the brain, such as intracranial volume) and cognitive reserve (referring to lifetime experiences, such as education) that offer protection against brain pathology, and compensatory mechanisms via which the brain actively compensates for clinical deterioration in the presence of pathology (Figure 2D) [316].

The concept of brain reserve states that individuals with a larger brain reserve tolerate a greater burden of pathology before clinical symptoms arise than do individuals with lower brain reserve [317]. One study reported a lower risk of dementia in patients with a larger brain volume [318] and another study reported that patients with WMH who had no cortical atrophy had a lower risk of dementia than did those with cortical atrophy [319]. Structural network efficiency can also be viewed as a form of brain reserve, whereby more efficient networks might protect against clinical deterioration in the presence of brain pathology. In other words, patients with highly efficient networks (for example, networks with many connections between nodes) could cope well with pathology, as these individuals are able to compensate for the disruption of white matter tracts by using alternative connections. This hypothesis is supported by the finding that patients with SVD who did not develop dementia had higher global network efficiency than did patients who converted to dementia, independent of SVD markers, including WMH volume, presence of lacunes and microbleeds, and brain atrophy [297].

The concept of cognitive reserve is that high intellectual enrichment or high cognitive reserve (usually operationalized as IQ and level of education) offers increased protection against age-related brain pathology [316, 317, 320-322]. Specifically, in the context of cognitive impairment associated with SVD, several studies reported that high cognitive reserve attenuated the negative effects of SVD on cognition [317, 320, 323-328]. Higher cognitive reserve was associated with a slower rate of decline in processing speed, executive functions and memory, independently of WMH severity, which supports the hypothesis that cognitive reserve protects against the clinical manifestations of SVD and could partly explain the variation in cognitive performance in patients with a similar burden of SVD markers [329].
Methodological considerations

Several methodological considerations must be addressed regarding the studies discussed in this article. We will discuss factors related to neuroimaging and to study design, as well as possible solutions to tackle these methodological issues in future studies.

The reproducibility of SVD imaging markers is a major concern, especially across different centers [145]. One review identified several aspects — from acquisition of imaging data to processing — that influence the reproducibility [145]. Quantification of SVD markers depends on magnetic field strength, choice of MRI sequence, image resolution, and the segmentation method used, the latter of which can range from qualitative visual rating scales to quantitative automatic segmentation routines [145, 146]. With the publication of the Standards for Reporting Vascular Changes on Neuroimaging (STRIVE), which provides definitions of visible SVD features and recommendations for image acquisition, analyses and scientific reporting to reduce the large variation between studies, a higher consistency between studies has been reached [5]. Nonetheless, variation between studies is still considerable, hampering progress in our understanding of how SVD might contribute to clinical symptoms.

In connectivity analyses, the presence of SVD lesions, such as WMH, affects the reconstruction of white matter fiber tracts with the use of DTI data and consequently affects the investigation of remote effects via white matter tracts connected to the lesion or the assessment of structural connectivity. In functional connectivity, brain activity is measured by blood-oxygen-level dependent imaging that relies on regional differences in cerebral blood flow, which is often affected in patients with SVD. Additionally, cerebral blood flow can be affected by antihypertensive treatment [339].

With regards to the design of the discussed studies, most are cross-sectional and consequently cannot elaborate on causality or on the directionality of the associations. Furthermore, studies might be susceptible to selection bias, as patients with the most severe SVD lesion load are often institutionalized and might not have been able to participate. Finally, study populations differ between different studies — ranging from pure SVD to SVD with concomitant AD pathology and from population-based studies to studies including patients with symptomatic lacunar stroke — and this variability should be taken into account when interpreting results on the clinical consequences of SVD.
Future directions

Comprehensive studies with in-depth phenotyping of patients are warranted to further unravel the mechanisms by which SVD might lead to clinical deficits. Studies should include patients of a younger age, as currently often patients above age 60 are studied, which would enable elaboration on the factors involved in the origin of SVD. Future studies should also take into account all neuroimaging markers of SVD and include advanced multimodal structural and functional magnetic resonance sequences with high spatial resolution. Moreover, studies with longitudinal designs, including those with short inter-scan intervals of weeks or months, are of utmost importance to elucidate the directionality of associations and the sequence of events from focal SVD towards global remote effects and clinical symptoms. Furthermore, rather than solely focusing on vascular dementia or AD as isolated diseases, the field would benefit from a focus on the interaction of SVD pathology with other neurodegenerative pathologies. Finally, the pathological correlates of MRI-defined SVD lesions should be further investigated.

Conclusion

SVD is associated with a remarkable variation of clinical symptoms that differ both in nature and in severity. In this Review, we elaborate on how SVD affects the brain and we provide a framework to explain the remarkable disparities between patients. SVD markers visible on conventional MRI only reveal the tip of the iceberg with regards to the changes associated with the disease. Despite an identical appearance on MRI, SVD lesions are pathologically heterogeneous and a cascade of events seems to spread from the initial SVD lesion to remote areas, with an as yet unknown time course. Furthermore, SVD disturbs structural and functional network integrity, especially in central connections, thereby disrupting efficient communication in brain networks. Consequently, even a small focal lesion can lead to widespread effects. In addition, reserve and compensatory mechanisms allow patients to maintain cognitive and motor performance at the pre-morbid level until these mechanisms are exhausted and functional performance drops. Finally, other neurodegenerative pathologies can be present and probably contribute to the clinical presentation.

Although SVD lesions mostly occur in subcortical areas, they exert their effect throughout the brain. Consequently, the clinical notion of SVD being a focal subcortical disease must be reconsidered. Following the identification of SVD markers on MRI, clinicians should be aware that the entire brain is affected, despite the presence of focal subcortical lesions. Hence, what we see, is not what we get. This notion has also become apparent in other neurological diseases, for instance in multiple sclerosis which clinical course is increasingly better understood from the perspective of a global, rather than a focal brain disease [340].

In conclusion, advances in structural and functional imaging and network analyses have increased our understanding of the clinical symptoms in SVD beyond the classic spectrum, and of the heterogeneity of these symptoms between individuals. Future prospective studies with in-depth phenotyping of patients and multimodal structural and functional MRI with high temporal and spatial resolution will shed light on the sequence of events in SVD from the very first MRI manifestation of focal SVD to its global remote effects.
Review criteria
Articles were selected from PubMed. To select articles on SVD we used the following search terms appearing in title and abstract: "cerebral small vessel disease", "cerebral microangiopathy", "white matter hyperintensities", "leukoaraiosis", "lacunar stroke", "lacunar infarct", "perivascular spaces" or "microbleeds". We combined these searches with search terms covering the topics in this Review, including "cognition", "motor", "cerebral cortex", "network" or "connect". We only included articles in English and focused on articles published within the past decade to discuss the most recent scientific findings. Furthermore, reference lists of cited articles and articles in our personal databases were screened for eligibility.
Chapter 9
Cognitive consequences of regression of cerebral small vessel disease

Submitted as:
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Cognitive consequences of regression of cerebral small vessel disease
Abstract

Background: Recent studies have shown that neuroimaging markers of cerebral small vessel disease (SVD) can also regress over time. If, and how, regression affects cognitive outcome remains to be established. We therefore investigated the cognitive consequences of regression of SVD markers in a cohort of elderly with SVD.

Methods: 276 participants of the RUN DMC study underwent neuroimaging and cognitive assessments at three time points over 8.7 years. We semi-automatically assessed WMH volumes and manually rated lacunes and microbleeds. We analyzed differences in cognitive decline and accompanying brain atrophy between participants with regression, progression and stable SVD by ANOVA.

Results: Of 276 participants (mean age 62.5±7.7 years; 59.1% male), 56 participants (20.3%) showed regression of SVD markers: 31 (11.2%) showed WMH regression, 10 (3.6%) vanishing lacunes and 27 (9.8%) vanishing microbleeds. Participants with regression showed a decline in overall cognition, memory, psychomotor speed and executive function similar to stable SVD. In addition, participants with WMH regression showed less cognitive decline compared to those with WMH progression (mean difference [95% confidence interval]; 29 [-0.2, 0.56]; p<0.035 for cognitive index and 42 [0.8, 0.76]; p=0.010 for memory), and participants with vanishing lacunes showed less decline in psychomotor speed compared to participants with incident lacunes (48 [0.1, 0.96]; p=0.043), although significance was lost after adjustments. Loss of total brain, gray matter and white matter volume did not differ between participants with SVD regression and stable SVD, while participants with SVD progression showed more volume loss of total brain (mean difference -11.9 [-21.1, -2.8]; p=0.06) and gray matter (-6.7 [-13.1, -2.0]; p=0.041) compared to stable SVD, though significance disappeared after adjustments.

Conclusions: Regression of SVD markers was associated with similar cognitive decline compared to stable SVD and did not accompany brain atrophy, suggesting that SVD regression follows a relatively benign clinical course.

Introduction

Markers of cerebral small vessel disease (SVD) include white matter hyperintensities (WMH), lacunes and microbleeds and are frequently observed on neuroimaging in older individuals [5]. SVD has been recognized as the most important vascular contributor to the development of cognitive decline and dementia [3].

Progression of SVD has been considered a continuous progressive process, but increasing evidence suggests that SVD progression is a dynamic process, sometimes interrupted by regression of SVD [59, 61, 110, 345, 346]. Specifically, a decrease over time in WMH volume, number of lacunes and number of microbleeds has been reported [345]. A neuroimaging study in patients with minor stroke observed WMH volume decline in 37 percent of participants [346] and we previously observed SVD regression in 20 percent of participants [110], suggesting that SVD regression might be a true phenomenon, with unknown clinical implications.

We therefore examined the cognitive consequences of SVD regression in a cohort of 276 older adults with SVD, using neuroimaging and cognitive assessments at three time points over nine years. We additionally tested whether SVD regression accompanied global brain atrophy.

Methods

Study population
The RUN DMC study prospectively investigates risk factors and clinical consequences of SVD in 503 non-demented older adults with SVD [148]. In the present study, we included 276 participants with repeated MRI assessment of sufficient quality [110]. The data that support the findings of this study are available from the corresponding author upon request.
Cognitive function
All participants underwent neuropsychological assessment at three time points using validated cognitive tasks [228]. We calculated a compound score for global cognitive function (cognitive index) and for three cognitive domains: memory, psychomotor speed and executive function. Raw scores of all time points were transformed into z-scores based on the baseline mean and standard deviation (SD).

Neuroimaging
MR images were acquired at three time points on 1.5-Tesla MRI and included T1-weighted 3D MP-RAGE, FLAIR and T2*-weighted gradient echo sequences [110, 148]. We calculated total brain, gray matter (GM) and white matter (WM) volumes using SPM12 (http://www.fil.ion.ucl.ac.uk/spm/) [110]. WMH volumes were calculated semi-automatically and lacunes and microbleeds were rated manually, according to the STRIVE criteria [5, 110]. All images were visually checked for co-registration and segmentation artefacts and volumes were corrected for inter-scan differences and normalized to baseline intracranial volume (ICV).

Statistical analyses
We compared baseline characteristics between participants with SVD regression, progression and stable SVD using univariate analyses. We defined SVD regression as >0.25ml WMH volume decline or having a vanishing lacune or microbleed, and SVD progression as >1SD WMH progression or at least one incident lacune or microbleed, according to our previous study [110]. We defined ‘stable SVD’ as a change in WMH volume between -0.25ml and +1SD and having no incident or vanishing lacunes or microbleeds. We assessed differences in cognitive decline and in brain atrophy between participants with SVD regression, progression and stable SVD by one-way ANOVA with Bonferroni-correction, additionally adjusted for age and sex.

Results
56 participants (20.3%) showed regression of SVD markers: 31 participants (11.2%) showed WMH regression, 10 (3.6%) vanishing lacunes and 27 (9.8%) vanishing microbleeds [110]. Characteristics of participants with SVD regression are presented in Table 1. Stratified by time-period, 42 participants (15.2%) showed regression during the first time-interval, 30 participants (10.9%) during the second time-interval, and 23 participants (8.3%) during the overall follow-up.

<table>
<thead>
<tr>
<th>Table 1 Baseline characteristics of participants with SVD regression</th>
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<td>Microbleeds, n</td>
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</table>

Data represent mean ± SD, number (n) of participants (%) or median (IQR). Comparisons were performed by ANOVA, Chi-square or Mann-Whitney-U test. ***p<0.001, **p<0.01, *p<0.05 versus participants with stable SVD; †††p<0.001, †p<0.05 versus participants with regression. n: number of participants; MMSE: Mini-Mental State Examination.

Participants with regression showed similar cognitive decline compared to those with stable SVD, during the nine-year follow-up (Figure 1). Participants with SVD progression showed more cognitive decline compared to those with stable SVD (WMH: mean difference [95% confidence interval] -0.23 [-0.56, -0.02]; p=0.017 for cognitive index; -3.4 [-5.9, -0.9]; p=0.004 for memory; and -2.9 [-5.1, -0.6]; p=0.006 for executive function; lacunes: -0.21 [-0.41, -0.01]; p=0.036 for executive function; microbleeds: -0.22 [-0.42, -0.02]; p=0.024 for cognitive index; -2.9 [-5.4, -0.4]; p=0.019 for memory and -2.4 [-4.7, -0.2]; p=0.029 for executive function), although significance was lost after adjustments for age and sex. In addition, participants with WMH regression showed less cognitive decline compared to those with WMH progression (-2.9 [-4.2, -0.6]; p=0.035 for cognitive index and -2.9 [-4.8, -0.6]; p=0.010 for memory), and participants
Figure 1 Cognitive consequences of SVD regression

A. WMH

- Cognitive Index
- Memory
- Psychomotor speed
- Executive function

Cognitive decline over the overall nine-year follow-up (2006-2015) per cognitive domain, separately for:
A) participants with stable WMH (light gray; n=208), WMH regression (dark gray; n=31) and WMH progression (black; n=37) at any time-period; B) participants without incident or vanishing lacunes (light gray; n=219), vanishing lacunes (dark gray; n=10) and incident lacunes (black; n=47) at any time-period; and C) for participants without incident or vanishing microbleeds (light gray; n=212), vanishing microbleeds (dark gray; n=27) and incident microbleeds (black; n=37) at any time-period. Bars represent mean decline in z-score with standard errors as whiskers. Statistical differences are analyzed by one-way ANOVA, followed by a Bonferroni-correction. \(^{\text{**}}p<0.001, \^{\text{*}}p<0.01, \text{^p}<0.05\) for participants with progression versus participants with stable SVD, unadjusted; \(\dagger p<0.05\) for participants with regression versus participants with stable SVD, unadjusted. Significance was lost after adjustments for age and sex.
with vanishing lacunes showed less decline in psychomotor speed compared to participants with incident lacunes (48.01 .96; p=.043), although significance was lost after adjustments.

Loss of total brain, gray matter and white matter volume did not differ between participants with SVD regression and stable SVD, while participants with SVD progression showed more volume loss of total brain (mean difference -11.9 [21.1, -2.8; p=.006] and gray matter (-6.7 [13.1, -20]; p=.041) compared to those with stable SVD (Table 2), although significance disappeared after adjustments.

### Discussion

We observed regression of SVD markers in a considerable proportion of participants who showed similar cognitive decline compared to participants with stable SVD. In addition, SVD regression did not accompany brain atrophy.

WMH regression has recently been related to fewer composite recurrent cerebrovascular events [346], but its associations with cognitive performance have not been investigated. We had two hypotheses on how SVD regression might affect cognitive outcome [345]. First, SVD regression might accompany brain atrophy and thereby lead to impaired clinical outcome. This hypothesis is less likely, since both brain atrophy and cognitive decline of participants with SVD regression were comparable to participants with stable SVD. Second, SVD regression might reflect resolution of transient white matter damage and thereby account for recovery of clinical symptoms [28, 345]. Our observations of comparable cognitive decline between participants with regression and stable SVD might be supportive of the second hypothesis. Of note, is that differences between the three SVD groups (progression, regression, stable) lost significance after adjustments, possibly due to reduced statistical power in relatively small groups. Our results should thus be interpreted with caution and require verification in larger studies.

Our findings might have clinical implications, as they suggest that future therapeutic strategies targeting vascular contributions to dementia may be particularly interested in regression of SVD. Previously observed associations between blood pressure reduction and WMH regression in patients with minor stroke [346] suggest that anti-hypertensive treatments might lead to regression of SVD, though future (intervention) studies are required to assess the role of vascular risk factor control on SVD regression and recovery of clinical symptoms.

Strengths of this study include the long follow-up of participants with SVD and the use of neuroimaging assessments at three time points. Study limitations include the possibility that regression of SVD markers might have a radiological explanation, for example by slight changes in neuroimaging protocols between baseline and first follow-up which might have led to an overestimation of WMH regression.[110] However, by taking into account the third MRI assessment and reslicing follow-up FLAIR to baseline images, we were able to capture most of this possible bias. Besides, due to the long-term follow-up a considerable proportion of participants was not able to complete all follow-up assessments, reducing power in statistical analyses.

In conclusion, our findings of comparable cognitive decline between participants with regression and stable SVD might suggest that SVD regression has a relative benign cognitive outcome. Future studies are required to validate these findings and to assess the role of vascular risk factor control on SVD regression and possible recovery of clinical symptoms.
Chapter 10

Memory decline in elderly with cerebral small vessel disease explained by temporal interactions of white matter hyperintensities with hippocampal atrophy

In press as:
van Leijsen EMC, Tay J, van Uden IWM, Kooijmans ECM, Bergkamp MI, van der Holst HM, Ghafoorian M, Norris DG, Kessels RPC, Markus HS, Tuladhar AM, de Leeuw FE

Memory decline in elderly with cerebral small vessel disease explained by temporal interactions of white matter hyperintensities with hippocampal atrophy

Hippocampus. 2018
Abstract

Background: White matter hyperintensities (WMH) constitute the visible spectrum of cerebral small vessel disease (SVD) markers and are associated with cognitive decline, although they do not fully account for memory decline observed in individuals with SVD. We hypothesize that WMH might exert their effect on memory decline indirectly by affecting remote brain structures such as the hippocampus. We investigated the temporal interactions between WMH, hippocampal atrophy and memory decline in older adults with SVD.

Methods: 503 participants of the RUNDMC study underwent neuroimaging and cognitive assessments up to 3 times over 8.7 years. We assessed WMH volumes semi-automatically and calculated hippocampal volumes (HV) using FreeSurfer. We used linear mixed effects models and causal mediation analyses to assess both interaction and mediation effects of hippocampal atrophy in the associations between WMH and memory decline, separately for working memory and episodic memory.

Results: Linear mixed effect models revealed that the interaction between WMH and hippocampal volumes explained memory decline (working memory: $\beta=0.067; 95\% CI[0.024–0.111]; p<0.01$; episodic memory: $\beta=0.061; 95\% CI[0.025–0.098]; p<0.01$), with better model fit when the WMH*HV interaction term was added to the model, for both working memory (likelihood ratio test, $\chi^2(1)=9.3, p<0.01$) and for episodic memory (likelihood ratio test, $\chi^2(1)=10.7, p<0.01$). Mediation models showed that both baseline WMH volume ($\beta=0.170; p=0.001$) and hippocampal atrophy ($\beta=0.126; p=0.009$) were independently related to episodic memory decline, but the effect of baseline WMH on episodic memory decline was not mediated by hippocampal atrophy ($p$-value indirect effect: 0.572).

Conclusions: Memory decline in elderly with SVD was best explained by the interaction of WMH and hippocampal volumes. The relationship between WMH and memory was not causally mediated by hippocampal atrophy, suggesting that memory decline during aging is a heterogeneous condition in which different pathologies contribute to the memory decline observed in elderly with SVD.

Introduction

White matter hyperintensities (WMH) are frequently observed on neuroimaging in older adults [6] and constitute an important radiological marker of cerebral small vessel disease (SVD) [5]. WMH have been associated with cognitive deficits in virtually every domain, including working memory and episodic memory [3, 43]. Working memory relies on prefrontal and parietal cortical regions that are largely affected in SVD [347, 348]. The role of WMH in episodic memory deficits, however, is less well understood, as episodic memory performance is mainly supported by structures in the medial temporal lobes and especially the hippocampus [47, 48], which are typically unaffected by WMH [49].

Several studies have reported associations between WMH and hippocampal volumes [186, 335, 349-352] and have found a cumulative effect of WMH and hippocampal atrophy on the degree of cognitive performance [3, 334, 335], while others have reported that these pathologies are independent processes that both affect cognition adversely [327, 338]. Thus far, studies have not thoroughly investigated the interactions of WMH, hippocampal atrophy and memory decline longitudinally. This is especially important as age is an important risk factor for all of these three phenomena and WMH progresses exponentially over time [110].

There is increasing awareness that SVD exerts its clinical effects by affecting remote brain structures [49, 147], suggesting that disruptions in white matter connections due to WMH might lead to secondary hippocampal atrophy and a concomitant memory decline in patients with SVD. A recent cross-sectional neuroimaging study in patients with Alzheimer’s Disease (AD) showed that WMH contributed indirectly to memory deficits by contributing to temporal lobe atrophy [353]. Prospective studies, however, would be required to elaborate on the directionality of the associations.

In this paper we specifically examined the temporal interactions between WMH and hippocampal atrophy for these two memory systems longitudinally, using three neuroimaging and cognitive assessments over nine years in an SVD cohort. Specifically, we tested two hypotheses as to how WMH and hippocampal atrophy might affect memory decline (Figure 1). First, we tested whether WMH and hippocampal atrophy interacted in predicting memory deficits. Second, we tested whether the effect of WMH on memory decline was mediated by hippocampal atrophy.
Methods

Study population
This study is part of the RUN DMC study, a prospective cohort study among 503 non-demented older adults with SVD, aged between 50 and 85 years, that investigates risk factors and clinical consequences of SVD. Symptoms of SVD include both acute symptoms, such as transient ischemic attack (TIA) or lacunar syndromes, and subacute manifestations such as cognitive and motor (gait) disturbances [354]. As the onset of SVD is often insidious, clinically heterogeneous, and typically with mild symptoms, the selection of participants with SVD was based on neuroimaging characteristics, including WMH and lacunes [174]. The detailed study protocol has been published previously [148]. In short, 503 independently living older adults with SVD, without dementia, were included for baseline assessment in 2006. Inclusion criteria were age between 50 and 85 years and presence of SVD on neuroimaging (i.e. WMH and/or lacunes). Subsequently, the above mentioned acute and subacute clinical symptoms of SVD were assessed. Of these 503 participants, 361 underwent repeated MRI assessment at first follow-up in 2011, and 296 participants at second follow-up in 2015 [110]. Of those, 7 participants were excluded because of insufficient scan quality at baseline, 15 participants at first follow-up, and 7 at second follow-up, yielding a sample of 496 participants for longitudinal analyses at baseline, 346 at first follow-up, and 289 at second follow-up. Thus, in total 1131 observations could be used for linear mixed effect analyses. In total, 263 participants underwent repeated cognitive and neuroimaging assessments of sufficient quality at all three time-points and could be used for longitudinal analyses (Supplemental Figure 1). The Medical Review Ethics Committee region Arnhem-Nijmegen approved the study and all participants gave written informed consent.

Cognitive function
Cognitive performance was measured using an extensive neuropsychological test battery during all three waves of data collection[228]. In the present study, we used the immediate and delayed recall of the Rey Auditory Verbal Learning Test (RAVLT) [355] and Rey Complex Figure Task (RCFT) [356] as well as Speed-Accuracy Trade-Off (SAT) scores of the 2-letter and 3-letter subtasks of the Paper-Pencil Memory Scanning Task (PPMST) [196]. Episodic memory is a compound score of the mean of the z-scores of the three learning trials and the delayed recall of the RAVLT and the mean of the z-scores of the immediate recall trial and the delayed recall trial of the RCFT. Cognitive decline over time was calculated for each participant individually, by subtracting baseline scores from the follow-up scores.

Vascular risk factors
We assessed the presence of hypertension, smoking, alcohol use, body mass index, diabetes and hypercholesterolemia using standardized questionnaires, as described previously [148]. Hypertension was defined as the use of antihypertensive agents and/or systolic blood pressure greater than or equal to 140 mmHg and/or diastolic blood pressure greater than or equal to 90 mmHg. Diabetes was defined as treatment with diabetic medication and hypercholesterolemia as the use of lipid-lowering drugs [148].

Neuroimaging protocols
MR images were acquired at three time-points on 1.5-Tesla MRI (2006: Siemens, Magnetom Sonata; 2011 and 2015: Siemens, Magnetom Avanto) and included the following whole brain scans: T1-weighted 3D MPRAGE MRI (isotropic voxel size 1.0 mm³), a FLAIR sequence (2006: voxel size 0.5×0.5×5.0 mm, interslice gap 1.0mm; 2011 and 2015: voxel size 0.5×0.5×2.5 mm; interslice gap 0.5 mm) and a DTI sequence (2006: isotropic voxel size 2.5 mm³, 4 unweighted scans, 30 diffusion weighted scans at b=900 s/mm²; 2011 and 2015: isotropic voxel size 2.5 mm³, 8 unweighted scans, 60 diffusion weighted scans at b=900 s/mm²). Full acquisition details have been described previously [110, 148, 229] and can be found in the Supplementary Methods.

Brain volumetry
We assessed grey matter (GMV), white matter (WMV) and CSF volumes using SPM12 (http://www.fil.ion.ucl.ac.uk/spm/) unified segmentation routines on the T1 MPRAGE images corrected for WMH, as has been described in detail elsewhere [110].

White matter hyperintensities
WMH volumes were calculated by a semi-automatic WMH segmentation method, which has been described previously [150]. Segmentations were visually checked for segmentation errors by one trained rater, blinded for clinical data. WMH volumes were corrected for inter-scan-differences in ICV and then normalized to compound scores for working memory and episodic memory. Working memory is a compound score of the SAT scores of the 2-letter and 3-letter subtasks of the PPMST [196]. Episodic memory is a compound score of the mean of the z-scores of the three learning trials and the delayed recall of the RAVLT and the mean of the z-scores of the immediate recall trial and the delayed recall trial of the RCFT.
baseline ICV. We calculated individual annualized WMH progression rates using linear mixed effects (LME) models based on all available time-points. The individual WMH progression rates were extracted and used for further analyses.

**Hippocampal volumes**
Hippocampal volume segmentations were automatically processed with the longitudinal stream [357] in FreeSurfer 5.3 (http://surfer.nmr.mgh.harvard.edu/). In short, the T1-weighted images from all three time-points were first processed separately using the standard processing stream. Subsequently, an unbiased within-subject template was created from all time-points for each subject using the longitudinal processing stream, and several processing steps were then initialized with common information from the within-subject template, to increase the reliability of the segmentation of brain regions over time [357]. For those participants who were only able to complete one or two neuroimaging assessments, we ran the same longitudinal pipeline using the available T1-weighted images, to ensure that all images underwent the same processing steps [358]. All segmentations were visually checked for segmentation accuracy at each time-point and manually adjusted when necessary. We used LME models to estimate individual annualized hippocampal atrophy rates based on all available time-points, with negative values reflecting more hippocampal atrophy. The individual hippocampal atrophy rates were extracted and used for further analyses.

**Statistical analysis**
All statistical analyses were carried out in R 3.4.2 (https://www.r-project.org/). Change in WMH, HV and cognitive performance over time was tested using repeated measures ANOVA. We visualized the change in both working memory and episodic memory over time according to quartiles of baseline WMH and hippocampal volumes. We analyzed WMH and hippocampal volumes in quartiles of their distribution and tested continuous linear trend per stratum. We additionally displayed the change in working and episodic memory according to the interaction between WMH and hippocampal volumes. We therefore created 4 groups: WMH+/HV+, WMH-/HV+, WMH+/HV- and WMH-/HV-. Differences between these groups were calculated using one-way ANOVA with post-hoc Bonferroni-correction.

To test whether WMH and hippocampal volumes interacted in predicting memory deficits (Figure 1 – Hypothesis I), we fitted linear mixed effects (LME) models with memory performance as the dependent variable using ‘lme4’ version 1.1-14 in R.

We tested two hypotheses on how white matter hyperintensities and hippocampal atrophy might affect memory decline: I. WMH and hippocampal atrophy interact in predicting memory decline; II. The effect of WMH on memory decline is mediated via hippocampal atrophy.

Figure 1 Illustrated the tested hypotheses

I. Interaction

II. Mediation

We tested two hypotheses on how white matter hyperintensities and hippocampal atrophy might affect memory decline: I. WMH and hippocampal atrophy interact in predicting memory decline; II. The effect of WMH on memory decline is mediated via hippocampal atrophy.

[154]. All analyses were performed separately for working and episodic memory, LME models allow for the simultaneous modeling of fixed (population-average) and random (subject-specific) effects, allowing us to examine memory decline in the entire population while accounting for individual differences in rates of memory decline. We fitted four models: (1) a null model including baseline age, sex and the level of education at baseline. Time between baseline and follow-up assessments was added as a fixed effect and a subject-specific random intercept and slope, such that the fixed effect coefficient estimates group-level memory decline per year, while the random terms are the individual trajectories of memory decline over time. In addition, we included a quadratic term for time, as WMH were found to progress nonlinearly over time [110]. In the second model (2) we additionally added WMH volumes of all available time-points and the interaction between WMH and time-squared, indicating the effect of nonlinear WMH progression on memory decline. In the third model (3) we added hippocampal volumes of all available time-points and the interaction between HV and linear time to the null model, to estimate the effect of hippocampal atrophy over time on memory decline. (4) In the fully-specified model we additionally added an interaction term between WMH and HV. We compared model fit between the four models using a likelihood ratio test, and we evaluated the change in Akaike information criterion (AIC) and Bayesian information criterion (BIC). For both indexes, smaller values indicate better fit.
We performed additional analyses on the temporal interactions between WMH, HV and memory decline. For this, we first used LME models to estimate individual annualized WMH progression and hippocampal atrophy rates based on all available time-points. The individual WMH progression and hippocampal atrophy rates were extracted and used for further analyses. Using ‘lavaan’ version 0.5-23.1097 in R [359], we estimated the effects of baseline WMH volume, WMH progression, baseline hippocampal volume and hippocampal atrophy on memory decline, separately for working and episodic memory decline. We also tested the effect of the interaction term between baseline WMH and hippocampal volumes on memory decline. Analyses were adjusted for baseline age, sex and level of education at baseline. We compared model fit between the models with and without the interaction term using a likelihood ratio test.

To test whether interactions between WMH and HV in predicting memory deficits were specifically attributed to hippocampal atrophy, we performed the same analyses with global gray matter atrophy instead of hippocampal atrophy. Hence, we compared four models: in the first model (1) we added WMH and the interaction between WMH and quadratic time only. In the second model (2) we additionally added HV, the interaction between HV and time, and the interaction term between WMH and HV. In the third model (3) we added GMV, the interaction between GMV and time, and an interaction term between WMH and GMV to the first model, to estimate the effect of global gray matter atrophy on memory decline. We additionally fitted a fourth model (4), in which we added both HV and GMV together with their interaction terms, to estimate the relative strengths of hippocampal and global gray matter atrophy in explaining memory decline.

To test whether hippocampal atrophy mediated the association between WMH and memory decline (Figure 1, Hypothesis II), we performed mediation analyses using ‘lavaan’ version 0.5-23.1097 in R [359]. For this, we first used LME models to estimate individual annualized hippocampal atrophy rates based on all available time-points. The individual hippocampal atrophy rates were extracted and used for further analyses. Using lavaan, we estimated the direct effect of baseline WMH volume on memory decline and the indirect effect of baseline WMH volume on memory decline via hippocampal atrophy, separately for working and episodic memory decline.

**Results**

Baseline characteristics of the study population are presented in Table 1. Mean age at baseline was 65.7 (SD 8.8) years and 57% of the participants were male. WMH and hippocampal volumes and cognitive performance over time is shown in Supplementary Table 1. Higher WMH volumes were related to both lower working memory and lower episodic memory performance (working memory: $\beta=-0.295; 95\%CI [-0.348 - -0.241]; p<0.001$; episodic memory: $\beta=-0.271; 95\%CI [-0.324 - -0.217]; p<0.001$) and higher hippocampal volumes were related to both higher working memory and higher episodic memory performance (working memory: $\beta=0.307; 95\%CI [0.253 - 0.359]; p<0.001$; episodic memory: $\beta=0.392; 95\%CI [0.341 - 0.440]; p<0.001$), modified by age (Figure 2). Change in both working memory and episodic memory over time according to quartiles of baseline WMH and hippocampal volumes is shown in Supplementary Figure 2. We performed additional analyses on the temporal interactions between WMH, HV and memory decline. For this, we first used LME models to estimate individual annualized WMH progression and hippocampal atrophy rates based on all available time-points. The individual WMH progression and hippocampal atrophy rates were extracted and used for further analyses. Using ‘lavaan’ version 0.5-23.1097 in R [359], we estimated the effects of baseline WMH volume, WMH progression, baseline hippocampal volume and hippocampal atrophy on memory decline, separately for working and episodic memory decline. We also tested the effect of the interaction term between baseline WMH and hippocampal volumes on memory decline. Analyses were adjusted for baseline age, sex and level of education at baseline. We compared model fit between the models with and without the interaction term using a likelihood ratio test.

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<table>
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<td>Lacunes, nr of participants</td>
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<td>WMH volume, ml</td>
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<td>Hippocampus volume, ml</td>
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Data represent number of participants (%), mean ± SD or median (IQR).
volumes as well as their interaction was graphically displayed in Figure 3. We observed more decline in the WMH−HV− group compared to both WMH+/HV− and WMH+/HV+ groups.

Memory decline explained by interactions between WMH and hippocampal atrophy

The results of the mixed effect models are shown in Table 2. Both models with WMH only and HV only provided significantly better fits in comparison to the null model, for both working memory (WMH only versus null model: likelihood ratio test, \(\chi^2(2)=7.3, p<0.05\); HV only versus null model: \(\chi^2(2)=9.8, p<0.01\)) and for episodic memory (WMH only versus null model: \(\chi^2(2)=28.3, p<0.001\); HV only versus null model: \(\chi^2(2)=74.4, p<0.001\)). The models with HV alone fitted better than the models with WMH alone, not only for episodic memory (likelihood ratio test, \(\chi^2(0)=46.1, p<0.001\)), but also for working memory (likelihood ratio test, \(\chi^2(0)=2.4, p<0.001\)). Importantly, including the WMH*HV interaction term significantly improved the model, for both working memory (likelihood ratio test model with both WMH & HV versus full model with WMH*HV interaction term, \(\chi^2(1)=9.3, p<0.01\)) and for episodic memory (likelihood ratio test model with both WMH & HV versus full model with WMH*HV interaction term, \(\chi^2(1)=10.7, p<0.01\)). The WMH*HV interaction term was significantly associated with memory function (working memory: \(\beta=0.067; 95\%CI [0.024 – 0.111]; p<0.01\); episodic memory: \(\beta=0.061; 95\%CI [0.025 – 0.098]; p<0.01\)). More hippocampal atrophy was associated with lower episodic memory performance (\(\beta=0.017; 95\%CI [0.009 – 0.025]; p<0.001\)).

The results of the interaction analyses on decline in working memory and episodic memory are shown in Figure 4. Lower baseline hippocampal volumes (\(\beta=0.132; p<0.001\)) and higher baseline WMH volumes (\(\beta=0.164; p<0.001\)) were associated with more episodic memory decline. More hippocampal atrophy was associated with more episodic memory decline (\(\beta=0.120; p<0.001\)). Including the WMH*HV interaction term significantly improved the model, for both working memory (likelihood ratio test, \(\chi^2(1)=6.0, p<0.05\)) and episodic memory (likelihood ratio test, \(\chi^2(1)=10.1, p<0.01\)).

Additional analyses on global gray matter atrophy revealed a significant interaction effect of WMH*GMV for working memory (\(\beta=0.090; 95\%CI [0.004 – 0.177]; p<0.05\)), though the addition of both hippocampal and gray matter atrophy to the model rendered the WMH*GMV interaction term non-significant (\(\beta=0.027; 95\%CI [0.077 – 0.131]; p<0.05\)) where the WMH*HV interaction term remained significant (\(\beta=0.059; 95\%CI [0.007 – 0.111]; p<0.05\)). There was no significant interaction effect of WMH*GMV for episodic memory (\(\beta=0.073; 95\%CI [-0.001 – 0.147]; p>0.05\)).
Figure 3  Graphical displays of working memory and episodic memory over time according to baseline WMH and hippocampal volumes and their interaction.

Table 2  Fixed effects results from interaction analyses explaining memory function by the interaction of WMH and hippocampal atrophy.

| Fixed effects results from linear mixed effect models explaining memory function, separately for working and episodic memory. In the null model (1) we estimated the effects of age, sex and education as well as the linear and quadratic effects of temporal progression on memory. We then adopted a data-driven approach to determine the role of baseline WMH as well as nonlinear WMH progression (2) and baseline HV as well as linear hippocampal atrophy (3) in contributing to memory deficits. In the full model (4) we estimated the effect of the interaction between WMH and HV on memory. Data represent standardized estimates with confidence intervals and statistical significance: *p<0.05; **p<0.01; ***p<0.001. Models were compared using likelihood ratio tests, with smaller AIC and BIC values indicating better model fit. WMH: white matter hyperintensities; HV: hippocampal volume.
Figure 4: Diagrams showing interaction analyses explaining memory decline by the interaction of WMH and hippocampal volumes.

The diagrams present standardized estimates (with p-values) for all associations, separately for working memory decline (left) and episodic memory decline (right). The statistical significance of the WMH*HV interaction is presented in the centre of the diagram. Analyses were performed using Lavaan, adjusted for age, sex, education and baseline memory performance.

Figure 5: Diagrams showing statistical mediation analyses of the relationship between WMH and memory decline by hippocampal atrophy.

The diagrams present standardized estimates (with p-values) for all direct associations, separately for working memory (left) and episodic memory (right). The statistical significance of the direct and indirect paths is presented in the centre of the diagram. Analyses were performed using Lavaan, adjusted for age, sex and education. The direct effects of both WMH and hippocampal atrophy on episodic memory decline are significantly different from zero, though the indirect effect of WMH on episodic memory decline via hippocampal atrophy is not, suggesting that the effect of WMH on episodic memory decline is not causally mediated by hippocampal atrophy.
Mediation of the associations between WMH and memory decline by hippocampal atrophy

The results of the causal mediation analyses are shown in Figure 5, separately for working memory and episodic memory decline. Decline in working memory performance over time was not associated with baseline WMH volume ($\beta = 0.022; p=0.707$) or hippocampal atrophy ($\beta = 0.036; p=0.306$) after adjusting for age, sex, and education. The direct effects of both baseline WMH volume ($\beta = -0.170; p=0.001$) and hippocampal atrophy ($\beta = 0.126; p=0.009$) on episodic memory decline were significantly different from zero. The effect of baseline WMH on episodic memory decline was not mediated by hippocampal atrophy ($p$-value indirect effect: 0.572).

Discussion

We observed that memory decline in individuals with SVD was best explained by an interaction of WMH and hippocampal atrophy, rather than these two variables independently. In addition, we showed that the association between WMH and episodic memory decline was not causally mediated by hippocampal atrophy. Finally, we demonstrated that these effects were specific to hippocampal atrophy, rather than general grey matter atrophy. Together, our findings suggest that memory decline in patients with SVD is a heterogeneous condition to which different pathologies contribute.

Our findings that memory decline could be best explained by the interaction of WMH and hippocampal atrophy are consistent with imaging studies showing an additive effect of WMH and hippocampal atrophy on the level of cognitive decline [3, 334, 335], although there are also studies that have reported that AD and vascular pathologies are independent processes [327]. These variable findings might be due to differences in the relative proportion of cerebrovascular and AD pathologies, with both pathologies being present in many older adults with dementia [360]. Our study adds to these findings by describing the temporal interactions between both pathologies and memory decline longitudinally, over a long follow-up of almost nine years. We found that memory decline could be best explained by the interaction of WMH and hippocampal atrophy and that the associations between WMH and hippocampal atrophy were not causally mediated by hippocampal atrophy, suggesting that WMH and hippocampal atrophy synergistically affect memory decline. Moreover, we found that this effect of hippocampal atrophy was not just the result of global brain atrophy, as the effect of hippocampal atrophy was stronger than the effect of global gray matter atrophy.

Interestingly we observed an interaction between WMH and hippocampal atrophy for working memory, although the relative involvement of the hippocampus itself was lower for working memory performance than for episodic memory performance. While it has been argued that working memory processing should not rely on the hippocampus [48, 348], and we hypothesized that working memory performance would be related to WMH specifically, some degree of long-term encoding has been demonstrated during working memory tasks related to the specific task demands [361]. That is, working memory tasks are rarely "process pure", but may also include components that promote incidental long-term encoding (e.g., maintaining the target items in the PPMT over a longer period of time).

Several mechanisms might be proposed for the association between WMH and memory decline. WMH might exert their clinical effects in a direct way by disconnecting white matter tracts, or indirectly through incident stroke [122] or by affecting remote brain structures [49, 147]. Alternatively, vascular risk factors might lead to cognitive decline in patients with SVD through other mechanisms, such as inflammatory responses, hormonal dysregulation or damage to neurotransmitter systems [362-364]. Finally, memory decline in patients with SVD might also be explained by the interaction of SVD pathology with other neurodegenerative pathologies, such as seen in AD [3, 14, 187, 360]. Our observations that WMH and hippocampal atrophy interact in explaining memory decline in patients with SVD support this hypothesis. This is in line with a study in patients with AD showed that reduced network integrity was associated with SVD severity, specifically in networks important for cognition [333]. Animal studies have suggested that neurovascular dysfunction could potentiate the production of amyloid beta [336], suggesting that cerebrovascular pathology might be a risk factor for AD, although longitudinal evidence on the directionality of associations between vascular and Alzheimer's pathology in humans remains limited.

The presence of an interaction effect in the absence of a causal mediation effect suggests that WMH and hippocampal atrophy are independent processes that synergistically affect memory decline. These findings suggest that age-related memory decline is a heterogeneous condition in which different pathologies contribute to the memory decline observed in elderly with SVD.

Major strengths of this study include the large number of participants with SVD included in the study and the longitudinal design of our study with three neuroimaging assessments over nine years, which enabled us to study temporal interactions between WMH progression, hippocampal atrophy and memory decline beyond cross-sectional associations. Several methodological issues and
limitations deserve consideration. First, slight changes in neuroimaging protocols between baseline and first follow-up might be a potential source of bias. To minimize effects of changes in FLAIR sequence we resliced follow-up FLAIR images to match the slice thickness of baseline images using linear interpolation. Besides, we calculated WMH volumes for odd and even slices separately to determine the effects of change in slice thickness of the FLAIR sequence. This revealed similar results. Furthermore, hippocampal volumes were calculated using subject templates as part of the longitudinal segmentation pipeline, reducing the risk of bias in longitudinal volume calculations. Another limitation might be that learning effects have led to an underestimation of memory decline in our study population. We limited these possible learning effects by the use of alternative versions of the memory tasks during the second follow-up assessment. Due to the long-term follow-up of our study, a proportion of the participants was unable to complete the entire follow-up. This attrition bias might have led to an underestimation of the effects, since those lost to follow-up had more severe WMH, smaller hippocampal volumes, and were cognitively more impaired already at baseline. However, by using mixed effects models we could also take into account the participants with one or more missing values, thereby limiting the effect of the attrition bias. Finally, as we did not have pathological confirmations on cerebrovascular or AD pathologies, we used WMH and hippocampal volumes as markers of these two diseases.

In conclusion, memory decline in elderly with SVD was best explained by the interaction of WMH with hippocampal atrophy. The relationship between WMH and memory was not causally mediated by hippocampal atrophy, suggesting that memory decline during aging is a heterogeneous condition in which different pathologies contribute to the memory decline observed in elderly patients with SVD.

Supplementary material

Supplementary methods

Neuroimaging protocols

MR images were acquired at three time-points on 1.5-Tesla MRI (2006: Siemens, Magnetom Sonata; 2011 and 2015: Siemens, Magnetom Avanto) and included the following whole brain scans: T1-weighted 3-dimensional MPRAGE (isotropic voxel size 1.0 mm$^3$), FLAIR (2006: voxel size 0.5x0.5x5.0 mm, interslice gap 1.0 mm; 2011 and 2015: voxel size 0.5x0.5x2.5 mm; interslice gap 0.5 mm), a transversal T2*-weighted gradient echo sequence (voxel size 1.3x1.0x5.0 mm, interslice gap 1.0mm) and DTI (2006: isotropic voxel size 2.5 mm$^3$, 4 unweighted scans, 30 diffusion weighted scans at b=900 s/mm$^2$; 2011 and 2015: isotropic voxel size 2.5 mm$^3$, 8 unweighted scans, 60 diffusion weighted scans at b=900 s/mm$^2$) [110, 148, 229]. The same head coil was used at all three time-points.

Brain volumetry

Grey matter (GM), white matter (WM) and CSF probability maps were computed using SPM12 (http://www.fil.ion.ucl.ac.uk/spm/) unified segmentation routines on the T1 MPRAGE images [110]. Additionally, we used the WMH masks to correct the segmentation images, since several brain regions with WMH damage were initially misclassified. All WMH voxels were given mean WM intensity and these corrected T1 images were segmented using SPM12. All images were visually checked for co-registration and segmentation artefacts. GM, WM and CSF volumes (GMV, WMV and CSFV) were computed by summing all voxels belonging to that tissue class multiplied by voxel volume in mL. Intracranial volume (ICV) was determined by summing GMV, WMV and CSFV and total brain volume (TBV) by summing GMV and WMV. To account for inter-scan-effects we corrected for differences in ICV between baseline and follow-up. We normalized all volumes to baseline ICV to account for head size [149].

White matter hyperintensities

WMH volumes were calculated by a semiautomatic WMH segmentation method [110, 150]. Segmentations were visually checked for segmentation errors by one trained rater, blinded for clinical data. WMH volumes were corrected for inter-scan-differences in ICV and then normalized to baseline ICV. Besides, we calculated WMH volumes for odd and even slices separately to determine the effects of change in slice thickness of the FLAIR sequence.
**Supplementary Figure 1** Flowchart

**Baseline (2006)**
- Participants with MRI: n=503
  - 49 participants deceased

**Participants eligible for follow-up 1 (2011)**
- n=454
  - 93 participants without MRI in 2011
    - Lost to follow-up: n=2
    - Unable to visit research centre: n=54
    - MRI contra-indications: n=37
  - Note: These 93 participants were again contacted for second follow-up assessment in 2015.
  - 43 participants deceased

**Follow-up 1 (2011)**
- Participants with MRI: n=361
  - n=411
  - 115 participants without MRI in 2015
    - Unable to visit research centre: n=65
    - MRI contra-indications: n=50
  - 20 participants excluded in longitudinal study
    - MRI assessment in 2015 but not 2011: n=15
    - Insufficient scan quality (2006): n=7
    - Insufficient scan quality (2011): n=15
    - Insufficient scan quality (2015): n=7
  - Participants with MRI in 2006 & 2011 & 2015: n=263

**Supplementary Table 1** WMH and hippocampal volume and memory performance over time

<table>
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<tbody>
<tr>
<td>WMH volume, ml</td>
<td>2.3 (0.8 – 6.1)</td>
<td>2.8 (1.2 – 7.7)</td>
<td>4.7 (2.0 – 11.5)</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Hippocampal volume, ml</td>
<td>7.9 ± 0.9</td>
<td>7.6 ± 1.0</td>
<td>7.4 ± 1.1</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Cognitive performance</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Working memory, z-score</td>
<td>0.21 ± 0.88</td>
<td>-0.19 ± 0.90</td>
<td>-0.41 ± 1.00</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Episodic memory, z-score</td>
<td>0.18 ± 0.77</td>
<td>-0.26 ± 0.79</td>
<td>0.15 ± 0.99</td>
<td>p&lt;0.001</td>
</tr>
</tbody>
</table>

WMH and hippocampal volumes and memory performance over time for all participants with repeated neuroimaging and cognitive assessments at three time-points (n=263), displayed as median (IQR) or mean ± SD. Z-scores are calculated based on the mean and SD from the baseline study population. Significant decline was tested using repeated measures ANOVA.
Chapter 11

Longitudinal changes in rich club organization and cognitive decline in cerebral small vessel disease

Submitted as:
van Leijen EMC, van Uden IWM, Bergkamp MI, Ghafoorian M, van der Holst HM, Platel B, Norris DG, Claassen JAHR, Kessels RPC, de Leeuw FE, Tuladhar AM

Longitudinal changes in rich club organization and cognitive decline in cerebral small vessel disease
CHAPTER 11

LONGITUDINAL CHANGES IN RICH CLUB ORGANIZATION AND COGNITIVE DECLINE

Introduction

Cerebral small vessel disease (SVD) is considered the most important vascular contributor to the development of cognitive impairment and dementia [3, 4, 41]. Markers of SVD include white matter hyperintensities (WMH), lacunes and microbleeds [5] and are present on neuroimaging in virtually every individual over 60 [6], although in a highly variable degree. All these conventional SVD markers have been associated with cognitive impairment, but exactly how these associations result in cognitive decline or dementia is hitherto incompletely understood [5, 365].

There is increasing awareness that SVD exerts its clinical effects by disrupting white matter connections [53, 366, 367]. That is, structural network connectivity might mediate the associations between conventional SVD markers and cognitive deficits by disrupting large-scale brain networks [53, 54]. Structural brain connectivity can be assessed using Diffusion Tensor Imaging (DTI)-based whole-brain tractography techniques [368]. Additional insights into the extent and location of structural network disruption in SVD have been provided by rich club analyses, referring to the presence of nodes that are both highly connected to the network and highly interconnected with each other, hence called rich clubs [55, 56]. Due to their central role in brain networks and high connectivity with other nodes, rich clubs play a pivotal role in the integration of information [55], and damage to rich club connections might therefore have a more widespread effect on network functioning compared with damage to peripheral connections in a network.

Several cross-sectional studies in patients with SVD have shown that reduced structural network integrity, reflected by decreased global efficiency, was related to increased cognitive impairment [53, 54, 295] and to an increased risk of future dementia [297]. In addition, associations between conventional MR markers of SVD and decreased cognitive functions were, at least in part, mediated through network disruption [53, 54, 289]. In particular, reduced connectivity was predominantly observed for connections between the rich club nodes rather than a generalized, random reduction of white matter connectivity [57]. Moreover, rich club connectivity strength mediated the association of WMH with processing speed and executive functioning, such that higher rich club connectivity strength was associated with better cognitive performance [57]. However, how SVD-related disturbances in rich club organization progress over time and how this relates to subsequent cognitive decline is unknown, but could provide new insights into the temporal course and location of disruptions of white matter connections.

Abstract

Background: Cerebral small vessel disease (SVD) is considered the most important vascular contributor to the development of cognitive impairment and dementia. There is increasing awareness that SVD exerts its clinical effects by disrupting white matter connections, predominantly disrupting connections between rich club nodes, a set of highly connected and interconnected regions. However, exactly how SVD-related disturbances in rich club organization progress over time and how this relates to subsequent cognitive decline is unknown. Here we examined the progression of disturbances in rich club organization in older adults with SVD and how it is associated with conventional SVD markers and cognitive decline. We additionally investigated associations of baseline network measures with dementia.

Methods: In 270 participants of the RUN DMC study, we performed diffusion tensor imaging (DTI) and cognitive assessments longitudinally. Rich club organization was examined in structural networks derived from DTI followed by deterministic tractography.

Results: Global efficiency (p<0.05), network density (p<0.01) and strength of rich club connections (p<0.001) declined from baseline to follow-up. Decline in global network efficiency was associated with worse cognitive index and psychomotor speed ($\beta=0.116$ and $\beta=0.146$; $p<0.05$). Decline in strength of peripheral connections was associated with a decline in overall cognition ($\beta=0.164$; $p=0.01$), psychomotor speed ($\beta=0.151$; $p=0.05$) and executive function ($\beta=0.117$; $p=0.05$). Baseline network measures were reduced in participants with dementia at follow-up, and the association between WMH and dementia was causally mediated by global efficiency (indirect p-value=0.037) and peripheral connection strength (indirect p-value=0.040).

Conclusions: SVD-related disturbances in rich club organization progress over time, which in turn are associated with decline in psychomotor speed and executive function. The effect of WMH on dementia was causally mediated by global network efficiency and the strength of peripheral connections, suggesting an important role for network disruption in causing cognitive decline and dementia in older adults with SVD.
We therefore longitudinally examined changes in structural network organization in 270 participants with SVD. The specific aims of this study were (1) to assess how SVD-related disturbances in structural network, and specifically rich club organization, progress over time; (2) to study whether the decline in structural network organization is related to the progression of conventional SVD markers; (3) to relate this decline in network organization to cognitive decline; and (4) to examine whether baseline alterations in network organization are associated with incident dementia and whether these alterations mediate the association between conventional SVD markers with dementia.

Methods

Study population
This study was part of the Radboud University Nijmegen Diffusion tensor and Magnetic resonance imaging Cohort (RUN DMC) study, a prospective cohort study of 503 older adults with SVD that investigates risk factors and clinical consequences of SVD. The detailed study protocol has been published previously [148]. In the present study, we only used data available from the first (2011) and second (2015) follow-up assessments and excluded data from the baseline (2006) assessment due to slight changes in scanner protocol between 2006 and 2011. In the rest of this article, we will refer to the 2011 assessment as ‘baseline’ and to the 2015 assessment as ‘follow-up’. Of the 503 participants (during the 2006 examination), 329 participants were available for baseline (2011) analyses. In addition, 281 participants underwent repeated neuroimaging assessments [110], 11 of whom were excluded because of neuroimaging artefacts, yielding 270 participants for the longitudinal analyses. The Medical Review Ethics Committee region Arnhem-Nijmegen approved the study and all participants gave written informed consent.

Cognitive assessment
Cognitive performance was measured using an extensive neuropsychological test battery during all waves of data collection, as has been described previously [228]. Raw scores of all time-points were transformed into z-scores based on the mean and standard deviation (SD) of the baseline study population. We calculated Speed–Accuracy Trade-Off (SAT) scores wherever appropriate. Cognitive decline over time was calculated for each participant individually, by subtracting baseline scores from the follow-up scores.

We calculated a compound score for global cognitive function (cognitive index) as well as for three cognitive domains: memory, psychomotor speed and executive function. For the cognitive index, we calculated the mean of the z-scores of all tests from the neuropsychological test battery. To measure memory, we used the immediate and delayed recall of the Rey Auditory Verbal Learning Test (RAVLT) [355] and the Rey Complex Figure Task (RCFT) [356], as well as Speed–Accuracy Trade-Off (SAT) scores of the 2- and 3-letter subtasks of the Paper-Pencil Memory Scanning Task (PPMST) [196]. Psychomotor speed was calculated as the mean of the z-scores of the 1-letter subtask of the PPMST, the reading and color naming tasks of an adapted version of the Stroop Test [369] and the Symbol Digit Substitution Task (SDST) [370]. For executive function, we calculated the interference score of the Stroop Test by dividing SAT-scores of the color-word task by the mean SAT-scores of the reading and color naming tasks of the Stroop Test [371], the verbal fluency task [372] and SAT-scores of the Verbal Series Attention Test (VSAT) [373]. To account for possible material-specific practice effects, parallel versions of the RAVLT, RCFT and verbal fluency test were used for the follow-up assessment.

Dementia diagnosis
Dementia case finding was extensively described previously [225]. In short, dementia was diagnosed after outpatient evaluation of the individual patient findings at the Radboud Alzheimer Center memory clinic, or by a consensus diagnosis by a panel consisting of a neurologist, clinical neuropsychologist and a geriatrician with expertise in dementia, who reviewed all available cognitive assessments and medical records. The diagnosis of dementia was based on the DSM-IV-TR criteria [227]. In total, 23 out of 329 participants were diagnosed with dementia at follow-up.

Vascular risk factors
We recorded the presence of hypertension, smoking, alcohol use, diabetes and hypercholesterolemia at baseline by standardized questionnaires, as described previously [148]. We defined hypertension as the use of antihypertensive agents and/or systolic blood pressure greater than or equal to 140 mm Hg and/or diastolic blood pressure greater than or equal to 90 mm Hg [148].

MRI acquisition
MR images were acquired at two time points (2011 and 2015) on the same 1.5-Tesla Siemens Magnetom Avanto scanner and included the following whole brain scans: T1-weighted 3D MPRAIE imaging (isotropic voxel size 1.0 mm³), a FLAIR sequence (voxel size 0.5x0.5x2.5 mm; interslice gap 0.5 mm) and a DTI sequence (isotropic voxel size 2.5 mm³), 8 unwighted scans, 60 diffusion weighted scans at b=900 s/mm²). Full acquisition details have been described previously [110, 148].
Network measures
Graph theoretical measures were calculated from the structural network using the Brain Connectivity Toolbox [52]. These measures included: (1) node degree, representing the number of connections of a node; (2) network density, defined as the ratio between the number of connections present and the number of total possible connections in a network; (3) total network strength, computed as the sum of all connection strengths in a network; (4) global efficiency, expressed as the average inverse of the shortest path length between two nodes.

Rich club measures
Rich club regions included the bilateral superior frontal gyrus, precuneus, superior parietal gyrus and the insula. This selection of rich club nodes was based on the literature and the selection of these nodes as rich club nodes has been validated by previous studies [375, 376]. The connections of the network were then classified for further analysis [55, 56]: connections between the rich club nodes were classified as rich club connections; connections to the rich club nodes as feeder connections and connections between the non-rich club nodes as peripheral connections. The strength of these three types of connections was calculated as the average of the edge weights for that group.

Statistical analysis
To assess how SVD-related disturbances in structural network organization progress over time, we calculated differences in network density, network strength, global connectivity and strength of rich club, feeder and peripheral connections over time using repeated measures ANOVA. We additionally analyzed whether the changes in rich club organization differed between participants with mild versus severe WMH. Therefore, we stratified WMH severity based on median split of baseline WMH volumes. Differences between participants with mild versus severe WMH were calculated using one-way ANOVA, adjusted for age and sex.

To study the associations between conventional SVD markers (i.e. WMH and presence of lacunes and microbleeds) and structural network measures, we performed linear regression analyses, adjusted for age and sex. In addition, we assessed whether the decline in structural network organization was affected by the progression of conventional SVD markers. We therefore performed linear regression analyses using WMH progression, incident lacunes and microbleeds and difference (Δ) scores of network measures, with adjustments for age and sex.
Additionally, we aimed to relate the decline in network organization to cognitive decline. Therefore, we performed linear regression analyses, separately for decline in cognitive index, memory, psychomotor speed and executive function, adjusted for age, sex and education.

Finally, to examine whether baseline alterations in network organization are associated with dementia status at follow-up, we analyzed differences in network measures for participants with and without dementia using one-way ANOVA, adjusted for age, sex and education. To assess whether these network alterations mediated the association between conventional SVD markers with dementia, we additionally performed mediation analyses using ‘lavaan’ version 0.5-23.1097 in R [359]. Using lavaan, we estimated the direct effect of baseline WMH volume on the development of dementia and the indirect effect of baseline WMH volume on the development of dementia via structural network measures, separately for global efficiency and strength of rich club, feeder and peripheral connections. Statistical analyses were performed using R 3.5.2 (https://www.r-project.org/) and SPSS Statistics version 20.

Results

Baseline characteristics of the study population are presented in Table 1. Mean age was 67.9 (SD 7.8) years and mean follow-up duration was 3.4 (SD 0.2) years.

Progression of rich club organization over time, by SVD severity

We first assessed if and how disturbances in rich club organization progress over time. The progression of rich club organization over time is shown in Figure 1. The strength of rich club connections declined over time (mean difference [95% confidence interval]: -0.44 [-0.63 – -0.26]; p<0.001), in contrast to the strength of feeder (mean difference: -0.03 [-0.073 – 0.023]; p=0.303) and peripheral connections (mean difference: -0.02 [-0.040 – 0.003]; p=0.094). In terms of global network measures, both network density and global efficiency declined over time. Network disturbances were most pronounced in participants with severe WMH (Figure 1). In addition, the strength of peripheral connections declined in participants with severe baseline WMH, but not in participants with mild baseline WMH (Figure 1).

Effects of SVD markers on changes in rich club organization

To assess whether disruptions in rich club organization are affected by the severity and progression of conventional SVD markers, we performed linear regression analyses (Table 2). The strength of rich club connections was affected by WMH volume ($\beta$=-0.189; p<0.001) and the number of lacunes ($\beta$=-0.067; p<0.01). The strength

Table 1 Characteristics of the study population

<table>
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<tr>
<th>Demographics</th>
<th>Baseline 2011 (n=270)</th>
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<tbody>
<tr>
<td>Age, years</td>
<td>67.9 ± 7.8</td>
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<tr>
<td>Male sex, number of participants (%)</td>
<td>162 (59.6)</td>
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<td>MMSE score</td>
<td>28.4 ± 1.8</td>
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<td>Education, years</td>
<td>10.1 ± 1.5</td>
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<tr>
<th>Vascular risk factors</th>
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<td>Hypertension, number of participants (%)</td>
<td>162 (59.6)</td>
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<tr>
<td>Diabetes, number of participants (%)</td>
<td>37 (13.6)</td>
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<td>Hypercholesterolemia, number of participants (%)</td>
<td>124 (45.6)</td>
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<td>Smoking, ever, number of participants (%)</td>
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<td>Alcohol, glasses/week</td>
<td>3.8 ± 4.0</td>
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<td>Body mass index, kg/m²</td>
<td>27.8 ± 4.2</td>
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<th>Imaging characteristics</th>
<th>Baseline 2011 (n=270)</th>
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<tr>
<td>Total brain volume, ml</td>
<td>1066.2 ± 77.7</td>
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<tr>
<td>Grey matter volume, ml</td>
<td>610.6 ± 50.1</td>
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<tr>
<td>White matter volume, ml</td>
<td>455.7 ± 44.0</td>
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<td>WMH volume, ml</td>
<td>2.8 (1.3 – 7.8)</td>
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<td>Lacunes, number of participants (%)</td>
<td>60 (25.4)</td>
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<td>Microbleeds, number of participants (%)</td>
<td>47 (17.3)</td>
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<tr>
<td>NAWM MD, 10⁻³ mm²/s</td>
<td>0.84 ± 0.04</td>
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<tr>
<td>NAWM FA</td>
<td>0.38 ± 0.02</td>
</tr>
</tbody>
</table>

Data represent mean ± SD or number of participants (%). WMH volume was expressed as median (IQR). MMSE: Mini-Mental State Examination; WMH: white matter hyperintensities; NAWM: normal appearing white matter; MD: mean diffusivity; FA: fractional anisotropy.

of feeder and peripheral connections was affected by WMH volume and by the number of lacunes and microbleeds ($p<0.001$ for all SVD markers). The progression of conventional SVD markers was not associated with changes in rich club, feeder or peripheral connections.

Effects of rich club organization on cognitive performance

The results from linear regression analyses on the associations between longitudinal changes in rich club organization and cognitive decline are shown in Table 3. The degree of reduction in global efficiency and network strength were associated with greater decline in cognitive index (global efficiency: $\beta$=0.116; $p<0.05$; network strength: $\beta$=0.147; $p<0.01$) and psychomotor speed (global
efficiency: $\beta=0.146$; $p<0.05$; network strength: $\beta=0.160$; $p<0.01$). With respect to disruptions in rich club organization, the decline in peripheral connection strength was associated with decline in cognitive index ($\beta=0.164$; $p<0.01$), psychomotor speed ($\beta=0.151$; $p<0.05$) and executive function ($\beta=0.117$; $p<0.05$). Decline in rich club or feeder connection strength was not associated with decline in cognitive performance.

**Associations between baseline network characteristics and dementia**

Of the 329 participants, 23 participants had been diagnosed with dementia at follow-up. We examined whether the degree of network organization at baseline examination was associated with dementia at follow-up and whether these alterations mediated the association between conventional SVD markers with dementia. Participants with dementia had, at baseline, lower total network

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**Figure 1** Progression of rich club organization over time

Progression of rich club organization from baseline to follow-up. Data indicate mean connection strength ± SEM for the study population (black), and additionally for patients with mild WMH (blue) and with severe WMH (red). WMH volumes are stratified based on median split of WMH volumes in 2011. All network measures are statistically different for patients with severe versus mild WMH (p<0.001 for all network measures). Statistical differences between baseline and follow-up have been calculated using repeated measures ANOVA. Differences between participants with mild versus severe WMH have been calculated using one-way ANOVA, adjusted for age and sex. *p<0.05; **p<0.01; ***p<0.001.

**Table 2** Associations between SVD markers and rich club organization

<table>
<thead>
<tr>
<th></th>
<th>Rich club strength</th>
<th>Feeder strength</th>
<th>Peripheral strength</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>-.500***</td>
<td>-.322***</td>
<td>-.474***</td>
</tr>
<tr>
<td></td>
<td>[.587, -.414]</td>
<td>[.403, -.241]</td>
<td>[.547, -.400]</td>
</tr>
<tr>
<td>Sex</td>
<td>.238***</td>
<td>-.038</td>
<td>.038</td>
</tr>
<tr>
<td></td>
<td>[.095, .382]</td>
<td>[.172, .096]</td>
<td>[.083, .160]</td>
</tr>
<tr>
<td>Time, years</td>
<td>-.040</td>
<td>.046</td>
<td>.025</td>
</tr>
<tr>
<td></td>
<td>[.118, .038]</td>
<td>[.027, .120]</td>
<td>[.042, .091]</td>
</tr>
<tr>
<td>WMH</td>
<td>-.189***</td>
<td>-.336***</td>
<td>-.270***</td>
</tr>
<tr>
<td></td>
<td>[.278, -.101]</td>
<td>[.419, -.254]</td>
<td>[.344, -.195]</td>
</tr>
<tr>
<td>WMH progression</td>
<td>-.016</td>
<td>-.041</td>
<td>-.036</td>
</tr>
<tr>
<td></td>
<td>[.094, .063]</td>
<td>[.115, .032]</td>
<td>[.102, .031]</td>
</tr>
<tr>
<td>Lacunes</td>
<td>-.067***</td>
<td>-.089***</td>
<td>-.108***</td>
</tr>
<tr>
<td></td>
<td>[.116, -.018]</td>
<td>[.136, -.043]</td>
<td>[.150, -.066]</td>
</tr>
<tr>
<td>Incident lacunes</td>
<td>.021</td>
<td>.002</td>
<td>.014</td>
</tr>
<tr>
<td></td>
<td>[.024, .067]</td>
<td>[.041, .044]</td>
<td>[.025, .053]</td>
</tr>
<tr>
<td>Microbleeds</td>
<td>-.005</td>
<td>-.033***</td>
<td>-.034***</td>
</tr>
<tr>
<td></td>
<td>[.024, .013]</td>
<td>[.050, .015]</td>
<td>[.049, -.018]</td>
</tr>
<tr>
<td>Incident microbleeds</td>
<td>.003</td>
<td>-.0002</td>
<td>-.003</td>
</tr>
<tr>
<td></td>
<td>[.015, .021]</td>
<td>[.017, .017]</td>
<td>[.018, .013]</td>
</tr>
</tbody>
</table>

Associations of the conventional SVD markers WMH, lacunes and microbleeds with structural network measures. Data are displayed as standardized betas [95% confidence intervals], analyzed using linear regression analyses. *p<0.05; **p<0.01; ***p<0.001.
density (mean difference [95% confidence interval]: -0.012 [-0.019 – -0.005]; p=0.001), lower network strength (mean difference: -21.0 [-38.0 – -4.0]; p=0.015) and lower global efficiency (mean difference: -1.2 [-2.1 – -0.30]; p=0.009) as compared with the group without dementia. Moreover, participants with dementia also showed, at baseline, lower strength of peripheral connections (Figure 2; mean difference: -0.21 [-0.37 – -0.05]; p=0.009). No differences were observed for rich club (mean difference: -0.13 [-1.2 – 1.5]; p=0.848) and feeder connection strength (mean difference: -0.37 [-0.76 – 0.01]; p=0.059) between participants with and without dementia. Mediation analyses revealed no direct effect of WMH on dementia, in contrast with the indirect effects of WMH on dementia via global efficiency (p=0.037) and peripheral connection strength (p=0.040)(Figure 3), suggesting that the effect of WMH on dementia is causally mediated by global network efficiency and the strength of peripheral connections.

### Table 3 Rich club organization and cognitive decline

<table>
<thead>
<tr>
<th>Δ Cognitive Index</th>
<th>Δ Memory</th>
<th>Δ Psychomotor speed</th>
<th>Δ Executive function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Global efficiency</td>
<td>.116*</td>
<td>.004</td>
<td>.146*</td>
</tr>
<tr>
<td></td>
<td>[.003, .229]</td>
<td>[-.115, .124]</td>
<td>[.027, .266]</td>
</tr>
<tr>
<td>Network strength</td>
<td>.147**</td>
<td>.042</td>
<td>.160**</td>
</tr>
<tr>
<td></td>
<td>[.036, .258]</td>
<td>[-.076, .160]</td>
<td>[.042, .278]</td>
</tr>
</tbody>
</table>
| Δ Connection strength
| Rich club        | .024    | .022                | .074                |
|                  | [-.087, .136] | [-.140, .096]      | [-.044, .194]       |
| Feeder           | .059    | .005                | .115                |
|                  | [.053, .172] | [-.123, .113]      | [.004, .234]        |
| Peripheral       | .164**  | .061                | .151*               |
|                  | [.054, .274] | [-.056, .178]      | [.034, .268]        | [.001, .233] |

Longitudinal associations between network measures and cognitive decline. Data are displayed as standardized betas [95% confidence intervals]. Statistical differences were analyzed using linear regression analyses, adjusted for age, sex and education. *p<0.05; **p<0.01; ***p<0.001.

Network characteristics at baseline, separately for participants with (dark grey, n=23) and without dementia (light grey, n=306). Top: The global network measures (network density, network strength and global efficiency) were reduced in participants with dementia. Bottom: Strength of rich club, feeder and peripheral connections in participants with and without dementia. Statistical differences were analyzed using one-way ANOVA, adjusted for age, sex and education. *p<0.05; **p<0.01; ***p<0.001.

Network characteristics at baseline stratified by dementia status at follow-up.
Figure 3  Diagrams showing statistical mediation analyses of the relationship between WMH and dementia by structural network measures

The diagrams present standardized estimates (with p-values) for all direct associations, separately for global efficiency and strength of rich club, feeder and peripheral connections. The statistical significance of the direct and indirect paths is presented in the centre of the diagram. Analyses were performed using lavaan, adjusted for age, sex and education. The direct effect of WMH on dementia is not significantly different from zero, while the indirect effects of WMH on dementia via global efficiency and peripheral connection strength are, suggesting that the effect of WMH on dementia is causally mediated by global network efficiency and the strength of peripheral connections.
Discussion

In this longitudinal study, we investigated the progression of structural network connectivity and rich club organization over time in participants with SVD. SVD-related disturbances in rich club organization progressed significantly over 3.4 years, predominantly in participants with severe WMH. Declines in global network efficiency and peripheral, but not rich club or feeder, connection strength were associated with cognitive decline and dementia. The effect of WMH on dementia was causally mediated by global network efficiency and the strength of peripheral connections, suggesting an important role for global network, rather than rich club disruption in causing cognitive decline and dementia in elderly with SVD.

Our study provides evidence that SVD-related disturbances in rich club organization progress over time, which in turn is related to cognitive decline. Previous cross-sectional studies have shown reductions in network global efficiency in participants with SVD that mediated the relationship between conventional MRI markers of SVD and cognitive impairment or dementia [53, 54, 289, 295, 297]. In particular, reduced connectivity was predominantly observed for rich club connections, mediating the association of WMH with processing speed and executive functioning [57]. However, differentiating causality from association is impossible in cross-sectional studies. Two previous longitudinal studies have reported associations between declines in global efficiency and cognitive performance in patients with cerebral amyloid angiopathy (CAA) [377] and in patients with severe symptomatic SVD [378]. However, to our knowledge, no longitudinal studies have addressed the progression of disturbances in rich club organization over time in patients with SVD and its relation to subsequent cognitive decline. Our longitudinal findings support the hypothesis that conventional MRI markers of SVD (such as WMH, lacunes and microbleeds) cause cognitive decline and dementia via disruption of complex brain networks.

Several mechanisms can be hypothesized that may explain the progression of network disturbances over time. First, the location of incident SVD might be consistent with the location of disrupted connections (i.e. WMH progression or incident lacunes or microbleeds might target specific connections and thereby disrupt white matter connections). However, we showed that, on top of baseline SVD, the progression of conventional SVD markers was not significantly associated with the strength of rich club, feeder or peripheral connections (Table 2). We therefore consider this hypothesis as less likely. An alternative explanation might be the high metabolic demand of especially the rich club nodes and connections. It has been argued that especially the rich club nodes have a high rate of metabolic activity and that the long fibers connecting the rich club nodes require higher levels of energy consumption [50, 379]. As damage in SVD is presumably caused by ischemia, the progression of small vessel damage might preferentially affect the highly metabolic rich club connections.

Disturbances in rich club organization were predominantly observed in participants with severe baseline WMH, both in rich club connections and in feeder as well as peripheral connections. These findings suggest an important role for global network function in older adults with SVD, rather than rich club disruption specifically, further supporting the notion that SVD should be considered a global rather than a focal disease [580].

Interestingly, we did not observe any associations between decline in rich club connection strength and decline in cognitive performance. While rich club connection strength was significantly associated with cognitive performance in cross-sectional analyses, cognitive decline over time was only associated with decline in peripheral connection strength, rather than with a decline in rich club or feeder connection strength. Although we are not aware of any studies investigating the preferential role of specific connections for cognitive function in elderly with SVD longitudinally, these results are in contrast to what we would expect based on findings from several cross-sectional studies reporting an important role for rich club connections in cognitive processes, specifically for psychomotor speed and executive function [57, 381]. There may be several explanations for not finding this association. First, it might be that the cognitive domains measured in our study reflect localized rather than global cognitive functions, for which proper network function and integration is required. However, since we observed associations between rich club connection strength and psychomotor speed and executive function – known to rely on the integration of distributed brain areas – rather than with memory performance in cross-sectional analyses, we consider this hypothesis less likely. Second, the selection of rich club nodes was based on node degree in healthy controls; although this selection has been validated in several studies [375, 376] and these nodes were among the highest ranked nodes in our study population (data not shown), the rich club organization in our study population was already disrupted at baseline due to their SVD [57]. Possibly, the contribution of decline in rich club connection strength to cognitive decline, in addition to the baseline disruption of rich club connections, is limited relative to peripheral connections. Third, it might be that initial disruptions of the rich club connections are followed by secondary disruptions of the feeder or peripheral connections and that impairments in...
peripheral connections only lead to clinical overt symptoms after a certain threshold of structural network disruption is reached [379]. Finally, it might be that cortical structures have an important role in causing cognitive decline and dementia. Gray matter atrophy in frontal brain areas, for example, might explain executive dysfunction in participants with SVD [382]. This hypothesis was supported by previous studies reporting that lower cortical thickness in fronto-temporal regions was related to cognitive deficits independent of WMH [289, 383].

Our finding that baseline measures of global efficiency and peripheral connections are impaired in participants who developed dementia is clinically relevant, because it suggests that network parameters might be useful as markers to predict cognitive decline and the risk of progression to dementia. Additionally, the finding that the effect of WMH on dementia is causally mediated by global network efficiency and the strength of peripheral connections provides additional insights into the underlying mechanisms of cognitive symptoms attributable to SVD. Altogether, these findings suggest that the structural network acts as a mediator between conventional SVD markers and cognitive outcome and may allow identification of individuals at high risk of developing dementia.

Major strengths of the study were the large cohort of individuals covering a wide range of the SVD spectrum, the detailed phenotyping of the patients, including the diagnosis of dementia according to a standardized approach in all patients and the availability of longitudinal neuroimaging data obtained from the same scanner without upgrade or change over the full data collection period. However, several methodological issues should also be considered. First, the identification of structural networks was based on DTI acquired at 1.5 Tesla with relatively few diffusion directions and deterministic streamlining using tensor reconstruction models, limiting the identification of long-distance fibers and the reconstruction of white matter tracts in a complex white matter architecture due to noise and partial volume effects [384]. Although high-resolution imaging and more advanced tractography methods are required to provide more detailed information about the white matter architecture, the consistency of our findings on impaired global efficiency in SVD and its relation with cognitive performance with other studies [53, 54, 295] indicate that our network analyses in participants with SVD are reliable. A second consideration is the selection of rich club nodes. Our main findings are based on a rich club defined from the eight nodes with highest degrees in healthy controls. Although these nodes were among the highest ranked nodes in our study population (data not shown) and have been validated in several studies [375, 376], this might have influenced the results from our longitudinal analyses. However, it should be noted that defining rich club nodes based on individuals would also have caused bias, since participants with severe SVD would have fewer connections and less interconnected nodes, and we would have investigated different rich clubs across participants. Future studies are should investigate how the definition of the rich club influences rich club properties, especially in longitudinal studies and in patients with severe SVD. Third, it might as well be that our observed changes in rich club organization and cognitive performance over time are not solely attributable to SVD, but also to the effects of normal aging or to other pathologies such as neurodegeneration or Alzheimer’s disease, or interaction with these pathologies. However, the associations between conventional SVD markers with measures of rich club organization and the causal mediations of the associations between WMH and dementia via structural network properties indicate that the disruption of structural networks is important in explaining cognitive decline in elderly with SVD.

In conclusion, SVD causes disturbances in rich club organization, which in turn were associated with declines in psychomotor speed and executive function. The effect of WMH on dementia was causally mediated by global network efficiency and the strength of peripheral connections, suggesting an important role for global, rather than rich club network disruption in causing cognitive decline and dementia in elderly with SVD.
Part V
Summary and discussion
Chapter 12

Summary
The aim of this thesis was to gain insights into the time course, the etiology and the cognitive consequences of cerebral small vessel disease. In this chapter I will summarize the main findings.

Temporal dynamics of cerebral small vessel disease
In Part II of this thesis, I described the change of SVD markers over time, or, the “temporal dynamics” of SVD. The course of SVD has usually been described as a linear progressive process, mainly due to the availability of studies with only two neuroimaging assessments.

In Chapter 2, I elaborated on the dynamic course of SVD progression by reviewing the existing literature on progression of SVD over time. Where regression of SVD markers was previously often attributed to measurement error or classified as ‘no progression’, SVD regression appears to be a true phenomenon with clinical implications.

In Chapter 3, I showed the temporal dynamics of SVD, using three neuroimaging assessments over a period of nine years in the RUN DMC study. Progression of all SVD markers (i.e. WMH, lacunes and microbleeds) occurred in a non-linear fashion, accelerating over time consistent with a quadratic course. In addition, SVD progression was predominantly seen in participants with moderate or severe WMH at baseline, whereas participants with mild baseline WMH rarely showed progression over a period of nine years. Interestingly, progression of SVD markers was alternated by regression in 20.3% of participants: 11.2% showed WMH regression, 3.6% vanishing lacunes and 9.8% vanishing microbleeds, suggesting that SVD progression is sometimes interrupted by regression of SVD.

These findings suggest a paradigm shift: traditionally seen as a continuous progressive process, it should rather be considered a dynamic and highly heterogeneous process, sometimes with regression of SVD, which might also imply heterogeneity in etiology and cognitive consequences of SVD.

The etiology of cerebral small vessel disease
In Part III of this thesis, I elaborated on the etiology of SVD. To gain additional insights into the underlying mechanisms of SVD, I used diffusion tensor imaging to infer on the microstructural integrity of the white matter as well as biomarkers in blood to assess their associations with severity and progression of SVD.
In Chapter 4, I showed that impaired microstructural integrity in the NAWM preceded conversion into WMH and that WM microstructural integrity declined over time. Adding an additional time point with a total follow-up of nine years enabled us to distinguish between NAWM converting into WMH within the first and the second time-period. This revealed that baseline MD values were higher in NAWM areas converting into WMH within five years than in NAWM areas that converted into WMH between five and nine years. Moreover, participants with severe baseline WMH showed more loss of structural integrity compared with participants with mild WMH in all areas of the WM, including the remaining NAWM and WMH. The continuously ongoing decline of microstructural integrity within the WM underlines that WMH progression visible on conventional FLAIR imaging is only the “tip of the iceberg” with underlying loss of microstructural integrity occurring years before WMH can be detected on conventional MRI.

In Chapter 5, I assessed the associations between amyloid β levels in blood plasma with severity and progression of SVD. Our study showed that higher plasma Aβ levels were associated with neuroimaging markers of SVD at the cross-sectional level and with progression of SVD over a follow-up of almost nine years. The longitudinal design of our study enabled us to carbohydrates the directionality of the associations between plasma Aβ levels and cerebrovascular pathology. In our study, progression of WMH and incident microbleeds and lacunes were associated with higher baseline plasma Aβ levels of one or more of the peptides: plasma Aβ40 levels were elevated in participants with WMH progression (mean 194.6 vs. 182.9 pg/ml; p<0.05), both Aβ38 and Aβ40 were elevated in participants with incident lacunes (Aβ38 24.5 vs. 22.5 pg/ml; p<0.05; Aβ40 194.9 vs. 181.2 pg/ml; p<0.01), and Aβ42 in participants with incident microbleeds (62.8 vs. 60.4 pg/ml; p<0.05). Altogether, our findings that higher plasma Aβ levels were not only associated with severity of SVD markers but also with progression of SVD markers, suggest that amyloid β pathology might contribute to the development and progression of SVD.

In Chapter 6, I analyzed a second blood marker, serum Neurofilament Light Chain (NfL), in relation to neuroimaging markers of SVD. Serum NfL levels were elevated in participants with SVD (p=1e-05 compared with healthy controls). In addition, serum NfL levels were associated with all SVD markers, in particular Mean Diffusivity (R²=0.21, p=1e-15). Serum NfL levels were independently related to psychomotor speed (R²=0.06, p=4.8e-08), the cognitive domain most often affected in patients with SVD, suggesting that serum NfL might complement established MRI markers in assessing SVD burden.

In Chapter 7, I additionally analyzed whether serum NfL levels were associated with future white matter damage. Serum NfL levels were associated with future MRI markers of SVD (WMH: β=0.173; 95%CI [0.062-0.327]; p=0.004 and MD: β=0.165; 95%CI [0.048-0.334]; p=0.009) and with future psychomotor speed (β=0.135; 95%CI [-0.226, -0.039]; p=0.005). Together, our findings that serum NfL levels are associated with future microstructural tissue damage and future cognitive performance, suggest that serum NfL may potentially serve as a biomarker for disease monitoring and outcome in SVD.

Cognitive consequences of cerebral small vessel disease

In Part IV, I elaborated on the cognitive consequences of SVD. Where SVD was previously thought to be mainly associated with psychomotor speed, the spectrum of cognitive symptoms attributable to SVD seems to be much broader than previously thought and cannot solely be explained by the severity and location of SVD markers on neuroimaging. In this part, I therefore examined additional explanations for the remarkable variation of SVD-related cognitive symptoms, first by reviewing the existing literature and second by studying additional MRI markers (remote effects, network disruptions and interactions with neurodegeneration).
In Chapter 9, I described the cognitive consequences of regression of SVD markers over time. Regression of SVD markers was observed in 20.3% of participants. SVD regression did not accompany global brain atrophy and was associated with similar cognitive decline compared to participants with stable SVD. In addition, participants with WMH regression showed less cognitive decline compared to those with WMH progression (mean difference [95%CI]: 0.29 [0.02, 0.56]; p=0.035 for cognitive index and 0.42 [0.08, 0.76]; p=0.010 for memory), and participants with vanishing lacunes showed less decline in psychomotor speed compared to participants with incident lacunes (0.48 [0.01, 0.96]; p=0.043), although significance was lost after adjustments. Together, our findings of comparable cognitive decline between participants with regression and stable SVD might suggest that SVD regression has a relative benign cognitive outcome.

In Chapter 10, I elaborated on memory deficits in participants with SVD. Memory deficits are not often observed in patients with SVD and cannot be completely explained by the location of SVD, but are rather thought to result from hippocampal atrophy. I therefore examined the interaction of SVD with hippocampal atrophy. Memory decline in individuals with SVD was best explained by the interaction of WMH with hippocampal volumes, rather than these two variables independently (AIC model with both WMH & HV 1996.6 versus AIC full model with WMH*HV interaction term 1987.9, likelihood ratio test, \(\chi^2(1)=10.7, p<0.01\)). The WMH*HV interaction term was significantly associated with memory function (\(\beta=0.061; 95\%CI [0.025 – 0.098]; p<0.01\)). Mediation models showed that both WMH volume (\(\beta=0.170; p=0.001\)) and hippocampal atrophy (\(\beta=0.126; p=0.009\)) were independently related to memory decline, but the effect of WMH on memory decline was not causally mediated by hippocampal atrophy (p-value indirect effect: 0.372), suggesting that WMH and hippocampal atrophy are independent processes that synergistically affect memory decline. Moreover, we found that this effect of hippocampal atrophy was not just the result of global brain atrophy, as the effect of hippocampal atrophy was stronger than the effect of global gray matter atrophy. These findings suggest that memory decline in patients with SVD is a heterogeneous condition to which different pathologies contribute.

In Chapter 11, I assessed the importance of decline in structural network connectivity, and specifically in rich club connection strength, for cognitive performance. SVD-related disturbances of rich club organization progressed over time (mean difference -0.44; 95%CI [-0.63 – -0.26]; p<0.001), predominantly in participants with severe baseline WMH. Declines of rich club, feeder and peripheral connection strength were affected by the severity of SVD, but not by the progression of SVD. A greater decline in peripheral connection strength, rather than rich club or feeder connection strength, was associated with a greater decline in cognitive index (\(\beta=0.164; p<0.01\)), psychomotor speed (\(\beta=0.151; p<0.05\)) and executive function (\(\beta=0.117; p<0.05\)). Moreover, participants with dementia showed lower global efficiency and peripheral connection strength compared with participants without dementia, and the association between WMH and dementia was causally mediated via global efficiency and peripheral connection strength, suggesting an important role for network disruption in causing cognitive decline and dementia in elderly with SVD.
Chapter 13
Discussion and future perspectives
The aim of this thesis was to gain insights into the time course, the etiology and the cognitive consequences of cerebral small vessel disease (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 on the risk factors and clinical consequences of SVD using three neuroimaging and cognitive assessments over nine years in elderly with SVD.

In this chapter I will discuss some methodological considerations with respect to the study design and to the validity, inference and generalizability of the results. Thereafter, I will discuss the main findings of this thesis, both in context of existing literature and with respect to possible clinical implications of these findings. Finally, I will discuss some future perspectives within the field.

Methodological considerations

Study design
The RUN DMC study is a prospective hospital-based cohort study on the causes and consequences of SVD among 503 non-demented older adults, aged between 50 and 85 years, with signs of SVD on neuroimaging [148]. As the onset of SVD is often insidious, clinically heterogeneous, and typically with mild symptoms, the selection of participants with SVD was based on neuroimaging characteristics, including WMH and lacunes [174]. Accordingly, all individuals aged between 50 and 85 years who were referred to the Department of Neurology between 2002 and 2006 and underwent routine diagnostic brain imaging for various reasons, including stroke, transient ischemic attack and mood or cognitive complaints, were selected for participation. All participants were free of dementia at baseline and were invited for repeated neuroimaging and cognitive assessments for three times over nine years: baseline assessment has been performed in 2006, first follow-up in 2011 and second follow-up in 2015.

Validity of results
The internal validity is a property of a study reflecting the extent to which the associations measured in a study are truly caused by the exposure [385]. In this section, I will discuss the precision of measurements and three types of bias that are related to the validity of the results of this thesis: selection bias (including attrition bias), information bias (including measurement error from cognitive assessments and neuroimaging analyses), and confounding.
CHAPTER 13 DISCUSSION AND FUTURE PERSPECTIVES

Precision of measurements

Precision reflects the amount of random error in a study and thereby affects the reproducibility of a study [385]. Random error often results in imprecise effect estimates, reflected by broad confidence intervals. Precision can be improved by increasing the size of a study and by measuring the outcome and determinants by means of validated procedures. In the RUN DMC study, we reduced random error by the inclusion of a large number of participants with SVD. In addition, we rated SVD according to standardized procedures [5], segmented WMH semi-automatically, calculated brain volumes using the latest segmentation routines of SPM12 and visually checked for artefacts and segmentation errors, to more precisely measure the change in SVD markers, thereby minimizing the risk of misclassification and optimizing the accuracy of measurements. We therefore believe that the studies reported in this thesis have a high reproducibility. Yet, despite these efforts to optimize the measurements, precision could have been further improved. Some analyses included subgroups that contained only a small number of participants (for example in Chapter 9 where we analyzed cognitive decline separately in subgroups of progression, regression and stable SVD), resulting in large random variation in the estimations, reflected by wide confidence intervals. Besides, precision of SVD measurements including more detailed characterization of SVD and visualization of underlying mechanisms of conversion of NAWM into SVD could be improved by more advanced neuroimaging protocols.

Selection bias

Selection bias can occur when participation is related to the outcome of the study, i.e. the relation between determinants and outcome is different for participants and those who do not participate in the study [385]. In the RUN DMC study, selection bias may have occurred during the initial inclusion in the study at baseline and during follow-up. During the initial baseline selection, the RUN DMC had a high response of 71.3%. However, those who did not participate in the study were older (response rate in individuals between 50 and 60 years was 82% compared to 56.5% in individuals aged between 70 and 85 years) and had more severe WMH (measured by the Age Related White Matter Changes scale [386]) than participants. This might have led to an underestimation of the effects, since age and SVD severity are important predictors for cognitive impairment and those who did not participate in the study thus likely would have had more SVD-related cognitive deficits.

Additionally, attrition bias might have occurred due to the long follow-up of our study, which is a special form of selection bias occurring due to selective drop-outs in longitudinal studies. In the RUN DMC study, 54.9% of baseline participants underwent repeated neuroimaging assessments at three time-points over the nine-years follow-up. A considerable proportion of our study population could not complete follow-up assessments, because they were deceased, were unable to visit our research centre or developed contra-indications for MRI acquisition. This might have influenced our results on SVD progression and cognitive decline as participation during follow-up most likely will have depended on the outcome of our study (i.e. progression of SVD). This probably may have led to an underestimation of the strength of the associations.

Information bias

Information bias may occur by not properly defined determinants or outcomes or improper methods to collect data on them, which may lead to misclassification of these variables [385]. In the RUN DMC study, the risk of information bias was reduced by the single-centre design of the study, which allowed us to systematically and uniformly perform clinical assessments. Furthermore, the assessments of SVD neuroimaging markers, blood levels and cognitive performance were performed independently and blinded to each other, further limiting the risk of information bias. Information bias might, however, have arisen from both cognitive assessments and neuroimaging acquisition and analyses, which will be discussed in the following sections. With respect to misclassification due to imaging measures, I will focus on the assessment of SVD markers, longitudinal neuroimaging analyses, microstructural integrity and structural networks including rich club organization.

The assessment of cognitive decline was vulnerable to misclassification, as cognitive performance was measured at three time-points over nine years and cognitive assessments have been performed by different researchers at both follow-up assessments. Although we feel that these effects are limited due to extensive trainings from the researcher who performed the previous assessments, this might have lead to either an underestimation or an overestimation of the effects. Additionally, learning effects might have led to an underestimation of the true memory decline in our study population, especially since those who were able to complete both follow-up assessments performed significantly better on cognitive tasks at baseline compared to those who did not complete all follow-up assessments [228]. We limited these possible learning effects by the use of alternative versions of the memory tasks during the second follow-up assessment.

WMH volumes were calculated by a semi-automatic WMH segmentation method, enabling us to study WMH quantitatively which was much more sensitive than qualitative rating scales. The segmentations from all three time-points were
visually checked for segmentation errors by one trained rater blinded for clinical data, thereby optimizing the accuracy of measurements and minimizing the risk of misclassification. Both number and location of lacunes and microbleeds were rated manually on FLAIR/T1-weighted and T2*-weighted MRI scans according to the STRIVE criteria [5] by two trained raters blinded for clinical data with excellent inter- and intra-rater reliability [152], limiting the risk of information bias. Furthermore, brain volumes were calculated using the newest segmentation routines of SPM12 on T1-weighted images corrected for WMH to reduce the risk of misclassification. Besides, hippocampal volumes were calculated using subject templates as part of the longitudinal segmentation pipeline in FreeSurfer 5.3. Moreover, all images were visually checked for artefacts and segmentation errors, reducing the risk of bias in volume calculations.

The assessment of progression of SVD markers over time, however, could have been biased, since the assessment of SVD markers has not been performed blinded to scanning date as to assess true WMH progression or regression and true incident or vanishing lacunes or microbleeds. Besides, neuroimaging analyses in participants with severe SVD (especially in combination with severe brain atrophy and large ventricles) are more vulnerable to measurement errors than analyses in healthy controls, especially because most standard atlases are based on healthy controls and registrations to these atlases are more difficult for participants with severe SVD than for participants with mild SVD. In addition, so-called partial volume effects (PVE) might have reinforced this potential bias, that is, NAWM voxels around the border of existing WMH or lacunes can contain a fraction of signal intensity from these WMH or lacunes that affects measurements in that voxel. Since participants with severe SVD are more prone to PVE than participants with mild SVD, this might have led to an overestimation of SVD, especially in participants with severe SVD.

A major source of potential bias in especially the longitudinal neuroimaging analyses within the RUN DMC study, is the upgrade of the MRI scanner and the slight adjustment of the FLAIR sequence between baseline and first follow-up. Changes in PVE between baseline and follow-up together with the reduced slice thickness of FLAIR images at follow-up might have led to erroneously higher rates of WMH progression and incident lacunes. We limited the risk of bias first by reslicing follow-up to baseline FLAIR images before rating lacunes and secondly by calculating WMH volumes for odd and even slices separately, which revealed similar results. Additionally, the scanner differences might also have lead to differences in co-registrations of all images to MNI space between baseline and first and second follow-up images. However, we consider this unlikely because co-registrations of all images to MNI space have been performed with great caution using an intermediate subject-template and the most robust and accurate registration routines [162]. Moreover, the availability of the three time-points enabled us to validate findings in the other follow-up periods. For example, in our analyses on alterations in microstructural integrity preceding WMH progression (Chapter 4), the results for the first follow-up and the overall time-interval were comparable with the results for the second follow-up period in which scanner and sequence protocols remained identical. Thus, although we could not completely exclude misclassification due to scanner changes, our efforts to estimate and minimize the effects of possible interscan effects suggest a limited effect of information bias.

To assess the microstructural integrity of the white matter we used diffusion tensor imaging, which is based on the diffusion of water molecules. However, as most applications on the diffusion tensor model have been based on regions with parallel white matter fibers, the use of diffusion tensor imaging in our study population might have led to misclassification, since the white matter architecture in older adults with SVD is more complex [384]. This might influence the interpretation of the microstructural integrity of the white matter, especially with respect to underlying pathophysiological mechanisms, because these associations have also been based on findings in parallel white matter fibers. The diffusion measures have been associated with, amongst others, axonal membrane integrity, demyelination, fiber density and coherence of fiber orientation in the white matter [387, 388], although the interpretation of underlying mechanisms remains complex and can differ along the spectrum of SVD severity.

The identification of structural networks was based on DTI acquired at 1.5 Tesla and deterministic streamlining based on tensor reconstruction models [389, 390]. That is, streamlines were reconstructed by following the principal direction in each voxel, not taking into account multiple fiber orientations. Although this method is considered to provide reliable information on the major white matter tracts, this might have led to misclassification because it has difficulties with the identification of long distance fibers and the reconstruction of white matter tracts in a complex white matter architecture with crossing fibers due to noise and partial volume effects [384]. Although high resolution imaging and more advanced tractography methods are required to provide more detailed information about the white matter architecture, the consistency of our findings on impaired global efficiency in SVD and its relation with cognitive performance with other studies [53, 54, 295] indicate that our network analyses in participants with SVD are reliable. Another consideration in our network analyses is the selection of rich
Confounding occurs when the effect between the determinant and outcome is confused with the effect of an external factor that is associated with both the determinant and the outcome. For example, age is an important confounder that we adjusted for in our studies, since age was significantly associated with all determinants and outcomes under study including all SVD markers, plasma Aβ and serum NfL levels and cognitive performance.

Of note is that the confounder should not be an intermediate factor in the causal pathway leading to the outcome variable [385]. Adjusting for an intermediate factor would result in an underestimation of effects, since part of the true effect is caused by the intermediate factor. However, whether to adjust for a certain intermediate factor highly depends on the research question. That is, in etiological questions, where one would want to unravel the underlying mechanisms of an outcome, one should not adjust for the intermediate factor as it underestimates the true association strength between determinant and outcome. In prognostic questions, on the other hand, one may choose to adjust for an intermediate factor as to estimate the independent effect of the determinant on the prognosis. For example, in Chapter 4 where we assessed the associations between plasma Aβ levels with severity and progression of SVD, we additionally adjusted for hypertension. The purpose of adjusting here was to estimate the independent associations with SVD. The associations between plasma Aβ levels and severity and progression of SVD were independent of hypertension, suggesting that the associations with SVD could not be attributed to the presence of hypertension alone.

Causal inference
Another topic that deserves consideration with respect to the interpretation of our results, is causal inference. That is, a precisely measured association between a determinant and an outcome that is free of bias, does not necessarily imply that the outcome is caused by the determinant. Reverse causality refers to the possibility that the direction of cause-and-effect might be opposite of the common presumption.

One of the main strengths of the RUN DMC study, is the prospective design of the study with repeated neuroimaging and cognitive assessments. Where cross-sectional studies can only examine associations between brain structures and cognitive performance and hence are limited in making inferences regarding causality, the longitudinal design of our study enables us to examine the change in neuroimaging markers in relation to the change in cognitive performance in order to provide more insights on temporal precedence between brain changes and cognitive impairments. Hence, confusion of cause and effect is unlikely.

Still, the prospective design of our study does not preclude reverse causality, since we performed three cross-sectional measurements and do not have additional information on levels of determinants and outcomes in the past or in between measurements. Especially in the studies on blood markers it was difficult to establish a temporal relationship, since blood samples were collected and analyzed at baseline only and we were unable to perform repeated plasma Aβ or serum NfL measurements. As a consequence, we could not assess the influence of changes in plasma Aβ or serum NfL levels over time on SVD markers. However, reverse causality is unlikely, because we performed repeated measurements of the outcome and assessed associations between blood markers at baseline and progression of SVD over time.

Generalizability of results
External validity reflects the extent to which the results of a study can be generalized to populations beyond the study sample [385]. The RUN DMC study is a single-center hospital-based cohort study, including individuals between 50 and 85 years of age without dementia at baseline, covering the whole spectrum of SVD. The prevalence of vascular risk factors and SVD markers on neuroimaging in our population is comparable to community-dwelling populations, where SVD markers were observed on neuroimaging in over 90% of individuals over 60 years of age [6]. We therefore believe that the results based on the RUN DMC study are generalizable to the older population. However, as the RUN DMC study is hospital-based rather than population-based and only includes older adults with SVD, the generalizability to individuals younger than 50 years and to healthy older adults without SVD might be limited.
General discussion of the main findings

Temporal dynamics of cerebral small vessel disease
A proper understanding of the time course of the disease is an important prerequisite to capture the complexity of underlying mechanisms and consequences of SVD. SVD progression so far has been considered a linear continuous process, without actual proof of concept. In this thesis, we assessed the temporal dynamics of SVD, using three neuroimaging assessments over a period of nine years.

Non-linear progression
The use of three rather than two neuroimaging assessments allowed us to better study the time course of SVD progression (Chapter 3). While the average progression in our study was comparable with other studies [67, 68, 82, 90, 97, 119, 125, 130], the use of three imaging assessments now allowed us to show that SVD progression accelerated over time: incidence of lacunes and microbleeds was higher in the second compared to the first follow-up period and including a quadratic term for follow-up duration improved the model for WMH progression.

Although we would need more than three time-points to further study exponential functions, our findings provide evidence for non-linear temporal dynamics of SVD progression and suggest that a quadratic course of SVD progression over time is plausible. Moreover, progression of SVD was predominantly seen in participants with moderate to severe baseline SVD, whereas participants with mild baseline SVD rarely showed progression, not even after nine years (Chapter 3). These findings do not support a hypothesized ceiling effect in which WMH progression reaches a certain threshold [3], as we also observed progression in those at high age and with severe SVD. The exponential progression of SVD can be explained by findings from our and previous studies, showing that, besides age, baseline lesion load may predict SVD progression [60-62, 97].

Our data show that SVD progression is a highly variable and exponential process, predominantly seen in participants with severe baseline SVD. However, at the same time, even the oldest participants with mild baseline SVD rarely show progression over a time course of nine years, indicating that SVD is a more complex and highly heterogeneous disease process than previously thought. The inter-individual variability in SVD progression suggest heterogeneity in etiology of mild versus severe SVD and stresses the need for personalized treatment approaches. Still, we would require an even longer follow-up to exclude the possibility that all participants with mild baseline WMH would ultimately progress to severe WMH.

Moreover, future studies should also focus on patients with a younger age than have been done so far, enabling us to elaborate on the factors involved in the origin of SVD, rather than of its progression.

Regression of cerebral small vessel disease
Imaging assessments at three time-points also allowed us to identify regression of SVD markers followed by progression, in a cohort that on average showed progression (Chapter 3). SVD regression has also been described by a few previous studies with two imaging assessment, that reported a decline in WMH volume [28, 59-62] or a decrease in number of lacunes [63, 64] or microbleeds [65, 66]. This is probably an underestimation of the real prevalence of SVD regression, since regression of SVD markers was often attributed to measurement error or classified as 'no progression', and because regression within a certain time window was possibly compensated by progression thereafter (or vice versa) and two imaging assessments do not allow disentangling of episodes with regression from those with progression. SVD regression might thus be a true phenomenon with clinical implications. This observation provides further evidence that SVD does not gradually evolve but is a dynamic process, with progression interrupted by regression in some.

The observed regression of SVD markers might have several explanations. SVD regression might depend on methodological issues or imaging artefacts, but might as well be the result of biological processes. Methodologically, it is possible that WMH regression within a certain time window is compensated by WMH progression thereafter (or vice versa) in a cohort that on average shows progression. WMH regression might therefore have been revealed in our study by adding a third neuroimaging assessment and could have been missed in studies using two neuroimaging assessments with a long interval. In addition, it is possible that, due to partial volume effects, SVD markers can be rated at baseline imaging but are not longer visible at follow-up imaging. Biologically, it might be that SVD regression is accompanied by brain atrophy and thereby lead to impaired clinical outcome, as we observed several lacunes that became incorporated into the ventricles (Chapter 3). However, brain atrophy in participants with SVD regression was comparable to the atrophy in participants with stable SVD (Chapter 8), making this hypothesis less likely. Alternatively, SVD regression might reflect reversibility of white matter damage (i.e. before permanent axonal injury or demyelination has occurred) and thereby account for recovery of clinical symptoms. Recently developed WMH might include areas of tissue edema and reduction in tissue edema at a later stage could then lead to reduced WMH volume, since the signal change on T2 FLAIR is not just due to permanent myelin loss or axonal damage but may also be due to (reversible) shifts in water content [28].
After acute infarction the blood-brain barrier might be disturbed, causing leakage of cerebral fluid into the white matter, which may recover afterwards [140, 141]. Besides, it can be that lacunes collapsed into small lacunes that can be missed by brain imaging [64]. The disappearance of microbleeds may also be explained by clearance of hemosiderin-containing macrophages, if we consider the pathology of microbleeds as hemosiderin pigment accumulations in macrophages adjacent to ruptured atherosclerotic microvessels [66, 144]. Participants with SVD regression showed comparable cognitive decline compared to participants with stable SVD (Chapter 8), which might be supportive of the hypothesis that SVD regression reflects reversibility of white matter damage. These findings of comparable cognitive decline between participants with regression and stable SVD might suggest that SVD regression has a relative benign cognitive outcome, although future studies are required to validate these findings and to assess the role of vascular risk factor control on SVD regression and possible recovery of clinical symptoms.

Together, these findings suggest a paradigm shift on how SVD progression should be considered: traditionally seen as a continuous progressive process, it should rather be considered a dynamic and highly heterogeneous process, sometimes interrupted by regression. The different progression profiles between participants with mild versus severe SVD might imply heterogeneity in etiology and cognitive consequences.

The etiology of cerebral small vessel disease
The underlying mechanisms of the development and progression of SVD are not yet fully understood. In this thesis, we used advanced neuroimaging techniques and biomarkers in blood to further elaborate on the underlying mechanisms of SVD. In this section, I will discuss three mechanisms that might contribute to the development and progression of SVD: disruption of the blood-brain-barrier, axonal damage and amyloid pathology.

Blood-brain-barrier disruption
Increasing evidence suggests that blood-brain-barrier disruption due to endothelial dysfunction might be an important factor in the pathogenesis of SVD [105, 160, 342, 391-394]. That is, disruption of the blood-brain-barrier might lead to tissue damage and edema, which has been found to be among the pathological substrates of SVD on neuroimaging. Our findings of elevated plasma Aβ levels in participants with severe SVD (Chapter 5) would support a role of blood-brain-barrier disruption in SVD, since elevated Aβ levels in plasma might be caused by disruption of the blood-brain-barrier. That is, a more permeable blood-brain-barrier might lead to increased efflux of Aβ into blood along with the Aβ concentration gradient. Our finding that plasma Aβ levels were elevated in participants with hypertension further supports this mechanism, since hypertension is thought to be a major cause of blood-brain-barrier breakdown [395]. Furthermore, some evidence exists of an exponential rather than a linear decrease in blood-brain-barrier function with higher age [391], which might explain the exponential course of SVD progression (Chapter 3).

Proof of a causal role of blood-brain-barrier disruption in the pathogenesis of SVD also comes from a neuroimaging study in patients with lacunar stroke showing increased blood-brain-barrier permeability not only in regions of WMH but also in NAWM [393], although longitudinal studies investigating the conversion of these NAWM regions into WMH would be required to truly estimate a causal relationship. In addition, we observed associations of elevated baseline plasma Aβ levels with progression of SVD markers. Although these findings are suggestive of a causal relationship, future longitudinal studies using quantitative contrast-enhanced MRI – to visualize blood-brain-barrier permeability – are required to assess whether increased blood-brain-barrier permeability is involved in the progression of SVD.

Axonal damage
Further insights into the underlying mechanisms of SVD have been gained by the assessment of the microstructural integrity of the white matter. Impaired microstructural integrity in the normal appearing white matter, reflected by increased mean diffusivity (MD) and decreased fractional anisotropy (FA), preceded conversion into WMH and continued to decline over time (Chapter 4). This decline of microstructural integrity within the white matter implies an underlying disease process in the on conventional MRI normal appearing white matter, with gradual loss of microstructural integrity. These results are in line with previous longitudinal studies showing changes in baseline DTI measures that were related to incident WMH at follow-up [31, 32]. This confirms the hypothesis that WMH progression visible on conventional FLAIR imaging is only the “tip of the iceberg” with underlying loss of microstructural integrity that can only be visualized using more advanced neuroimaging techniques. Moreover, the observation of impaired microstructural integrity in remaining NAWM in participants with severe baseline SVD underlines that NAWM is not as normal one would expect from conventional MRI and might be an explanation for the observation that WMH progression was most pronounced in participants with severe SVD at baseline (Chapter 3).
 CHAPTER 13 DISCUSSION AND FUTURE PERSPECTIVES

The interpretation of impaired white matter microstructural integrity is challenging, especially with respect to underlying pathophysiological mechanisms. Alterations in diffusion measures have been associated with axonal membrane integrity and are often interpreted as impaired white matter integrity [387, 388], though recent studies have shown that diffusion alterations in SVD were mostly driven by increased extracellular free water [396]. In this thesis, we assessed the associations between neurofilament light chain (NFL), a blood marker for neuroaxonal damage, and diffusion measures both cross-sectionally (Chapter 6) and longitudinally (Chapter 7) in participants with SVD. The strong associations between NFL and mean diffusivity provide evidence for axonal damage as underlying mechanism for diffusion alterations, though imaging-pathology combination studies are required to reveal the exact pathophysiological mechanisms.

Interestingly, baseline MD values in NAWM areas converting into WMH within five years were similar to MD values in WMH (Chapter 4), suggesting that additional mechanisms are involved in the final conversion of NAWM into WMH, e.g. inflammation or small acute infarctions. A role for inflammation in SVD has been suggested by previous studies indicating that inflammatory mediators might affect the permeability of the blood-brain barrier [397]. Evidence for acute WMH progression has been provided by a longitudinal neuroimaging study among five subjects with moderate to severe WMH with sixteen weekly MRI assessments, reporting nine small acute infarcts in three participants which approached the imaging characteristics of WMH in the weeks thereafter [398]. A follow-up of the RUN DMC study, the RUN DMC InTENse study, is designed to elaborate on the role of these small acute infarctions in the pathogenesis of SVD, using repeated MRI assessments monthly during ten consecutive months among participants with mild to severe SVD.

Amyloid pathology

To reveal further insights into the underlying mechanisms of SVD, we investigated the associations between plasma Aβ with both severity and progression of SVD markers. Our findings that elevated plasma Aβ levels were not only associated with severity of SVD cross-sectionally but also predicted the progression of SVD over time (Chapter 5), suggest that amyloid pathology might contribute to the development and progression of SVD. However, as plasma Aβ might only partly reflect amyloid pathology, quantitative measures of amyloid pathology using positron emission tomography (PET) are required to further elucidate the role of amyloid pathology in SVD.

Several mechanisms could explain the associations between plasma Aβ levels and SVD. Plasma Aβ might enhance endothelium-dependent vasoconstriction, leading to cerebral hypoperfusion, which in turn might result in WMH and lacunes [181-183]. Alternatively, an inverse relation could also explain the associations between plasma Aβ levels and SVD, i.e. that cerebral hypoperfusion – or reduced cerebral blood flow – promotes overproduction of Aβ and its secretion into the circulation [184], although the associations between plasma Aβ levels and progression of SVD markers over time support the first mechanism. Furthermore, amyloid has been shown to increase the permeability of the blood-brain-barrier [399], further supporting the notion that amyloid pathology might contribute to the pathogenesis of SVD. Besides, the associations between plasma Aβ and SVD might also be explained by mixed age-related pathologies, i.e. by interactions of SVD with Alzheimer’s type neurodegeneration. This hypothesis has been supported by previous studies reporting presence of WMH in AD patients [186, 187]. Finally, it might also be that cerebrovascular and amyloid pathologies are caused by shared risk factors such as hypertension. We observed that plasma Aβ levels were elevated in participants with hypertension, which, in turn, was associated with SVD markers. Together with previously reported associations of plasma Aβ with hypertension [34, 36, 172, 173] and SVD markers [33-36, 176], these results suggest that amyloid pathology might also contribute to the development and progression of hypertension based SVD.

Taken together, additional insights into the underlying mechanisms of SVD have been gained by both diffusion tensor imaging and blood biomarkers. Impaired microstructural integrity preceded conversion into WMH, suggesting an underlying disease process in conventional MRI normal appearing white matter, with gradual loss of microstructural integrity. Inter-individual differences in microstructural integrity suggest heterogeneity of both NAWM and WMH, which might explain the exponential progression in participants with severe baseline SVD as well as the cognitive variability observed in patients with similar SVD severity. The combination of these diffusion measures with serum NFL and plasma Aβ provides evidence for blood-brain-barrier disruption, axonal damage and amyloid pathology as underlying mechanisms of SVD, though future studies are required to further elucidate the underlying pathophysiological mechanisms in the origin of SVD.

Cognitive consequences of cerebral small vessel disease

The spectrum of cognitive deficits attributable to SVD is broad and cannot be fully explained by the severity and location of SVD markers. In this thesis, I elaborated on mechanisms that might explain the cognitive variability observed in patients...
investigate a causal role of specific disruption of rich club connections in cognitive decline and dementia, we performed longitudinal mediation analyses on the associations between SVD markers, rich club organization and cognitive decline and dementia (Chapter 10). Interestingly, the decline in strength of peripheral connections, rather than rich club or feeder connections, was associated with cognitive decline and dementia. Although we are not aware of any studies investigating the preferential role of specific connections for cognitive function in elderly with SVD longitudinally, these results are in contrast to what we would expect based on the findings from several cross-sectional studies reporting an important role for rich club connections in cognitive processes [57, 58, 381]. There may be several explanations for this discrepancy. First, the selection of rich club nodes was based on node degree in healthy control subjects [375, 376], whereas the rich club organization in our study population was already disrupted at baseline due to their SVD [57], which might have influenced our results. In addition, it might be that initial disruptions of the rich club connections are followed by secondary disruptions of the feeder or peripheral connections and that impairments in peripheral connections only lead to clinical overt symptoms after a certain threshold of structural network disruption is reached [379]. Future longitudinal studies are required to ascertain the extrapolation of the concept of rich clubs to patients with SVD.

SVD might also exert its effects on cognitive performance by affecting remote brain structures. That is, SVD might lead to global brain atrophy and consequent cognitive decline by inducing a cascade of events that spread from visible SVD markers to remote brain areas, possibly via axonal loss by anterograde or retrograde degeneration [4, 155]. Our findings that WMH progression was associated with subsequent white matter and total brain atrophy (Chapter 2), provide evidence for remote effects of SVD. These findings have been corroborated by others, who reported associations of SVD with brain atrophy and cortical thinning [49, 147]. In addition, these cortical changes were associated with cognitive decline and, moreover, mediated the associations between WMH and cognitive deficits. A cross-sectional study reported negative associations between WMH and cortical thickness [289], which in turn mediated the associations between WMH and cognitive deficits. Furthermore, several longitudinal studies showed that brain atrophy mediated the relationship between presence of WMH and cognitive decline [109, 291], providing evidence that SVD exerts its effect on cognition by affecting remote brain structures.

The variability in cognitive symptoms between patients with apparently similar degrees of SVD might also be explained by the presence of other neurodegenera-
tive pathologies, such as Alzheimer’s Disease (AD). Co-occurrence of both cerebrovascular and neurodegenerative pathologies has been reported previously, for example by studies reporting higher WMH volumes in patients with AD [400-403]. Our findings that memory decline could be best explained by the interaction of WMH with hippocampal atrophy, while the association between WMH and memory decline was not causally mediated by hippocampal atrophy (Chapter 9), suggest that WMH and hippocampal atrophy synergistically affect memory decline. In addition, several neuroimaging studies also reported a synergistic effect between WMH and markers indicative of AD pathology (e.g. hippocampal volume) on cognitive performance [334, 335], while others have suggested that cerebrovascular pathology and AD pathology negatively affect cognition independently of each other [327, 338]. Together, these findings indicate that cognitive decline in patients with SVD is a heterogeneous clinical phenotype to which different pathologies contribute. Future studies combining neuroimaging with pathology would be required to unravel the true pathophysiological mechanisms underlying cognitive deficits in SVD and should thereby take into account both cerebrovascular and neurodegenerative pathologies.

Brain resilience
In the previous sections, I discussed mechanisms that might explain the spectrum of cognitive deficits observed in patients with SVD. Still, these mechanisms cannot fully explain the variation in cognitive deficits, as many individuals remain functionally independent despite a considerable burden of SVD. Conversely, an alternative approach might be to take into account brain resilience, which is the capacity to tolerate a certain degree of brain damage before clinical symptoms become manifest. That is, participants with larger brain volumes or higher levels of education might tolerate a greater burden of SVD pathology before cognitive symptoms arise than participants with smaller brain volumes or lower levels of education. This hypothesis was supported by studies reporting lower risk of dementia in patients with a larger brain volume [318] and slower rates of cognitive decline in participants with higher cognitive reserve [329], independently of SVD severity. Thus, by protecting against SVD-related cognitive deterioration via reserve and compensatory mechanisms, the concept of brain resilience might explain the variation of cognitive deficits observed in patients with apparently equal SVD severity.

To conclude, SVD markers with a rather homogeneous appearance on conventional neuroimaging are in fact highly heterogeneous, and might additionally affect brain tissue beyond the commonly recognized visible SVD by inducing a cascade of events that spread from the initial lesion to remote brain areas, which might explain the heterogeneity in cognitive symptoms in patients with apparently similar SVD burden. To fully understand the spectrum of cognitive deficits in patients with SVD, one should also take into account the interaction of cerebrovascular and neurodegenerative pathologies as well as the concept of brain resilience.

Clinical relevance

Biomarkers
The findings in this thesis are clinically relevant as differences in microstructural integrity between remaining NAWM and incident WMH suggest that it would be possible to predict which NAWM voxels will convert into WMH, based on baseline MRI values. Our observation that impaired microstructural integrity at baseline was associated with more extensive WMH progression over nine years, implies that measures of microstructural integrity can possibly be used as biomarker for WMH progression. Besides, the associations between serum NfL with neuroimaging markers of SVD, and especially with measures of microstructural integrity, implies that serum NfL might complement established MRI markers in assessing SVD burden. In addition, our findings that plasma Aβ levels were associated with both presence and progression of SVD markers, suggest that plasma Aβ might serve as inexpensive and non-invasive measure for identifying individuals with increased risk for progression of SVD.

The longitudinal design of our study enabled us to elaborate on the directionality of associations. That is, we were able to examine baseline markers in relation to the severity and progression of SVD over time, thereby providing more insights on temporal precedence. Hence, measures of microstructural integrity, together with plasma Aβ and serum NfL levels, might serve as promising biomarkers for future SVD, possibly able to identify individuals at high risk for SVD progression, years before SVD can be detected on conventional MRI.

However, none of these markers is specific for SVD, since Aβ has also been associated with AD [404], and NfL with multiple sclerosis [37] and neurodegenerative disorders [38], including AD [39] and frontotemporal dementia [40]. Since cerebrovascular and neurodegenerative pathologies often co-occur in older individuals, plasma Aβ and serum NfL might offer the possibility to assess the combined effect of these pathologies on brain integrity. Perhaps, a combination of neuroimaging and blood markers would be able to increase the specificity, which would be an interesting angle for future studies.
Personalized treatment

Since SVD is considered the most important vascular contributor to cognitive decline and the development of dementia, it is important to identify those at risk as early as possible, preferably before irreversible damage has occurred. Our findings on inter-individual variability in SVD progression and underlying mechanisms are a major step forward in developing personalized treatment approaches. Those with severe SVD – already at baseline – had more microstructural white matter damage, even in the on conventional MRI normal appearing white matter, with subsequent exponential progression of their SVD. These findings may suggest that these patients are also at highest risk of developing cognitive decline or dementia. This may provide a window of opportunity to deploy intervention strategies, already before SVD becomes visible on conventional neuroimaging, to prevent or postpone cognitive symptoms. In order to facilitate personalized treatment, future studies should further elaborate on the inter-individual differences in SVD and its cognitive consequences, especially aiming to identify patients at high risk of developing cognitive decline or dementia in early phases.

Treatment strategies of SVD should target risk factors or factors involved in the pathogenesis of SVD. Hypertension, diabetes mellitus, hypercholesterolemia, obesity, smoking, and lack of physical exercise, are major vascular risk factors for SVD and have been associated with an increased risk of cognitive impairment and dementia [14-26]. Since all these vascular risk factors are modifiable, either by medication or life-style interventions, they are important targets for treatment or prevention strategies. In addition, there is growing evidence for a causal role of blood-brain-barrier disruption due to endothelial dysfunction in the pathogenesis of SVD, suggesting that therapeutic strategies targeting vascular contributions to dementia may also be interested in targeting endothelial function. Furthermore, the concurrence of cerebrovascular pathologies with neurodegenerative pathologies in the development of cognitive decline and dementia implies that treatment should not be solely focused on targeting vascular contributions to dementia, but might rather require a multi-domain intervention.

Thus far, randomized trials investigating the effect of intervention of vascular risk factors on progression of SVD and cognitive decline have revealed mixed results [75, 161, 211, 405, 406]. Hence, future randomized trials are required, particularly focusing on multi-domain interventions in populations at high risk of SVD progression and developing dementia. Novel treatments might target the brains’ microvascular endothelium with endothelin antagonists or nitric oxide donors [161], although randomized controlled trials are required. For now, there is evidence of a stringent control of vascular risk factors according to current guidelines, until further data from randomized controlled trials are available.

Future perspectives

Future studies are required to further unravel the etiology of SVD and the mechanisms by which SVD leads to cognitive deficits (Box). These studies should include advanced multimodal structural and functional MRI with high temporal and spatial resolution. To elaborate on the factors involved in the origin of SVD, the pathological correlates of neuroimaging markers of SVD should be further investigated and studies should include patients of a younger age than have been done thus far, preferably already from early adulthood. Moreover, studies with longitudinal designs are of utmost importance to elaborate on the directionality of associations and the sequence of events from local SVD towards remote effects eventually leading to widespread cognitive symptoms in patients with SVD. Furthermore, rather than solely focusing on vascular or Alzheimer’s dementia as isolated diseases, the field would benefit from a focus on the interaction of SVD pathology with other neurodegenerative pathologies.

Concluding remarks

Cerebral small vessel disease is highly heterogeneous, reflected in inter-individual variability in the temporal dynamics, etiology and cognitive consequences. SVD might exert its effects throughout the brain by inducing a cascade of events that spread from the visible SVD markers to remote brain areas, which might explain the variability in cognitive symptoms in patients with apparently similar SVD burden.
Next steps in the field

Research in early phases
Studies on causes and consequences of SVD currently mainly focus on elderly individuals over 50 years of age. However, in order to improve our understanding on the causes of SVD, studies should also focus on patients from a younger age than have been done so far, enabling us to elaborate on the factors involved in the origin of SVD.

Longitudinal studies
Studies with longitudinal designs are of utmost importance to shed more light on the causality or directionality of associations and the sequence of events from local SVD towards global remote effects eventually leading to widespread cognitive symptoms in patients with SVD.

Standardized methods
Better SVD identification methods are needed that are both reliable and less time-consuming. Ideally, robust standardized SVD segmentation should be developed to identify SVD markers in various study populations. Besides, standardized methods for analyzing and reporting structural connectivity should be developed. This would enhance robustness and generalization of findings.

Multimodal imaging
Multimodal imaging is required to assess various neuroimaging parameters such as T1, FLAIR, MD, FA, connectivity and perfusion contemporary in whole brain white and gray matter. The use of different imaging sequences will aid to assess coherence between different findings, e.g. correlations between SVD markers and structural or functional connectivity loss.

High field imaging
Using high field strength MRI, one will become capable of directly visualizing small intracerebral arteries and veins, as well as the vascular wall of intracerebral arteries, perivascular spaces, and microvascular lesions including microbleeds and microinfarcts. Hence, future studies using high field imaging are required to offer new perspectives on underlying mechanisms of SVD.

High frequency scanning
Our understanding of SVD is mainly based on cross-sectional data or longitudinal studies with typically inter-scan intervals of several years. Rather, longitudinal studies with much shorter inter-scan intervals of weeks or months might tremendously enhance our understanding of the sequence of events from focal SVD towards remote effects, thereby providing insights in SVD as a dynamic global brain disease.

Biomarker studies
There is a need for combining neuroimaging, including amyloid PET imaging, with biomarkers from serum and CSF, mainly to elaborate on both vascular and neurodegenerative pathologies in the brain, as this would shed light on how these pathologies interact in causing clinical symptoms.

Pathology studies
Future studies should also target the connection between neuroimaging findings and neuropathological changes. Revealing underlying pathological substrates of SVD markers would improve our understanding of the heterogeneity observed between and within neuroimaging markers of SVD.

Animal studies
The use of animal models (e.g. rodents) might enhance our understanding of the pathogenesis of SVD. Furthermore, animal models might be particularly useful to investigate potential biomarkers and therapeutic approaches for SVD. Particular attention should be paid on the translation of findings, as this is often hampered by the small proportion of white relative to gray matter in rodents compared to humans and the acute induction of risk factors. Instead, animal models should allow for a chronic development over a life-time course.

Focus on mixed age-related pathologies
Future studies are required to shed more light into the longitudinal interactions between vascular, neurodegenerative and Alzheimer’s pathologies. Especially the interaction between SVD and these other factors needs to be further investigated, rather than solely focusing on “Vascular Dementia” or “Alzheimer’s Disease” who are clinical diagnoses likely representing mixtures of underlying pathologies, in order to fully understand the role of SVD in clinical presentation.

Clinical trials
Clinical (intervention) trials are required to test whether (vascular) risk factor control, drug treatment and lifestyle modifications can prevent or minimize the transition of SVD into a global brain disease ultimately leading to widespread clinical symptoms.
Chapter 14
Summary in Dutch
Nederlandse samenvatting
Schade aan de kleine bloedvaten in de hersenen wordt in het Engels ‘small vessel disease’ genoemd. De veronderstelde gevolgen van deze schade aan de kleine hersenvaatjes kunnen worden gezien op MRI scans als witte stofafwijkingen, lacunes (kleine herseninfarcten) en microbloedingen (kleine hersenbloedingen). Small vessel disease komt veel voor op latere leeftijd en is de belangrijkste vasculaire oorzaak voor cognitieve achteruitgang en dementie.

De studies in dit proefschrift zijn gebaseerd op de Radboud Universiteit Nijmegen Diffusie tensor en Magnetische resonantie beeldvorming Cohort (RUN DMC) studie, een prospectieve cohort studie bedoeld om de oorzaken en gevolgen van small vessel disease te onderzoeken. Hierin onderzochten we 503 mensen tussen de 50 en 85 jaar met small vessel disease, waarbij we op drie momenten over een periode van negen jaar (2006, 2011 en 2015) MRI scans hebben gemaakt en cognitief onderzoek hebben verricht. In dit proefschrift onderzocht ik eerst de verandering van small vessel disease in de tijd, om daarna verder in te gaan op onderliggende mechanismen en cognitieve gevolgen van small vessel disease. In dit hoofdstuk vat ik de belangrijkste bevindingen van mijn proefschrift samen.

Het dynamische beloop van small vessel disease in de tijd
In Deel II van dit proefschrift onderzocht ik het tijdsverloop van small vessel disease, met name omdat het in kaart brengen van het tijdsverloop van een ziekte zou kunnen helpen om de onderliggende mechanismen en klinische consequenties beter te begrijpen.

Hoofdstuk 2 bevat een literatuurstudie naar de toename en afname van small vessel disease. Hoewel small vessel disease in de loop der jaren gemiddeld meestal toeneemt, wordt afname van small vessel disease ook vaak beschreven. Deze afname werd meestal gerapporteerd als een meetfout of beschreven als ‘geen toename’, maar zou op basis van de resultaten van onze literatuurstudie eerder moeten worden beschouwd als een echt fenomeen.

De dynamiek van small vessel disease binnen de RUN DMC studie heb ik beschreven in Hoofdstuk 3. In de meeste studies werd verandering van small vessel disease gemeten op twee tijdpunten, daarbij uitgaand van een lineaire toename. Middels het gebruik van drie MRI scans konden we deze hypothese voor het eerst daadwerkelijk testen. We vonden dat de toename van small vessel disease niet lineair verliep, maar exponentieel volgens een kwadratische curve. De toename was het grootst in deelnemers die op baseline al ernstige small vessel disease hadden; deelnemers met milde small vessel disease op baseline toonden nauwelijks progressie. Daarnaast zagen we dat deze gemiddelde toename in ongeveer een
viervijfde van de deelnemers afgewisseld werd met afname van small vessel disease in een andere periode. In plaats van een continue lineair toenemend proces, zou small vessel disease dus moeten worden beschouwd als een zeer dynamisch proces, waarbij toename soms ook kan worden afgewisseld door afname.

De etiologie van small vessel disease

In Deel III van dit proefschrift onderzocht ik de onderliggende mechanismen van small vessel disease. Dat deed ik aan de hand van geavanceerde MRI methoden en aan de hand van markers in bloed.

In Hoofdstuk 4 onderzocht ik de kwaliteit van witte stof vóór het ontstaan van witte stofafwijkingen. Met een bepaalde MRI scan, de zogenaamde DTI scan, konden we de kwaliteit – ook wel genoemd: ‘microstructurele integriteit’ – van de witte stof beoordelen. We vonden dat de microstructurele integriteit van witte stof aangedaan was, zelfs jaren voor er afwijkingen te zien waren op MRI scans. De microstructurele integriteit was meer aangedaan in deelnemers met ernstige small vessel disease, in alle gebieden, inclusief de normaal liggende witte stof en de witte stofafwijkingen. Deze heterogeniteit zou kunnen verklaren dat deelnemers met ernstige small vessel disease in de loop van de jaren aanzienlijk meer toename hebben dan degenen met slechts milde small vessel disease. Daarnaast zou deze heterogeniteit ook kunnen verklaren waarom een individu met ernstig small vessel disease in de loop van de jaren aanzienlijk meer toename heeft dan degenen met slechts milde small vessel disease. Sommigen hebben daar geen last van; bij anderen leidt het tot een ziekteverschijnsel dat, naast vasculaire risicofactoren als hypertensie, een oorzaak van small vessel disease. Verder onderzoek is nodig om te bepalen of Aβ zou kunnen dienen als biomarker om al in een vroege fase mensen op te sporen met een hoog risico op toename van small vessel disease.

In Hoofdstuk 6 heb ik vervolgens onderzocht of small vessel disease geassocieerd was met de concentraties van amyloid beta (Aβ) in bloed, een eiwit dat, naast vasculaire risicofactoren als hypertensie, een oorzaak van small vessel disease zou kunnen zijn. Hogere concentraties van Aβ in plasma waren geassocieerd met zowel de ernst als de toename van small vessel disease, hetgeen suggerereert dat Aβ betrokken is bij de ontwikkeling van small vessel disease. Verder onderzoek is nodig om te bepalen of Aβ zou kunnen dienen als biomarker om al in een vroege fase mensen op te sporen met een hoog risico op toename van small vessel disease.

In Hoofdstuk 8 bevat een literatuurstudie naar mechanismen voor cognitieve problemen in patiënten met small vessel disease. Hierin schepp ik een nieuw kader voor de variatie in cognitieve problemen: lokale aanwezigheid van small vessel disease leidt tot globale verschijnselen. Nieuwe beeldvormende onderzoeken hebben aangetoond dat er een gehele manifestaties van small vessel disease hun effect door de gehele hersenen kunnen hebben. De locatie waar de schade ontstaat, kan dus ook op andere locaties leiden tot verminderde functie – en dus tot andere ziekteverschijnselen leiden dan op grond van de locatie van zichtbare small vessel disease te verwachten zou zijn.
In Hoofdstuk 9 heb ik de gevolgen van afname van small vessel disease voor het cognitief functioneren beschreven. Eerder lieten we zien dat een op de vijf deelnemers naast toename ook afname van small vessel disease liet zien, maar de klinische gevolgen hiervan waren nog niet bekend. Enerzijds dachten we dat afname van small vessel disease gepaard zou gaan met atrofie van de hersenen en zo zou kunnen leiden tot meer cognitieve problemen. We vonden echter geen bewijs voor deze hypothese: afname van small vessel disease ging niet gepaard met globale atrofie van de hersenen. Anderzijds dachten we dat afname van small vessel disease een weerspiegeling kon zijn van omkeerbare witte stofschade en daarmee een relatief gunstig effect zou hebben op het cognitief functioneren. We vonden geen verschillen in cognitieve achteruitgang tussen deelnemers met afname van small vessel disease en deelnemers waarvan de hoeveelheid small vessel disease gelijk bleef. Daarnaast vonden we minder cognitieve achteruitgang in deelnemers met afname van witte stofafwijkingen dan in deelnemers met toename van witte stofafwijkingen en minder achteruitgang van handelingssnelheid in deelnemers met afname van lacunes dan in deelnemers met nieuwe lacunes, hoewel deze verschillen niet significant waren na correctie voor leeftijd. Verder onderzoek is nodig om de effecten van afname van small vessel disease op cognitieve achteruitgang vast te stellen.

In Hoofdstuk 10 zocht ik een verklaring voor de geheugenproblemen in patiënten met small vessel disease. Geheugenproblemen kunnen niet goed verklaard worden door de locatie van small vessel disease, maar worden vaak toegeschreven aan atrofie van de hippocampus. In dit hoofdstuk heb ik daarom de interactie tussen small vessel disease en hippocampusatrofie onderzocht. We konden de geheugenproblemen in deelnemers met small vessel disease beter verklaren door de interactie van witte stofafwijkingen met hippocampusatrofie dan door deze variabelen apart. Daarnaast vonden we dat het effect van witte stofafwijkingen op geheugenachteruitgang niet causaal gemedieerd werd door hippocampusatrofie, hetgeen suggereert dat witte stofafwijkingen en hippocampusatrofie onafhankelijke processen zijn die samen een groter effect hebben op geheugenproblemen dan allebei apart.

Tot slot heb ik de rol van achteruitgang van hersenverbindingen bij cognitieve problemen onderzocht in Hoofdstuk 11. Specifiek heb ik daarbij gekeken naar de ‘centrale stations’ van de hersengebieden, de zogeheten ‘rich clubs’, en naar onderlinge verbindingen tussen deze gebieden. Dit netwerk van hersenverbindingen wordt gezien als de spil van de communicatie in de hersenen en speelt een belangrijke rol in het cognitief functioneren. We zagen dat de kwaliteit van specifiek deze rich club verbindingen achteruit was gegaan na negen jaar, met name in deelnemers met ernstige small vessel disease. Daarnaast vonden we dat de achteruitgang van de kwaliteit van perifere verbindingen, maar niet van de rich club verbindingen, geassocieerd was met achteruitgang van het cognitief functioneren. Bovendien hadden deelnemers met dementie een minder efficiënt hersennetwerk en verminderde kwaliteit van de perifere verbindingen dan deelnemers zonder dementie en was het effect van witte stofafwijkingen op dementie causaal gemedieerd door de globale efficiëntie en kwaliteit van de perifere verbindingen. Deze bevindingen impliceren dat verstoringen van hersenverbindingen een belangrijke rol speelt in het ontstaan van cognitieve achteruitgang en dementie in mensen met small vessel disease.

Conclusie
Small vessel disease is een heterogene ziekte, wat tot uiting komt in het dynamische tijdsverloop van small vessel disease, de onderliggende mechanismen en de variatie aan cognitieve problemen in mensen met small vessel disease. Met het nieuwe concept ‘lokale aanwezigheid, globale verschijnselen’ kunnen de cognitieve problemen als gevolg van small vessel disease beter worden begrepen: small vessel disease leidt niet alleen tot problemen door lokale hersenschade, maar kan door het beschadigen van hersenverbindingen ook leiden tot schade aan verder weg gelegen hersengebieden en via die weg leiden tot cognitieve problemen.
Part VI
Appendices
LIST OF ABBREVIATIONS

List of abbreviations

Aβ = amyloid beta
AD = Alzheimer’s Disease
AIC = akaike information criterion
ANTS = advanced neuroimaging tools
APOE = apolipoprotein E
ARWMC = age-related white matter changes
ASC = area under the curve
B = Baseline
CAA = cerebral amyloid angiopathy
CADASIL = cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy
CAVIA = Cerebral Amyloid Angiopathy: Vascular Imaging and fluid markers of Amyloid deposition
CI = confidence interval
CSF = cerebrospinal fluid
CSFV = cerebrospinal fluid volume
DAN = dorsal attention network
DMN = default mode network
DTI = diffusion tensor imaging
DWT = diffusion-weighted imaging
ELISA = enzyme-linked colorimetric immunosorbent assay
FA = fractional anisotropy
FPCN = frontoparietal control network
FLAIR = fluid-attenuated inversion recovery
FSL = Functional Magnetic Resonance Imaging of the Brain (FMRIB) Software Library
FU = follow-up
GM = gray matter
GMV = gray matter volume
GRE = gradient recalled echo
HC = healthy controls
ICV = intracranial volume
IQR = interquartile range
MB = microbleed
MD = mean diffusivity
MMSE = Mini-Mental State Examination
MPRAGE = magnetization-prepared rapid gradient echo
MRI = magnetic resonance imaging
mRS = modified Rankin scale
NAWM = normal appearing white matter
NFL = neurofilament light chain
NIHSS = National Institutes of Health stroke scale
OR = odds ratio
PVE = partial volume effects
ROC = receiver operating characteristics
RUN DMC = Radboud University Nijmegen Diffusion tensor and Magnetic resonance imaging Cohort
SD = standard deviation
SPM = statistical parametric mapping
STRIVE = Standards for Reporting Vascular Changes on Neuroimaging
SVD = cerebral small vessel disease
TBV = total brain volume
TIA = transient ischemic attack
TBSS = tract-based spatial statistics
WM = white matter
WMH = white matter hyperintensities
WMV = white matter volume
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* Equal contributions

Other publications


Accepted


Submitted


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* Equal contributions
Curriculum vitae

Esther van Leijsen was born on November 13, 1989 in Tilburg. She attended grammar school at the St. Odulphuslyceum in Tilburg and graduated in 2008. She studied Biomedical Sciences in Nijmegen and obtained her Bachelor's degree in 2012 after finishing her Bachelor internship at the department of Neurophysiology (Dr. D. Schubert). In September 2012, she started the Research Master Cognitive Neuroscience at the Donders Institute for Brain, Cognition and Behavior, with the specialization ‘Learning, memory and plasticity’. Meanwhile, she followed the Radboud Honours Programme Reflections on Science, in which she investigated a complex social issue as member of the international and interdisciplinary think tank European Culture. She performed her master internship on longitudinal brain changes and cognitive consequences in cerebral small vessel disease at the department of Neurology at the Radboud university medical center (Prof. dr. FE de Leeuw). After obtaining her Master's degree in Cognitive Neuroscience, she started her PhD project in November 2014, which resulted in this thesis. She has presented her results on many international conferences, for which she received multiple international awards: in 2016, she has been awarded the Young Investigator Award of the European Stroke Organisation as well as the Junior Investigator Award of the International CAA Association, and in 2018, she has received a Young Investigator Award commendation letter of the European Stroke Organisation. She is currently enjoying her work as researcher cybercrime at the Research and Documentation Centre (WODC) of the Ministry of Justice and Security.
**Woord van de paranimfen**

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*De paranimfen*
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Oma & Opa Luijckx, u vormt een prachtig voorbeeld voor mij. Ik heb dierbare herinneringen aan de etentjes en aan alle kerstdiners die “nu echt voor de laatste keer” in Restaurant De Braak waren. Ik vind het heel bijzonder dat u vandaag bij mijn promotie aanwezig kunt zijn.

Jokelien en Fred, ik meen het echt als ik zeg dat ik je geen betere schoonouders had kunnen wensen. Wat een warm welkom in de familie. En wat is het fijn dat jullie zo meelevend. Dus doe voorzichtig, niet de chauffeur afleiden, we hebben maar één jokfred… Ewout, hippe Amsterdammer! Jouw discipline en passie voor wielrennen zijn bewonderenswaardig. Dankjewel voor je oprechte interesse. Sjoerd, wat een humor heb jij! Ik hoop dat ik nog heel vaak onder de tafel mag liggen door die goede grappen van jou.

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Lieve Im, wat ben ik blij met een vriendin als jij! Eigenlijk heb ik er met jou gewoon een derde zus bij, want wij lijken stiekem echt heel veel op elkaar. Daarnaast ben jij het levende bewijs dat telepathie daadwerkelijk bestaat; op de een of andere manier weet jij altijd precies wanneer je mij een berichtje moet sturen. Ik ben heel trots op je en vind het echt super lief dat je speciaal uit München bent gekomen om er vandaag bij te zijn. Zo gaan we onze tweemaandelijkse bezoeken nog bijna halen ook!

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Dissertations of the disorders of movement research group Nijmegen

Vascular disorders of movement – The Radboud Stroke centre

- Liselore Snaphaan. Epidemiology of post stroke behavioral consequences. Radboud University Nijmegen, 12 March 2010
- Anouk GW van Norden. Cognitive function in elderly individuals with cerebral small vessel disease. Radboud University Nijmegen, 30 November 2011
- Anil M. Tuladhar. The disconnected brain: mechanisms of clinical symptoms in small vessel disease. Radboud University Nijmegen, 4 October 2016
- Ingeborg W.M. van Uden. Behavioural consequences of cerebral small vessel disease: an MRI approach. Radboud University Nijmegen, 14 February 2017
- Renate M. Arntz. The long-term risk of vascular disease and epilepsy after stroke in young adults. Radboud University Nijmegen, 16 February 2017
- Helena M. van der Holst. Mind the step in cerebral small vessel disease. Brain changes in motor performance. Radboud University Nijmegen, 5 April 2017
- Joyce Wilbers. Long-term neurovascular complications in cancer patients. Radboud University Nijmegen, 25 September 2017
- Frank G. van Rooij. Transient neurological attacks. Neuroimaging, etiology, and cognitive consequences. Radboud University Nijmegen, 14 June 2018
- Tessa van Middelaar. Memory under pressure: blood pressure management to prevent dementia. Radboud University Nijmegen, 5 November 2018

Parkinson Center Nijmegen (ParC)

- Jasper E. Visser. The basal ganglia and postural control. Radboud University Nijmegen, 17 June 2008
- W. Farid Abdo. Parkinsonism: possible solutions to a diagnostic challenge. Radboud University Nijmegen, 7 October 2009
- Lars B. Oude Nijhuis. Modulation of human balance reactions. Radboud University Nijmegen, 29 November 2010
- Rick C.G. Helmich. Cerebral reorganization in Parkinson's disease. Radboud University Nijmegen, 24 May 2011
Charlotte A. Haaxma. New perspectives on preclinical and early stage Parkinson’s disease. Radboud University Nijmegen, 6 December 2011

Johanna G. Kalf. Drooling and dysphagia in Parkinson’s disease. Radboud University Nijmegen, 22 December 2011

Anke H. Snijders. Tackling freezing of gait in Parkinson’s disease. Radboud University Nijmegen, 4 June 2012

Bart F.L. van Nuenen. Cerebral reorganization in premotor parkinsonism. Radboud University Nijmegen, 22 November 2012

Wandana Nanhoo-Mahabier. Freezing of physical activity in Parkinson’s disease, the challenge to change behavior. Radboud University Nijmegen, 13 February 2013

Marlies van Nimwegen. Promotion of physical activity in Parkinson’s disease, the challenge to change behavior. Radboud University Nijmegen, 6 March 2013

Arlène D. Speelman. Promotion of physical activity in Parkinson’s disease, feasibility and effectiveness. Radboud University Nijmegen, 6 March 2013


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Marjolein R. Arts. Improving diagnostic accuracy in parkinsonism. Radboud University Nijmegen, 27 June 2014

Maartje Louter. Sleep in Parkinson’s disease. A focus on nocturnal movements. Radboud University Nijmegen, 13 February 2015

Frederick Anton Meijer. Clinical Application of Brain MRI in Parkinsonism: From Basic to Advanced Imaging. Radboud University Nijmegen, 23 June 2015


Martijn van der Eijk. Patient-centered care in Parkinson’s disease. Radboud University Nijmegen, 1 December 2015


Merel M. van Gilst. Sleep benefit in Parkinson’s disease. Radboud University Nijmegen, 13 April 2016

Arno M. Janssen. Transcranial magnetic stimulation – measuring and modeling in health and disease. Radboud University Nijmegen, 2 June 2016

Non-Parkinsonian disorders of movement

Sacha Vermeer. Clinical and genetic characterization of autosomal recessive cerebellar ataxias. Radboud University Nijmegen, 5 April 2012

Susanne T. de Bo. Hereditary spastic paraplegias in the Netherlands. Radboud University Nijmegen, 20 December 2013

Catherine C.S. DeLour. Unraveling primary focal dystonia. A treatment update and new pathophysiological insights. Radboud University Nijmegen, 7 January 2014

Ella MR Fonteyn. Falls, physiotherapy, and training in patients with degenerative ataxias. 29 June 2016

Brit S Hofland. Investigating the role of the cerebellum in idiopathic focal dystonia. 22 March 2018

Neuromuscular disorders of movement

Mireille van Beekvelt. Quantitative near infrared spectroscopy (NIRS) in human skeletal muscle. Radboud University Nijmegen, 24 April 2002

Johan Hiel. Ataxia telangiectasia and Nijmegen Brokage syndrome, neurological, immunological and genetic aspects. Radboud University Nijmegen, 23 April 2004

Gerald JD Hengstman. Mysitis specific autoantibodies, specificity and clinical applications. Radboud University Nijmegen, 21 September 2005

M. Schillungs. Fatigue in neuromuscular disorders and chronic fatigue syndrome, a neurophysiological approach. Radboud University Nijmegen, 23 November 2005


J. Kalma. From prevalence to predictors of fatigue in neuromuscular disorders. The building of a model. Radboud University Nijmegen, 31 October 2006

E. Cup. Occupational therapy, physical therapy and speech therapy for persons with neuromuscular diseases, an evidence based orientation. Radboud University Nijmegen, 5 July 2011

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