Brain atrophy in patients with arterial disease
The SMART-MR study

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De SMART-MR studie
(met een samenvatting in het Nederlands)

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Chapter 3.2

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Chapter 4.1

van Raamt AF, Appelman APA, Mali WPTM, van der Graaf Y; SMART Study Group. Arterial blood flow to the brain in patients with vascular disease: the SMART study. *Radiology* 2006;240:515-21.

Chapter 4.2

Appelman APA, van der Graaf Y, Vincken KL, Tiehuis AM, Witkamp TD, Mali WPTM, Geerlings MI. Total cerebral blood flow, white matter lesions and brain atrophy: the SMART-MR study. *J Cereb Blood Flow Metab* 2008;28:633-639.

Chapter 4.3

Appelman APA, van der Graaf Y, Vincken KL, Mali WPTM, Geerlings MI; for the SMART Study Group. The association between lower cerebral blood flow and executive impairment is modified by white matter lesions. The SMART-MR study. *Submitted*

Contents

1

General introduction

Brain atrophy is often observed on magnetic resonance imaging (MRI) in the elderly.[1] Cortical brain atrophy is characterized by widening of the sulci and narrowing of the gyri, while subcortical brain atrophy is characterized by enlargement of the ventricles. This decrease in brain volume is the result of neurodegenerative processes, which are a contributing factor in the majority of cognitive diseases. The extent and rate of progression of brain atrophy and ventricular enlargement are associated with cognitive deterioration and conversion to Alzheimer's disease.[2,3]

In the last decade population-based studies have shown that the role of vascular disease in the development of cognitive decline is more important than was thought before.[4] Cerebrovascular disease and vascular risk factors including hypertension, hyperlipidemia, diabetes, obesity, and smoking are associated with an increased risk of cognitive impairment and dementia.[5-10] Vascular risk factors are also associated with increased risk of clinical and subclinical pathology in the brain, including infarcts, white matter lesions and brain atrophy.[11-16] Cognitive impairment due to neurodegeneration and due to vascular disease were traditionally regarded as separate clinical and pathophysiological entities. However, it is nowadays recognized that vascular pathology and neurodegenerative processes may not act independently in the pathogenesis of cognitive decline[17,18] and more research is needed to understand the role of vascular pathology in the development of brain atrophy and cognitive deterioration.

One of the hypotheses is that vascular pathology is directly involved in the pathogenesis of brain atrophy and subsequent cognitive impairment. Cerebrovascular lesions and brain atrophy are often found simultaneously on MRI and associations between vascular disease and cognitive deterioration are supposedly mediated by ischemic vascular lesions.[19] In addition, cerebral hypoperfusion may also be one of these mechanisms through which vascular disease contributes to cognitive decline.[20,21]

Brain atrophy and vascular pathology can both be visualized on MRI. The development of new segmentation methods has made it possible to reliably estimate volumes of white and gray matter, sulcal and ventricular cerebrospinal fluid spaces and white matter lesions. These methods have several advantages compared with visual assessment of brain atrophy and white matter lesion load. Contrary to visual rating scales, volumetric assessments are not impeded by ceiling effects. Furthermore, segmentation methods are more precise and therefore more likely to detect small differences in brain volumes which are not visible to the human eye. Finally, volumetric measurements can be used as continuous variables in statistical analysis and thereby increase the power to detect associations between white matter lesions, brain atrophy and cognitive performance.

Brain perfusion can also be assessed using imaging techniques, but the majority of perfusion measurement techniques are impractical in large populations due to their complexity and their use of ionizing radiation or contrast agents.[22] Therefore, large scale epidemiologic studies assessing cerebral blood flow are scarce. Total cerebral blood flow can be measured with two-dimensional phase-contrast magnetic resonance angiography by summing the blood flow in the internal carotid and basilar arteries.[23-25] The actual perfusion of cerebral tissue cannot be measured with this technique, but it gives a reliable estimate of the amount of blood that reaches the brain and is therefore

considered an indirect measurement of global brain perfusion. Moreover, since it is now possible to accurately calculate total brain volume with segmentation analysis, a measure of mean brain perfusion can be obtained by dividing total cerebral blood flow by total brain volume. This fast and simple non-invasive technique may be especially useful to investigate the role of cerebral blood flow in the etiology of brain atrophy and subsequent cognitive decline.
The research described in this thesis is performed using data from the SMART-MR study; a prospective cohort study within the Second Manifestations of ARTerial disease (SMART) study.[26] In the SMART study patients with symptomatic atherosclerotic disease or risk factors for atherosclerosis are screened for additional risk factors and severity of atherosclerosis. Since patients with atherosclerotic disease are at increased risk of developing brain atrophy and subsequent cognitive decline, the SMART cohort may be a useful population to examine associations between vascular pathology on MRI and brain atrophy. Between May 2001 and December 2005, an MR investigation of the brain was added to the baseline examination of SMART as part of the SMART-MR study in patients who were included with manifest coronary artery disease, cerebrovascular disease, peripheral arterial disease or abdominal aortic aneurysm. Neuropsychological testing was introduced in the SMART-MR study in January 2003. In total 1309 patients were included in the SMART-MR study. The extensive information on vascular risk factors and extent of subclinical atherosclerosis, in combination with measurements of total cerebral blood flow, volumetric assessment of brain and white matter lesions and the results of the neuropsychological tests allow us to further elucidate associations between vascular pathology, cerebral blood flow, brain atrophy and cognitive functioning.

Aim of this thesis

The overall aim of this thesis is to gain further insight in the contribution of vascular pathology to the development of brain atrophy and cognitive decline. The first objective of this thesis is to estimate brain volumes and cerebrovascular lesions on MRI in a large cohort of patients with manifest arterial disease. The second objective is to investigate whether white matter lesions and lacunar infarcts, as indicators of cerebral-small vessel disease, are associated with brain atrophy and whether white matter lesions and lacunar infarcts are associated with cognitive impairment, independent of brain atrophy. The third objective is to investigate the associations between cerebral blood flow, brain atrophy and cognitive functioning.

Outline of this thesis

First, in **chapter 2** we estimate total brain, cortical gray matter, ventricular and white matter lesion volumes for patients with manifest arterial disease. We also describe the prevalence of asymptomatic cortical, subcortical, lacunar and infratentorial infarcts for this population, and we investigate age- and sex-related differences in brain volumes and silent brain infarcts.

In **chapter 3** we investigate the association of white matter lesions and lacunar infarcts with brain atrophy and cognitive functioning. In **chapter 3.1** we systematically review the literature for studies that investigated the association of white matter lesions with measures of global brain atrophy and measures of medial temporal lobe atrophy. In **chapter 3.2** we investigate the independent association of white matter lesions and lacunar infarcts with global, cortical and subcortical brain atrophy in patients with manifest arterial disease. In **chapter 3.3** we investigate whether white matter lesions and lacunar infarcts are independently associated with executive functioning and memory performance, and assess whether these associations are mediated by global, cortical or subcortical brain atrophy.
In **chapter 4** we study the associations between total cerebral blood flow, brain atrophy and cognitive functioning. In **chapter 4.1** we investigate whether age, sex, vascular risk factors and location of arterial disease are associated with total cerebral blood flow. We will also compare total cerebral blood flow values from our study population with those from another study that were obtained in a sample of the general population. In **chapter 4.2** we examine the relationship between cerebral blood flow and global and subcortical brain atrophy, and investigate whether white matter lesions modify the relation between cerebral blood flow and brain atrophy. In **chapter 4.3** we investigate whether cerebral blood flow is associated with executive functioning and memory performance, and also investigate whether white matter lesions modify the relation between cerebral blood flow and cognitive performance.
In **chapter 5** we will discuss our findings, and try to answer the question to what extent cerebral small-vessel disease and lower cerebral blood flow contribute to the development of brain atrophy and cognitive decline. The results of the studies that are performed in this thesis will be summarized in **chapter 6**.

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2

Brain volumes and cerebrovascular lesions in patients with arterial disease

Background and aim

Recently, volumetric estimates of brain volumes and white matter lesions (WML), and prevalence of silent brain infarcts on MRI according to age and sex have been reported for the general population. Here, we report brain volumes and cerebrovascular lesions on MRI in a large cohort of patients with symptomatic arterial disease.

Methods

Within the SMART-MR study, a cohort study among patients with clinically manifest arterial disease, cross-sectional analyses were performed in 1044 patients (mean age of men and women 58 ± 10 years, 79% male). Brain segmentation was used to quantify volumes of cortical gray matter, white matter, sulcal and ventricular cerebrospinal fluid, and WML. Volumes were expressed as percentage of the intracranial volume (ICV). Brain infarcts were rated visually by an investigator and neuroradiologist. We used linear regression analysis, adjusted for vascular risk factors, to investigate the associations of age and sex with brain volumes and WML volume.

Results

Total brain volume was 78.8% of ICV in men and 79.9% in women, which decreased with 0.18% per year. Cortical gray matter volume also decreased with age, but stronger in men than in women. Ventricular volume was 2.16% of ICV in men and 1.83% in women and increased with age, similarly in men and women. WML volume also increased with older age, but more strongly in men than in women. Silent brain infarcts were present in 14% of men and women, and increased to 24% of subjects aged 65 years or older.

Conclusions

This study provides estimates of brain volumes and cerebrovascular lesions for patients with manifest arterial disease. The results suggest that the decrease in brain volumes with increasing age is comparable with findings from the general population. However, vascular pathology on MRI, as indicated by white matter lesions, silent brain infarcts, and subcortical brain atrophy, is more common in this population.

Introduction

Brain atrophy, characterized by widening of the sulci, narrowing of the gyri, and enlargement of the ventricles is a common finding on magnetic resonance imaging (MRI) in the elderly.[1] In healthy individuals, global and regional brain volume decline starts at a slow rate in early adulthood and accelerates in older age.[2,3] The extent and rate of progression of global and regional brain atrophy are associated with cognitive decline and development of dementia.[4-6] In elderly people, white matter lesions and silent brain infarcts are also commonly found on MRI.[7-10] They are also associated with future cognitive decline and dementia.[10-13] Evidence has accumulated that vascular risk factors, including hypertension, hyperlipidemia, diabetes mellitus, obesity, large amounts of alcohol, and cigarette smoking, play an important role in the etiology of white matter lesions, silent brain infarcts and brain atrophy, and future cognitive decline and dementia.[7,14-23]

With recently developed methods for automated segmentation of brain structures, it is possible to make accurate estimations of brain volumes and volume of white matter lesions. Recent studies on basis of these techniques reported brain volumes and volumes of white matter lesions in the general elderly population.[24-26] However, studies are also needed that examine brain volumes and cerebrovascular lesions on MRI in patients with a high burden of atherosclerotic disease, since this group is, in particular, at high risk of developing brain atrophy, white matter lesions, silent brain infarcts, and subsequent cognitive decline.

In the present study, we estimated brain volumes, white matter lesion volume, and presence of silent brain infarcts on MRI in a large cohort of patients with vascular disease. We also examined sex and age differences in brain volumes and cerebrovascular lesions on MRI in this cohort.

Methods

SMART-MR Study

The present study is a cross-sectional study within the SMART-MR study, a prospective cohort study within the Second Manifestations of ARTerial disease (SMART) study.[27] All eligible patients, newly referred to the University Medical Center Utrecht with symptomatic atherosclerotic disease or risk factors for atherosclerosis, are screened for additional risk factors and severity of atherosclerosis. The baseline examination is performed during a one day visit to our medical center and includes a physical examination, ultrasonography of the carotid arteries, and blood and urine sampling. Risk factors, medical history, and functioning are assessed with questionnaires that the patients fill in before their visit to the medical center.

Between May 2001 and December 2005, an MR investigation of the brain was added to the baseline examination as part of the SMART-MR study. The objective of the SMART-MR study is to investigate causes and consequences of brain changes on MRI in patients with vascular disease. Patients were eligible for an MRI of the brain if

they were included with manifest coronary artery disease, cerebrovascular disease, peripheral arterial disease or an abdominal aortic aneurysm (AAA), and if they had no MR contraindications. Coronary artery disease was defined as myocardial infarction, coronary artery bypass graft surgery or percutaneous transluminal coronary angioplasty in the past or at inclusion. Patients with a transient ischemic attack (TIA) or stroke at inclusion and patients who reported stroke in the past were considered to have cerebrovascular disease. Peripheral arterial disease was defined as surgery or angioplasty of the arteries supplying the lower extremities in history or intermittent claudication or rest pain at inclusion. AAA was defined as present AAA (distal aortic anteroposterior diameter ≥3 cm) or previous AAA surgery. The SMART study and SMART-MR study were approved by the ethics committee of our institution and written informed consent was obtained from all participants.

Magnetic resonance imaging protocol

The MR investigations were performed on a 1.5-Tesla whole-body system (Gyroscan ACS-NT, Philips Medical Systems, Best, the Netherlands). The protocol consisted of a transversal T1-weighted gradient-echo sequence (repetition time (TR)/echo time (TE): 235/2 ms; flip angle, 80°), a transversal T2-weighted turbo spin-echo sequence (TR/TE: 2200/11 ms and 2200/100 ms; turbo factor 12), a transversal T2-weighted fluid attenuating inverse recovery (FLAIR) sequence (TR/TE/inversion time (TI): 6000/100/2000 ms) and a transversal inversion recovery (IR) sequence (TR/TE/TI: 2900/22/410 ms) (field of view (FOV) 230 x 230 mm; matrix size, 180 × 256; slice thickness, 4.0 mm; slice gap, 0.0 mm; 38 slices).

Brain segmentation

We used the T1-weighted gradient-echo, the IR sequence and the FLAIR sequence for brain segmentation. The probabilistic segmentation technique has been described elsewhere, and has been proven to be very reliable with similarity indices exceeding 0.8 for all segmented tissue and cerebrospinal fluid volumes, indicating an excellent agreement between the results of the segmentation program and manual segmentation.[28] In short, two preprocessing steps were performed. The first step was an intra-patient rigid registration in order to compensate for motion and scan variations.[29] The second preprocessing step was an automatic skull-stripping of the T1 image,[30] in order to define a proper region of interest for the segmentation process. The actual segmentation of the MR-images was done with a statistical method called k-Nearest Neighbor (KNN) classification.[28] The result of the classification method is a probability value for each voxel that quantifies the amount of a specific tissue type contained in that voxel. Total volumes were calculated by multiplying these probabilities by the number and volumes of the voxels (4.0 x 0.9 x 0.9 mm). The segmentation program distinguishes cortical gray matter, white matter, sulcal and ventricular cerebrospinal fluid (CSF), and lesions. Subcortical gray matter was not segmented separately, but was included in the white matter volume. White matter lesions (WML) and brain infarcts are classified as 'lesion' volume, since the

segmentation program cannot distinguish between them. Therefore the results of the segmentation analysis were visually checked and a further distinction was made into WML and infarct volumes by manually assigning the lesion volumes to one of these two categories (see below).
Total brain volume was calculated by summing the volumes of gray and white matter and, if present, the volumes of WML or infarcts. Total intracranial volume (ICV) was calculated by summing the total brain volume and the volumes of the sulcal and ventricular CSF. To avoid incorporation of neuronal tissue and CSF outside the cranium, an investigator determined the slice that included the foramen magnum. As a result, the total brain volume includes the cerebrum, brainstem and cerebellum.

Brain volumes

The brain volumes that were distinguished were total brain volume, ventricular volume, and cortical gray matter volume. Total brain volume was expressed by brain parenchymal fraction (BPF), an indicator for global brain atrophy, which represents the percentage of the ICV that is occupied by brain tissue.[31] Ventricular enlargement, an indicator of subcortical brain atrophy, was assessed with the ventricular fraction (VF) and was calculated as the percentage ventricular volume of the total ICV. Cortical gray matter volume was assessed with the cortical gray matter fraction (GMF) and was calculated as the percentage cortical gray matter volume of the total ICV. A lower GMF indicates cortical atrophy.

White matter lesion volume

Volumes of WML obtained with the segmentation program were summed to obtain the total volume of WML. We made no distinction between deep and periventricular WML, since it has been shown that deep, periventricular and total WML are highly correlated with each other, and it has been suggested that categorical distinctions between periventricular and deep WML are arbitrary.[32] We normalized WML volumes for intracranial volume to correct for differences in head size,[31] by dividing total WML volume by ICV. As a result, WML volume is expressed as percentage of ICV.

Brain infarcts

The whole brain, including cortex, brainstem, and cerebellum, was visually searched for infarcts by two trained investigators and a neuroradiologist. All raters were blinded for the history and diagnosis of the patient. Discrepancies in rating were re-evaluated in a consensus meeting. Infarcts were defined as focal hyperintensities on T2-weighted images of at least 3 mm in diameter. Infarcts located in the white matter also had to be hypointense on T1-weighted images in order to distinguish them from WML. Dilated perivascular spaces were distinguished from infarcts on the basis of their location (along perforating or medullary arteries, often symmetrical bilaterally, usually in the lower third of the basal ganglia or in the centrum semiovale), form (round/oval), and the absence of gliosis.[20] The location, affected flow territory and type were scored for every infarct. Brain infarcts were categorized as cortical infarcts, lacunar

infarcts, large subcortical infarcts and infratentorial infarcts. An infarct in the cortex was defined as cortical irrespective whether it involved the subcortical white matter. We defined lacunar infarcts as infarcts sized 3 to 15 mm in diameter and located in the subcortical white matter, thalamus or basal ganglia. Large subcortical infarcts had the same characteristics as lacunar infarcts, but were sized >15 mm and were located exclusively subcortical, i.e. not confluent with cortical infarcts. Infratentorial infarcts were located in the brainstem or cerebellum.

Vascular risk factors

During the patient's visit at the medical center, an overnight fasting venous blood sample was taken to determine glucose and lipid levels. Height and weight were measured without shoes and heavy clothing, and the body mass index (BMI) was calculated (kg/m^2). Systolic and diastolic blood pressures (mmHg) were measured twice with a sphygmomanometer and the average of the two measures was calculated. Hypertension was defined as mean systolic blood pressure ≥140 mmHg or mean diastolic blood pressure ≥95 mmHg or use of antihypertensive drugs. Diabetes mellitus was defined as glucose ≥7.0 mmol/L or use of oral antidiabetic drugs or insulin. Hyperlipidemia was defined as total cholesterol >5.0 mmol/L, low-density lipoprotein cholesterol >3.2 mmol/L or use of lipid lowering drugs. Smoking habits and alcohol intake was assessed with questionnaires. Smoking and alcohol intake were categorized as never, former, or current. Patients who had quit smoking or drinking during the past year were assigned to the category current smoking or alcohol intake. Ultrasonography was performed to measure the intima-media thickness (IMT) (mm) in the left and right common carotid arteries, represented by the mean value of six measurements.[33]

Study sample

A total of 1309 patients was included in the SMART-MR study. Of these, 192 patients had missing MR sequences needed for the segmentation analyses due to a temporary change in MR protocol. Also, MR data were irretrievable for 19 patients; the FLAIR sequence was missing in 14 patients; and 40 patients had missing data due to motion or other artifacts. As a result, segmentation data were available for 1044 patients.

Data analysis

First, frequency of vascular risk factors, location of vascular disease, and extent of atherosclerosis was calculated for the total study population and for men and women separately. Second, brain volumes, WML volume, and presence and location of brain infarcts were calculated for the total study population and for men and women separately. Third, linear regression analysis was used to estimate the association of age and sex with brain volumes (BPF, VF and GMF) and WML volume. WML volume was natural log transformed to obtain a normal distribution. In the first model age (as a continuous variable) and sex (women vs. men) were entered, and in the second model additional adjustments were made for smoking (never, former, current), alcohol

intake (never, former, current), body mass index (kg/m^2), hypertension (ever vs. never), hyperlipidemia (yes vs. no), diabetes mellitus (yes vs. no), intima-media thickness (mm), and location of vascular disease (cerebrovascular disease yes vs. no; coronary artery disease yes vs. no; peripheral arterial disease yes vs. no; and AAA yes vs. no). In the third model, we entered the interaction between age and sex and age squared to the fully adjusted model, to investigate whether the association between age and brain volumes differed between men and women and whether a decrease in brain volume accelerated with age.[2] Graphic representations were made of the predicted values from the linear regression model with age, sex, the interaction between age and sex, and age squared, for men and women. Finally, the prevalence of asymptomatic (silent) infarcts on MRI was calculated for three age groups (<55 years, 55-64 years, and 65 years or older). Asymptomatic infarcts were defined as infarcts on MRI in patients without TIA or stroke at inclusion or patients who did not report stroke in the past.

Results

Table 1 presents the baseline frequency of location of vascular disease, vascular risk factors, and extent of atherosclerosis for the total study population, and for men and women separately. Two hundred and twenty women (21%) and 824 (79%) men were included in the study. The mean age in men and women was almost identical (58 ± 10 years). The majority of the study population had coronary artery disease (58%). Cerebrovascular disease was present in 23% of the study population. Peripheral arterial disease was present in 22% of the study population, and an AAA in 9%. The cumulative percentage exceeds 100% because some patients had vascular disease at more than one location. Men more often had a history of coronary heart disease, while women more often had peripheral arterial disease. Women more often had hypertension and more often had never smoked than men. In the total study population, the majority (58%) was former smokers.
Table 2 presents the brain volumes and WML volume for men and women. Men had larger ICV and larger uncorrected total brain volume, but smaller brain volumes and larger ventricles after correcting for ICV than women. **Table 3** presents the brain volumes and cerebrovascular lesions on MRI in the total study population (n=1044) and in patients without a history of cerebrovascular disease (n=808). The mean intracranial volume in the total study population was 1462 ± 129 ml and the mean total brain volume, including the cerebellum and brainstem, was 1155 ± 107 ml. Total brain volume and cortical gray matter volume were slightly smaller in patients with a history of cerebrovascular disease, and ventricular volume, and WML volume were slightly larger in these patients. In 26.3% of the total population an infarct was seen on MRI, and silent infarcts were present in 14.4% of the population. There were no sex differences in presence of silent infarcts (14.4% in men and 14.3% in women).

Table 1 Characteristics of the study population

	Men n=824	Women n=220	All n=1044
Disease location			
Cerebrovascular disease, %	22	24	23
Coronary artery disease, %	62	43	58
Peripheral arterial disease, %	20	32	22
Abdominal aortic aneurysm, %	10	6	9
Risk factors			
Age (years)	58.5 ± 10.2	58.6 ± 10.4	58.6 ± 10.2
Body mass index (kg/m^2)	26.7 ± 3.5	26.9 ± 4.6	26.7 ± 3.7
Systolic blood pressure (mmHg)	141 ± 20	143 ± 22	141 ± 21
Diastolic blood pressure (mmHg)	82 ± 11	81 ± 11	82 ± 11
Hypertension, %	48	63	51
Hyperlipidemia, %	79	77	79
Diabetes mellitus, %	21	18	20
Smoking			
Never, %	15	23	17
Former, %	60	49	58
Current or recently quit, %	25	28	25
Alcohol intake			
Never, %	11	34	16
Former, %	9	11	9
Current or recently quit, %	80	55	75
Extent of atherosclerosis			
Intima-media thickness (mm)	0.94 ± 0.32	0.91 ± 0.26	0.93 ± 0.31

Characteristics presented as mean ± SD, unless otherwise specified

Table 2 Brain volumes and white matter lesion volume in men and women

	Men n=824	Women n=220
Age (years), mean ± SD	58.5 ± 10.2	58.6 ± 10.4
Intracranial volume (ICV) (ml)	1501 ± 109	1316 ± 87
Total brain volume (ml)	1183 ± 97	1052 ± 79
Total brain volume (% of ICV)	78.8 ± 2.9	79.9 ± 2.6
Ventricular volume (% of ICV)	2.16 ± 1.00	1.83 ± 0.87
Cortical gray matter volume (% of ICV)	35.9 ± 3.5	37.2 ± 3.0
White matter lesion volume (% of ICV), median	0.11	0.13
(25th-75th percentile)	(0.06, 0.24)	(0.08, 0.25)

Characteristics presented as mean ± SD, unless otherwise specified

Table 3 Brain volumes and cerebrovascular lesions on MRI in the total study population and in patients without clinically manifest cerebrovascular disease

	Total study population	Patients without clinically manifest cerebrovascular disease
	n=1044	n=808
Intracranial volume (ICV) (ml)	1462 ± 129	1464 ± 131
Total brain volume (ml)	1155 ± 107	1161 ± 109
Total brain volume (% of ICV)	79.0 ± 2.9	79.3 ± 2.8
Ventricular volume (% of ICV)	2.09 ± 0.98	2.01 ± 0.94
Cortical gray matter volume (% of ICV)	36.2 ± 3.5	36.5 ± 3.4
White matter lesion volume (% of ICV), median	0.12	0.10
(25^{th}-75^{th} percentile)	(0.07, 0.24)	(0.06, 0.21)
Infarcts		
Any infarct, %	26.3	14.4
Cortical, %	11.1	3.7
Large subcortical, %	0.9	0.0
Lacunar, %	17.7	10.0
Infratentorial, %	5.8	3.6
Cerebellum, %	3.5	2.2
Brainstem, %	2.5	1.4

Characteristics presented as mean ± SD, unless otherwise specified

Table 4 shows the results of the linear regression analysis of the association between age, sex and brain volumes. As can be seen, total brain volume and cortical gray matter volume significantly decreased with older age, and ventricular volume and WML volume significantly increased with older age. In addition, women had larger brain and cortical gray matter volumes and smaller ventricular volumes than men, after taking ICV into account. Compared with men, women had 1.14% (95%CI 0.82 to 1.47) higher BPF and 1.37% (95%CI 0.92 to 1.82) higher GMF. Also, women had 0.34% (95%CI 0.21 to 0.47) lower VF than men, indicating that men had more global, cortical and subcortical atrophy than women. These findings were similar after adjusting for vascular risk, although the sex differences became somewhat smaller for BPF and GMF (**Table 4**, Model II). However, women had slightly higher WML volume than men, although this difference was not significant after adjusting for vascular risk and disease location (**Table 4**, Model II). When the interaction between age and sex was entered to the model, significant interactions were present for GMF and WML, indicating that the decrease in GMF and increase in WML with older age was greater in men than in women (**Table 4**, Model III). Furthermore, age squared was significantly associated with BPF, VF and WML volume, indicating that the decrease in BPF and the increase in VF and WML accelerated with older age. **Figures 1 - 4** show the individual

Table 4 Results of linear regression analyses of the associations of age and sex with brain volumes and WML volume

		BPF	VF	GMF	WML†
		β (95%CI)*	β (95%CI)*	β (95%CI)*	β (95%CI)*
Model I	Age (per year)	-0.18 (-0.20 to -0.17)	0.05 (0.04 to 0.05)	-0.17 (-0.19 to -0.15)	0.05 (0.04 to 0.05)
	Women vs. men	1.14 (0.82 to 1.47)	-0.34 (-0.47 to -0.21)	1.37 (0.92 to 1.82)	0.13 (0.01 to 0.27)
Model II	Age (per year)	-0.17 (-0.18 to -0.15)	0.04 (0.03 to 0.05)	-0.15 (-0.17 to -0.13)	0.04 (0.04 to 0.05)
	Women vs. men	1.11 (0.76 to 1.45)	-0.37 (-0.51 to -0.23)	1.28 (0.80 to 1.76)	0.10 (-0.05 to 0.24)
Model III	Age^2	-0.002 (-0.003 to -0.001)	0.001 (0.001 to 0.002)	0.001 (-0.001 to 0.002)	0.001 (0.001 to 0.002)
	Age * sex	-0.029 (-0.060 to 0.003)	0.000 (-0.013 to 0.013)	-0.098 (-0.142 to -0.054)	0.016 (0.003 to 0.030)

Model I: Age and sex adjusted
Model II: Additionally adjusted for smoking, alcohol intake, BMI, hypertension, hyperlipidemia, diabetes mellitus, intima-media thickness, and disease location
Model III: Model II plus age^2, and interaction between age and sex (men are coded 1 and women are coded 0)
* β represents % difference in BPF, VF, GMF, and WML (as % of ICV) per year increase in age and mean difference for women relative to men
† natural log transformed WML
BPF = brain parenchymal fraction; VF = ventricular fraction; GMF = gray matter fraction; WML = white matter lesions; CI = confidence interval

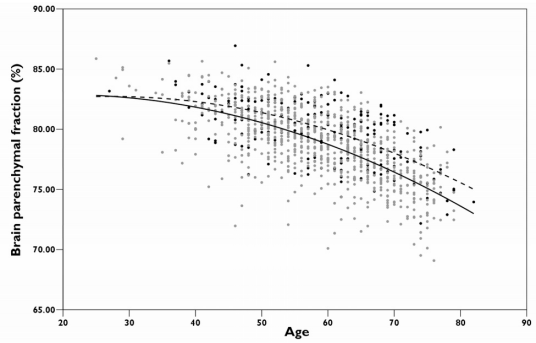

Figure 1 Individual data points and predicted values of the regression model with age, sex, the interaction between age and sex, and age squared for brain parenchymal fraction for men and women. The best fit of the regression model is quadratic.
Grey dots = raw data for men, solid line = best fit of regression model for men, black dots = raw data for women, dashed line = best fit of regression model for women

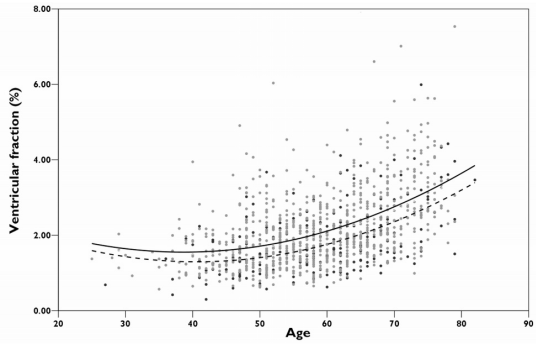

Figure 2 Individual data points and predicted values of the regression model with age, sex, the interaction between age and sex, and age squared for ventricular fraction for men and women. The best fit of the regression model is quadratic.
Grey dots = raw data for men, solid line = best fit of regression model for men, black dots = raw data for women, dashed line = best fit of regression model for women

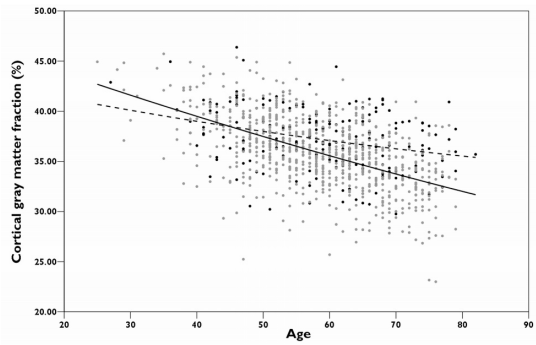

Figure 3 Individual data points and predicted values of the regression model with age, sex, the interaction between age and sex, and age squared for cortical gray mater fraction for men and women. The best fit of the regression model is linear.
Grey dots = raw data for men, solid line = best fit of regression model for men, black dots = raw data for women, dashed line = best fit of regression model for women

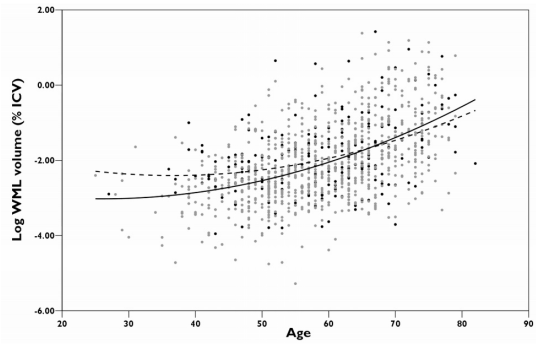

Figure 4 Individual data points and predicted values of the regression model with age, sex, the interaction between age and sex, and age squared for natural log transformed WML volume (as % of ICV) for men and women. The best fit of the regression model is quadratic.
Grey dots = raw data for men, solid line = best fit of regression model for men, black dots = raw data for women, dashed line = best fit of regression model for women. WML = white matter lesion, ICV = intracranial volume

data points and predicted values of the regression models for BPF, VF, GMF and WML according to age and sex for the regression models with age, sex, age*sex, and age squared. When we repeated the analyses after excluding patients with any infarct on MRI (26.3% of 1044), the results from the linear regression models did not materially change (data not shown).

Table 5 presents the number of silent brain infarcts across three age groups. In the youngest age group (<55 years) silent infarcts were present in 8% of the patients; this percentage increased to 24% in patients of 65 years or older. There were no significant differences in presence of silent infarcts between men and women. In the youngest age group 7.3% of men and 12.3% of women had silent infarcts; in the middle age group 13.0% of men and 12.5% of women had silent infarcts, and in the oldest age group 26.1% of men and 18.2% of women had silent infarcts. Asymptomatic cortical infarcts were present in 1.3% of patient of <55 years, and this percentage increased to 7.4% in patients 65 years or older. Asymptomatic lacunar infarcts were present in 5.9% of patients <55 years and in 18.2% of patients of 65 years or older. Asymptomatic infratentorial infarcts also increased with age; from 1.6% in the youngest age category to 6.5% in the oldest age category. This increase in prevalence of infratentorial infarcts with age was primarily caused by an increase in cerebellar infarcts. Asymptomatic large subcortical infarcts were not found in our study population.

Table 5 Presence of silent brain infarcts in 3 age groups among 808 patients without clinically manifest cerebrovascular disease

	<55 years n=305	55 – 64 years n=272	65 years or older n=231	All n=808
Any infarct, n (%)	25 (8.2)	35 (12.9)	56 (24.2)	116 (14.4)
Cortical, n (%)	4 (1.3)	9 (3.3)	17 (7.4)	30 (3.7)
Large subcortical *, n (%)	0 (0)	0 (0)	0 (0)	0 (0)
Lacunar, n (%)	18 (5.9)	21 (7.7)	42 (18.2)	81 (10.0)
Infratentorial, n (%)	5 (1.6)	9 (3.3)	15 (6.5)	29 (3.6)
Cerebellum, n (%)	3 (1.0)	4 (1.5)	11 (4.8)	18 (2.2)
Brainstem, n (%)	2 (0.7)	5 (1.8)	4 (1.7)	11 (1.4)

* located exclusively subcortical, and not confluent with cortical infarct

Discussion

We estimated brain volumes, white matter lesion volume, and presence of silent brain infarcts on MRI in a large cohort of patients with vascular disease. We also examined sex and age differences in brain volumes and cerebrovascular lesions on MRI in this cohort. To our knowledge, this is the first study reporting brain volumes and cerebrovascular lesions on MRI in a population of patients with manifest arterial

disease. The large number of patients included and the wide age range of the sample make this cohort unique. The size of this vascular cohort and the segmentation technique used made precise estimates possible. The segmentation technique also allowed us to differentiate between total brain volume, ventricular volume, cortical gray matter volume and white matter lesion volume. Also, since the whole brain was scanned, we could differentiate between different types of infarcts, including infarcts in the cerebellum and brainstem.

Total brain volume, expressed as percentage of intracranial volume, decreased with a mean of 0.18% per year. This decrease accelerated with older age, similarly in men and women. A recent study reported a mean decrease of 0.23% total brain volume per year based on thirteen studies.[2] The Framingham Heart Study found a mean decrease of 0.21% per year in subjects on average 60 years, and this decrease also accelerated with age.[24] Recent findings from the Rotterdam Scan Study showed a linear decrease of 0.31% total brain volume per year in subjects with an average age of 73 years.[25] In our study ventricular fraction, an indicator of subcortical atrophy, was 2.16% in men and 1.83% in women, and increased in men and women with a mean of 0.05% per year, and this increase also accelerated with older age. Few other studies reported volumetric measurements of the ventricles, but it appears that the ventricular volumes in our population are much larger than found in the general population in the same age range.[24] Cortical gray matter fraction, an indicator of cortical atrophy, showed a linear decrease with age, and this decrease was stronger in men than in women (decrease per year; 0.27% for men vs. 0.17% for women). This linear decrease in cortical gray matter has also been found in other studies,[24,34] while others did not find a decrease with age[25] or found a nonlinear decrease.[2] These findings may not be fully comparable though, since other studies analyzed total gray matter volume including subcortical gray matter volume.

Consistent with findings from several community-based studies[7] we observed that WML volume steeply increased with age. The mean increase of the log transformed WML volume in our population was 0.05% per year, which is highly comparable with findings from the Rotterdam Scan Study (0.066% in men and 0.061% in women)[25] and the Framingham Heart Study (0.05% in men and 0.06% in women).[24] This increase accelerated with older age, which was also found in the Framingham Heart Study,[24] but not in the Rotterdam Scan Study. However, compared with their estimates, in our population the mean WML volumes were higher. This is most likely explained by the fact that our population consisted of patients with vascular disease. Some studies observed sex differences, with women having more WML than men.[8,35] We observed that women at younger age had larger WML volumes than men, but at older age this sex difference disappeared.

Silent brain infarcts were present in 14.4% of our population. This percentage is higher than the 7% that was recently observed in a general population with a mean age of 63 years,[26] but fell well within the range reported in a recent systematic review[10] that found an overall prevalence of 8% to 28% depending on age in the general elderly population. Similar to other studies, the presence of silent infarcts in our study steeply increased with increasing age. In patients of 65 years or older, 24% had silent infarcts.

This estimate is higher than findings in elderly from the Rotterdam Scan Study who were 75 years or older,[26] and the Northern Manhattan Study,[36] suggesting that the prevalence of silent brain infarcts in our population is much higher when compared with the general population. We did not find a sex difference, but this may be due to the fact that all patients had manifest arterial disease. As expected, lacunar infarcts were the majority of silent infarcts (10.0%), although asymptomatic cortical infarcts (3.7%) and infratentorial infarcts (3.6%) were also not uncommon. A recent study in a multi-ethnic cohort observed a much higher prevalence of infratentorial infarcts (9%), which was explained by a higher prevalence of infarcts in black and Hispanic subjects.

Taken together, the results of this study suggest that the decrease in brain volumes with increasing age is comparable with findings from the general population. However, vascular pathology on MRI, as indicated by white matter lesions, silent brain infarcts, and subcortical brain atrophy, is more common in this population.

A limitation of our study is its cross-sectional design. Therefore, we do not know to what extent age and sex differences represent a decline within subjects or cohort or survival effects. Also, we do not know to what extent our study population is representative of the typical patient with vascular disease. Our cohort consists of a large number of patients who were consecutively included in the study, and who represent a wide variety of patients with vascular disease in different locations of the vascular tree. The majority of patients with manifest vascular disease will be referred to a secondary care clinic, and as such this cohort may be representative of a typical patient with vascular disease. Another limitation may be that it is difficult to verify if silent infarcts are really clinically asymptomatic. We tried to minimize misclassification by excluding patients who reported a TIA in the past, and we defined infarcts on MRI as silent infarcts only if patients had no TIA or stroke at inclusion, as confirmed by a neurologist, or if patients did not report stroke in the past.

Cardiovascular and cerebrovascular diseases are the top contributors of mortality worldwide. Also, because these patients will live longer as a result of improved treatment possibilities, cardiovascular and cerebrovascular diseases are expected to become a major cause of disability. Since vascular disease is involved in the etiology of brain changes on MRI and development of dementia, patients with cardiovascular and cerebrovascular disease can be considered a high-risk group, not only for disability and mortality but also for cognitive decline. New imaging and segmentation techniques make it possible to reliably estimate brain volumes and cerebrovascular lesions. Future studies adopting these techniques should examine different populations, preferably with follow-up examinations, to provide insight in what can be considered normal brain changes and who will develop pathological brain changes.

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3.1

White matter lesions and brain atrophy A systematic review

Background

White matter lesions (WML) and brain atrophy are often found on MRI in the elderly. Shared vascular risk factors may be an explanation for their concomitance. However, disturbances of white matter integrity could also be involved in the pathogenesis of brain atrophy.

Objectives

To systematically review studies that investigated the relation between WML and brain atrophy on MRI and to investigate whether there is sufficient evidence that this relation is independent of shared risk factors.

Data sources

We searched PubMed for studies published between 1980 and October 2007, combining search terms for WML with search terms for brain atrophy.

Study selection and data extraction

Articles that studied the relation between WML and brain atrophy were included if they met the following criteria; (1) published in English, (2) original study, (3) MRI used for imaging, (4) assessment methods for WML and brain atrophy specified, and (5) a sample size of at least 20 participants. For every study we recorded type and age of the study population, type and assessment of WML and brain atrophy, and the variables for which adjustments were made in the analyses.

Results

We identified 48 studies that met our inclusion criteria. A significant relation between WML and brain atrophy was found in 37 out of 48 studies. The source of the study population (e.g. clinic or population based) did not affect this relation. However, only 10 studies adjusted for shared risk factors.

Conclusions

The majority of studies found an association between WML and brain atrophy, but it is not yet clear if this association is independent of shared risk factors.

Introduction

In healthy individuals, global and regional brain volume decline starts at a slow rate in early adulthood and accelerates in older age.[1-3] The rate of this decline varies considerably among individuals.[3] This is of clinical importance since the extent and rate of progression of global and regional brain atrophy are associated with future cognitive deterioration and conversion to dementia.[4-6]

Although brain atrophy may be caused by specific neurological diseases, it is often found on MRI in elderly without apparent neurological symptoms. Evidence has accumulated that vascular factors play an important role in the etiology of brain atrophy and development of dementia. These factors include hypertension,[7,8] hyperlipidemia,[8] diabetes mellitus,[9] obesity,[10] large amounts of alcohol,[11] and cigarette smoking.[8,9]

In addition to brain atrophy, the presence and severity of white matter lesions (WML) increase with age.[12-14] They too are associated with future cognitive decline and dementia.[15-18] Furthermore, vascular risk factors, including hypertension,[12,14,19-23] diabetes mellitus,[24] obesity,[25,26] and smoking [14,21] are also associated with WML.

Already more than 100 years ago, Alois Alzheimer and Otto Binswanger described the concomitant presence of subcortical vascular pathology and pronounced atrophy of the white matter with enormously enlarged ventricles in post-mortem studies.[27] More recently, concomitant brain atrophy and WML are frequently observed in elderly people on magnetic resonance imaging (MRI). However, it is unknown which factors may explain the co-occurrence of WML and brain atrophy. One possibility is that their coexistence may be explained by shared vascular risk factors or other shared factors associated with aging. Another possibility is that the disturbances of white matter integrity can contribute to the pathogenesis of brain atrophy by causing ischemic damage to axons, oligodendrocytes and other glial cells.[28]

In this study we systematically reviewed available studies that investigated the relation between WML and global or regional brain atrophy on MRI to assess whether there is sufficient evidence to conclude that WML and brain atrophy are associated with each other independent of shared risk factors. Finally, we discussed the proposed mechanisms that could underlie the relation between WML and brain atrophy.

Methods

We searched PubMed for studies published in English between 1980 and October 2007. Since several synonyms exist for WML and brain atrophy we made search terms for WML and brain atrophy and entered them as follows: ('search term for WML' AND 'search term for brain atrophy'). We combined this search with limits for language and publication date (**Box 1**). The last search was performed on October 31st, 2007. Additional literature was obtained from reference lists of relevant articles. One investigator (APAA) screened all titles and abstracts and all full-text articles were evaluated by two investigators independent from each other (APAA and LGE). A consensus meeting was held in case of disagreement. Since we used a broad search

term we expected a large number of irrelevant articles. Therefore, we defined the following exclusion criteria on the basis of which abstracts could be discarded; case reports and reviews, studies performed in a pediatric study population, and studies in which only computed tomography (CT) was used for imaging. We also excluded studies that investigated hyperintensities on MRI with other known causes (hematological disorders, metabolic or toxic causes, non-infectious inflammatory or autoimmune diseases, infectious causes, genetic disorders, radiotherapy or chemotherapy, and head trauma).

Box 1 Search terms and limits used for retrieval of relevant articles

Search term for white matter lesions:
("leukoaraiosis"[MeSH] OR leukoaraiosis[TIAB] OR leuko-araiosis[TIAB] OR white matter hyperintense[TIAB] OR white matter hyperintensity[TIAB] OR white matter hyperintensities[TIAB] OR white matter lesion[TIAB] OR white matter lesions[TIAB] OR white matter change[TIAB] OR white matter changes[TIAB] OR white matter abnormality[TIAB] OR white matter abnormalities[TIAB] OR white matter signal[TIAB] OR white matter signals[TIAB])
Search term for brain atrophy:
(((ventricle[TIAB] OR ventricles[TIAB] ventricular[TIAB] OR sulcus[TIAB] OR sulci[TIAB] OR sulcal[TIAB]) AND (enlargement[TIAB] OR size[TIAB] OR dilatation[TIAB] OR expansion[TIAB])) OR (("atrophy"[MeSH] OR atrophy[TIAB]) AND ("brain"[MeSH] OR brain[TIAB] OR cerebral[TIAB] OR hippocampus[TIAB] OR hippocampal[TIAB] OR cortical[TIAB] OR subcortical[TIAB] OR entorhinal cortex[TIAB] OR medial temporal lobe[TIAB])))
Limits
English[language] AND ("1980/01/01"[EDAT] :"2007/10/31"[EDAT])

[TIAB] = Title/Abstract; [MeSH] = Medical Subjects Heading; [EDAT] = Entrez date; the date the citation was added to PubMed

Of all potentially relevant articles we retrieved the full-text version. Data was extracted from articles if they met the following criteria; (1) the relation between WML and brain atrophy was studied (2) published in English, (3) original study, (4) MRI used for imaging, (5) assessment methods for WML and brain atrophy specified, (6) sample size of at least 20 participants per study group.

Of all relevant studies, we recorded the source population, the design (cross-sectional or longitudinal), the sample size and the mean age of the participants. Next, we described the types of WML and measures of brain atrophy that were investigated and the methods that were used to assess WML and atrophy. Due to heterogeneity in the assessment of WML and brain atrophy, and due to variation in the units of measurements it proved difficult to compare the measures of effect across studies quantitatively. Therefore, we decided to summarize the results qualitatively and recorded whether the relation between WML and measures of atrophy was statistically significant (p-value <0.05). If different subtypes of WML or brain atrophy were investigated within one study, we recorded the results separately. Finally, we checked whether adjustments were made for potential shared risk factors in the analyses and recorded the variables for which adjustments were made.

Results

Our search strategy resulted in 689 articles. As expected, the majority of these articles were not relevant for this review because we used a broad search term. After screening of the titles and abstracts we retrieved the full-text version of 145 articles. Of these, 41 met the inclusion criteria and 7 additional relevant articles were identified through reference lists of the included articles. The most important reason why articles were excluded was because WML and brain atrophy were both studied as the determinant or as the outcome of interest, but the relation between them was not investigated.

Since the source populations were very heterogeneous, we classified the 48 included articles into three categories: general population, subjects with cognitive impairment, and patients with vascular disease. The general population category consisted of studies that investigated a community-dwelling population or studies that were performed in healthy controls or in patients without neurological or psychiatric disease. In the category of patients with cognitive impairment study populations were included with (probable) Alzheimers disease, mixed dementia or cognitive impairment without dementia. In one study also patients with Lewy body dementia were included [29]. Studies investigating patients with vascular disease were further divided into a group of patients with vascular risk factors and a group with clinically manifest cerebrovascular disease.

We also distinguished studies that investigated indicators of global brain atrophy from studies that investigated indicators of regional brain atrophy. Measures of global brain atrophy included total brain atrophy, cortical gray matter atrophy, white matter atrophy, and ventricular enlargement. The main measure of regional atrophy was medial temporal lobe atrophy (MTA), including atrophy of the hippocampus, amygdala, and entorhinal cortex.

Studies in which several study populations were investigated and studies reporting on the relation between WML and both global and regional brain atrophy were described in all relevant categories.

General population

The characteristics and results of the 20 general population studies (17 cross-sectional and 3 longitudinal) are summarized in **Table 1**. One study is included twice in **Table 1**, since both cross-sectional and longitudinal results were reported.[30] Thirteen studies involved a community-based study sample,[9,13,20,31-40] while 5 studies involved healthy controls or hospital personnel.[30,41-44] Two studies included neurologically healthy patients with different amounts of WML.[45,46] The size of the study populations varied considerably, with three studies having a very large number of subjects (>1500),[9,13,33] while 10 studies had fewer than 150 subjects,[30,32,36,39-45] and 7 studies investigated between 200 and 600 subjects.[20,31,34,35,37,38,46]

Table 1 Studies investigating the relation between white matter lesions and brain atrophy in subjects from the general population

First author, Year of publication	Study population	N	Mean age ± SD (Range)	WML	WML assessment
Cross-sectional studies – Measures of global brain atrophy					
Mirsen, 1991 [43]	Healthy controls	45	70 ± 10	PVWML DWML	Ordinal (0-1) Ordinal (0-4)
Agartz, 1992 [42]	Healthy adults	76	46 ± 18	PVWML DWML	Ordinal (0-2)
Breteler, 1994 [39]	Non-demented elderly	90	74 ± 6	Total WML	Ordinal (0-2)
Christiansen, 1994 [40]	Healthy volunteers	142	(21 – 80)	Total WML	Ordinal (0-11)
DeCarli, 1995 [44]	Healthy individuals	51	52 ± 20	Total WML	Volumetric
Ylikoski, 1995 [36]	Neurologically non-diseased elderly	128	72 (55 – 85)	PVWML DWML	Ordinal (0-1)
Yue, 1997 [13]	Population-based	3660	72 (>65)	Total WML	Ordinal (0-9)
Swan, 2000 [20]	Community-dwelling, white elderly men free of severe cognitive impairment	383	73 ± 3	Total WML	Volumetric
Longstreth, 2000 [9]	Community-dwelling elderly without stroke or TIA	3255	> 65	Total WML	Ordinal (0-9)
Mosley, 2005 [33]	General population	1538	63 ± 5	Total WML	Ordinal (0-9)
Rossi, 2006 [45]	Neurologically healthy	133	57 ± 10	Mainly anterior WML Mainly posterior WML	Ordinal (0-3)

BMI = body mass index; DWML = deep white matter lesions; ECG = electrocardiogram; HDL = High Density Lipoprotein; IMT = intima media thickness; MMSE = mini mental state examination;

Atrophy	Atrophy assessment	Results	Significant relation	Adjustments
Sulcal widening	Ordinal (0-5)	Control subjects with PVWML showed more ventricular enlargement then those without	No	No
Ventricular enlargement			Yes	
Sulcal widening			No	
Ventricular enlargement			No	
Sulcal widening	Ordinal (1-3)	No association between WML and sulcal size or ventricular enlargement	No	Age
Ventricular enlargement			No	
Sulcal widening			No	
Ventricular enlargement			No	
Ventricular volume	Volumetric	WML associated with ventricular enlargement	Yes	Age and sex
Cerebral hemispheres	Volumetric	No correlation between WML and size of the hemispheres or ventricles	No	No
Ventricular volume			No	
Total brain volume	Volumetric	WML associated with total brain atrophy and ventricular enlargement	Yes	Age
Ventricular volume			Yes	
Sulcal widening	Ordinal (0-3)	PVWML and DWML are associated with central brain atrophy	No	Age, sex, social class, MMSE, hypertension, diabetes, coronary heart disease, cardiac failure, cardiac arrhythmia, silent infarcts
Ventricular enlargement			Yes	
Sulcal widening			No	
Ventricular enlargement			Yes	
Sulcal enlargement	Ordinal (0-9)	WML were associated with ventricular enlargement	No	Age, sex and race
Ventricular enlargement			Yes	
Total brain volume	Volumetric	Only a marginal association between total brain volume and WML when treated as categorical variables (e.g., more or less than the median) was observed ($p < 0.11$)	No	No
Sulcal widening	Ordinal (0-9)	WML related to sulcal and ventricular size	Yes	Age, race, education, heart failure, smoking, alcohol, use of insulin or anti/diabetic medication, albumin, use of estrogens, abnormality on ECG, IMT, and stratified for sex
Ventricular enlargement			Yes	
Cortical atrophy	Ordinal (0-9)	WML correlated with cortical atrophy and ventricular enlargement	Yes	No
Ventricular enlargement	Ordinal (0-9)		Yes	
Frontal gray matter	Volumetric	Anterior WML (n=39) associated with frontal atrophy, while posterior (n =14) WML associated with diffuse atrophy	Yes	Age and sex
Cortical gray matter			Yes	

MTA = medial temporal lobe atrophy; PVWML = periventricular white matter lesions; SD = standard deviation; WML = white matter lesions

Table 1 Continued.

First author, Year of publication	Study population	N	Mean age ± SD (Range)	WML	WML assessment
Wen, 2006 [38]	Normal elderly	397	63 ± 1	PVWML DWML	Volumetric
Ikram, 2007 [37]	Non-demented elderly	490	73 ± 8	Total WML	Volumetric
Cross-sectional studies – Measures of regional brain atrophy					
O'Brein, 1997 [41]	Neurologically healthy volunteers	39	72	PVWML DWML	Ordinal (0-3)
Korf, 2004 [32]	Population-based	543	82	Total WML	Ordinal (0-9)
van der Flier, 2005 [46]	Non-disabled elderly people	581	74 ± 5	Total WML	Ordinal (0-1)
den Heijer, 2005 [35]	Non-demented elderly	511	73 ± 8	PVWML DWML	Ordinal (0-9) Ordinal
Du, 2006 [30]	Cognitively normal elderly	42	74 ± 8	Total WML	Volumetric
Longitudinal studies – Measures of global brain atrophy					
Enzinger, 2005 [31]	Subjects without neuropsychiatric disease	201	60 ± 6	Total WML	Ordinal (0-3)
Schmidt, 2005 [34]	Elderly without neuropsychiatric disease	329	60 ± 6	Total WML	Volumetric
Longitudinal studies – Measures of regional brain atrophy					
Du, 2006 [30]	Cognitively normal elderly	42	74 ± 8	Total WML	Volumetric

BMI = body mass index; DWML = deep white matter lesions; ECG = electrocardiogram; HDL = High Density Lipoprotein; IMT = intima media thickness; MMSE = mini mental state examination;

Atrophy	Atrophy assessment	Results	Significant relation	Adjustments
Cortical gray matter	Volumetric	DWML correlated with cortical gray matter loss and ventricular and sulcal enlargement. PVWML correlated with cortical gray matter loss only.	Yes	Sex
Ventricular size			No	
Sulcal size			No	
Cortical gray matter			Yes	
Ventricular size			Yes	
Sulcal size			Yes	
Total brain volume	Volumetric	Persons with WML had smaller brain volumes and less white matter volumes. Gray matter did not decrease with increasing WML	Yes	Age and sex
Gray matter volume			No	
White matter volume			Yes	
Hippocampus	Ordinal (0-3)	PVWML correlated with hippocampal atrophy	Yes No	No
Hippocampus	Volumetric	There was no significant difference in WML grade between individuals with and without hippocampus atrophy	No	Age
MTA	Ordinal (0-1)	There was a correlation between the presence of severe WML and the presence of MTA	Yes	No
Hippocampus	Volumetric	Persons with more WML had smaller hippocampal and amygdalar volumes	Yes	Age, sex, blood pressure, use of antihypertensive medication, cholesterol/HDL ratio, BMI and smoking
Amygdala			No	
Hippocampus			Yes	
Amygdala			Yes	
Hippocampus	Volumetric	WML volumes were not associated with volumes of entorhinal cortex and hippocampus	No	Age
Entorhinal cortex			No	
Total brain volume	Volumetric	WML score at baseline was associated with brain atrophy during follow-up	Yes	Brain volume at baseline, age and HbA1c
Total brain volume	Volumetric	Increasing WML volume was associated with brain parenchymal loss	Yes	Age, sex, education, BMI, hypertension, diabetes, cardiac disease and total cholesterol
Hippocampus	Volumetric	WML volumes at baseline had no effect on atrophy rates of entorhinal cortex and hippocampus	No	Age
Entorhinal cortex			No	

MTA = medial temporal lobe atrophy; PVWML = periventricular white matter lesions; SD = standard deviation; WML = white matter lesions

Table 2 Studies investigating the relation between white matter lesions and brain atrophy in subjects with cognitive impairment

First author, Year of publication	Study population	N	Mean age ± SD (Range)	WML	WML assessment
Cross-sectional studies – Measures of global brain atrophy					
Mirsen, 1991 [43]	AD	30	73 ± 7	PVWML DWML	Ordinal (0-1) Ordinal (0-4)
Fazekas, 1996 [48]	Probable AD	30	65 ± 7	PVWML Total WML	Thickness Volumetric
Decarli, 1996 [59]	Probable AD	26	74 ± 7	PVWML DWML	Ordinal (0-3)
Fein, 2000 [49]	Elderly subjects with lacunes and a spectrum of cognitive impairment, a control group of subjects with probable AD, and cognitively healthy controls	58/ 29/ 37	75	Total WML	Volumetric
Hirono, 2000 [60]	AD	76	76 ± 7	Total WML	Volumetric
Barber, 2000 [29]	AD / Lewy body	25/27	77 ± 7	PVWML DWML	Ordinal (0-5)
Mungas, 2001 [50]	Cognitively normal, cognitively impaired and demented elderly	90/37/ 30	74 ± 8	Total WML	Volumetric
Bigler, 2002 [47]	Patients with AD, VaD, MCI, other neuropsychiatric disorders and normal controls	195	82 (67 - 97)	PVWML DWML	Ordinal (0-3)
Capizzano, 2004 [61]	Probable AD	81	70 ± 8	Total WML	Volumetric
Tullberg, 2004 [62]	CIND / Demented	30/26	78 ± 9	Frontal WML Total WML	Volumetric

AD = Alzheimer's disease; CIND = cognitively impaired not demented; DWML = deep white matter lesions; MMSE = mini mental state examination; MTA = medial temporal lobe atrophy;

Atrophy	Atrophy assessment	Results	Significant relation	Adjustments
Sulcal widening	Ordinal (0-5)	No relation between PVWML and DWML with measures of atrophy	No	No
Ventricular enlargement			No	
Sulcal widening			No	
Ventricular enlargement			No	
Ventricular enlargement	Volumetric	PVWML were associated with ventricular enlargement	Yes	No
			No	
Brain volume	Volumetric	No difference in brain or ventricular volumes between AD patients with (PVWML or DWML >2) or without WML (PVWML or DWML <2)	No	No
Ventricular volume			No	
Brain volume			No	
Ventricular volume			No	
Cortical gray matter	Volumetric	WML volume correlated with cortical gray matter atrophy in all subjects	Yes	No
Total brain volume	Volumetric	No association between WML volume and normalized brain volume	No	Age, sex, education and duration of symptoms
Total brain volume	Volumetric	PVWML were related to ventricular dilatation in all subjects	Yes	Age
Ventricular volume			Yes	
Total brain volume			No	
Ventricular volume			No	
Cortical gray matter	Volumetric	WML volume was associated with smaller cortical gray matter volume	Yes	Volume of lacunes
Gray matter	Volumetric	PVWML were associated with white matter volume loss	No	Age
White matter			Yes	
Gray matter			No	
White matter			No	
Cortical gray matter	Volumetric	WML related to cortical gray matter volume loss in the frontal, temporal, parietal and occipital lobes	Yes	Age, hypertension and MMSE
Cortical gray matter	Volumetric	Increased WML in the frontal region were associated with reduced frontal cortical gray matter in all subjects	Yes	Unknown
			Yes	

parieto-occ. WML = Parieto-occipital WML; PVWML = periventricular white matter lesions; SD = standard deviation; WML = white matter lesions

Table 2 Continued I.

First author, Year of publication	Study population	N	Mean age ± SD (Range)	WML	WML assessment
Du, 2005 [58]	AD / Mixed dementia	50/13	77 ± 6	Total WML	Volumetric
Bracco, 2005 [53]	Probable AD	86	72 ± 7	Total WML	Ordinal (0-18)
Lunetta, 2007 [56]	AD-affected individuals and their unaffected siblings	424	73 ± 9	Total WML	Ordinal (0-100)
Cross-sectional studies – Measures of regional brain atrophy					
Fein, 2000 [49]	Elderly subjects with lacunes and a spectrum of cognitive impairment, a control group of subjects with probable AD, and cognitively healthy controls	58/29/37	75	Total WML	Volumetric
Mungas, 2001 [50]	Cognitively normal, cognitively impaired and demented elderly	90/37/30	74 ± 8	Total WML	Volumetric
Bigler, 2002 [47]	Patients with AD, VaD, MCI, other neuropsychiatric disorders and normal controls	195	82 (67 - 97)	PVWML DWML	Ordinal (0-3)
de Leeuw, 2004 [63]	Probable AD	179	68 ± 9	Frontal WML Temporal WML Parieto-occ. WML Infratentorial WML	Ordinal (0-2)
Du, 2005 [58]	AD / Mixed dementia	50/13	77 ± 6	Total WML	Volumetric
Korf, 2005 [54]	Probable AD	159	68 ± 9	Total WML	Ordinal (0-30)
van der Flier, 2005 [55]	AD / MCI / elderly without memory impairment	41/20/28	74 ± 7	Total WML	Volumetric
van de Pol, 2007 [51]	Participants of a randomized, double-blind, placebo-controlled trial of galantamine in MCI	896	70 ± 9	Total WML	Ordinal (0-30)
Bombois, 2007 [52]	MCI patients attending a memory clinic	170	68 (46 – 87)	PVWML DWML	Ordinal
Lunetta, 2007 [56]	AD-affected individuals and their unaffected siblings	424	73 ± 9	Total WML	Ordinal (0-100)

AD = Alzheimer's disease; CIND = cognitively impaired not demented; DWML = deep white matter lesions; MMSE = mini mental state examination; MTA = medial temporal lobe atrophy;

Atrophy	Atrophy assessment	Results	Significant relation	Adjustments
Cortical gray matter	Volumetric	WML related to cortical gray matter atrophy in all subjects	Yes	Age, sex, group effect and lacunes
Cortical atrophy	Ordinal (0-3)	No difference in cortical atrophy between groups of patients with different WML grade	No	No
Cortical atrophy	Ordinal (0-100)	WML correlated with cortical atrophy	Yes	No
Hippocampus	Volumetric	WML volume did not correlate with hippocampal volume	No	No
Hippocampus	Volumetric	WML volume was associated with hippocampal volume	Yes	Volume of lacunes
Hippocampus	Volumetric	WML did not correlate with hippocampal volume	No No	Age
MTA	Ordinal (0-4)	WML in the frontal and parieto-occipital regions are related to more hippocampal atrophy	Yes No Yes No	Age, sex, MMSE, hypertension and cortical atrophy
Entorhinal cortex Hippocampus	Volumetric	WML are not related to hippocampal atrophy and EC atrophy in all subjects	No No	Age, sex, group effect and lacunes
MTA	Ordinal (0-4)	More MTA in patients with WML compared to patients without WML	Yes	Age
MTA	Volumetric	There was a significant correlation between medial temporal lobe volume and WML volume	Yes	No
MTA	Ordinal (0-4)	MTA was weakly associated with WML	Yes	Age and sex
MTA	Ordinal	Age and MTA were independently associated with PVWML	Yes No	Age, gender, vascular risk factors (unspecified) and educational level
MTA	Ordinal	WML correlated with MTA	Yes	No

parieto-occ. WML = Parieto-occipital WML; PVWML = periventricular white matter lesions; SD = standard deviation; WML = white matter lesions

Table 2 Continued II.

First author, Year of publication	Study population	N	Mean age ± SD (Range)	WML	WML assessment
Staekenborg, 2007 [57]	AD	111	70 ± 9	Total WML	Ordinal (0-3)
Longitudinal studies – Measures of regional brain atrophy					
de Leeuw, 2006 [65]	AD	35	66 ± 9	PVWML DWML	Ordinal (0-1)
van de Pol, 2007 [64]	Participants of a randomized, double-blind, placebo-controlled trial of galantamine in MCI	323	69 ± 9	Total WML	Ordinal (0-30)

AD = Alzheimer's disease; CIND = cognitively impaired not demented; DWML = deep white matter lesions; MMSE = mini mental state examination; MTA = medial temporal lobe atrophy;

Table 3a Studies investigating the relation between white matter lesions and brain atrophy in subjects with vascular risk factors

First author, Year of publication	Study population	N	Mean age ± SD (Range)	WML	WML assessment
Cross-sectional studies – Measures of global brain atrophy					
Meguro, 1992 [69]	Patients with cerebrovascular risk factors without neurological abnormalities	52	72	PVWML	Volumetric
Meguro, 1993 [70]	Patients with cerebrovascular risk factors without neurological abnormalities	52	(59 – 81)	PVWML	Volumetric
Wiseman, 2004 [67]	Hypertensive subjects normotensive subjects	103 51	77 ± 4	PVWML Basal ganglia WML Total WML	Ordinal (0-2) Ordinal (0-30) Ordinal (0-54)
Cross-sectional studies – Measures of regional brain atrophy					
Wiseman, 2004 [67]	Hypertensive subjects normotensive subjects	103 51	77 ± 4	PVWML Basal ganglia WML Total WML	Ordinal (0-2) Ordinal (0-30) Ordinal (0-54)
Longitudinal studies – Measures of global brain atrophy					
Firbank, 2007 [71]	Hypertensive elderly normotensive elderly	68 27	77 ± 4 75 ± 2	Total WML	Volumetric

DWML = deep white matter lesions; MTA = medial temporal lobe atrophy; PVWML = periventricular white matter lesions; SD = standard deviation; WML = white matter lesions

Atrophy	Atrophy assessment	Results	Significant relation	Adjustments
MTA	Ordinal (0-4)	Presence of MTA was associated with presence of WML	Yes	No
MTA	Ordinal (0-4)	Presence and progression of PVWML are associated with progression of MTA	Yes No	Age, sex, duration of follow up, and blood pressure
Hippocampus	Volumetric	Baseline WML were not associated with rate of hippocampal atrophy	No	Age and sex

parieto-occ. WML = Parieto-occipital WML; PVWML = periventricular white matter lesions; SD = standard deviation; WML = white matter lesions

Atrophy	Atrophy assessment	Results	Significant relation	Adjustments
Global brain atrophy Ventricular enlargement	Volumetric	WML related to global brain atrophy	Yes Yes	Age and aortic arch calcification
Global brain atrophy Sulcal enlargement Ventricular enlargement	Volumetric	Correlation between WML and brain atrophy and sulcal and ventricular enlargement	Yes Yes Yes	No
Whole brain	Volumetric	No correlation between WML and total brain volume	No No No	No
Hippocampus	Volumetric	No correlation between WML and hippocampal volume	No No No	No
Global brain atrophy	Volumetric	There was no association between atrophy rate and either degree of WML at baseline, or change in WML severity	No	Age, sex, smoking, glucose, cholesterol, baseline and change in WML and baseline and inter MRI blood pressure

Table 3b Studies investigating the relation between white matter lesions and brain atrophy in subjects with symptomatic vascular disease

First author, Year of publication	Study population	N	Mean age ± SD (Range)	WML	WML assessment
Cross-sectional studies – Measures of global brain atrophy					
Pohjasvaara, 2000 [66]	Post-stroke	337	70 ± 8	Mean WML score	Ordinal (0-3)
Grau-Olivares, 2007 [73]	Patients with lacunar stroke with and without MCI	40	71 ± 12	Total WML	Ordinal (0-30)
Cross-sectional studies – Measures of regional brain atrophy					
Pohjasvaara, 2000 [66]	Post-stroke	337	70 ± 8	Mean WML score	Ordinal (0-3)
Firbank, 2007 [68]	Stroke survivors without post-stroke dementia	79	80 ± 4	Temporal Parietal Frontal Occipital Total WML	Volumetric
Longitudinal studies – Measures of global brain atrophy					
Walters, 2003 [72]	Patients with a first TIA without cognitive impairment	60	72 ± 7	Total WML	Ordinal (0-60)
Firbank, 2007 [68]	Stroke survivors without post-stroke dementia	41	80 ± 4	Temporal Parietal Frontal Occipital Total WML	Volumetric

MTA = medial temporal lobe atrophy; SD = standard deviation; WML = white matter lesions

Atrophy	Atrophy assessment	Results	Significant relation	Adjustments
Cortical brain atrophy	Ordinal (0-3)	The mean WML score was higher in the patients having any moderate or severe cortical atrophy or any moderate or severe central atrophy	Yes	No
Central brain atrophy			Yes	
Gray matter atrophy	Volumetric	In patients with MCI, total WML were correlated with frontal and temporal gray matter atrophy. These correlations were not found in patients without MCI	Yes	No
MTA	Ordinal (0-3)	The mean WML score was higher in the patients having any moderate or severe medial temporal lobe atrophy	Yes	No
MTA	Ordinal (0-4)	MTA was correlated with both total and temporal WML	Yes	No
			No	
			No	
			No	
			Yes	
Total brain volume	Volumetric	Baseline WML were associated with progression of total brain atrophy	Yes	Age, systolic and diastolic blood pressure
Ventricular enlargement	Volumetric	Total WML at baseline correlated with rate of ventricular enlargement	No	No
			No	
			No	
			No	
			Yes	

Although younger adults were included in 4 studies,[40,42,44,45] the mean age of the study populations was above 60 years in the remaining studies. The majority of studies used visual rating scales to quantify the amount of WML, while volumetric assessment of WML was used in 5 studies.[30,34,37,38,44] In contrast, brain atrophy was assessed with volumetric measurements in the majority of studies.[18,30-32,34,35,37,38,40,44,45]

Of the 13 cross-sectional studies that investigated global atrophy, 10 found a significant relation between more WML and more global brain atrophy.[9,13,33,36-39,43-45] This relation was also observed in the two longitudinal studies investigating global atrophy.[31,34] Of the 5 cross-sectional studies that examined regional brain atrophy 3 studies found that WML were associated with more medial temporal lobe atrophy and with smaller hippocampal and amygdalar volumes.[35,41,46] However, in the 2 other cross-sectional studies there was no association between WML and hippocampal atrophy,[32] or hippocampal and entorhinal volumes.[30] Also, WML volume at baseline was not associated with the atrophy rates of entorhinal cortex and hippocampus during follow-up.[30]

In 7 of the 20 general population studies no adjustments were made for age,[20,33,38,40,41,43,46] and shared risk factors were only considered in 4 of these studies.[9,34-36]

Subjects with cognitive impairment

Nineteen cross-sectional studies[29,43,47-63] and two longitudinal studies[64,65] were performed in patients with cognitive impairment. Characteristics and results of these studies are presented in **Table 2**. Study populations consisted of patients referred for evaluation of cognitive impairment or dementia[29,43,47,48,52-55,57,60,61] or of patients who were recruited at specialized dementia centers.[50,58,62,63,65] Two studies were performed in MCI patients participating in a clinical trial,[51,64] one study was part of a large genetic study in AD patients and their nondemented siblings,[56] and in one study participants were selected because they met the criteria for probable AD and did or did not show severe WML on MRI.[59] Finally, one study was performed in a heterogeneous group of patients with lacunes and a spectrum of cognitive impairment, in patients with probable AD without lacunes and a control group of individuals without cognitive impairment and without lacunes.[49] The size of the study populations also varied considerably. Three studies had more than 300 subjects,[51,56,64] while eleven studies had less than 100 subjects,[29,43,48,53,55,58-62,65] and 7 studies investigated between 100 and 200 subjects.[47,49,50,52,54,57,63] The mean age of the study populations in this category was high, varying between 65 years[48] and 82 years.[47]

Visual rating scales were used for the assessment of WML in 13 studies[29,43,47,51-54,56,57,59,63-65] and for the assessment of brain atrophy in 9 studies,[43,51-54,56,57,63,65] while volumetric assessment of WML was used in 8 studies[48-50,55,58,60-62] and of brain atrophy in 12 studies.[29,47-50,55,58-62,64]

In five studies[47,49,50,56,58] both global and regional brain atrophy were investigated, and therefore these studies are presented twice in **Table 2**. The relation between WML and global brain atrophy was assessed in 13 cross-sectional studies. In 9 of these, WML were associated with more global brain atrophy and with smaller cortical gray matter

volumes.[29,47-50,56,58,61,62] In the other 4 studies these relations were not found.[43,53,59,60] The relation between WML and measures of regional brain atrophy was investigated in 11 cross-sectional studies and in two longitudinal studies. A significant association between WML and atrophy of the hippocampus or medial temporal lobe was found in 7 cross-sectional studies,[50-52,54-57] although in one of these studies only periventricular WML, and not deep WML, were associated with medial temporal lobe atrophy.[52] In another cross-sectional study[63] a relation was found between WML in the frontal and parieto-occipital regions and more hippocampal atrophy. In three cross-sectional studies[47,49,58] no relation was found between total WML volume or WML ratings and volumes of the hippocampus or entorhinal cortex. In one longitudinal study presence and progression of periventricular WML, but not deep WML, were associated with progression of medial temporal lobe atrophy.[65] In the other longitudinal study, total WML volume at baseline was not associated with rate of hippocampal atrophy.[64]
In ten studies no adjustments were made for potential shared risk factors in the relation between WML and atrophy.[43,48-50,53,55-57,59,62] Although adjustments were made for age or sex in the remaining 11 studies, only three considered hypertension or blood pressure as a possible shared risk factor.[61,63,65] In one study[52] adjustments were made for vascular risk factors, but these were not specified.

Patients with vascular risk factors or symptomatic vascular disease

The characteristics and results of the 4 studies performed in patients with vascular risk factors are presented in **Table 3a** and the characteristics and results of the 4 studies performed in patients with symptomatic cerebrovascular disease are given in **Table 3b**. Of these 8 studies, 5 examined the cross-sectional and 3 the longitudinal relation between WML and atrophy. Two cross-sectional studies[66,67] examined global as well as regional atrophy. Furthermore, one study[68] reported the cross-sectional relation between WML and medial temporal lobe atrophy and the longitudinal relation between WML and ventricular enlargement.
The four studies that investigated a population with vascular risk factors recruited their patients from outpatient clinics[67,69,70] or from a trial on antihypertensive drugs[71] The studies that investigated patients with manifest cerebrovascular disease included stroke-survivors 3 months after discharge,[66,68] patients with a transient ischemic attack,[72] and patients with a lacunar stroke.[73] The mean age of all study populations was above 70 years. WML were measured volumetrically in four studies[68-71] and with a rating scale in the other four.[66,67,72,73] In one study[66] global and regional brain atrophy were rated visually, while in six other studies[67,69-73] brain atrophy was measured volumetrically. In one study[68] medial temporal lobe atrophy was assessed with a rating scale, while ventricular enlargement was assessed quantitatively.
In two cross-sectional studies, performed patients with vascular risk factors, associations were found between larger volumes of periventricular WML and more global brain atrophy and both sulcal and ventricular enlargement.[69,70] However, in two other cross-sectional studies in hypertensive patients and normotensive controls no associations between WML and total brain volume or hippocampal volume were

found.[67] In a longitudinal study in hypertensive and normotensive elderly there was no association between global atrophy rate and degree of WML at baseline, or change in WML severity during follow-up.[71]
In the patients with stroke or TIA, cross-sectional associations were found between more WML and more cortical, central and medial temporal lobe atrophy.[66,68,72,73] The relation between WML and ventricular enlargement was also found in the longitudinal study.[68]
In three studies[69,71,72] the analyses were adjusted for age. In one of these studies[72] adjustments were made for systolic and diastolic blood pressure, while in another study[71] adjustments were made for other vascular risk factors as well. In the remaining 5 studies, no adjustments were made for age, sex or other possible shared vascular risk factors.

Discussion

We included 48 articles that investigated the relation between white matter lesions and measures of global or regional brain atrophy. A significant relation between WML and global brain atrophy was found in 12 out of 15 general population studies, 9 out of 13 studies in patients with cognitive impairment and in 6 out of 8 studies performed in patients with vascular risk factors or symptomatic cerebrovascular disease. A significant relation between WML and medial temporal lobe atrophy including atrophy of the hippocampus, amygdala and entorhinal cortex, was found in 3 out of 6 general population studies, 9 out of 13 studies in patients with cognitive impairment and in 2 out of 3 studies performed in patients with vascular risk factors or symptomatic cerebrovascular disease.
The clinical observation that WML and brain atrophy are often observed simultaneously on MRI is thus confirmed by the majority of the retrieved articles. However, most of the studies did not adjust for possible shared risk factors. Moreover, in 22 of the 48 studies no adjustments were made for age. From these studies, it is not possible to conclude that the relation between WML and brain atrophy is independent of shared risk factors. However, when we look at the studies that did adjust for age and other risk factors 9 out of 10 studies found that WML were associated with measures of brain atrophy, providing at least some evidence that the relation is independent of shared risk.
To determine whether WML are a risk factor for development of brain atrophy, prospective studies are needed. Eight studies examined the longitudinal relation between WML and atrophy. Of these, one study did not adjust for age or other possible shared risk factors, 3 adjusted for age or age and sex, and 4 studies also adjusted for a small number of shared risk factors. Of the 7 longitudinal studies that adjusted for age or other factors, 4 found that WML were significantly associated with progression of brain atrophy, whereas 3 did not find an association. Thus, from the existing longitudinal studies we can not conclude that WML are a risk factor for brain atrophy.

An interesting pattern was observed when we compared the results of studies investigating global atrophy with the results of studies investigating regional brain atrophy. The majority of studies found an association between WML and global brain atrophy, which remained in the majority of the studies after adjustment for age and vascular risk factors. This association was also present in the majority of the longitudinal studies. The studies that did not find an association between WML and global atrophy generally had smaller sample sizes and used less accurate rating scales, which may have resulted in a low power to detect a significant relationship. In comparison, the results from the studies investigating the association between WML and measures of regional brain atrophy were less consistent. Interestingly, from the 12 studies that adjusted for age or other factors, all five studies that used the MTA score found a significant association with WML, while the seven studies that used volumetric measurements of the hippocampus did not, except for one study, find an association with WML. Since the MTA score not only visually assesses volume of the medial temporal lobe, but also volume of the temporal horn of the lateral ventricle and the choroid fissure, it is plausible that concomitant ventricular enlargement accounts for the observed association between WML and MTA score. Support for this explanation comes from a study that found that the MTA score correlated better with the volume of the lateral ventricle than with the volume of the medial temporal lobe itself.[74]

Although we can not conclude from the existing evidence that WML is a causal risk factor for brain atrophy, several mechanisms could account for such a causal link. The association between WML and subcortical brain atrophy may be explained by loss of myelin, axons, and oligodendrocytes and other glial cells in the subcortical white matter as a direct result of ischemic damage due to the underlying small-vessel disease.[28,75] In contrast, the association of WML with cortical gray matter atrophy can not be explained by direct tissue loss, as WML are not located in the cortex. Possibly, ischemic damage to the axons in the subcortical white matter leads to deafferentation of cortical–subcortical connections resulting in cortical neurodegeneration.[58] Similar mechanisms may also explain the relation between WML and atrophy of the hippocampus and amygdala, and consequently the medial temporal lobe.[35,63] Ischemia due to the cerebral small-vessel disease may damage the axons in the white matter, which eventually may lead to shrinkage of the hippocampus as a result of Wallerian degeneration.[76] A second explanation is that an impaired autoregulation due to the microangiopathy in combination with the luminal narrowing reduces the cerebral blood flow.[77] Since the hippocampus and amygdala are especially sensitive to hypoxia and ischemia,[78,79] this ischemia may eventually induce loss of the neurons in these brain structures.[80]

Given the biological plausibility, more research is warranted. Future studies should preferably assess WML as well as brain atrophy volumetrically. Visual rating scales are impeded by floor and ceiling effects and their reliability is often limited.[81] Volumetric measurements offer a more reliable, sensitive, and also objective alternative to visual rating scales. In addition, quantitative measurements of WML and brain volumes should be normalized for head size.[82] This facilitates comparison between studies and also helps in overcoming the difficulty of interpreting the strength of the relation. Analyses

on the association between WML and atrophy should at least take the effects of age and sex into account, but should also address shared vascular risk factors. Another factor that should be considered is the presence or number of lacunar infarcts. Lacunar infarcts are also considered to be caused by cerebral small-vessel disease and are also often found on MRI in the elderly.[83,84] In patients with Alzheimer's disease and mixed dementia lacunar infarcts may be associated with subcortical brain atrophy.[58,85] Furthermore, in studies that examine the association between WML and atrophy of the medial temporal lobe or structures herein, global brain atrophy should be taken into account to assess whether an association is explained by global brain atrophy. Finally, to properly address the issue of the directionality of the association between WML and brain atrophy, future studies should have a longitudinal design.

In conclusion, the majority of studies found an association between WML and global brain atrophy, but it is yet uncertain if this association is independent of shared risk factors. Further studies on the relation between WML and brain atrophy are therefore warranted and should make proper adjustments in the analysis and preferably be longitudinal in design, and use volumetric assessment of WML and brain atrophy.

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3.2

White matter lesions lacunar infarcts and brain atrophy

Objective

To investigate the independent association of white matter lesions (WML) and lacunar infarcts (LI) with brain atrophy on MRI.

Methods

Within the SMART-MR study, a cohort study among patients with manifest arterial disease, cross-sectional analyses were performed in 840 patients (mean age 58±10 years, 80% male) without cortical, large subcortical or infratentorial infarcts. Brain segmentation was used to quantify volumes of brain tissue, cerebrospinal fluid and WML. Total brain volume, ventricular volume and cortical gray matter volume were divided by intracranial volume to obtain brain parenchymal fraction (BPF), ventricular fraction (VF) and cortical gray matter fraction (GMF). Location and number of infarcts were rated visually.

Results

Mean ± SD BPF was 79.3 ± 2.8%, mean ± SD VF was 2.01 ± 0.95%, and mean ± SD GMF was 36.6 ± 3.3%. Linear regression analyses, adjusted for age, sex, vascular risk factors, intima-media thickness and LI showed that in patients with moderate to severe WML (upper quartile) BPF was lower (-0.51%; 95%CI -0.93 to -0.08), VF was higher (0.48%; 95%CI 0.31 to 0.65) and GMF was lower (-1.48%; 95%CI -2.07 to -0.88) than in patients with few WML (lower quartile). Presence of LI was associated with lower BPF (-0.52%; 95%CI -0.96 to -0.07) and higher VF (0.25%; 95%CI 0.07 to 0.42), but not with GMF, independent of WML and other potential confounders.

Conclusions

WML are associated with global, subcortical and cortical brain atrophy, whereas LI are associated with global and subcortical atrophy, but not with cortical atrophy, suggesting an independent role for WML and LI in the pathogenesis of brain atrophy.

Introduction

Brain atrophy, characterized by widening of the sulci, narrowing of the gyri, as well as enlargement of the ventricles, is a common finding on MRI in the elderly.[1] This is of clinical importance, since the extent and rate of progression of brain atrophy and ventricular enlargement are associated with cognitive deterioration and conversion to Alzheimer's disease.[2,3] Identification of factors that contribute to increased brain atrophy is therefore important. Several vascular risk factors, including hypertension,[4,5] hyperlipidemia,[4] diabetes mellitus,[6] obesity,[7] alcohol consumption,[8,9] and cigarette smoking[4,6] are associated with brain atrophy, suggestive of an important role of vascular disease in the pathogenesis of brain atrophy.

White matter lesions (WML) and lacunar infarcts (LI), considered the result of cerebral small-vessel disease, are also often found on MRI in the elderly.[10,11] They too are associated with vascular risk factors[11-13] and impaired cognitive functioning. WML and LI are the primary type of brain lesions in subcortical vascular dementia[14] and are associated with cognitive decline in non-demented elderly.[11,15]

It has been hypothesized that cerebral small-vessel disease plays a role in the pathogenesis of brain atrophy. Several studies observed associations of WML and LI with measures of brain atrophy on MRI.[6,16,17] However, since few studies adjusted for shared vascular risk factors, an association between cerebral small-vessel disease and brain atrophy does not necessarily imply a direct relation. Furthermore, as WML and LI are often not studied simultaneously, it remains unclear to what extent they are independently associated with brain atrophy. Finally, since WML and LI differ with respect to their cerebral localization[18] and their affect on cognitive performance,[15] their relation with brain atrophy may also differ.

The purpose of the present study was to investigate if WML and LI were independently associated with global, cortical and subcortical brain atrophy, independent of shared vascular risk factors.

Methods

Study population

The present study is a cross-sectional study within the SMART-MR study, a prospective cohort study within the Second Manifestations of ARTerial disease (SMART) study.[19] All eligible patients, newly referred to the University Medical Center Utrecht with symptomatic atherosclerotic disease or risk factors for atherosclerosis, are screened for additional risk factors and severity of atherosclerosis. The baseline examination is performed during a one day visit to our medical center and includes a physical examination, ultrasonography of the carotid arteries, and blood and urine sampling. Risk factors, medical history, and functioning are assessed using questionnaires that the patients fill in before their visit to the medical center.

Between May 2001 and December 2005, an MR investigation of the brain was added to the baseline examination as part of the SMART-MR study.[20] The objective of the

SMART-MR study is to investigate causes and consequences of brain changes on MRI in patients with vascular disease. Patients were eligible for an MRI of the brain if they were included with manifest coronary artery disease, cerebrovascular disease, peripheral arterial disease or an abdominal aortic aneurysm (AAA) and if they had no MR contraindications. Coronary artery disease was defined as myocardial infarction, coronary artery bypass graft surgery or percutaneous transluminal coronary angioplasty in the past or at inclusion. Patients with a transient ischemic attack (TIA) or stroke at inclusion and patients who reported stroke in the past were considered to have cerebrovascular disease. Peripheral vascular disease was defined as surgery or angioplasty of the arteries supplying the lower extremities in history or intermittent claudication or rest pain at inclusion. Present AAA (distal aortic anteroposterior diameter ≥3 cm) or previous AAA surgery was the criterion for AAA. Coronary artery disease was present in 59%, cerebrovascular disease in 23%, peripheral arterial disease in 22% and an AAA in 9% of these patients. The cumulative percentage exceeds 100% because patients can have symptomatic vascular disease at more than one location. The SMART study and SMART-MR study were approved by the ethics committee of our institution and written informed consent was obtained from all participants.

Magnetic resonance protocol

The MR investigations were performed on a 1.5-T whole-body system (Gyroscan ACS-NT, Philips Medical Systems, Best, the Netherlands). The protocol consisted of a transversal T1-weighted gradient-echo sequence (repetition time (TR)/echo time (TE): 235/2 ms; flip angle, 80°), a transversal T2-weighted turbo spin-echo sequence (TR/TE: 2200/11 ms and 2200/100 ms; turbo factor 12), a transversal T2-weighted fluid attenuating inverse recovery (FLAIR) sequence (TR/TE/inversion time (TI): 6000/100/2000 ms) and a transversal inversion recovery (IR) sequence (TR/TE/TI: 2900/22/410 ms) (field of view (FOV) 230 x 230 mm; matrix size, 180 × 256; slice thickness, 4.0 mm; slice gap, 0.0 mm; 38 slices).

Brain segmentation

We used the T1-weighted gradient-echo and IR sequence and the FLAIR sequence for brain segmentation. The probabilistic segmentation technique has been described elsewhere.[21] In short, two preprocessing steps were performed. The first step was an intra-patient rigid registration in order to compensate for motion and scan variations.[22] The second preprocessing step was an automatic skull-stripping of the T1 image,[23] in order to define a proper region of interest for the segmentation process. The actual segmentation of the MR-images was done with a statistical method called k-Nearest Neighbor (KNN) classification.[21] The result of the classification method is a probability value for each voxel that quantifies the amount of a specific tissue type contained in that voxel. Total volumes were calculated by multiplying these probabilities by the number and volumes of the voxels (4.0 x 0.9 x 0.9 mm). The segmentation program distinguishes cortical gray matter, white matter, sulcal and ventricular cerebrospinal fluid (CSF) and lesions. Subcortical gray matter was not segmented separately, but was included in the white matter volume.

Both WML and infarcts are classified as 'lesion' volume, since the segmentation program cannot distinguish between them. Therefore, the results of the segmentation analysis were visually checked and a further distinction was made into WML and infarct volumes by manually assigning the lesion volumes to one of these two categories.
Total brain volume was calculated by summing the volumes of gray and white matter and, if present, the volumes of WML or infarcts. Total intracranial volume (ICV) was calculated by summing the total brain volume and the volumes of the sulcal and ventricular CSF. To avoid incorporation of neuronal tissue and CSF outside the cranium, an investigator (APAA) determined the slice that included the foramen magnum. As a result, the total brain volume includes the cerebrum, brainstem and cerebellum.

Brain atrophy

Brain parenchymal fraction (BPF), an indicator for global brain atrophy, represents the percentage of the ICV that is occupied by brain tissue.[24] Ventricular enlargement, an indicator for subcortical brain atrophy, was assessed with the ventricular fraction (VF) and was calculated as the percentage ventricular volume of the total ICV. Cortical atrophy was assessed with the cortical gray matter fraction (GMF) and was calculated as the percentage cortical gray matter volume of the total ICV. Lower BPF indicates more global brain atrophy, higher VF indicates more subcortical brain atrophy and lower GMF indicates more cortical brain atrophy.

White matter lesions

The volumes of WML obtained with the segmentation program were summed to obtain the total volume of WML. We made no distinction between deep and periventricular WML, since deep, periventricular and total WML are highly correlated with each other,[25] and it was suggested that categorical distinctions between periventricular and deep WML are arbitrary.[25] According to current guidelines,[24] WML volumes were normalized for intracranial volume to correct for differences in head size.

Infarcts

The whole brain, including cortex, brainstem, and cerebellum, was visually searched for infarcts by two investigators and a neuroradiologist. Discrepancies in rating were re-evaluated in a consensus meeting. All raters were blinded for the history and diagnosis of the patient. Infarcts were defined as focal hyperintensities on T2-weighted images of at least 3 mm in diameter. Hyperintensities located in the white matter also had to be hypointense on T1-weighted images, in order to distinguish them from WML. Dilated perivascular spaces were distinguished from infarcts on the basis of their location (along perforating or medullary arteries, often symmetrical bilaterally, usually in the lower third of the basal ganglia or in the centrum semiovale), form (round/oval), and the absence of gliosis.[26] The location, affected flow territory and type were scored for every infarct. We defined LI as infarcts sized 3 to 15 mm and located in the subcortical white matter, thalamus or basal ganglia.

Vascular risk factors

The subjects' height and weight were measured, and the body mass index (BMI) was calculated (kg/m^2). Systolic and diastolic blood pressures (mmHg) were measured twice with a sphygmomanometer and the means were calculated. A fasting venous blood sample was taken to determine glucose and lipid levels. Diabetes mellitus was defined as glucose ≥7.0 mmol/L or use of oral antidiabetic drugs or insulin. Hyperlipidemia was defined as total cholesterol >5.0 mmol/L, low-density lipoprotein cholesterol >3.2 mmol/L or use of lipid lowering drugs. Smoking was assessed with the use of pack-years. To calculate pack-years of smoking, the average number of cigarettes smoked per day was divided by 20 and then multiplied by the number of years of cigarette smoking. Alcohol consumption was divided into three categories: never, past, and current. Patients who had quit drinking during the past year were assigned to the category current alcohol intake. Ultrasonography was performed to measure the intima-media thickness (IMT) (mm) in the left and right common carotid arteries, represented by the mean value of six measurements.[27]

Study sample

A total of 1309 patients were investigated in the SMART-MR study. Segmentation data were missing in 265 patients (missing MR sequences needed for the segmentation analyses, due to a temporary change in MR protocol (n=192), MR data irretrievable (n=19), missing FLAIR sequence (n=14), motion or other artifacts (n=40)). There were no differences in age, sex and vascular risk factors between patients with and without segmentation results.
For the purpose of the present study we excluded 170 patients with one or more cortical, large subcortical or infratentorial infarcts, because they are considered the result of large-vessel disease and the brain volume decrease in these patients is the direct effect of tissue loss in the infarcted region. In 34 patients not all cardiovascular risk factors were assessed. Consequently, the analyses were performed in 840 patients.

Statistical analysis

White matter lesions

The association of WML with measures of brain atrophy was assessed with analysis of covariance (ANCOVA) and linear regression analyses. First, we calculated mean measures of brain atrophy adjusted for age and sex for quartiles of normalized WML. Second, we performed stepwise linear regression analyses to investigate to what extent the relation between WML and measures of brain atrophy was explained by vascular risk factors, extent of subclinical atherosclerosis and LI. In the first model, we adjusted for age and sex. In the second model, we also adjusted for systolic and diastolic blood pressure, diabetes mellitus, hyperlipidemia, smoking, alcohol use, BMI and IMT. In the third model, we additionally adjusted for presence of LI to investigate if the relation between WML and brain atrophy was independent of presence of LI.

Lacunar infarcts

The association of LI with measures of brain atrophy was assessed with ANCOVA and linear regression analyses. First, we investigated the association between the presence of LI and measures of brain atrophy. The regression analyses with LI as independent variable and measures of brain atrophy as dependent variable were performed in three steps. In the first model, we adjusted for age and sex. In the second model, we also adjusted for systolic and diastolic blood pressure, diabetes mellitus, hyperlipidemia, smoking, alcohol use, BMI and IMT. In the third model we also adjusted for WML.
Second, we investigated whether a dose-response relation existed for the association of LI with atrophy. For this purpose we categorized patients in 3 groups: no LI (n=740), one LI (n=57), and two or more LI (n=43). For every patient category we calculated the mean measures of brain atrophy, adjusted for age, sex, vascular risk factors, IMT and WML. In all analyses the 95% confidence intervals are given. SPSS version 14.0 (Chicago, Ill, USA) was used to analyze our data.

Results

In the total study sample (n=840), the mean BPF was 79.3 ± 2.8%, the mean VF was 2.01 ± 0.95%, and the mean GMF was 36.6 ± 3.3%. Patient characteristics according to WML volume, for patients with and without LI, and for the total study sample are given in **Table 1**. Patients with moderate to severe WML (upper quartile) were older and more frequently had diabetes mellitus. Furthermore, systolic blood pressure and IMT were higher in patients with moderate to severe WML. LI were more prevalent in patients with moderate to severe WML compared with patients with no or few WML (25% vs. 8%), and in patients with LI median WML volume was higher than in patients without LI (3.6 ml vs. 1.5 ml). Similar distribution of risk factors was found for patients with and without LI.

White matter lesions

The mean measures of brain atrophy adjusted for age and sex for every quartile of WML are given in **Figure 1**. Pairwise comparisons revealed no difference in BPF between the three lower quartiles of WML. However, subjects in the upper quartile of WML had lower BPF compared to the lowest (-0.74%; 95%CI; -1.17 to -0.31), second (-0.61%; 95%CI; -1.04 to -0.19) and third (-0.93%; 95%CI; -1.33 to -0.53) quartile of WML (test for linear trend; $P < 0.001$) (**Figure 1a**), indicating that subjects with moderate to severe WML had more global brain atrophy. Similar results were found for ventricular enlargement. The VF was higher in the upper quartile of WML compared to the lowest (0.56%; 95%CI; 0.40 to 0.73), second (0.67%; 95%CI; 0.51 to 0.84) and third quartile (0.55%; 95%CI; 0.39 to 0.71) (test for linear trend; $P < 0.001$) (**Figure 1b**). With regard to cortical gray matter atrophy, small amounts of WML were already associated with more atrophy. Compared to the lowest quartile of WML, GMF was lower in the second (-0.96%; 95%CI; -1.49 to -0.43),

Table 1 Patient characteristics according to white matter lesions volume, presence of lacunar infarcts and total study sample (n=840)

	White matter lesion volume		Lacunar infarcts		All patients
	Lowest three quartiles (n=630)	Upper quartile (n=210)	None (n=740)	One or more (n=100)	(n=840)
Age (years) *	55.8 ± 9.6	64.8 ± 8.6	57.3 ± 10.0	63.1 ± 10.1	58.0 ± 10.1
Male gender †	80	78	79	84	80
Smoking (pack-years) ‡	18 (6, 32)	18 (5, 35)	18 (6, 32)	20 (7, 35)	18 (6, 32)
Alcohol consumption †					
Never	14	21	16	13	16
Past	9	10	9	9	9
Current	77	69	75	78	75
Body mass index (kg/m^2) *	26.8 ± 3.8	26.7 ± 3.5	26.8 ± 3.7	26.2 ± 3.3	26.7 ± 3.7
Systolic blood pressure (mmHg) *	138 ± 20	147 ± 22	139 ± 20	149 ± 21	140 ± 20
Diastolic blood pressure (mmHg) *	81 ± 10	82 ± 11	81 ± 11	83 ± 10	81 ± 11
Diabetes mellitus †	17	26	18	27	19
Hyperlipidemia †	81	76	80	80	80
Intima-media thickness (mm) *	0.88 ± 0.27	1.01 ± 0.28	0.89 ± 0.27	1.01 ± 0.27	0.91 ± 0.28
White matter lesions volume (ml) ‡	1.2 (0.8, 1.8)	5.6 (4.0, 8.7)	1.5 (0.9, 2.8)	3.6 (1.7, 8.7)	1.6 (0.9, 3.1)
One or more lacunar infarcts †	8	25	…	…	12

* Mean ± SD, † percentage, ‡ median (25th percentile, 75th percentile)

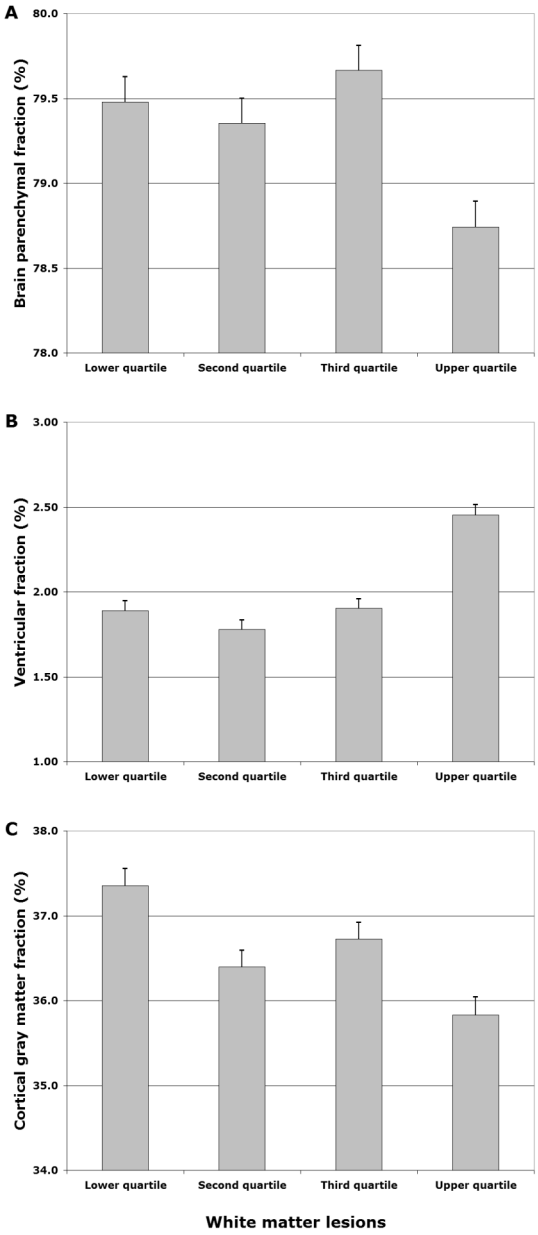

Figure 1 Mean measures of brain atrophy (SEs) for quartiles of WML, adjusted for age and sex. (Test for linear trend for BPF; $P < 0.001$, VF; $P < 0.001$, and GMF; $P < 0.001$) SE = standard error; WML = white matter lesions

third (-0.63%; 95%CI; -1.19 to -0.08) and upper (-1.53%; 95%CI; -2.12 to -0.93) quartile (test for linear trend; P < 0.001) (**Figure 1c**). Additional adjustment for vascular risk factors, IMT and LI attenuated the associations between WML and all measures of atrophy, but the relations remained statistically significant (**Table 2**).

Table 2 Results of linear regression analyses with quartiles of WML as independent variables and measures of brain atrophy as dependent variable (n=840)

		Regression coefficients (95%CI)		
		BPF	VF	GMF
Model I *	Lower quartile	(reference)	(reference)	(reference)
	Second quartile	-0.13% (-0.52 to 0.27)	-0.11% (-0.26 to 0.04)	-0.96% (-1.49 to -0.43)
	Third quartile	0.19% (-0.22 to 0.60)	0.01% (-0.15 to 0.17)	-0.63% (-1.19 to -0.08)
	Upper quartile	-0.74% (-1.17 to -0.31)	0.56% (0.40 to 0.73)	-1.53% (-2.12 to -0.93)
Model II †	Lower quartile	(reference)	(reference)	(reference)
	Second quartile	-0.13% (-0.51 to 0.25)	-0.12% (-0.27 to 0.04)	-0.97% (-1.49 to -0.45)
	Third quartile	0.30% (-0.10 to 0.70)	-0.01% (-0.17 to 0.15)	-0.54% (-1.09 to -0.01)
	Upper quartile	-0.60% (-1.03 to -0.18)	0.54% (0.37 to 0.71)	-1.47% (-2.05 to -0.88)
Model III ‡	Lower quartile	(reference)	(reference)	(reference)
	Second quartile	-0.12% (-0.50 to 0.26)	-0.12% (-0.28 to 0.03)	-0.97% (-1.49 to -0.46)
	Third quartile	0.33% (-0.06 to 0.72)	-0.02% (-0.18 to 0.13)	-0.54% (-1.09 to -0.00)
	Upper quartile	-0.51% (-0.93 to -0.08)	0.48% (0.31 to 0.65)	-1.48% (-2.07 to -0.88)

* Adjusted for age and sex
† Additionally adjusted for systolic and diastolic blood pressure, diabetes mellitus, hyperlipidemia, smoking, alcohol use, body mass index and intima-media thickness
‡ Additionally adjusted for presence of one or more lacunar infarcts
BPF = brain parenchymal fraction; CI = confidence interval; GMF = gray matter fraction; VF = ventricular fraction; WML = white matter lesions

Lacunar infarcts

The results of the linear regression analyses with LI as independent variable and measures of brain atrophy as dependent variables are given in **Table 3**. Compared to patients without LI, patients with one or more LI had a lower BPF and a higher VF after adjustment for age and sex, indicating more global and more subcortical brain atrophy. Additional adjustment for vascular risk factors and extent of subclinical atherosclerosis attenuated the association between LI and BPF slightly. Adding WML to the model attenuated the association further, but presence of LI remained significantly associated with more global and more subcortical atrophy. In contrast, there was no relation between LI and GMF.
Figure 2a shows the adjusted mean BPF according to number of LI. Subjects with an increase in number of LI had increasingly lower BPF (test for linear trend; P = 0.008),

Table 3 Results of linear regression analyses with one or more lacunar infarcts as independent variable and measures of brain atrophy as dependent variable (n=840)

	One ore more lacunar infarcts		
	Regression coefficients (95%CI)		
	BPF	VF	GMF
Model I *	-0.82% (-1.25 to -0.38)	0.48% (0.31 to 0.66)	-0.29% (-0.89 to 0.31)
Model II †	-0.69% (-1.11 to -0.26)	0.46% (0.29 to 0.64)	-0.18% (-0.77 to 0.41)
Model III ‡	-0.52% (-0.96 to -0.07)	0.25% (0.07 to 0.42)	0.13% (-0.49 to 0.74)

* Adjusted for age and sex
† Additionally adjusted for systolic and diastolic blood pressure, diabetes mellitus, hyperlipidemia, smoking, alcohol use, body mass index and intima-media thickness
‡ Additionally adjusted for normalized WML volume
BPF = brain parenchymal fraction; CI = confidence interval; GMF = gray matter fraction; VF = ventricular fraction; WML = white matter lesions

compared to patients without LI. BPF was lower in patients one LI (-0.18%; 95%CI; -0.72 to 0.36), and in patients with two or more LI (-0.41%; 95%CI; -0.96 to 0.14). **Figure 2b** gives the adjusted mean VF according to number of LI. Subjects with more LI had a higher VF (test for linear trend; P = 0.017). Compared to patients without LI, patients with one LI had a 0.30% (95%CI; 0.09 to 0.52) higher VF, and patients with two or more LI had a 0.16% (95%CI; -0.11 to 0.42) higher VF. **Figure 2c** gives the adjusted mean GMF according to number of LI. There was no relation between number of LI and GMF (test for linear trend; P = 0.26) and there were no significant differences in GMF between subjects with no, one, two or more LI.

Discussion

In a large population of patients with manifest arterial disease, WML were associated with more global, subcortical and cortical brain atrophy, independent of shared risk factors and presence of lacunar infarcts. Furthermore, lacunar infarcts were associated with more global and subcortical brain atrophy, independent of shared risk factors and volume of WML. Lacunar infarcts were not associated with cortical brain atrophy in this study.

Before we interpret our findings, strengths and limitations have to be discussed. The strengths of this study are the large number of patients investigated and the volumetric assessment of WML and measures of brain atrophy. This made it possible to obtain precise estimates of the relation between markers of cerebral small-vessel disease and brain atrophy. Second, since we assessed WML as well as LI in our study it was possible to investigate their independent relation with brain atrophy. Third, the segmentation of different brain tissue types and CSF spaces allowed us not only to investigate global brain atrophy, but also to differentiate between cortical and subcortical brain atrophy. Fourth, the extensive information available on several risk

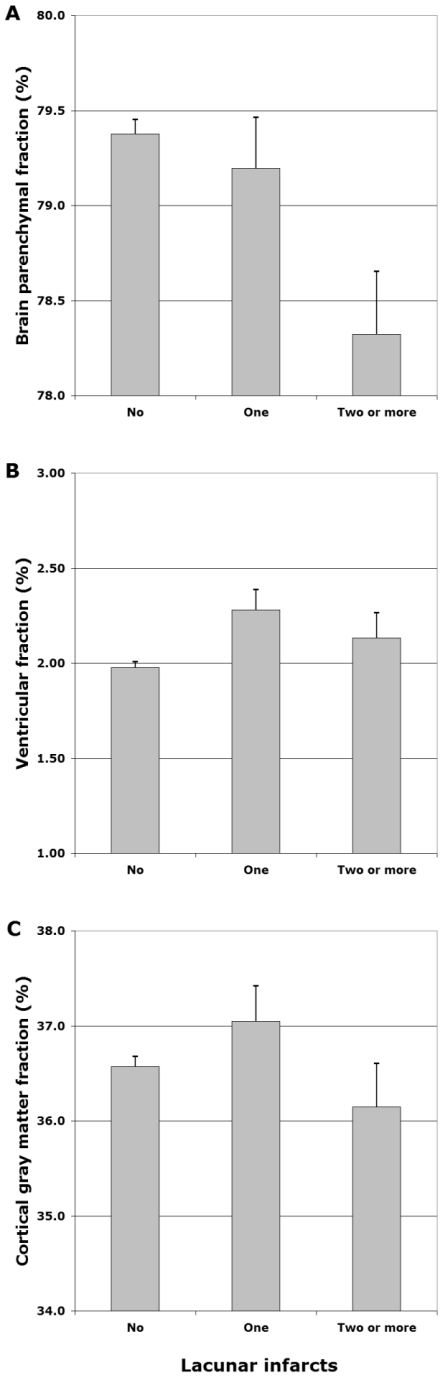

Figure 2 Adjusted mean measures of brain atrophy (SEs) for patients with no, one and two or more lacunar infarcts. Adjustments were made for age, sex, systolic and diastolic blood pressure, diabetes mellitus, hyperlipidemia, smoking, alcohol use, body mass index, intima-media thickness and WML volume. (Test for linear trend for BPF; $P < 0.008$, VF; $P < 0.017$, and GMF; $P = 0.28$)
SE = standard error; WML = white matter lesions

factors and IMT made it possible to adjust for potential shared risk factors and extent of subclinical atherosclerosis.

A limitation of our study is the cross-sectional design, which makes it not possible to discern cause from consequence. However, several lines of evidence suggest that it is more likely that cerebral small-vessel disease precedes the development of brain atrophy. First, cerebral white matter is more vulnerable to cerebral ischemia than gray matter.[28] Since WML are thought to be the result of ischemia,[28] they are more likely to evolve earlier than gray matter atrophy. Second, a prospective study found that in nondemented elderly extensive WML at baseline were associated with a greater loss of brain volume at follow-up.[29] Third, brain atrophy is commonly found in patients with CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy). Since microangiopathy is the primary mechanism in this inherited small-vessel disease,[30] the brain atrophy in these patients is most likely the result of the small-vessel disease.[31]

We observed that WML were related to a decrease in cortical gray matter volume as well as an increase in ventricular size. The association between WML and larger ventricular size can be explained by ex vacuo enlargement of the ventricles due to loss of subcortical white matter as a result of ischemic changes. However, the association with cortical gray matter atrophy cannot be explained by direct tissue loss, since WML are not located in the cortex, but may be explained by deafferentation of cortical–subcortical connections.

The presence of LI was associated with an increase in ventricular size, but not with a decrease in cortical gray matter atrophy. Similar results have been found by other investigators. In patients with Alzheimer disease, presence of LI has been associated with ventricular enlargement, but not with cortical atrophy.[17] The absence of a relation between LI and cortical brain atrophy has also been found in a group of patients with Alzheimer's disease and mixed dementia.[32] Since LI are located in subcortical gray and white matter and are caused by occlusion of arteriosclerotic vessels,[33] direct tissue loss in the infarcted subcortical region will result in subcortical brain atrophy. LI are less likely than WML to interrupt the cortical–subcortical connections in the subcortical white matter since the majority of LI are located in the subcortical gray matter nuclei,[11] and therefore LI may not result in cortical gray matter loss. Still, we cannot exclude the possibility that when multiple LI are present they may lead to cortical gray matter atrophy. In our population only 43 patients had 2 or more LI and it is possible that we were not able to find this association in this population.

In summary, in patients with arterial disease WML were associated with global brain atrophy, ventricular enlargement and cortical atrophy, independent of shared vascular risk factors. Lacunar infarcts were associated with global brain atrophy and ventricular enlargement, but not with cortical atrophy. Our findings suggest a differential role for WML and LI in the development of brain atrophy. Future studies should investigate whether these findings can be generalized to the general population and whether WML and LI are independent risk factors for brain atrophy in a longitudinal design.

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3.3

White matter lesions lacunar infarcts and cognitive functioning

Objective

To investigate the independent association of white matter lesions (WML) and lacunar infarcts (LI) with cognitive performance and to investigate whether brain atrophy mediates these associations.

Methods

Within the SMART-MR study, a cohort study among patients with manifest arterial disease, cross-sectional analyses were performed in 522 patients (mean age 57 ± 10 years, 76% male) without cortical, large subcortical, or infratentorial infarcts. Brain segmentation was used to quantify volumes of brain tissue, cerebrospinal fluid and WML. Infarcts were rated visually. Total brain volume, ventricular volume and cortical gray matter volume were divided by intracranial volume to obtain indicators of global, subcortical and cortical brain atrophy. WML volume was also normalized for intracranial volume. Neuropsychological tests assessing executive functioning (EXEC) and memory (MEM) were performed and raw scores were transformed into z-scores. We used linear regression analyses, adjusted for age, sex, education, intelligence, and vascular risk factors to investigate the association between WML, number of LI and cognitive performance.

Results

One standard deviation higher WML volume was associated with worse EXEC (β = -0.12; 95%CI -0.20 to -0.04), independent of LI and other covariates. Subcortical, but not global or cortical brain atrophy, attenuated this association (β for EXEC = -0.09; 95%CI -0.18 to 0.02). Presence of two or more LI was associated with worse EXEC (β = -0.48; 95%CI -0.87 to -0.09), independent of WML, other covariates and measures of brain atrophy. WML and LI were not associated with worse memory performance.

Conclusions

In patients with manifest arterial disease, WML and LI are independently associated with worse executive functioning. These associations remained essentially the same after adjustment for measures of brain atrophy, although subcortical brain atrophy partly explained the association between WML and executive performance.

Introduction

Vascular cognitive impairment is used to describe a range of cognitive disorders related to vascular disease.[1] A major subtype of vascular cognitive impairment is subcortical ischemic vascular disease. Subcortical ischemic vascular disease is characterized by white matter lesions (WML) and lacunar infarcts (LI) on MRI, and is associated with a specific cognitive profile involving preserved memory with impairments in attentional and executive functioning.[2]

Neurodegenerative processes, characterized on MRI as widening of the sulci, narrowing of the gyri, as well as enlargement of the ventricles, are also involved in the pathogenesis of cognitive deterioration. In non-demented persons, the extent and rate of progression of global, cortical and subcortical brain atrophy are associated with cognitive deterioration and conversion to Alzheimer's disease.[3,4]

Cognitive impairment due to neurodegeneration and due to vascular disease were traditionally regarded as separate clinical and pathophysiological entities. However, there is increasing evidence that there is an overlap in the pathways by which vascular pathology and neurodegenerative processes contribute to cognitive decline.[5] Several studies observed associations between WML and progression of brain atrophy,[6-8] suggesting that cerebral small-vessel disease may lead to cognitive impairment through brain atrophy.

The objective of our study was to investigate whether WML and LI were associated with executive functioning and memory performance, independent of brain atrophy, in patients with manifest arterial disease. Furthermore, we investigated whether global, cortical or subcortical brain atrophy explained these associations.

Methods

SMART-MR Study

The present study is a cross-sectional study within the SMART-MR study, a prospective cohort study within the Second Manifestations of ARTerial disease (SMART) study.[9] All eligible patients, newly referred to the University Medical Center Utrecht with symptomatic atherosclerotic disease or risk factors for atherosclerosis, are screened for additional risk factors and severity of atherosclerosis. The baseline examination is performed during a one day visit to our medical center and includes a physical examination, ultrasonography of the carotid arteries, and blood and urine sampling. Risk factors, medical history, and functioning are assessed using questionnaires that the patients fill in before their visit to the medical center.

Between May 2001 and December 2005, an MR investigation of the brain was added to the baseline examination as part of the SMART-MR study.[10] The objective of the SMART-MR study is to investigate causes and consequences of brain changes on MRI in patients with vascular disease. Patients were eligible for an MRI of the brain if they were included with manifest coronary artery disease, cerebrovascular disease, peripheral arterial disease or an abdominal aortic aneurysm (AAA) and if they

had no MR contraindications. Coronary artery disease was defined as myocardial infarction, coronary artery bypass graft surgery or percutaneous transluminal coronary angioplasty in the past or at inclusion. Patients with a transient ischemic attack (TIA) or stroke at inclusion and patients who reported stroke in the past were considered to have cerebrovascular disease. Peripheral vascular disease was defined as surgery or angioplasty of the arteries supplying the lower extremities in history or intermittent claudication or rest pain at inclusion. Present AAA (distal aortic anteroposterior diameter ≥3 cm) or previous AAA surgery was the criterion for AAA. Neuropsychological testing was introduced in the SMART-MR study starting January 2003, and was performed on the same day as the MR and other investigations. The SMART study and SMART-MR study were approved by the ethics committee of our institution and written informed consent was obtained from all participants.

Magnetic resonance protocol

The MR investigations were performed on a 1.5-T whole-body system (Gyroscan ACS-NT, Philips Medical Systems, Best, the Netherlands). The protocol consisted of a transversal T1-weighted gradient-echo sequence (repetition time (TR)/echo time (TE): 235/2 ms; flip angle, 80°), a transversal T2-weighted turbo spin-echo sequence (TR/TE: 2200/11 ms and 2200/100 ms; turbo factor 12), a transversal T2-weighted fluid attenuating inverse recovery (FLAIR) sequence (TR/TE/inversion time (TI): 6000/100/2000 ms) and a transversal inversion recovery (IR) sequence (TR/TE/TI: 2900/22/410 ms) (field of view (FOV) 230 x 230 mm; matrix size, 180 × 256; slice thickness, 4.0 mm; slice gap, 0.0 mm; 38 slices).

Brain segmentation

We used the T1-weighted gradient-echo, IR sequence and FLAIR sequence for brain segmentation. The probabilistic segmentation technique has been described elsewhere.[11] Two preprocessing steps were performed. The first step was an intra-patient rigid registration in order to compensate for motion and scan variations.[12] The second preprocessing step was an automatic skull-stripping of the T1 image,[13] in order to define a proper region of interest for the segmentation process. The actual segmentation of the MR-images was performed by k-Nearest Neighbor (KNN) classification.[11] The result of the classification method is a probability value for each voxel that quantifies the amount of a specific tissue type contained in that voxel. The segmentation program distinguishes cortical gray matter, white matter, sulcal and ventricular cerebrospinal fluid (CSF) and lesions. Subcortical gray matter was not segmented separately, but was included in the white matter volume. Total volumes were calculated by multiplying these probabilities by the number and volumes of the voxels (4.0 x 0.9 x 0.9 mm).
WML and infarcts are classified as 'lesion' volume, since the segmentation program cannot distinguish between them. Therefore, the results of the segmentation analysis were visually checked and a further distinction was made into WML and infarct volumes by manually assigning the lesion volumes to one of these two categories.

Total brain volume was calculated by summing the volumes of gray and white matter and, if present, the volumes of WML and infarcts. Total intracranial volume (ICV) was calculated by summing the total brain volume and the volumes of the sulcal and ventricular CSF.
To avoid incorporation of neuronal tissue and CSF outside the cranium, an investigator determined the slice that included the foramen magnum. This transversal slice was defined as the lowest position in the brain at which voxels could contribute to the different segmentations. As a result, total brain volume includes the cerebrum, brainstem and cerebellum.

Brain atrophy

Brain parenchymal fraction (BPF), an indicator for global brain atrophy, represents the percentage of the ICV that is occupied by brain tissue. Ventricular enlargement, an indicator for subcortical brain atrophy, was assessed with the ventricular fraction (VF) and was calculated as the percentage ventricular volume of the total ICV. Cortical atrophy was assessed with the cortical gray matter fraction (GMF) and was calculated as the percentage cortical gray matter volume of the total ICV. Lower BPF indicates more global brain atrophy, higher VF indicates more subcortical brain atrophy and lower GMF indicates more cortical brain atrophy.

White matter lesions

The volumes of WML obtained with the segmentation program were summed to obtain the total volume of WML. We made no distinction between deep and periventricular WML, since it has been shown that deep, periventricular and total WML are highly correlated with each other, and it has been suggested that categorical distinctions between periventricular and deep WML are arbitrary.[14] We normalized WML volumes for intracranial volume to correct for differences in head size,[15] by dividing total WML volume by ICV and multiplying this by the average ICV of the study population (1458 ml).

Infarcts

The whole brain, including cortex, brainstem, and cerebellum, was visually searched for infarcts by two investigators and a neuroradiologist. Discrepancies in rating were re-evaluated in a consensus meeting. All raters were blinded for the history and diagnosis of the patient. Infarcts were defined as focal hyperintensities on T2-weighted images of at least 3 mm in diameter. Hyperintensities located in the white matter also had to be hypointense on T1-weighted images in order to distinguish them from WML. Dilated perivascular spaces were distinguished from infarcts on the basis of their location (along perforating or medullary arteries, often symmetrical bilaterally, usually in the lower third of the basal ganglia or in the centrum semiovale), form (round/oval), and the absence of gliosis.[16] The location, affected flow territory and type were scored for every infarct. We defined LI as infarcts sized 3 to 15 mm and located in the subcortical white matter, thalamus or basal ganglia.

Neuropsychological assessment

Cognitive performance was assessed with a set of standard neuropsychological tests, sensitive to mild impairments. Verbal memory was assessed with the 15-word learning test (a modification of the Rey Auditory Verbal Learning test[17]). In 5 consecutive trials, an interviewer read out aloud a list of 15 words, after which the patients were asked to recall as many words as they could. The number of correctly recalled words for each trial is the immediate recall (range 0 to 15). After a period of approximately 25 minutes, during which other non-verbal tasks were performed, the patients were asked again to recall as many words as possible (delayed recall, range 0 to 15). Next, we calculated a retention score by dividing the number of words recalled after 25 minutes by the maximum number of words recalled during the immediate recall. A composite score for memory performance (MEM) was calculated by averaging the z-scores (individual test score minus mean test score divided by the standard deviation of that score) of the mean score of the 5 trials of the immediate recall, the z-score of the delayed recall, and the z-score of the retention score.

Executive functioning was assessed with the following three tests. The Visual Elevator test (subtest of the Test of Everyday Attention) is a timed test of 10 trials that measures mental flexibility.[18] Patients had to determine the floor on which a visually presented elevator was located. The elevator moves one floor each time and changes in direction when an arrow is presented. The timing score of the Visual Elevator test is equivalent to time per switch for correct items (seconds per switch). Second, the Brixton Spatial Anticipation test was used to assess the capacity to discover logical rules.[19] Patients were presented a booklet of 56 pages with on every page 10 circles of which one circle was filled. Patients had to predict which circle would be filled on the next page based on a pattern deduced from previous pages. In total this pattern changed eight times. The total number of errors made was scored. Third, the Verbal Fluency test was used to assess mental flexibility, shifting of attention, and employment of strategies.[20] For this test patients were asked to generate as many words as possible starting with the letter N within a one minute time-frame. Before calculating z-scores, the scores of the Visual Elevator test and Brixton Spatial Anticipation test were multiplied by minus one, so that on all tests a lower score denotes a worse performance. A composite score for executive functioning (EXEC) was estimated by averaging the z-scores of the Visual Elevator Test, the Brixton Spatial Anticipation test and the Verbal Fluency test.

Premorbid intellectual functioning was assessed using the Dutch version of the National Adult Reading Test (DART) in which patients had to read out loud a list of words with irregular pronunciation.[21] Educational level was divided into seven categories, graded from primary school to academic degree, according to the Dutch educational system.[18]

Vascular risk factors

During the patient's visit at the medical center, an overnight fasting venous blood sample was taken to determine glucose and lipid levels. Height and weight were measured

without shoes and heavy clothing, and the body mass index (BMI) was calculated (kg/m^2). Systolic and diastolic blood pressures (mmHg) were measured twice with a sphygmomanometer and the average was obtained. Hypertension was defined as mean systolic blood pressure ≥140 mmHg or mean diastolic blood pressure ≥95 mmHg or use of antihypertensive drugs. Diabetes mellitus was defined as glucose ≥7.0 mmol/L or use of oral antidiabetic drugs or insulin. Hyperlipidemia was defined as total cholesterol >5.0 mmol/L, low-density lipoprotein cholesterol >3.2 mmol/L or use of lipid lowering drugs. Smoking habits and alcohol intake were assessed using questionnaires. Pack-years of smoking was calculated by the average number of cigarettes smoked per day was divided by 20 and then multiplied by the number of years of cigarette smoking. Alcohol consumption was divided into three categories: never, past, and current. Patients who had quit drinking during the past year were assigned to the category current alcohol intake. Ultrasonography was performed to measure the intima-media thickness (IMT) (mm) in the left and right common carotid arteries, represented by the mean value of six measurements.[22] IMT is a marker for the extent of subclinical atherosclerosis.

Study sample

A total of 1309 patients were investigated in the SMART-MR study. Since neuropsychological testing was not introduced until 2003, data on cognitive performance was present of 831 patients. Of these 831 patients, segmentation data was missing in 226 patients (missing MR sequences needed for the segmentation analyses, due to a temporary change in MR protocol (n=179), MR data irretrievable (n=11), missing FLAIR sequence (n=8), motion or other artifacts (n=28)). Patients without available segmentation data were more often male (82% vs. 76%) and more often had diabetes (25% vs. 19%). Age and other vascular risk factors were comparable between patients with and without segmentation results.
For the purpose of the present study, we excluded 83 patients with cortical, large subcortical, or infratentorial infarcts on MRI from our study sample, because we were interested in the relation between small-vessel disease pathology on MRI and cognition. As a result the analyses were performed in 522 patients.

Statistical analysis

We used multiple linear regression analyses to investigate the associations between WML, LI and measures of cognitive function. Composite z-scores for EXEC and MEM were used as outcome variables. First, we investigated the association between WML, LI and cognitive performance. For this purpose normalized WML volume was entered in a first model, adjusted for age, sex, education and DART score. Since WML and LI are strongly associated with each other we included presence of LI as covariate in a second model to investigate if the relation between WML and cognitive performance could be explained by the concomitant presence of LI.
The association between LI and cognition was investigated similarly. For analyses, we categorized patients into three groups: patients with no LI (n=472), one LI (n=30), and two or more LI (n=20). In the first model we included one LI and two or more LI

as dummy variables and in the second model WML volume was entered as covariate. The regression coefficients of one LI and two or more lacunar LI represent the differences in cognitive performance compared to patients without LI.
Next, we investigated to what extent the associations of WML and LI with cognition could be explained by global, subcortical or cortical brain atrophy. This was done by repeating the linear regression analyses with additional adjustment for BPF, VF or GMF. Third, we repeated all analyses with additional adjustments for hypertension, diabetes mellitus, hyperlipidemia, BMI, pack-years of smoking, alcohol use and IMT, to investigate whether the associations of WML and LI with cognitive performance were independent of vascular risk factors and extent of subclinical atherosclerosis. In all analyses the 95% confidence intervals are given. SPSS version 14.0 (Chicago, Ill, USA) was used to analyze our data.

Results

Table 1 gives the baseline characteristics of our study sample. The mean age (SD) was 57 (10) years and the majority (76%) were men. LI on MRI were present in 50 patients (10%), of whom 30 had one LI and 20 patients had two or more LI. **Table 2** shows the raw scores of the neuropsychological tests. **Table 3** shows the associations of normalized WML volume with EXEC and MEM. Linear regression analysis with adjustment for age, sex, educational level, and DART score showed that an increase of one SD (3.4 ml) in normalized WML volume was associated with worse performance on EXEC (β= -0.14; 95%CI -0.22 to -0.06). Additional adjustment for presence of LI attenuated the association with EXEC slightly, but remained statistically significant. WML were not significantly associated with MEM. **Table 4** shows the associations of LI with EXEC and MEM. Presence of a single LI was not associated with worse performance on EXEC, but two or more LI were associated with a lower score on EXEC (β= -0.61; 95%CI -0.99 to -0.23), after adjustment for age, sex, educational level, and DART score. This association was also attenuated after adjustment for WML. LI were not associated with a lower MEM score.
Next, we investigated whether the associations of WML and LI with executive functioning could be explained by global, cortical or subcortical brain atrophy. Additional adjustment for BPF or GMF did not change the association of WML with EXEC (**Table 5**). However, after adjustment for VF the association between WML and EXEC attenuated, but an increase in WML volume of one SD remained significantly associated with a lower score on EXEC (β= -0.09; 95%CI -0.18 to -0.01). The association between LI and EXEC did not materially change after adjustment for measures of brain atrophy (**Table 6**). Finally, additional adjustment for vascular risk factors and IMT did not materially change the associations between WML, LI and executive functioning (data not shown).

Table 1 Patient characteristics (n=522)

Age, mean ± SD (years)	57 ± 10
Male gender (%)	76
Educational level, mean ± SD*	3.6 ± 1.8
Dutch Adult Reading Test score, mean ± SD	79 ± 17
Hypertension (%)	54
Diabetes mellitus (%)	19
Hyperlipidemia (%)	79
Body mass index (kg/m^2)	27 ± 4
Smoking, median (25th percentile, 75th percentile) (pack-years)	18 (6, 32)
Alcohol consumption (%)	
Never	17
Past	9
Current	74
Intima-media thickness, mean ± SD (mm)	0.9 ± 0.2
White matter lesions volume, median (25th percentile, 75th percentile) (ml)	1.5 (0.9, 2.9)
Any lacunar infarct (%)	10
One lacunar infarct (%)	6
Two or more lacunar infarcts (%)	4
Brain parenchymal fraction, mean ± SD	79 ± 3
Ventricular fraction, mean ± SD	2.0 ± 0.9
Cortical gray matter fraction, mean ± SD	37 ± 3

* Educational level was divided into seven categories, graded from primary school to academic degree, according to the Dutch educational system

Table 2 Raw scores for neuropsychological tests (n=522)

Memory (MEM)	
15-word learning task	
Immediate recall, mean (number of words)	7.6 ± 2.0
Delayed recall (number of words)	7.4 ± 2.9
Retention score (%)	72.1 ± 19.3
Executive functioning (EXEC)	
Visual elevator test, timing score (seconds per switch)	5.1 ± 2.2
Brixton spatial anticipation test (total number of errors)	18.2 ± 6.1
Verbal fluency (words with letter N, during 1 minute)	10.4 ± 4.2

A higher score on the 15-word learning task and the Verbal fluency task indicates a better performance. A higher score on the Visual elevator test and Brixton spatial anticipation test indicates a worse performance.

Table 3 Association of WML with Z-scores for executive functioning (EXEC) and memory (MEM) (n=522)

		EXEC		MEM	
		β	(95% confidence interval)	β	(95% confidence interval)
Model I	White matter lesions (SD)	-0.14	(-0.22 to -0.06)	-0.06	(-0.14 to 0.03)
Model II	White matter lesions (SD)	-0.12	(-0.20 to -0.04)	-0.05	(-0.13 to 0.04)

Model I: Adjusted for age, sex, educational level and Dutch Adult Reading Test score
Model II: Model I, additionally adjusted for lacunar infarcts
β represents the difference (95% confidence interval) in z-scores for executive functioning (EXEC) and memory (MEM) per standard deviation (3.4 ml) higher white matter lesion volume. A lower z-score denotes a worse performance.

Table 4 Association of number of LI with Z-scores for executive functioning (EXEC) and memory (MEM) (n=522)

		EXEC		MEM	
		β	(95% confidence interval)	β	(95% confidence interval)
Model I	No lacunar infarcts	(reference)		(reference)	
	One lacunar infarct	0.05	(-0.27 to 0.37)	0.16	(-0.18 to 0.50)
	Two or more lacunar infarcts	-0.61	(-0.99 to -0.23)	-0.30	(-0.70 to 0.11)
Model II	No lacunar infarcts	(reference)		(reference)	
	One lacunar infarct	0.09	(-0.23 to 0.42)	0.18	(-0.16 to 0.52)
	Two or more lacunar infarcts	-0.48	(-0.87 to -0.09)	-0.25	(-0.66 to 0.17)

Model I: Adjusted for age, sex, educational level and Dutch Adult Reading Test score
Model II: Model I, additionally adjusted for white matter lesion volume
β represents the difference (95% confidence interval) in z-scores for executive functioning (EXEC) and memory (MEM) compared with patients without lacunar infarcts. A lower z-score denotes a worse performance.

Table 5 Association of WML with z-scores for executive functioning (EXEC), adjusted for measures of brain atrophy (n=522)

		EXEC	
		β	(95% confidence interval)
Model I	White matter lesions (SD)	-0.12	(-0.20 to -0.04)
Model II	White matter lesions (SD)	-0.12	(-0.20 to -0.04)
Model III	White matter lesions (SD)	-0.09	(-0.18 to -0.01)
Model IV	White matter lesions (SD)	-0.12	(-0.21 to -0.04)

Model I: Adjusted for age, sex, educational level, Dutch Adult Reading Test score and lacunar infarcts
Model II: Model I, additionally adjusted for brain parenchymal fraction
Model III: Model I, additionally adjusted for ventricular fraction
Model IV: Model I, additionally adjusted for cortical gray matter fraction
β represents the difference (95% confidence interval) in z-scores for executive functioning (EXEC) and memory (MEM) per standard deviation (3.4 ml) higher white matter lesion volume. A lower z-score denotes a worse performance.

Table 6 Association of number of lacunar infarcts with z-scores for executive functioning (EXEC), adjusted for measures of brain atrophy (n=522)

		EXEC	
		β	(95% confidence interval)
Model I	No lacunar infarcts	(reference)	
	One lacunar infarct	0.09	(-0.23 to 0.42)
	Two or more lacunar infarcts	-0.48	(-0.87 to -0.09)
Model II	No lacunar infarcts	(reference)	
	One lacunar infarct	0.09	(-0.23 to 0.42)
	Two or more lacunar infarcts	-0.44	(-0.83 to -0.05)
Model III	No lacunar infarcts	(reference)	
	One lacunar infarct	0.13	(-0.20 to 0.46)
	Two or more lacunar infarcts	-0.47	(-0.86 to -0.08)
Model IV	No lacunar infarcts	(reference)	
	One lacunar infarct	0.13	(-0.21 to 0.47)
	Two or more lacunar infarcts	-0.44	(-0.85 to -0.04)

Model I: Adjusted for age, sex, educational level, Dutch Adult Reading Test score and white matter lesions
Model II: Model I, additionally adjusted for brain parenchymal fraction
Model III: Model I, additionally adjusted for ventricular fraction
Model IV: Model I, additionally adjusted for cortical gray matter fraction

Discussion

We found that larger white matter lesion volume and presence of multiple lacunar infarcts were associated with poorer executive functioning in a relatively young population of patients with manifest arterial disease. White matter lesions and lacunar infarcts were not associated with poorer verbal memory performance.

The association between WML and lower scores on executive tests attenuated after adjustment for ventricular enlargement, but remained statistically significant. Ventricular enlargement is most likely the result of ex vacuo enlargement due to subcortical brain atrophy. Subcortical brain atrophy may be an intermediate in the relation between WML and cognitive dysfunction, since ventricular enlargement is associated with executive impairment[23] and associations between WML and ventricular enlargement have been found,[24] which suggests that ischemic damage due to small-vessel pathology may be involved in the pathogenesis of subcortical brain atrophy.[25] Because of the cross-sectional design of our study, we cannot exclude the possibility that subcortical brain atrophy also is a confounder in the relation between WML and cognitive functioning and thus an independent risk factor for cognitive impairment. Nevertheless, the association between WML and executive functioning was still present after adjustment for ventricular enlargement. This suggests that WML also has an independent effect on cognitive functioning.

We also observed that WML were related to worse executive functioning independent of the presence of LI. Associations between WML and impairment in executive functioning have been observed in other studies.[26-28] Executive functioning is primarily controlled by the prefrontal cortex. The frontal lobes are extensively connected to other parts of the brain through axons that lie in the subcortical matter.[29,30] It is thought that WML interrupt these prefrontal-subcortical connections, which leads to impaired prefrontal lobe functioning characterized by executive dysfunction.[2]

Presence of multiple LI was also associated with executive impairment, but this association was independent of brain atrophy. As strategically located infarcts are strongly associated with cognitive impairment, it is possible that they already impair cognitive functioning in the absence of detectable brain atrophy. Furthermore, LI located in the subcortical gray matter nuclei are associated with impairments in several cognitive domains, including frontal functions such as execution.[31]

WML and LI were not associated with poorer memory performance. Memory performance as assessed with the 15-word learning test is mainly a function of structures in the medial temporal lobe and memory impairment is not a distinctive feature of subcortical ischemic vascular disease.[1,27] Although an association between WML and worse memory has been found in other studies,[23,32-34] this association disappeared after adjustment for medial temporal lobe atrophy,[33,34] suggesting that the memory impairment was caused by Alzheimer pathology and subsequent atrophy of the hippocampus. In this study, we had no data available on hippocampal atrophy, and therefore do not know whether WML were associated with hippocampal volume.

Strengths of our study are the large number of patients investigated and the volumetric assessment of WML and measures of brain atrophy. This made it possible to obtain precise estimates of the relation between brain changes on MRI and cognitive functioning, and resulted in a large power to detect associations. Second, since WML and LI were assessed simultaneously in our study it was possible to investigate their independent impact on executive functioning and memory. Furthermore, the segmentation of different brain tissue types and CSF spaces allowed us not only to investigate the role of global brain atrophy, but also to differentiate between cortical and subcortical brain atrophy.

In summary, white matter lesions and multiple lacunar infarcts were independently associated with worse executive functioning in a relatively young population of patients with manifest arterial disease. Part of the association between white matter lesions and executive impairment was explained by subcortical brain atrophy. White matter lesions and lacunar infarcts were not associated with memory. These findings suggest that white matter lesions and lacunar infarcts both impair cognitive functioning independent of brain atrophy, and highlights the importance of acknowledging that both WML and LI should be considered risk factors for cognitive deterioration.

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4.1

Total cerebral blood flow in patients with arterial disease

Purpose

To retrospectively investigate which characteristics are related to total arterial blood flow to the brain in patients with symptomatic vascular disease.

Materials and methods

The study was approved by the ethics committee of the authors' institution, and written informed consent was obtained. The total volume flow rate (tVFR) values in the internal carotid arteries and the basilar artery in 636 patients (536 men, 100 women; mean age, 58 years) with symptomatic vascular disease were measured with two-dimensional phase-contrast magnetic resonance angiography. Reference tVFR values in the general population were obtained from previous research involving 158 subjects (73 men, 85 women; mean age, 60 years).

Results

A higher tVFR was found in patients with symptomatic vascular disease, but this association was statistically significant in only those patients in the 7th decade of life. The mean tVFR decreased with increasing age (-3.4 ml/min per year; 95%CI: -4.3 to -2.5). Diabetes (-27.6 ml/min; 95%CI: -52.6 to -2.6) and increasing body mass index (BMI) (-2.8 ml/min per BMI unit; 95%CI: -5.3 to -0.2) were associated with lower tVFR. Patients with vascular disease in a cerebral location had lower tVFR values (-39.7 ml/min; 95%CI: -65.1 to -14.3) than did patients with symptomatic vascular disease elsewhere in the vascular tree.

Conclusions

Patients with symptomatic vascular disease had slightly higher arterial blood flow to the brain compared with the general population. The tVFR decreased with increasing age and increasing BMI, and patients with diabetes had lower tVFR values than did those without diabetes. Patients with vascular disease in a cerebral location had lower tVFR values than did those with symptomatic vascular disease at other arterial sites.

Introduction

The vascularization of the brain can be assessed at different levels. First, the blood supply through the large vessels can be measured with two-dimensional phase-contrast magnetic resonance (MR) angiography. With this simple noninvasive technique, the volume flow rate (expressed in milliliters per minute) can be measured in both the internal carotid arteries and the basilar artery. The volume flow rates measured in these vessels are then summed to calculate the total rate of volume flow (i.e., total volume flow rate (tVFR), in milliliters per minute) to the brain. By dividing this value throughout the entire brain volume, the mean cerebral perfusion can be calculated.
Second, at the brain tissue level, perfusion can be measured with different techniques such as positron emission tomography or perfusion MR imaging. These techniques can yield perfusion values in specific regions of the brain; that is, regional cerebral blood flow (rCBF) values (in milliliters per 100 g of brain tissue per minute). The cerebral blood flow in the entire brain should be similar to the tVFR corrected for the brain volume. Compared with the rCBF, the tVFR is simple and inexpensive to measure and can be easily measured in hundreds of patients. rCBF studies typically involve small sample sizes and thus have limited value in showing relationships between risk factors and flow. To show these relationships, large patient groups are required. Up to now, these smaller rCBF studies have been the only investigations available for comparison with the few tVFR studies.
A decrease in rCBF is associated with deterioration of cognitive function[1] and depression,[2] and it may indicate an increased risk of cerebral ischemia.[3] Several study investigators have studied the factors that influence rCBF. Contradicting results regarding the relationships between rCBF and the following variables have been described: older age,[4-9] male sex,[6,9] smoking,[6,10-12] high amounts of alcohol intake,[6,13] hypertension,[6-8,14-16] hyperlipidemia,[17,18] and diabetes.[19] Little is known about the arterial blood flow to the brain in patients with vascular disease and the association between this flow and vascular disease risk factors. Thus, the purpose of our study was to retrospectively investigate which characteristics are related to total arterial blood flow to the brain in patients with symptomatic vascular disease.

Materials and methods

Patients

All patients were participants in the Second Manifestations of Arterial Disease (SMART) Study, an ongoing single-center (University Medical Center Utrecht) prospective cohort study that began in September 1996. All eligible patients aged 18-79 years who were newly referred to our institution with symptomatic atherosclerosis or risk factors for atherosclerosis were screened for additional risk factors and severity of atherosclerosis. Definitions of the diseases that qualified patients for enrollment of their data in the study are reported elsewhere.[20] The data for a total of 636 patients (536 men, 100 women; mean age ± SD, 58 ± 10 years) were included in the study. Baseline characteristics of the patients are given in **Table 1**.

Table 1 Baseline characteristics of 636 patients

Characteristic	Value	Data missing
Age*	58.1 ± 9.9	0
Male sex	536 (84)	0
Smoking history		35
Never	113 (19)	…
Past	338 (56)	…
Current	150 (25)	…
Alcohol consumption		35
Never	86 (14)	…
Past	55 (9)	…
Current	460 (77)	…
Body mass index (kg/m^2)*	26.7 ± 3.7	8
Systolic blood pressure (mmHg)*	138 ± 19	39
Diastolic blood pressure (mmHg)*	81 ± 11	40
Hypertension	272 (49)	80
Diabetes mellitus	112 (19)	58
Hyperlipidemia	285 (46)	18
Plasma homocysteine level (μmol/L)*	14.0 ± 5.6	11
Cerebrovascular disease†	102 (16)	0
Coronary artery disease†	432 (68)	0
Peripheral arterial disease†	121 (19)	0
Abdominal aortic aneurysm†	67 (11)	0

Unless otherwise noted, data are numbers of patients, with percentages in parentheses.
* Mean value ± standard deviation.
† Patients with vascular disease at more than one location were assigned to more than one disease category.

In May 2001, MR angiography, including two-dimensional phase-contrast MR angiography, of the brain was added to the screening program for the enrolled patients with symptomatic vascular disease and without contradictions to MR angiography (i.e., pacemakers, claustrophobia, and/or pregnancy). The SMART Study was approved by the ethics committee of our institution, and written informed consent was obtained from all participants. The approval and consent included those for future retrospective analyses.

For the current study, the data of 636 patients with coronary artery disease, cerebrovascular disease, peripheral arterial disease, or abdominal aortic aneurysm for whom the results of MR angiography of the brain were available were included. Coronary artery disease was defined as myocardial infarction or having undergone coronary surgery or percutaneous transluminal coronary angioplasty in the past or at the time of inclusion in the study. Patients with stroke or transient ischemic attack

at inclusion and patients who reported having a stroke in the past were considered to have cerebrovascular disease. Peripheral arterial disease was defined as intermittent claudication or rest pain at inclusion or history of vascular surgery or angioplasty. The presence of abdominal aortic aneurysm or a history of previous surgery for it was the criterion for abdominal aortic aneurysm.

Patients with vascular disease at more than one location were assigned to more than one disease category. Patients who had internal carotid artery stenosis of 50% or greater or occlusion were excluded from the analyses. For comparison of the tVFR between the symptomatic group and the reference group, patients younger than 40 years were excluded because of their limited number (n=21). For the other analyses, they were included.

Vascular risk factors

At study enrollment, the subjects' risk factors were assessed by means of an extensive questionnaire and physical, ultrasonographic (US), and laboratory examinations. The subjects' height and weight were measured, and the body mass index (BMI, in kilograms per square meter) was calculated by dividing the weight by the height squared. Systolic and diastolic blood pressures (in millimeters of mercury) were measured twice with a sphygmomanometer. Hypertension was considered to be present when the mean systolic blood pressure was 160 mmHg or higher and/or the mean diastolic blood pressure was 95 mmHg or higher at study inclusion and/or antihypertensive treatment was being administered. A fasting venous blood sample was taken to determine glucose, lipid, and homocysteine levels. Diabetes mellitus was defined as a glucose level of 7.0 mmol/L or higher or the reported treatment for diabetes. Hyperlipidemia was defined as a total cholesterol level higher than 5.0 mmol/L, a low-density lipoprotein cholesterol level higher than 3.2 mmol/L, or the reported treatment for elevated cholesterol. Hyperhomocysteinemia was defined as a total homocysteine level of 16.3 μmol/L or higher in women or 18.8 μmol/L or higher in men.

The degree of extracranial stenosis of the internal carotid artery was assessed by using duplex US, in which the peak systolic velocity is translated into a degree of diameter reduction.[21] US was also performed to measure the intima-media thicknesses (in millimeters) in the left and right common carotid arteries, as represented by the mean value of six measurements. Smoking and alcohol consumption histories were divided into three categories: never, past, and current. Patients who had quit smoking or drinking during the past year were assigned to the current category.

Sample of the general population

Reference tVFR values were obtained from a study involving 250 adults (122 men, 128 women; age range, 19-88 years; mean age, 50 years) who had been examined with MR imaging of the brain by our research group.[22] Data on the 158 individuals (46% men; mean age, 60 years) who were aged 40-79 years among these 250 subjects were used for the current analysis. This population consisted of 79 individuals who

were first-degree relatives of patients with subarachnoid hemorrhage and had been screened for the presence of intracranial aneurysms and 79 elderly persons who were participating in a population-based study. The tVFR values in this group had been measured with ungated two-dimensional phase-contrast MR angiography by using the same technique used to examine the patient group in our current study.

Magnetic resonance angiography

The MR examinations were performed by using a 1.5-T whole-body system (Gyroscan ACS-NT; Philips Medical Systems, Best, the Netherlands). On the basis of findings on a localizer MR angiographic slab in the sagittal plane, a two-dimensional phase-contrast section was positioned at the level of the skull base to measure the volume flow in the internal carotid arteries and the basilar artery. The **Figure** illustrates the positioning of the two-dimensional phase-contrast section (16/9 [repetition time msec/echo time msec]; flip angle, 7.5°; section thickness, 5 mm; field of view, 250 x 250 mm; matrix size, 256 x 256; eight acquired signals; velocity sensitivity, 100 cm/sec) through the internal carotid arteries and the basilar artery. Postprocessing was performed by specialized MR technologists with more than 5 years of experience. Two images obtained with opposed, bipolar, velocity-encoding gradients were subtracted to achieve velocity sensitivity.

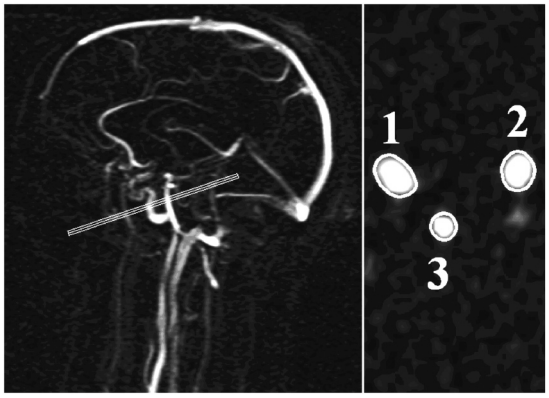

Figure Left: Sagittal localizer MR angiogram illustrates the positioning of a two-dimensional phase-contrast MR angiographic section (16/9, 7.5° flip angle) for measurement of the volume flow through the internal carotid arteries and the basilar artery. Right: Quantitative flow values were obtained by means of integration across manually drawn regions of interest (on corresponding two-dimensional phase-contrast MR angiographic image) that enclosed the vessels. 1=right-sided internal carotid artery, 2=left-sided internal carotid artery, 3=basilar artery. Line through the left image indicates the plane of the right image.

For each vessel, the spatial and time-averaged flow velocity was calculated from the phase-difference images by manually drawing a region of interest around the vessel. Special care was taken to ensure that the region of interest encompassed the entire lumen of the vessel without including too many stationary tissue voxels (**Figure**). The surrounding stationary tissue voxels included in the region of interest were not expected to affect accuracy, since such voxels do not carry flow. The flow velocity in

each vessel was multiplied by the cross-sectional area of the pixels in the region of interest to obtain the volume flow rate. Good agreement between repeated volume flow rate measurement postprocessing procedures was revealed for hand-drawn regions of interest in our research group, with a coefficient of variation of 5%.[23] The flow through the left and right internal carotid arteries and the basilar artery was summed to calculate the tVFR (in milliliters per minute).

Statistical analysis

The ages of the symptomatic patients were categorized by decade, as was done for the sample of the general population.[22] In each age group, the mean tVFR ± the standard deviation was calculated for the male and female subjects separately and for the group as a whole. Since the raw data of the sample of the general population were no longer available, differences in tVFR between populations and differences between the sexes were determined with an unpaired Student's *t*-test (CIA 1.0; BMJ Publishing, London, England) to result in a tVFR difference. A 99% confidence interval (CI) was calculated to adjust for multiple comparisons.

We assessed the effects of age, other risk factors, and vascular disease location on the tVFR by using linear regression analysis (SPSS 12.0.1; SPSS, Chicago, Ill) after checking whether the continuous variables were normally distributed. For each factor, we calculated the crude and age-adjusted regression coefficients, ß, which yield the slope of the regression fitted by the model and indicate the increase (positive value) or decrease (negative value) in tVFR, in milliliters per minute per unit of the independent variable. The crude regression coefficient was determined for all risk factors and vascular disease locations. We calculated an age-adjusted regression coefficient by including subject age in the model. For all test results, 95% CIs, being more informative than P values, were given.[24] A 95%CI that did not include the value of 0 had a P value of less than .05.

Results

The tVFR was higher in the patients with symptomatic vascular disease than in the reference group, among all age groups. However, the difference was significant in only those patients in the 7th decade of life; the difference in tVFR was 55 ml/min (95%CI: 12 to 98) (**Table 2**). A decrease in tVFR with increasing age was observed. No significant differences in tVFR between the male and female subjects were observed.

All continuous variables in the patient group were normally distributed. tVFR decreased with increasing age (**Table 3**).When we analyzed each risk factor separately with adjustments for age, diabetes (-27.6 ml/min; 95%CI: -52.6 to -2.6) and BMI (-2.8 ml/min per BMI unit; 95%CI: -5.3 to -0.2) were associated with a decrease in tVFR. Patients with cerebrovascular disease clearly had lower tVFR values than did those without it; this difference remained when age was taken into account (-39.7 ml/min; 95%CI: -65.1 to -14.3).

Table 2 Age-based MR Angiographic tVFR measurements in patients with symptomatic vascular disease and healthy subjects

	Patients with symptomatic vascular disease						
Age range (years)	No. of subjects[§]	tVFR (ml/min)[‡]			Healthy subjects[*]		tVFR difference (ml/min)[†]
		Men	Women	All subjects	tVFR (ml/min)[‡‖]	No. of subjects[§]	
40–49	104 (86/18)	654 ± 134	694 ± 101	660 ± 130	623 ± 116	41 (23/18)	37 (-24, 98)
50–59	231 (200/31)	627 ± 124	595 ± 101	623 ± 122	595 ± 102	26 (13/13)	28 (-37, 93)
60–69	193 (155/38)	598 ± 119	563 ± 92	591 ± 115	536 ± 99	59 (22/37)	55 (12, 98)[#]
70–79	87 (77/10)	569 ± 114	501 ± 153	562 ± 120	517 ± 110	32 (15/17)	45 (-19, 109)

[*] Source: reference 22.
[†] Difference in mean tVFR between symptomatic and healthy groups. Numbers in parentheses are 99% CIs.
[‡] Data are mean values ± standard deviations.
[§] Numbers in parentheses are numbers of men/women. Twenty-one patients younger than 40 years were excluded from this analysis.
[‖] Mean tVFR values for all subjects (male and female) in the healthy population.
[#] The difference between the symptomatic and healthy subjects was significant in the subjects aged 60–69 years.

Table 3 Crude and age-adjusted regression coefficients for individual risk factors and location of vascular disease, with tVFR as dependent variable

Factor	Crude regression coefficient	Age-adjusted regression coefficient
Age	-3.4 (-4.3 to -2.5)*	…
Male sex	23.0 (3.9 to 49.8)*	22.2 (-3.5 to 47.9)
Smoking history		
Never	0.0 …	0.0 …
Past	-3.1 (-29.6 to 23.3)	3.8 (-21.7 to 29.4)
Current	18.0 (2.6 to 33.4)*	13.7 (-1.2 to 28.6)
Alcohol consumption		
Never	0.0 …	0.0 …
Past	-4.0 (-48.3 to 40.2)	-13.6 (-55.9 to 28.7)
Current	6.9 (-7.4 to 21.2)	5.4 (-8.3 to 19.0)
Body mass index (kg/m^2)	-2.0 (-4.7 to 0.6)	-2.8 (-5.3 to -0.2)*
Systolic blood pressure (mmHg)	-0.6 (-1.2 to -0.1)*	-0.01 (-0.5 to 0.5)
Diastolic blood pressure (mmHg)	-0.2 (-1.1 to 0.8)	0.02 (-0.9 to 0.9)
Hypertension	-19.9 (-41.1 to 1.2)	-9.8 (-30.4 to 10.7)
Diabetes mellitus	-42.0 (-67.5 to -16.4)*	-27.6 (-52.6 to -2.6)*
Hyperlipidemia	10.5 (-9.5 to 30.4)	8.5 (-10.6 to 27.7)
Plasma homocysteine level (µmol/L)	-2.0 (-3.7 to -0.2)*	-0.6 (-2.3 to 1.1)
Cerebrovascular disease	-46.8 (-73.2 to -20.4)*	-39.7 (-65.1 to -14.3)*
Coronary artery	10.3 (-10.6 to 31.2)	6.0 (-14.1 to 26.1)
Peripheral arterial disease	21.4 (-3.5 to 46.2)	20.4 (-3.5 to 44.2)
Abdominal aortic aneurysm	-20.3 (-52.1 to 11.5)	7.8 (-23.6 to 39.3)

Numbers in parentheses are 95% CIs.
* Statistically significant value (CI does not include zero).

Discussion

Our study results show that the patients with vascular disease had a slightly higher tVFR overall than did the sample of the general population. In the patient group, the tVFR decreased with increasing age and increasing BMI and was lower in the patients with diabetes. Furthermore, the patients with vascular disease in a cerebral location had lower tVFR values than did the patients with vascular disease in other locations. To date, we are unable to predict which patients with risk factors for vascular disease will have symptomatic cerebrovascular disease. In our opinion, the population that should be targeted for research of cerebral blood flow is that of high-risk patients who have at least one manifestation of vascular disease. To prevent the presence of internal carotid artery stenosis from confounding the results, we restricted our study to patients who had neither 50% or greater stenosis nor occlusion at baseline.

We chose a reference group that was not entirely free of vascular disease but represented a sample of the general population. If the results were influenced by this decision, the difference in tVFR could have been only underestimated. In the reference group, the same two-dimensional phase-contrast MR imaging technique was used to measure the tVFR. In a study to compare methods of measuring blood flow volume, flow values differed widely among different techniques,[25] implying the need to use the same method for every consecutive or comparative flow measurement.

A comparison of the results obtained by different investigators revealed a wide range of tVFR values, which were in good agreement with the internal carotid artery values but slightly lower than the basilar artery values in our reference group.[22] In our opinion, since the methods used to measure the tVFR in our patient group and the reference group were exactly the same and the patient numbers were relatively high compared with those in many other studies, it was unlikely that the difference in flow between the two groups was based on chance.

Volume flow rate measurement in patients with vascular disease or in healthy control subjects is described in few small-sample studies.[26-29] The main focus in the literature is measurement of rCBF in patients with cerebrovascular disease rather than measurement of the total arterial flow to the brain.

We expected the patients with vascular disease to have a lower tVFR than the sample from the general population. In contrast, the tVFR was slightly higher in the patients with vascular disease. An explanation could be that the inflammatory process related to atherosclerosis results in elevated tVFR values. We have found no other studies in which greater flow in a similar patient group was reported.

In our study, the tVFR decreased 34 ml/min per decade. Investigators in a longitudinal study reported that rCBF decreases with increasing age and with progressive cerebrovascular disease.[6] The relationship between lower rCBF and increasing age has been reported in cross-sectional studies with healthy volunteers,[5,8,9,22] while another study revealed that the main relationship was increasing atherosclerosis with increasing age[4] and another study revealed no relationship between age and rCBF.[7]

The longer duration of diabetes was reported to be related to lower rCBF in a patient group with insulin-dependent diabetes.[30] Investigators in another study proposed that the relationship between diabetes and rCBF may accelerate the age-related reduction in rCBF.[19] Diabetes is also related to cerebral atrophy.[31] The arterial occlusive disease associated with diabetes, together with the lower brain volume due to atrophy, might explain the lower tVFR values in the patients with diabetes observed in our study. The observed lower flow in patients with increasing BMI could have been due to the high prevalence of diabetes in patients with a high BMI.

Our study results also show that cerebrovascular disease is related to lower tVFR. Since this was a cross-sectional study, it is impossible to say which came first: the cerebrovascular disease or the lower tVFR. However, because the relationship between increasing age and lower tVFR is well established and because in our study diabetes also was related to lower tVFR, it seems more probable that the lower tVFR was an effect of the progression of disease. Internal carotid artery stenosis cannot be an explanation for this relationship because patients with stenosis of 50%

or greater were excluded from this study. Since generalized atherosclerotic disease cannot account for the observed relationship, we speculate that more distally located obstructive disease in the cerebral vessels or the smaller brain volume necessitating blood after a stroke could have accounted for the correlation.

A limitation of our study was the absence of data on brain volume. For comparison of the rCBF data with our arterial flow data in particular, brain volume data were needed. The purpose of our study was not to compare our data with those obtained with other modes of measuring cerebral blood flow but rather to evaluate the determinants of the total rate of volume flow to the brain. It is known that the average weight of the cerebrum decreases with age.[32] We believe that in our analyses, after adjustments for age, our results were not confounded by brain volume differences. Differences in brain volume due to disease (for example, diabetes) could very well be an explanation for the observed results. However, this remains an assumption.

Another limitation was that all patients with transit ischemic attack or stroke at inclusion were considered to have cerebrovascular disease. Cardiovascular disease could have been the real cause of the transit ischemic attack or stroke. We did not have enough information to make this distinction. If the results were affected by this possible misclassification, then it could have caused some underestimation.

The patients included in our study were newly referred to our university hospital with a clinical manifestation of cerebral, cardiac, or peripheral vascular disease, or an abdominal aortic aneurysm. In our opinion, no selection bias occurred at study inclusion. However, the generalizability of our study findings could be confined to academic hospitals since patients with more severe disease are expected to visit these facilities.

Our study results show that patients with vascular disease have slightly higher tVFR values than do healthy subjects; that age, BMI, and diabetes are related to tVFR; and that patients with vascular disease in a cerebral location have lower tVFR values than do patients with symptomatic vascular disease elsewhere in the vascular tree. The cause of the lower tVFR values in the patients with cerebrovascular disease will have to be unraveled in future research, because the process of generalized atherosclerosis alone apparently cannot explain our results.

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4.2

Total cerebral blood flow white matter lesions and brain atrophy

Introduction

We investigated whether total cerebral blood flow (CBF) was associated with brain atrophy, and whether this relation was modified by white matter lesions (WML).

Methods

Within the SMART-MR study, a prospective cohort study among patients with arterial disease, cross-sectional analyses were performed in 828 patients (mean age 58 ± 10 yrs, 81% male) with quantitative flow, atrophy and WML measurements on MRI. Total CBF was measured with MR angiography and was expressed per 100 ml brain volume. Total brain volume and ventricular volume were divided by intracranial volume to obtain brain parenchymal fraction (BPF) and ventricular fraction (VF). Lower BPF indicates more global brain atrophy while higher VF indicates more subcortical brain atrophy.

Results

Mean CBF was 52.0 ± 10.2 ml/min per 100 ml, mean BPF was 79.2 ± 2.9% and mean VF was 2.03 ± 0.96%. Linear regression analyses showed that lower CBF was associated with more subcortical brain atrophy, after adjusting for age, sex, vascular risk factors, intima-media thickness and lacunar infarcts, but only in patients with moderate to severe WML (upper quartile of WML): change in VF per SD decrease in CBF 0.18%, 95%CI 0.02 to 0.34.

Conclusions

Our findings suggest that cerebral hypoperfusion in the presence of WML may be associated with subcortical brain atrophy.

Introduction

Brain atrophy increases with age and is a common finding on MRI in the elderly.[1] It is characterized by widening of the sulci, narrowing of the gyri, as well as enlargement of the ventricles. In cognitively healthy persons, as well as in patients who already experience cognitive problems, the extent and rate of progression of global brain atrophy and ventricular enlargement predict future cognitive deterioration and conversion to Alzheimer's disease.[2,3]

Although the etiology of brain atrophy remains incompletely understood, there are indications that hypoperfusion plays a role in its development. Hypoperfusion impairs the delivery of oxygen and nutrients to the brain and may subsequently trigger cerebral neurodegeneration. The role of hypoperfusion in the pathogenesis of brain atrophy is supported by experimental data from animal studies.[4] The brains of gerbils showed ventricular dilatation, cortical atrophy, and rarefaction of the white matter after clipping of the carotid arteries to cause stenosis.[5] Furthermore, in healthy and cognitively impaired humans, associations between a decrease in regional cerebral blood flow (CBF) and brain atrophy have been found.[6,7]

In healthy persons, the brain can compensate for hypoperfusion through the process of autoregulation by decreasing the resistance of the cerebral vascular bed and thereby maintaining an adequate cerebral perfusion. In patients who suffer from cerebral small-vessel disease (CSVD) the cerebral vasomotor reactivity is impaired, reducing the capacity to respond adequately to a decrease in cerebral perfusion.[8,9] As a result, the relation between hypoperfusion and brain atrophy may be more pronounced in patients with CSVD.

Measuring perfusion in large groups of patients is not feasible, since most techniques are too invasive or involve the use of ionizing radiation.[10] Two-dimensional phase-contrast MR angiography is a fast, noninvasive and reproducible method[11] that can be used to measure total CBF. A measure of mean brain perfusion can be obtained by dividing the total CBF by the total brain parenchymal volume.[12] The purpose of the present study was to investigate the association between total CBF adjusted for brain volume and global and subcortical brain atrophy. In addition, we assessed whether the presence of WML, as an indicator of CSVD, modified the relation between total CBF and brain atrophy. We hypothesized that a lower cerebral perfusion was associated with more brain atrophy and that this relation was more pronounced in patients with CVSD.

Methods

SMART and SMART-MR Study

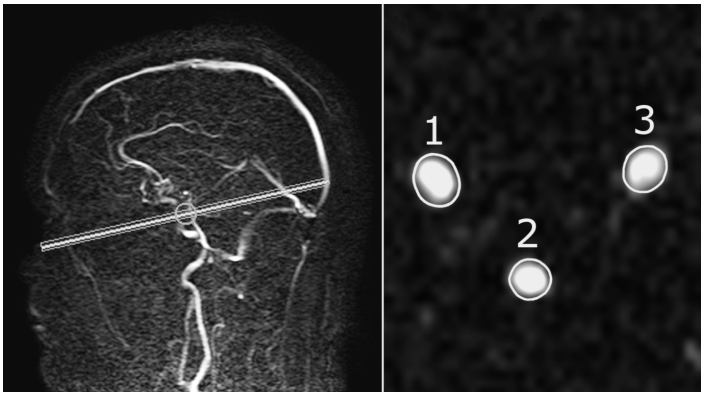
The Second Manifestations of ARTerial disease (SMART) study is an ongoing prospective cohort study that started in 1996. Since then, all eligible patients, aged 18-79 years, newly referred to the University Medical Center Utrecht with symptomatic atherosclerotic disease or risk factors for atherosclerosis, are screened for additional

risk factors and severity of atherosclerosis. The baseline examination comprises an extensive questionnaire, a physical and ultrasonographic examination, and blood and urine sampling. The objectives of the SMART study are to determine the prevalence of vascular risk factors and concomitant arterial disease at other sites and to study the incidence of future cardiovascular events and its predictors in these high-risk patients. Definitions of the diseases qualifying for enrollment were reported elsewhere.[13]
Between May 2001 and December 2005, as part of the SMART-MR Study, an MR investigation of the brain was added to the baseline examination in patients who were included with manifest coronary artery disease, cerebrovascular disease, peripheral arterial disease or an abdominal aortic aneurysm (AAA) and without MR contraindications. Coronary artery disease was defined as myocardial infarction, coronary artery bypass graft surgery or percutaneous transluminal coronary angioplasty in the past or at inclusion. Patients with a transient ischemic attack (TIA) or stroke at inclusion and patients who reported stroke in the past were considered to have cerebrovascular disease. Peripheral vascular disease was defined as surgery or angioplasty of the arteries supplying the lower extremities in history or intermittent claudication or rest pain at inclusion. Present AAA (distal aortic anteroposterior diameter ≥3 cm) or previous AAA surgery was the criterion for AAA. Coronary artery disease was present in 59%, cerebrovascular disease in 23%, peripheral arterial disease in 22% and an AAA in 9% of these patients. The cumulative percentage exceeds 100 % because patients can have vascular disease at more than one location. The SMART study and SMART-MR study were approved by the ethics committee of our institution and written informed consent was obtained from all participants.

Magnetic resonance imaging protocol

The MR investigations were performed on a 1.5-T whole-body system (Gyroscan ACS-NT, Philips Medical Systems, Best, the Netherlands). The protocol consisted of a transaxial T1-weighted gradient-echo sequence (repetition time (TR)/echo time (TE): 235/2 ms; flip angle, 80°), a transaxial T2-weighted turbo spin-echo sequence (TR/TE: 2200/11 ms and 2200/100 ms; turbo factor 12), a transaxial T2-weighted fluid attenuating inverse recovery (FLAIR) sequence (TR/TE/inversion time (TI): 6000/100/2000 ms) and a transaxial inversion recovery (IR) sequence (TR/TE/TI: 2900/22/410 ms) (field of view (FOV) 230 x 230 mm; matrix size, 180 × 256; slice thickness, 4.0 mm; slice gap, 0.0 mm; 38 slices).
Next, on the basis of a localizer MR angiographic slab in the sagittal plane, a two dimensional phase-contrast (2D-PC) section was positioned at the level of the skull base to measure the volume flow in the internal carotid arteries (ICA's) and the basilar artery (BA). **Figure 1** illustrates the positioning of the 2D-PC section through the ICA's and the BA (TR/TE: 16/9 ms; flip angle, 7.5°; slice thickness, 5.0 mm; FOV, 250 x 250 mm; matrix size, 256 x 256; eight acquired signals; velocity sensitivity, 100 cm/sec).

Figure 1 Left: Sagittal localizer MRA image illustrating the positioning of a two-dimensional phase-contrast MR angiographic slab used to measure the volume flow through the internal carotid arteries and basilar artery. Right: Quantitative flow values were obtained by integrating across manually drawn regions of interest that enclose the vessels (1 indicates right-sided internal carotid artery; 2 indicates basilar artery; 3 indicates left-sided internal carotid artery).



Measurement of total cerebral blood flow

Post-processing of the flow measurements was performed by one of the authors without knowledge of clinical or brain characteristics from the same patient. For each vessel, the spatial and time-averaged flow velocity was calculated from the phase-difference images by manually drawing a region of interest around the vessel (**Figure 1**). The average flow velocity in each vessel was multiplied by the cross-sectional area of the pixels in the region of interest (ROI) to obtain the volume flow rate. Good agreement between repeated volume flow rate measurement post-processing procedures was shown in our research group, with a coefficient of variation of 5%.[14,15] The flow through the left and right ICA's and BA were summed to calculate the total CBF (ml/min).
Part of the differences in total CBF between individuals can be attributed to differences in brain-size.[12] However, our objective was to investigate if a low total CBF, relative to a subject's brain volume, was associated with brain atrophy. Therefore we expressed total CBF per 100 ml brain parenchymal volume to obtain a measure of the mean total brain perfusion.[12]

Brain segmentation

Brain segmentation was performed using three different MR scans. The scans needed for this purpose were the T1-weighted gradient-echo and IR sequence and the FLAIR sequence. The probabilistic segmentation technique has been described elsewhere.[16] In short, two preprocessing steps were performed. The first step was an intra-patient rigid registration in order to compensate for motion and scan variations.[17]

The second preprocessing step was an automatic skull-stripping of the T1 image,[18] in order to define a proper ROI for the segmentation process. The segmentation of the MR-images was done with a statistical classification method called k-Nearest Neighbor (KNN) classification.[16] The result of the classification method is a probability value for each voxel that quantifies the amount of a specific tissue type contained in that voxel. Total volumes were calculated by multiplying these probabilities by the number and volumes of the voxels (4.0 x 0.9 x 0.9 mm). The segmentation program distinguishes gray matter, white matter, pericerebral cerebrospinal fluid (CSF), ventricles and lesions.

Both WML and infarcts are classified as 'lesion' volume, since the segmentation program cannot distinguish between them. Therefore, an investigator and neuroradiologist visually checked the results of the segmentation analysis and made a further distinction into WML and infarct volumes by manually assigning the lesion volumes to one of these two categories.

To avoid incorporation of neuronal tissue and CSF outside the cranium, an investigator determined the slice that included the foramen magnum. This was done by scrolling through the MR slices in a craniocaudal direction. The first slice caudal to the last slice that contained cerebellar tonsils was defined as the level of the foramen magnum. All volumes cranial to the foramen magnum were included in the segmentation results. As a result, the total brain volume includes the cerebrum, brainstem and cerebellum.

Assessment of brain atrophy

The brain tissue and CSF volumes that were obtained from the segmentation program were used for the assessment of brain atrophy. The total brain volume was calculated by summing the volumes of gray and white matter and, if present, the volumes of WML or infarcts. The total intracranial volume (ICV) was calculated by summing the total brain volume and the volumes of the sulcal and ventricular CSF.

Brain parenchymal fraction (BPF), an indicator for global brain atrophy, represents the percentage of the ICV that is occupied by brain tissue. Ventricular enlargement, an indicator for subcortical brain atrophy, was assessed with the ventricular fraction (VF) and was calculated as the percentage ventricular volume of the total ICV. A lower BPF indicates more global brain atrophy and a higher VF indicates more subcortical brain atrophy.

Assessment of white matter lesions

The volumes of WML obtained with the segmentation program were summed to obtain the total volume of WML. We made no distinction between deep and periventricular WML, since it has been shown that deep, periventricular and total WML are highly correlated with each other.[19] Furthermore, we chose to use total WML volume, since it has been suggested that categorical distinctions between periventricular and deep WML are arbitrary.[19] According to current guidelines,[20] WML volumes were normalized for intracranial volume to correct for differences in head size. Normalized WML (nWML) (ml) was calculated by dividing the patient's WML volume by the patient's ICV and multiplying by the mean ICV of the study population (1473 ml).

Assessment of infarcts

The whole brain, including cortex, brainstem, and cerebellum, was visually searched for infarcts independently by an investigator and neuroradiologist. Discrepancies in rating were re-evaluated in a consensus meeting. Both were blinded for the history and diagnosis of the patient. Infarcts were defined as focal lesions of at least 3 mm in diameter, with signal intensity corresponding to CSF: hyperintense on T2-weighted images and low signal on the FLAIR image. They were differentiated from WML on FLAIR, which were of high signal intensity. Dilated perivascular spaces were distinguished from infarcts on the basis of their location (along perforating or medullary arteries, often symmetrical bilaterally, usually in the lower third of the basal ganglia or in the centrum semiovale), form (round/oval), and the absence of gliosis.[21] The location, affected flow territory and type were scored for every infarct. We defined lacunar infarcts as infarcts sized 3 to 15 mm and located in the subcortical white matter, thalamus or basal ganglia.

Vascular risk factors

Risk factors were assessed by means of an extensive questionnaire and physical, laboratory and ultrasonographic examinations. The subjects' height and weight were measured, and the body mass index (BMI) was calculated (kg/m^2). Systolic and diastolic blood pressures (mmHg) were measured twice with a sphygmomanometer and the average of the two measurements was obtained. Hypertension was defined as mean systolic blood pressure ≥140 mmHg or mean diastolic blood pressure ≥95 mmHg or use of antihypertensive drugs. A fasting venous blood sample was taken to determine glucose and lipid levels. Diabetes mellitus was defined as glucose ≥7.0 mmol/L or use of oral antidiabetic drugs or insulin. Hyperlipidemia was defined as total cholesterol >5.0 mmol/L, low-density lipoprotein cholesterol >3.2 mmol/L or use of lipid lowering drugs. Smoking was assessed with the use of pack-years. To calculate pack-years of smoking, the average number of cigarettes smoked per day was divided by 20 and then multiplied by the number of years of cigarette smoking. Ultrasonography was performed to measure the intima-media thickness (IMT) (mm) in the left and right common carotid arteries, represented by the mean value of six measurements.[22]

Study sample

A total of 1309 patients was investigated in the SMART-MR study. Segmentation data were missing in 265 patients (missing MR sequences needed for the segmentation analyses, due to a temporary change in MR protocol (n=192), MR data irretrievable (n=19), missing FLAIR sequence (n=14), motion or other artifacts (n=40)). As a result, segmentation data were available for 1044 patients. There were no differences in age, sex and vascular risk factors between patients with and without segmentation results. Of the remaining 1044 patients, CBF measurements were missing in 71 patients (due to a missing Magneto-Optical Disc (n=50), flow measurement failed (n=14), and flow measurement not performed (n=7)). Patients without available flow measurements were more often female (36% vs. 20%) and more often had hypertension (64% vs. 51%). Other patient characteristics were comparable between patients with and

Table 1 Patient characteristics according to tertiles of normalized total CBF (ml/min/100 ml) (n=828)

	CBF (ml/min per 100 ml)		
	<47.4 (n=276)	47.4 - 54.9 (n=275)	>54.9 (n=277)
Normalized total CBF, mean ± SD (ml/min per 100 ml)	41.7 ± 5.1	51.2 ± 2.1	63.2 ± 6.9
Age, mean ± SD (years)	60.1 ± 10.1	58.1 ± 10.0	55.9 ± 10.2
Male gender, %	87	79	75
Smoking, median (25^{th} percentile, 75^{th} percentile) (pack-years)	16 (5, 32)	18 (5, 34)	21 (7, 33)
Body mass index, mean ± SD (kg/m^2)	27.0 ± 3.4	26.7 ± 3.9	26.4 ± 3.8
Hypertension, %	54	47	45
Diabetes mellitus, %	24	17	18
Hyperlipidemia, %	80	81	77
Intima-media thickness, mean ± SD (mm)	0.94 ± 0.31	0.91 ± 0.25	0.89 ± 0.26
WML volume, median (25^{th} percentile, 75^{th} percentile) (ml)	1.8 (0.9, 4.0)	1.5 (0.9, 3.0)	1.6 (0.9, 3.0)
One or more lacunar infarcts, %	14	10	11

CBF = cerebral blood flow; SD = standard deviation; WML = white matter lesions

without flow measurements. We excluded 114 patients with one or more cortical infarcts since the brain volume decrease in these patients is primarily the direct effect of tissue loss in the infarcted region. Finally, in 31 patients not all cardiovascular risk factors were assessed. Consequently, the analyses were performed in 828 patients.

Data analysis

We used linear regression analysis to investigate changes in BPF and VF per SD increase in CBF. The analyses were performed in four steps. In the first model we adjusted for age and sex. In the second model we additionally adjusted for hypertension, diabetes mellitus, smoking, body mass index, hyperlipidemia, intima-media thickness and presence of lacunar infarcts. In the third model we also adjusted for WML volume. Since the volume of WML was positively skewed we log transformed WML volume to normalize the distribution. In order to assess if the relation between CBF and measures of brain atrophy was modified in the presence of WML an interaction term was entered in the final model. If the regression analyses revealed an interaction between CBF and volumes of WML ($P < 0.05$) stratified analyses were performed according to volumes of WML. Patients were then categorized as having no or few WML (lower three quartiles of WML volume) or moderate to severe WML (upper quartile of WML volume). In all analyses the 95% confidence intervals are given. SPSS version 14.0 (Chicago, Ill, USA) was used to analyze our data.

Results

In the study sample of 828 patients, the mean normalized CBF (±SD) was 52.0 ± 10.2 ml/min per 100 ml, the mean BPF (±SD) was 79.2 ± 2.9% and the mean VF (±SD) was 2.03 ± 0.96%.

Patient characteristics according to tertiles of CBF are given in **Table 1**. Patients with a lower CBF were older and more often male. Vascular risk factors were also more prevalent in patients with a lower CBF; they had a higher BMI and more frequently hypertension and diabetes. Furthermore, IMT, a measure for subclinical atherosclerosis, was higher in patients with a lower CBF; volumes of WML were larger, and one or more lacunar infarcts on MRI were more often present.

The results for the linear regression analysis with BPF and VF as dependent variable are given in **Table 2**. No relation between CBF and BPF was found. Furthermore, no interaction between normalized CBF and WML volume was found (interaction term P = 0.11). A small but significant relation was found between an increase in CBF and a decrease in VF, independent of possible confounders and WML volume. When we included the interaction term in the model, a significant interaction between CBF and WML volume for VF was found (interaction term P < 0.001). Therefore the analyses were repeated across strata of WML.

The results of the stratified analysis are given in **Table 3**. In the group of patients with no or few WML (lower three quartiles of WML volume) no relation was observed between CBF and VF. However, in the group of patients with moderate to severe WML (upper quartile) there was a relation between an increase in CBF and a decrease in VF after adjusting for age and sex. This relation remained after additional adjustment for vascular risk factors, IMT and presence of one or more lacunar infarcts. In perspective of the mean VF of 2.03% a change in VF of 0.18% per SD increase in CBF implies a strong relation.

Discussion

Table 2 Results of linear regression analyses with CBF as independent variable and measures of brain atrophy as dependent variable (n=828)

	Brain parenchymal fraction		Ventricular fraction	
	β	(95% confidence interval)	β	(95% confidence interval)
Model I *	0.07%	(-0.08 to 0.22)	-0.08%	(-0.14 to -0.02)
Model II †	0.08%	(-0.07 to 0.22)	-0.08%	(-0.14 to -0.03)
Model III ‡	0.07%	(-0.07 to 0.21)	-0.07%	(-0.13 to -0.02)

* Adjusted for age and sex

† Additionally adjusted for hypertension, diabetes, smoking, body mass index, hyperlipidemia, intima-media thickness and presence of lacunar infarcts

‡ Additionally adjusted for log white matter lesion volume

The β represents the change (95% confidence interval) in measures of brain atrophy per standard deviation (10.2 ml/min per 100 ml) increase in CBF. Lower brain parenchymal fraction indicates more global brain atrophy while higher ventricular fraction indicates more subcortical brain atrophy.

CBF = cerebral blood flow

Table 3 Results of linear regression analyses with CBF as independent variable and ventricular fraction as dependent variable

	Ventricular fraction			
	No or few WML (n=621)		Moderate to severe WML (n=207)	
	β	(95% confidence interval)	β	(95% confidence interval)
Model I *	-0.04%	(-0.09 to 0.02)	-0.16%	(-0.31 to -0.01)
Model II †	-0.04%	(-0.10 to 0.01)	-0.18%	(-0.34 to -0.02)

* Adjusted for age and sex

† Additionally adjusted for hypertension, diabetes, smoking, body mass index, hyperlipidemia, intima-media thickness and presence of lacunar infarcts

The β represents the change (95% confidence interval) in ventricular fraction per standard deviation (10.2 ml/min per 100 ml) increase in CBF. Higher ventricular fraction indicates more subcortical brain atrophy. Patients in the lower three quartiles of WML volume (≤3.2 ml) were categorized as having no or few WML while patients in the upper quartile of WML volume (>3.2 ml) were categorized as having moderate to severe WML.

CBF = cerebral blood flow; WML = white matter lesions

Among patients with manifest arterial disease we observed that a lower total CBF was related to more subcortical, but not global, brain atrophy if moderate to severe WML were present. In patients with no or few WML, no relation was observed between total CBF and brain atrophy.

Strengths of our study are, first, the large number of patients included in this study, which made precise estimates possible. Second, we used an accurate segmentation program to calculate volumes of WML and quantitative measures of cerebral atrophy, rather than visual rating scales. Thereby we increased the power to detect a relation between CBF and measures of atrophy. Third, since brain volume measurements were available, we had the possibility to adjust the total CBF for brain size, thereby removing residual confounding due to differences in brain size between patients. Fourth, we had extensive information on cardiovascular risk factors and other possible confounders, which made it possible to determine unbiased estimates of the relation between CBF and measures of atrophy.

A limitation of our study is the cross-sectional nature, which makes it difficult to disentangle cause from consequence. Although we hypothesized that a decrease in CBF leads to brain atrophy, we cannot exclude the possibility that the lower total CBF was the result of a decrease in metabolic demand caused by brain atrophy. In healthy aging there is an age-associated decrease in brain volume,[23] primarily caused by a decrease in neuronal size and partly due to a reduction in numbers of neurons caused by apoptosis.[24-26] Since the metabolism of the neurons that remain is also reduced, a lower CBF is sufficient to maintain neuronal viability.[27] However, if the decrease in CBF were a consequence of brain atrophy, we would have expected to find a relation between global brain atrophy and CBF, because the regional CBF in the cortical gray matter is two to three times higher than in the cerebral white

matter. Furthermore, the metabolic activity is larger in the cortical gray matter, which contains the majority of cerebral neurons. Therefore, we think that it is unlikely that brain atrophy preceded a decrease in CBF.

A possible explanation for the observed relation between lower total CBF and subcortical brain atrophy may be the vascular architecture of the cerebral white matter. The cerebral white matter is primarily supplied by long medullary penetrating arteries, which originate from the large cerebral arteries and run towards the white matter perpendicular to the brain surface. These vessels give off short branches that irrigate the deep white matter. The periventricular white matter is supplied by perforating branches of the medial cerebral artery. As there are no anastomoses between the deep perforating arteries and the medullary arterioles, the periventricular white matter is an arterial borderzone and particularly susceptible to injury caused by a decrease in CBF.[28] As a result, cerebral hypoperfusion may result in ischemic damage in the white matter, which in turn, may lead to loss of brain parenchyma and ex vacuo ventricular enlargement. Our finding that a lower CBF was not related to ventricular enlargement in patients with no or few WML suggests that cerebral hypoperfusion alone may not be sufficient to induce subcortical brain atrophy.

WML are the result of CSVD and are associated with an increase in cerebrovascular resistance and an incapacity to respond to decreases in cerebral perfusion pressure.[9] Although several vascular risk factors may be involved in the pathogenesis of CSVD, hypertension is considered the most important one.[29-32] The chronic hemodynamic stress of hypertension causes thickening and hardening of the walls of arterioles with narrowing of the lumen. Therefore, only the combination of a decrease in CBF and an impaired autoregulation in the cerebral white matter may result in subcortical brain atrophy.

Since our study sample consisted of subjects suffering from atherosclerotic disease, we do not know if our results can be generalized to the general population. However, since the relation between CBF and ventricular enlargement was independent of severity of atherosclerosis and was not explained by the presence of vascular risk factors and lacunar infarcts, we could argue that all persons with a decrease in CBF in the presence of moderate to severe WML may be at risk of developing subcortical brain atrophy. As WML are common in the general population,[33] our result may be relevant for large groups of elderly.

Furthermore, although we do not know the dementia status of our study population, it is unlikely that our results can be explained by demented patients, since our study was performed in a relative young population with a mean age of 58 years. It is therefore probable that the subcortical brain atrophy in our study population is still in its early phase. If future studies can confirm that a decrease in CBF precedes the development of subcortical brain atrophy, it may be interesting to investigate if changing the CBF can prevent brain atrophy and subsequent cognitive decline.

In conclusion, we found that a lower total CBF was associated with ventricular enlargement, but only in the presence of moderate to severe WML. Prospective

studies are needed to investigate the directionality of the relation between total cerebral blood flow and brain atrophy and to confirm the hypothesis that a decrease in total CBF in the presence of WML precedes the development of subcortical brain atrophy and cognitive decline.

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4.3

Total cerebral blood flow white matter lesions and cognitive functioning

Background

It has been hypothesized that cerebral hypoperfusion contributes to cognitive deterioration. Patients with white matter lesions (WML) are more vulnerable to a decrease in cerebral blood flow (CBF) due to an impaired autoregulation. We investigated the association between total CBF and cognitive performance and assessed whether this relation was modified by WML.

Methods

Within the SMART-MR study, a cohort study among patients with manifest arterial disease, cross-sectional analyses were performed in 472 patients (mean age 57 ± 10 years, 77% male) without cortical and large subcortical infarcts. Total CBF was measured with MR angiography in the internal carotid arteries and basilar artery, and was expressed per 100 ml brain volume. Brain segmentation was used to obtain volumetric measurements of total brain volume and WML volume. Infarcts were rated visually. Neuropsychological tests assessing executive functioning and memory were performed and scores were transformed into composite z-scores for both domains. We used linear regression analyses, adjusted for age, sex, education and intelligence, to investigate the association between CBF and cognitive performance and the effect of WML on the association between CBF and cognition.

Results

Mean CBF was 51 ± 10 ml/min/100 ml. We found that WML modified the association between CBF and executive functioning (P for interaction < 0.001). Therefore, we repeated the analysis for patients with WML volumes above increasing cut-off points. The association between lower CBF and worse performance on executive functioning became stronger and significant with increasing volumes of WML. Additional adjustment for vascular risk factors, intima-media thickness and lacunar infarcts did not materially change the association between CBF and executive functioning. Lower CBF was not associated with worse memory performance, and WML did not modify this association.

Conclusions

In this population, lower cerebral blood flow is associated with worse executive performance, but only in the presence of WML. These findings suggest that patients with WML are more vulnerable to hypoperfusion-related cognitive impairment. Longitudinal studies are needed to determine whether a decrease in cerebral blood flow increases the risk for cognitive decline and dementia.

Introduction

Cognitive deterioration and dementia are the result of a complex interplay of several pathological processes. Although neurodegenerative processes are considered important,[1] vascular disease also plays an essential role in the development of cognitive decline and dementia.[2,3] Vascular risk factors including hypertension,[4-6] hyperlipidemia,[6,7] diabetes mellitus,[5,8,9] hyperhomocysteinemia,[10] obesity,[11,12] and smoking[13] are associated with an increased risk of dementia. The association between vascular disease and cognitive deterioration is supposedly mediated by ischemic vascular lesions and brain atrophy.[14] This has led to the hypothesis that cerebral hypoperfusion is one of the mechanisms by which vascular disease may contribute to cognitive deterioration.[15,16] This hypothesis is supported by findings from several studies. Regional cerebral blood flow (CBF) is lower in demented and cognitively impaired patients, compared with cognitively healthy controls.[17-19] In a large longitudinal population-based study blood flow velocity in the medial cerebral artery was lower in patients with dementia and in nondemented subjects a higher blood flow velocity was related to less cognitive decline over the preceding 6 years.[20] Another study observed that total CBF was lower in patients with dementia compared with subjects of the same age with optimal cognitive function.[21] Finally, several cardiovascular conditions, such as congestive heart failure, orthostatic hypotension, and coronary artery bypass graft surgery, are associated with cerebral hypoperfusion and also with cognitive impairment and dementia.[16]

Recently, we found that lower total CBF, as measured with two-dimensional phase-contrast magnetic resonance angiography (MRA), was associated with ventricular enlargement, but only in patients with moderate to severe white matter lesions (WML).[22] We hypothesized that in patients with WML cerebral vasomotor reactivity is impaired and the capacity to respond adequately to a decrease in cerebral perfusion thereby reduced.[23,24] Since ventricular enlargement is associated with cognitive deterioration,[25,26] lower total CBF may also be associated with cognitive impairment, and in particular in patients with WML.

The purpose of this study was to investigate the association between total CBF and cognitive performance. In addition, we assessed whether the extent of white matter lesions (WML) modified a relation between total CBF and cognition. We hypothesized that a lower CBF was associated with worse cognitive performance and that this association was more pronounced in patients with moderate to severe WML.

Methods

SMART-MR Study

The present study is a cross-sectional study within the SMART-MR study, a prospective cohort study within the Second Manifestations of ARTerial disease (SMART) study.[27] All eligible patients, newly referred to the University Medical Center Utrecht with symptomatic atherosclerotic disease or risk factors for atherosclerosis, are screened

for additional risk factors and severity of atherosclerosis. The baseline examination is performed during a one day visit to our medical center and includes a physical examination, ultrasonography of the carotid arteries, and blood and urine sampling. Risk factors, medical history, and functioning are assessed using questionnaires that the patients fill in before their visit to the medical center.
Between May 2001 and December 2005, an MR investigation of the brain was added to the baseline examination as part of the SMART-MR study. The objective of the SMART-MR study is to investigate causes and consequences of brain changes on MRI in patients with vascular disease. Patients were eligible for an MRI of the brain if they were included with manifest coronary artery disease, cerebrovascular disease, peripheral arterial disease or an abdominal aortic aneurysm (AAA) and if they had no MR contraindications. Coronary artery disease was defined as myocardial infarction, coronary artery bypass graft surgery or percutaneous transluminal coronary angioplasty in the past or at inclusion. Patients with a transient ischemic attack (TIA) or stroke at inclusion and patients who reported stroke in the past were considered to have cerebrovascular disease. Peripheral vascular disease was defined as surgery or angioplasty of the arteries supplying the lower extremities in history or intermittent claudication or rest pain at inclusion. Present AAA (distal aortic anteroposterior diameter ≥3 cm) or previous AAA surgery was the criterion for AAA. Neuropsychological testing was introduced in the SMART-MR study starting January 2003, and was performed on the same day as the MR and other investigations. The SMART study and SMART-MR study were approved by the ethics committee of our institution and written informed consent was obtained from all participants.

Magnetic resonance protocol

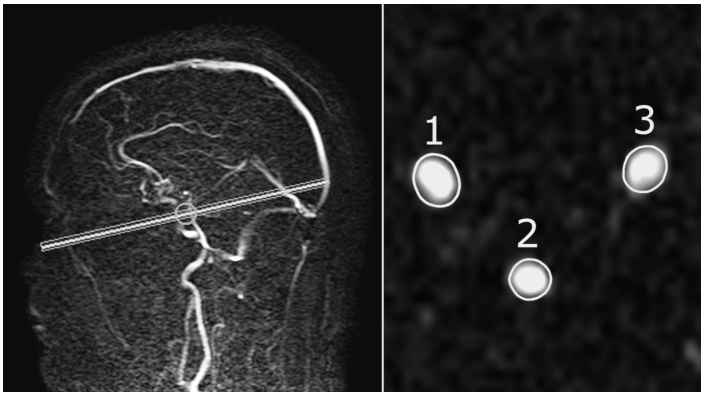
The MR investigations were performed on a 1.5-T whole-body system (Gyroscan ACS-NT, Philips Medical Systems, Best, the Netherlands). The protocol consisted of a transversal T1-weighted gradient-echo sequence (repetition time (TR)/echo time (TE): 235/2 ms; flip angle, 80°), a transversal T2-weighted turbo spin-echo sequence (TR/TE: 2200/11 ms and 2200/100 ms; turbo factor 12), a transversal T2-weighted fluid attenuating inverse recovery (FLAIR) sequence (TR/TE/inversion time (TI): 6000/100/2000 ms) and a transversal inversion recovery (IR) sequence (TR/TE/TI: 2900/22/410 ms) (field of view (FOV) 230 x 230 mm; matrix size, 180 × 256; slice thickness, 4.0 mm; slice gap, 0.0 mm; 38 slices).
On the basis of a localizer MR angiographic slab in the sagittal plane, a two-dimensional phase-contrast section was positioned at the level of the skull base to measure the volume flow in the internal carotid arteries (ICAs) and the basilar artery (BA). **Figure 1** illustrates the positioning of the two-dimensional phase-contrast section through the ICAs and the BA (TR/TE: 16/9 ms; flip angle, 7.5°; slice thickness, 5.0 mm; FOV, 250 x 250 mm; matrix size, 256 x 256; eight acquired signals; velocity sensitivity, 100 cm/sec).

Cerebral blood flow

Post-processing of the flow measurements was performed by one of the authors . For each vessel, the spatial and time-averaged flow velocity was calculated from the phase-difference images by manually drawing a region of interest around the vessel (**Figure 1**). The average flow velocity in each vessel was multiplied by the cross-sectional area of the pixels in the region of interest (ROI) to obtain the volume flow rate. Good agreement between repeated volume flow rate measurement post-processing procedures was shown in our research group, with a coefficient of variation of 5%.[28,29] The flow through the left and right ICAs and BA were summed to calculate the total CBF (ml/min).

We expressed total CBF per 100 ml brain parenchymal volume to obtain a measure of mean total brain perfusion, since part of the differences in total CBF between individuals can be attributed to differences in brain-size.[30]

Figure 1 Left: Sagittal localizer MRA image illustrating the positioning of a two-dimensional phase-contrast MR angiographic slab used to measure the volume flow through the internal carotid arteries and basilar artery. Right: Quantitativ flow values were obtained by integrating across manually drawn regions of interest that enclose the vessels (1 indicates right-sided internal carotid artery; 2 indicates basilar artery; 3 indicates left-sided internal carotid artery).



Brain segmentation

We used the T1-weighted gradient-echo, the IR sequence, and the FLAIR sequence for brain segmentation. The probabilistic segmentation technique has been described elsewhere.[31] In short, two preprocessing steps were performed. The first step was an intra-patient rigid registration in order to compensate for motion and scan variations.[32] The second preprocessing step was an automatic skull-stripping of the T1 image,[33] in order to define a proper region of interest for the segmentation process. The actual segmentation was done with a statistical method called k-Nearest Neighbor (KNN)

classification.[31] The result of the classification method is a probability value for each voxel that quantifies the amount of a specific tissue type contained in that voxel. Total volumes were calculated by multiplying these probabilities by the number and volumes of the voxels (4.0 x 0.9 x 0.9 mm). The segmentation program distinguishes cortical gray matter, white matter, sulcal and ventricular cerebrospinal fluid (CSF) and lesions.

Both WML and infarcts are classified as 'lesion' volume, since the segmentation program cannot distinguish between them. Therefore, an investigator and neuroradiologist visually checked the results of the segmentation analysis and made a further distinction into WML and infarct volumes by manually assigning the lesion volumes to one of these two categories.

Total brain volume was calculated by summing the volumes of gray and white matter and, if present, the volumes of WML or infarcts. The total intracranial volume (ICV) was calculated by summing the total brain volume and the volumes of the sulcal and ventricular CSF.

To avoid incorporation of neuronal tissue and CSF outside the cranium, an investigator determined the slice that included the foramen magnum. All volumes cranial to the foramen magnum were included in the segmentation results. As a result, the total brain volume includes the cerebrum, brainstem and cerebellum.

White matter lesions

The volumes of WML obtained with the segmentation program were summed to obtain the total volume of WML. We normalized WML volumes for intracranial volume to correct for differences in head size,[34] by dividing total WML volume by ICV and multiplying this by the average ICV of the study population (1460 ml).

Infarcts

The whole brain, including cortex, brainstem, and cerebellum, was visually searched for infarcts by two investigators and a neuroradiologist. Discrepancies in rating were re-evaluated in a consensus meeting. All raters were blinded for the history and diagnosis of the patient. Infarcts were defined as focal hyperintensities on T2-weighted images of at least 3 mm in diameter. Hyperintensities located in the white matter also had to be hypointense on T1-weighted images, in order to distinguish them from WML. Dilated perivascular spaces were distinguished from infarcts on the basis of their location (along perforating or medullary arteries, often symmetrical bilaterally, usually in the lower third of the basal ganglia or in the centrum semiovale), form (round/oval), and the absence of gliosis.[35] The location, affected flow territory and type were scored for every infarct. We categorized infarcts as cortical, large subcortical (>15 mm), infratentorial and lacunar infarcts (LI). We defined LI as infarcts sized 3 to 15 mm and located in the subcortical white matter, thalamus or basal ganglia.

Neuropsychological assessment

Cognitive performance was assessed with a set of standard neuropsychological tests, sensitive to mild impairments. Verbal memory was assessed with the 15-word learning

test (a modification of the Rey Auditory Verbal Learning test[36]). In 5 consecutive trials, an interviewer read out aloud a list of 15 words, after which the patients were asked to recall as many words as they could. The number of correctly recalled words for each trial is the immediate recall (range 0 to 15). After a period of approximately 25 minutes, during which other non-verbal tasks were performed, the patients were asked again to recall as many words as possible (delayed recall, range 0 to 15). Next, we calculated a retention score by dividing the number of words recalled after 25 minutes by the maximum number of words recalled during the immediate recall. A composite score for memory performance (MEM) was calculated by averaging the z-scores (individual test score minus mean test score divided by the standard deviation of that score) of the mean score of the 5 trials of the immediate recall, the z-score of the delayed recall, and the z-score of the retention score.

Executive functioning was assessed with the following three tests. The Visual Elevator test (subtest of the Test of Everyday Attention) is a timed test of 10 trials that measures mental flexibility.[37] Patients had to determine the floor on which a visually presented elevator was located. The elevator moves one floor each time and changes in direction when an arrow is presented. The timing score of the Visual Elevator test is equivalent to time per switch for correct items (seconds per switch). Second, the Brixton Spatial Anticipation test was used to assess the capacity to discover logical rules.[38] Patients were presented a booklet of 56 pages with on every page 10 circles of which one circle was filled. Patients had to predict which circle would be filled on the next page based on a pattern deduced from previous pages. In total this pattern changed eight times. The total number of errors made was scored. Third, the Verbal Fluency test was used to assess mental flexibility, shifting of attention, and employment of strategies.[39] For this test patients were asked to generate as many words as possible starting with the letter N within a one minute time-frame. Before calculating z-scores, the scores of the Visual Elevator test and Brixton Spatial Anticipation test were multiplied by minus one, so that on all tests a lower score denotes a worse performance. A composite score for executive functioning (EXEC) was estimated by averaging the z-scores of the Visual Elevator Test, the Brixton Spatial Anticipation test and the Verbal Fluency test.

Premorbid intellectual functioning was assessed using the Dutch version of the National Adult Reading Test (DART) in which patients had to read out loud a list of words with irregular pronunciation.[40] Educational level was divided into seven categories, graded from primary school to academic degree, according to the Dutch educational system.[37]

Vascular risk factors

During the patient's visit at the medical center, an overnight fasting venous blood sample was taken to determine glucose and lipid levels. Height and weight were measured without shoes and heavy clothing, and the body mass index (BMI) was calculated (kg/m^2). Systolic and diastolic blood pressures (mmHg) were measured twice with a sphygmomanometer and the average was obtained. Hypertension was defined as mean systolic blood pressure ≥140 mmHg or mean diastolic blood pressure

≥95 mmHg or use of antihypertensive drugs. Diabetes mellitus was defined as glucose ≥7.0 mmol/L or use of oral antidiabetic drugs or insulin. Hyperlipidemia was defined as total cholesterol >5.0 mmol/L, low-density lipoprotein cholesterol >3.2 mmol/L or use of lipid lowering drugs. Smoking habits and alcohol intake were assessed using questionnaires. Pack-years of smoking was calculated by the average number of cigarettes smoked per day was divided by 20 and then multiplied by the number of years of cigarette smoking. Alcohol consumption was divided into three categories: never, past, and current. Patients who had quit drinking during the past year were assigned to the category current alcohol intake. Ultrasonography was performed to measure the intima-media thickness (IMT) (mm) in the left and right common carotid arteries, represented by the mean value of six measurements.[41] IMT is a marker for the extent of subclinical atherosclerosis.

Study sample

A total of 1309 patients were investigated in the SMART-MR study. Since neuropsychological testing was not introduced until 2003, data on cognitive performance was present of 831 patients. Of these 831 patients, segmentation data was missing in 226 patients (missing MR sequences needed for the segmentation analysis, due to a temporary change in MR protocol (n=179), MR data irretrievable (n=11), missing FLAIR sequence (n=8) and motion or other artifacts (n=28)). Of the remaining 605 patients, CBF measurements were missing in 59 (missing Magneto-Optical Disc (n=47), flow measurement failed (n=7), and flow measurement not performed (n=5)). Age, sex, DART reading score and level of education were comparable for patients with (n=546) and without complete (n=285) data.
We calculated mean brain perfusion by dividing total CBF by total brain volume. In the presence of a large infarct this method may not reflect the mean brain perfusion due to large hardly perfused areas in the infarct. For this reason, we excluded 74 patients with cortical (n=54), large subcortical (n=5), or infratentorial infarcts (n=31) on MRI from our study sample. Patients with lacunar infarcts were not excluded since their volume rarely exceeds 0.5 ml.[42] Therefore, the analytical sample consisted of 472 patients.

Statistical analysis

We used linear regression analyses to investigate changes in composite z-scores for MEM and EXEC per SD decrease in CBF. In the first model we adjusted for age, sex, education and DART score. In the second model we additionally adjusted for systolic and diastolic blood pressure, diabetes mellitus, body mass index, hyperlipidemia, smoking, IMT and presence of lacunar infarcts.
We included an interaction term (CBF x normalized WML volume) in the model to investigate whether the association between CBF and cognitive performance was modified by WML volume. If the regression analyses revealed a significant interaction between CBF and volumes of WML ($P < 0.05$), we repeated the analyses for patients with WML volumes above increasing cut-off points, to investigate how the association

between CBF and cognitive functioning changed according to increasing volume of WML. In all analyses the 95% confidence intervals are given. SPSS version 14.0 (Chicago, Ill, USA) was used to analyze the data.

Results

In the study sample of 472 patients, the mean CBF ± SD was 51.4 ± 9.8 ml/min per 100 ml brain volume. The mean age ± SD was 57 ± 10 years and the majority (77%) was men. **Table 1** presents patient characteristics according to tertiles of CBF. Patients with a lower CBF were older, more often male, had a higher systolic blood pressure and more often had one or more lacunar infarcts on MRI. **Table 2** presents the mean raw scores of the neuropsychological tests according to tertiles of CBF. Patients in the lowest quartile of CBF had lower scores on the 15-word learning test and higher scores on the Brixton anticipation test and Visual elevator test, all indicating poorer performance.

Table 1 Patient characteristics according to tertiles of normalized total CBF (ml/min/100 ml) (n=472)

	CBF (ml/min per 100 ml)		
	<46.6 (n=158)	46.6 – 54.2 (n=156)	>54.3 (n=158)
Normalized total CBF, mean ± SD (ml/min per 100 ml)	41 ± 4	51 ± 2	62 ± 6
Age, mean ± SD (years)	60 ± 10	57 ± 10	54 ± 10
Male gender, %	86	73	72
Educational level, mean ± SD*	3.7 ± 2.0	3.7 ± 1.8	3.4 ± 1.6
Dutch Adult Reading Test score, mean ± SD	79 ± 18	80 ± 16	77 ± 17
Smoking, median (25^{th} percentile, 75^{th} percentile) (pack-years)	17 (6, 32)	14 (6, 31)	24 (9, 36)
Body mass index, mean ± SD (kg/m^2)	27 ± 3	27 ± 4	26 ± 4
Systolic blood pressure, mean ± SD	146 ± 20	141 ± 22	139 ± 20
Diastolic blood pressure, mean ± SD	84 ± 11	83 ± 12	82 ± 10
Diabetes mellitus, %	24	14	19
Hyperlipidemia, %	78	83	77
Intima-media thickness, mean ± SD (mm)	0.92 ± 0.27	0.90 ± 0.22	0.87 ± 0.24
WML volume, median (25^{th} percentile, 75^{th} percentile) (ml)	1.7 (1.0, 3.3)	1.3 (0.8, 2.4)	1.4 (0.8, 2.7)
One or more lacunar infarcts, %	15	7	8

* Educational level was divided into seven categories, graded from primary school to academic degree, according to the Dutch educational system

CBF = cerebral blood flow; SD = standard deviation; WML = white matter lesions

Table 2 Raw scores for neuropsychological tests according to tertiles of normalized total CBF (ml/min /100 ml) (n=472)

	CBF (ml/min/100 ml)		
	<46.6 (n=158)	46.6 – 54.2 (n=158)	>54.3 (n=158)
15-word learning task			
Immediate recall, mean (number of words)	7.3 ± 2.0	7.9 ± 1.9	7.9 ± 2.0
Delayed recall (number of words)	6.8 ± 2.7	7.9 ± 2.8	7.8 ± 3.0
Retention score (%)	68.6 ± 19.3	74.8 ± 19.5	73.5 ± 18.4
Visual elevator test, timing score (seconds per switch)	5.2 ± 2.6	4.9 ± 2.0	5.0 ± 2.0
Brixton spatial anticipation test (total number of errors)	18.4 ± 6.7	17.9 ± 6.0	17.3 ± 5.1
Verbal fluency (words with letter N, during 1 minute)	10.3 ± 4.7	10.7 ± 4.0	10.2 ± 4.0

A higher score on the 15-word learning task and the Verbal fluency task indicates a better performance. A higher score on the Visual elevator test and Brixton spatial anticipation test indicates a worse performance.

Table 3 presents the results for the linear regression analyses with z-scores for MEM and EXEC as dependent variable. A decrease in normalized CBF of one SD (9.8 ml/min per 100 ml) was not associated with worse performance on MEM (β= -0.04; 95%CI -0.13 to 0.04). Additional adjustment for systolic and diastolic blood pressure, diabetes, smoking, body mass index, hyperlipidemia, intima-media thickness and presence of lacunar infarcts did not materially change the results (β= -0.05; 95%CI -0.13 to 0.04). Furthermore, WML did not modify the association between CBF and MEM (interaction term P = 0.451).

Table 3 Change in z-scores for executive functioning (EXEC) and memory (MEM) per standard deviation decrease in CBF (n=472)

	MEM		EXEC	
	β	(95% confidence interval)	β	(95% confidence interval)
CBF (per SD decrease) *	-0,04	(-0.13 to 0.04)	-0,04	(-0.12 to 0.04)

*Adjusted for age, sex, educational level and Dutch Adult Reading Test score
The β represents the change (95% confidence interval) in z-scores for memory (MEM) and executive functioning (EXEC) per standard deviation (9.8 ml/min per 100 ml) decrease in CBF. A lower z-score denotes a worse performance.
CBF = cerebral blood flow

We did not find an association between CBF and performance in EXEC in the total study sample. However, as we included the interaction term in the model, a significant interaction between CBF and WML volume for EXEC was found (interaction term P < 0.001). Therefore we performed consecutive analyses in our study population, only including patients with WML volumes above increasing cut-off points. **Table 4** presents the results of these analyses. The association between lower CBF and worse performance on executive functioning became stronger with increasing volumes of WML. When we performed these analyses with additional adjustments for vascular risk factors, IMT and presence of lacunar infarcts the results remained essentially the same.

Table 4 Association between CBF and z-score for executive functioning (EXEC) for patients with WML volumes above increasing cut-off points

WML volume cut-off point	N	β	(95% confidence interval)
> 0.0 ml *	472	-0.04	(-0.12 to 0.04)
> 1.0 ml	316	-0.07	(-0.17 to 0.03)
> 2.0 ml	173	-0.10	(-0.24 to 0.05)
> 3.0 ml	112	-0.17	(-0.37 to 0.02)
> 4.0 ml	74	-0.27	(-0.52 to -0.02)
> 5.0 ml	63	-0.25	(-0.56 to 0.07)
> 6.0 ml	51	-0.26	(-0.60 to 0.09)
> 7.0 ml	42	-0.38	(-0.76 to 0.00)
> 8.0 ml	30	-0.58	(-1.08 to -0.09)
> 9.0 ml	22	-0.71	(-1.16 to -0.26)
> 10.0 ml	21	-0.71	(-1.16 to -0.26)
> 11.0 ml	20	-0.73	(-1.21 to -0.25)
> 12.0 ml	16	-0.71	(-1.32 to -0.10)

* Equals the total study population.
β represents the change (95% confidence interval) in z-score for EXEC per standard deviation (9.8 ml/min per 100 ml) decrease in CBF, adjusted for age, sex, educational level and Dutch Adult Reading Test score. A lower z-score denotes a worse performance.
CBF = cerebral blood flow; WML = white matter lesions

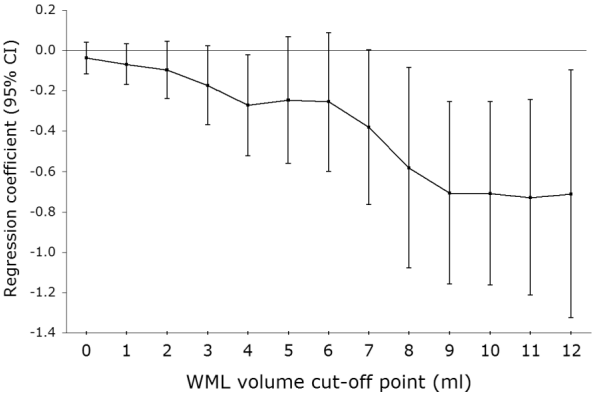


Figure 2 Graphic illustration of the association between cerebral blood flow and z-score for executive functioning for patients with WML volumes above increasing cut-off points. The regression coefficient (95%CI) represents the change in z-score for executive functioning per standard deviation (9.8 ml/min per 100 ml) decrease in cerebral blood flow, adjusted for age, sex, educational level and Dutch Adult Reading Test score. A lower z-score denotes a worse performance.
CI = confidence interval; WML = white matter lesions

Discussion

In a population of patients with manifest arterial disease we observed that lower total cerebral blood flow corrected for total brain volume was associated with worse executive functioning, and that this association was stronger with larger white matter lesion volumes. This association was independent of age, sex, education, intelligence, vascular risk factors, extent of subclinical atherosclerosis and presence of lacunar infarcts. A lower total CBF was not associated with worse performance on verbal memory, nor did WML modify this association.

The finding that the relation between CBF and executive functioning depended on the extent of WML suggests that lower CBF by itself is not sufficient to induce cognitive impairment. White matter lesions are the result of ischemic damage due to thickening and hardening of the walls of arterioles with narrowing of the lumen and are associated with an increase in cerebrovascular resistance and an impairment of vasomotor reactivity.[24] Possibly, only the combination of a decrease in CBF and an impaired autoregulation in the cerebral white matter may result in cognitive impairment. Recently, we found that lower total CBF was associated with subcortical brain atrophy.[22] This association also depended on the presence of WML, supporting our hypothesis that the relation between lower CBF and poorer executive functioning is the result of ischemic damage in the subcortical white matter.

The observation that lower CBF was associated with poorer executive functioning may be explained by the vascularization of the subcortical white matter. It is thought that disruption of frontal-subcortical circuits results in executive impairment.[43,44] The subcortical white matter that houses these frontal-subcortical circuits is especially vulnerable to ischemia. The cerebral white matter is, compared with the cortex, poorly vascularized by long penetrating and perforating arteries that give off short branches.[45] Furthermore, the cerebral white matter is an arterial borderzone, since the arteries that irrigate the white matter do not anastomose, and therefore are susceptible to injury caused by hypoperfusion.[46] As a result, cerebral hypoperfusion may result in ischemic damage in the white matter, which in turn, may lead to interruption of the frontal-subcortical circuits and consequently to impairment of executive functioning.

We did not find an association between lower CBF and memory performance, nor did WML modify this association. Our finding that lower CBF was only associated with subcortical brain atrophy, but not with global brain atrophy,[22] also suggests that a lower CBF mainly affects the subcortical white matter. It is possible that structures involved in memory functioning are less vulnerable to reduced CBF. However, it has been found that the hippocampus is more sensitive to hypoxia, compared with other cortical gray matter structures.[47,48] Another explanation for the absence of a relation between CBF and memory is that our study population is too young to find this relation. We therefore cannot exclude the possibility that in older subjects lower CBF can induce loss of neurons in the hippocampus, and eventually result in memory dysfunction.

Strengths of our study are the large number of patients investigated and the use of neuropsychological tests which are sensitive to mild impairments. Furthermore, as quantitative brain volume measurements were available, we had the possibility to adjust total CBF for brain size, thereby removing residual confounding due to differences in brain size between patients. The extensive information on vascular risk factors and extent of subclinical atherosclerosis allowed us to investigate whether the association between CBF and cognitive performance was independent of these possible confounders. A limitation of our study is the cross-sectional design. Therefore, we are unable to distinguish cause from consequence. Although we hypothesized that a decrease in total CBF precedes cognitive deterioration, we cannot exclude the possibility that the lower total CBF was the result of decreases in metabolic demand in the diseased brain.

Our study sample consisted of patients with atherosclerotic disease. Therefore, we do not know if our results can be generalized to the general population. Possibly, patients with arterial disease are more susceptible to the consequences of a decrease in CBF, and the association between CBF and cognitive performance is less apparent in subjects free of vascular disease. However, because the association between CBF and cognition was not explained by vascular risk factors and extent of subclinical atherosclerosis, and because WML are also prevalent in older subjects in the general population, our results may still be clinically relevant the population at large. Furthermore, since our study was performed in a relative young population with a mean age of 57 years, it is conceivable that the poorer cognitive performance in our study population is still in its early phase.

In conclusion, in a population of patients with arterial disease, lower total cerebral blood flow in combination with white matter lesions was related to poorer executive functioning. Cerebral blood flow was not associated with memory performance. These findings suggest that patients with WML are more vulnerable to hypoperfusion-related cognitive impairment. Longitudinal studies are needed to establish whether a decrease in total cerebral blood flow precedes cognitive decline.

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5

General discussion

Brain atrophy in patients with arterial disease

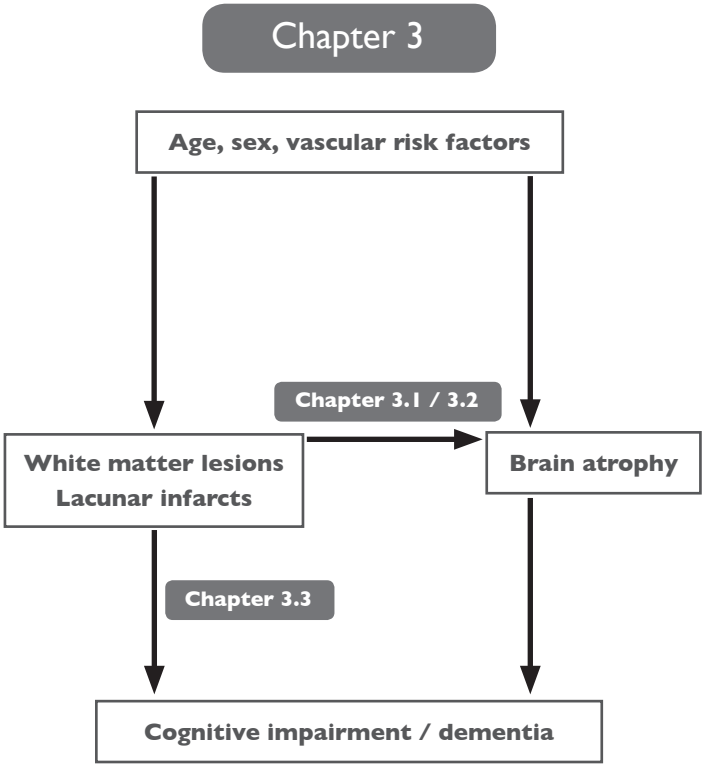
The overall aim of this thesis was to investigate the role of vascular pathology in the development of brain atrophy and subsequent cognitive decline in patients with arterial disease. Vascular risk factors have been related to brain atrophy, suggestive of an important role for vascular disease in the etiology of brain atrophy.[1-5] Patients with arterial disease can therefore be considered a population at high risk of developing brain atrophy and subsequent cognitive decline. The first objective of this thesis was to estimate brain volumes and cerebrovascular lesions on MRI within the SMART-MR study, a large cohort of patients with coronary heart disease, cerebrovascular disease, peripheral arterial disease and abdominal aortic aneurysm. We expected that in this population brain atrophy would be more severe as compared to the general population. Interestingly, we found no apparent differences in total brain volume and rate of decline in total brain volume when we compared our results with estimates obtained from literature from several large population-based studies with similar age range (**chapter 2**). However, subcortical brain atrophy, visualized on MRI as ventricular enlargement, and cerebrovascular pathology on MRI was more severe in our study population. Compared to estimates from several population-based studies, white matter lesion volume was larger, and the prevalence of asymptomatic infarcts, mainly lacunar infarcts, was higher.

Cerebral small-vessel disease and brain atrophy

The second objective of this thesis was to investigate whether white matter lesions and lacunar infarcts were associated with brain atrophy, and whether white matter lesions and lacunar infarcts were associated with cognitive impairment independent of brain atrophy (**chapter 3**) (**Figure 1**). White matter lesions and lacunar infarcts are considered to be the result of cerebral small-vessel disease. In elderly people, they are often observed on MRI in the presence of brain atrophy. Since brain atrophy and cerebral small-vessel disease are associated not only with older age but also with vascular risk factors, their co-occurrence may be explained by shared vascular risk factors or other shared factors associated with aging. However, it is also possible that the disturbances of white matter integrity due to the underlying small-vessel disease are a direct cause of brain atrophy by causing ischemic damage to axons, oligodendrocytes and other glial cells.[6,7]

Our systematic review of the literature showed that many investigators studied the association between white matter lesions and brain atrophy (**chapter 3.1**). The majority of these studies found significant associations between white matter lesions and measures of global, cortical and subcortical brain atrophy. The studies that did not find an association between WML and brain atrophy included a smaller number of subjects and used less accurate visual rating scales. This may have resulted in low statistical power to detect a significant relationship. Nevertheless, few studies investigated whether the observed association between white matter lesions and brain atrophy was independent of shared risk factors. Therefore, based on the current literature it remains difficult to establish whether white matter lesions contribute to the development of global brain atrophy.

Figure 1 In chapters 3.1 and 3.2 we investigated whether white matter lesions and lacunar infarcts were associated with brain atrophy independent of shared risk factors, and in chapter 3.3 we investigated whether white matter lesions and lacunar infarcts were associated with cognitive impairment independent of brain atrophy.



The association between white matter lesions and medial temporal lobe atrophy, which is considered to be one of the first structures affected in Alzheimer's disease, has also been investigated by many investigators (**chapter 3.1**). It is noteworthy that studies, which assessed medial temporal lobe atrophy visually, often found an association with white matter lesions, while almost all studies that used volumetric measurements of the hippocampus did not find an association. Visual rating of medial temporal lobe atrophy not only assesses the hippocampus, but also the size of the temporal horn of the lateral ventricle and the choroid fissure. It is thus possible that concomitant ventricular enlargement explains the observed associations between white matter lesions and visually assessed medial temporal lobe atrophy. Therefore, based on the available evidence we can not conclude that white matter lesions are associated with neurodegenerative processes in the medial temporal lobe.

Following the literature, we examined whether white matter lesions and lacunar infarcts were associated with brain atrophy measures (**chapter 3.2**). We found that white matter lesions were associated with total brain atrophy, cortical gray matter atrophy and ventricular enlargement, and that this association was independent of shared risk factors. These findings provide support for the hypothesis that microvascular pathology contributes to neurodegeneration. Although our cross-sectional design does not allow discerning cause from consequence, it is likely that white matter lesions precede brain atrophy. Findings from a longitudinal study, in which nondemented elderly with extensive white matter lesions at baseline experienced a substantially greater loss of brain volume during subsequent years than patients without white matter lesions, support the hypothesis that white matter lesion progression precedes loss in parenchymal volume.[8] Presumably, cerebral small-vessel disease leads to ex vacuo enlargement of the ventricles through loss of subcortical white matter as a result of ischemic changes. Subsequently, damage to the axons located in the region of the white matter lesions may cause deafferentation of cortical–subcortical connections and subsequent loss of cortical neurons.

We found that presence of one or more lacunar infarcts was associated with total brain atrophy and ventricular enlargement, but not with cortical atrophy (**chapter 3.2**). It could be argued that direct tissue loss in the infarcted subcortical gray or white matter explains the relation with ventricular enlargement. However, the volume of a lacunar infarct generally not exceeds 0.5 milliliter,[9] and the volume loss associated with the presence of lacunar infarcts that we observed was higher, suggesting that ischemic damage is not confined to the region visible on MRI. While white matter lesions were associated with cortical gray matter atrophy, lacunar infarcts were not. An explanation may be that lacunar infarcts mostly occur in the subcortical gray matter nuclei,[10] and they are therefore less likely than white matter lesions to interrupt the cortical–subcortical connections. Other studies that investigated the association between lacunar infarcts and brain atrophy also did not find an association between lacunar infarcts and cortical atrophy.[11,12] We cannot exclude the possibility, though, that lack of statistical power prevented finding a significant association, because only a small proportion of patients in our study population had multiple lacunar infarcts.

We then investigated whether white matter lesions and lacunar infarcts were associated with cognitive impairment, and whether an association was independent of brain atrophy. We observed that white matter lesions and the presence of multiple lacunar infarcts were both associated with worse executive functioning (**chapter 3.3**). The association of white matter lesions and lacunar infarcts with executive dysfunction may be explained by disruption of the frontal-subcortical circuits, and especially disruption of the connections to the prefrontal dorsolateral cortex which is involved in executive functioning.[13] Subcortical brain atrophy partly explained the association between white matter lesions and executive impairment. However, the association between lacunar infarcts and executive functioning was not explained by brain atrophy. Since lacunar infarcts located in the basal ganglia or thalamus are strongly associated with cognitive impairment,[14,15] it is possible that these strategic infarcts impair executive functioning in the absence of detectable brain atrophy.

White matter lesions and lacunar infarcts were not associated with worse memory performance (**chapter 3.3**). This is not surprising, since memory performance as assessed in our study is mainly a function of structures in the medial temporal lobe and memory impairment is not a distinctive feature of subcortical ischemic vascular disease.[16,17] Previous studies that did find associations between white matter lesions and poorer memory performance,[18,19] did not find this association after adjustment for medial temporal lobe atrophy,[18,19] suggesting that medial temporal lobe atrophy accounted for the association.

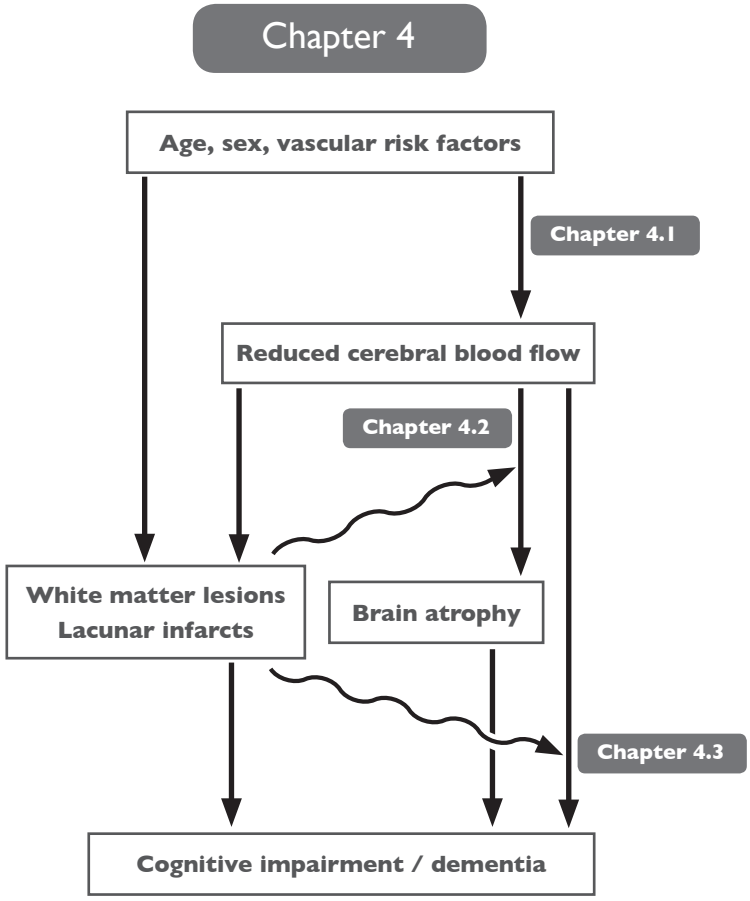
Cerebral blood flow and brain atrophy

The third objective of this thesis was to investigate the association between cerebral blood flow, brain atrophy and cognitive functioning (**chapter 4**) (**Figure 2**). We observed in our population that total cerebral blood flow, as measured with two-dimensional phase-contrast MR angiography in the carotid and basilar arteries, was comparable with subjects from the general population. Patients with a history of transient ischemic attack or stroke had a lower total cerebral blood flow compared with patients with arterial disease at other locations (**chapter 4.1**). In this study, we did not adjust for brain volume, and therefore we do not know whether the lower total cerebral blood flow in patients with cerebrovascular disease can be attributed to smaller brain volumes.

In the following studies we obtained a measure of mean brain perfusion by dividing total cerebral blood flow by total brain volume. Using this measure, we found that a lower cerebral blood flow was associated with subcortical brain atrophy, but not with total brain atrophy (**chapter 4.2**). These findings suggest that the vulnerability of the brain to a lower perfusion differs between brain regions. The vulnerability of subcortical white matter has been described earlier, and can mainly be attributed to the fact that the periventricular white matter is an arterial borderzone, due to its lack of anastomoses.[20] We also observed that the association between lower cerebral blood flow and subcortical brain atrophy was modified by the extent of white matter lesions. In patients with cerebral small-vessel disease the walls of the arterioles are thickened and hardened, and the lumen is narrowed. This leads to an impairment of cerebral vasomotor reactivity, reducing the capacity to respond adequately to a decrease in cerebral perfusion.[21,22] Therefore, the relation between hypoperfusion and brain atrophy may be more pronounced in patients with cerebral small-vessel disease.

Our observation of a selective vulnerability of subcortical brain tissue to a lower cerebral blood flow raised the question whether impairments in specific cognitive domains were associated with a lower cerebral blood flow. We found that a lower cerebral blood flow was associated with impairment in executive functioning, but not with memory performance (**chapter 4.3**). This finding is in correspondence with our finding in **chapter 4.2** that lower cerebral blood flow was associated with subcortical pathology. Possibly, a lower cerebral blood flow induces ischemic

Figure 2 In chapter 4.1 we investigated determinants of total cerebral blood flow. In chapters 4.2 and 4.3 we investigated the association of cerebral blood flow with brain atrophy and cognitive functioning, and whether white matter lesions modified these associations.



damage to the vulnerable subcortical white matter, which then disrupts subcortical-cortical connections and subsequently leads to executive impairment. White matter lesions are associated with executive impairment as well as with lower cerebral blood flow.[23] This makes it conceivable that white matter lesions are an intermediate in the association between cerebral blood flow and executive impairment. However, the role of hypoperfusion in the pathogenesis of brain atrophy and cognitive decline is probably far more complex, since we found that presence of white matter lesions strongly modified the association between lower cerebral blood flow and executive

impairment. Thus, lower cerebral blood flow may not only lead to ischemic damage in the subcortical white matter, but by causing impairment in autoregulatory processes patients may also become more vulnerable to this cerebral hypoperfusion. This hypothesis is also supported by our finding that white matter lesions and subcortical brain atrophy were more severe in patients with arterial disease, and that their progression accelerated with increasing age (**chapter 2**).

Brain atrophy or subcortical ischemic vascular disease?

The studies described in this thesis showed that white matter lesions, lacunar infarcts, and subcortical brain atrophy were highly prevalent in our study population, suggesting that subcortical vascular pathology, and not global brain atrophy, is a distinctive feature in patients with arterial disease. The role of, in particular, white matter lesions emerged to be complex. White matter lesions seem to contribute to development of brain atrophy, but they also appear to be and independent risk factor for impairment in executive functioning. Furthermore, white matter lesions may make persons more vulnerable to hypoperfusion.

The finding of a high prevalence of subcortical vascular pathology suggests that patients with arterial disease may be at increased risk of developing subcortical ischemic vascular disease. The term vascular cognitive impairment was recently coined to describe a range of cognitive disorders related to cerebrovascular disease,[17,24] with subcortical ischemic vascular disease, characterized by white matter lesions and lacunar infarcts, as a major subtype.[25] It was also proposed that vascular cognitive impairment is characterised by a specific cognitive profile involving preserved memory with impairments in attentional and executive functioning.[17,19]

Global and cortical brain atrophy appeared to be a less prominent feature of our population. Our estimates of brain volumes were very similar as found in the general population in the same age range, and global and cortical brain atrophy did not explain the associations of cerebral small-vessel disease and cerebral blood flow with cognitive impairment. It is interesting to note that our study population consisted of patients who were, with a mean age of 57 years, relatively young. At this age, the risk for dementia is low, and it is likely that the brain atrophy and cognitive impairment are in an early phase. We speculate that subcortical ischemic vascular disease is a contributing factor in the pathogenesis of cognitive decline and dementia at a stage before brain atrophy contributes to this process.

Our results are not only interesting from an etiologic point of view, but may also be clinically relevant. First, the vascular origin of subcortical ischemic brain damage makes it a potentially treatable target to prevent or slow down the development of brain atrophy and cognitive decline. This is especially important since vascular cognitive impairment may already be the most common form of cognitive impairment.[26] Also, the burden of vascular cognitive impairment will even further increase with the expected increase in vascular disease in the population.[27] Second, as our patients were relatively young their brain atrophy and cognitive impairment are probably at an

early stage. If interventions become available to prevent progression of brain atrophy and cognitive decline, they are more likely to be effective in early stages of the disease. Third, patients with manifest arterial disease are probably at high risk of developing vascular cognitive impairment, and therefore these patients are the most likely to benefit from intervening therapies.

Future research

With the studies described in this thesis we tried to provide further insight in the complex associations between vascular pathology, brain atrophy and cognitive decline. Obviously, since all studies in this thesis were cross-sectional it is not possible to draw conclusions about causality or the directionality of the associations. Longitudinal studies are needed to determine whether subcortical ischemic vascular disease indeed precedes brain atrophy, and whether lower cerebral blood flow precedes or results from cerebrovascular pathology.
Starting in January 2006 all patients from the SMART-MR study have been invited for a second visit to our medical center after a mean follow-up period of 4 years. All patients are invited for follow-up examinations, including risk factor assessment, an MRI of the brain, and cognitive testing. Furthermore, we have added an MR sequence for volumetric assessment of the hippocampus and have extended the neuropsychological test battery to investigate more cognitive domains. Finally, the dementia status will be determined for all participants. This follow-up study will allow us to further investigate causes and consequences of progression of vascular brain pathology on MRI, and may thereby help to better understand the underlying mechanisms of vascular cognitive impairment.

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6

Summary
Nederlandse samenvatting
Dankwoord
Curriculum Vitae
Appendix

Brain atrophy is often observed on magnetic resonance imaging (MRI) in the elderly. This is of clinical importance since the extent and rate of progression of brain atrophy are associated with future cognitive deterioration and conversion to Alzheimer's dementia. In the last decade population-based studies have shown that the role of vascular disease in the development of cognitive decline is more important than was thought before. Cognitive impairment due to neurodegeneration and due to vascular disease were traditionally regarded as separate clinical and pathophysiological entities. However, it is nowadays recognized that vascular pathology and neurodegenerative processes may not act independently in the pathogenesis of cognitive decline and more research is needed to understand the role of vascular pathology in the development of brain atrophy and cognitive deterioration.

The overall aim of this thesis was to gain further insight in the contribution of vascular pathology to the development of brain atrophy and cognitive decline. The first objective of this thesis was to estimate brain volumes and cerebrovascular lesions on MRI in a large cohort of patients with manifest arterial disease (**chapter 2**). The second objective was to investigate whether white matter lesions and lacunar infarcts, as indicators of cerebral-small vessel disease, are associated with brain atrophy and whether white matter lesions and lacunar infarcts are associated with cognitive impairment, independent of brain atrophy (**chapter 3**). The third objective was to investigate the associations between cerebral blood flow, brain atrophy and cognitive functioning (**chapter 4**).

With recently developed methods for automated segmentation of brain structures, it is possible to make accurate estimations of brain volumes and volume of white matter lesions. Recent studies on basis of these techniques reported brain volumes and volumes of white matter lesions in the general elderly population. In **chapter 2**, we estimated total brain, cortical gray matter, ventricular and white matter lesion volumes for 1044 patients (mean age 58 ± 10 years, 79% male) with arterial disease. We also described the prevalence of asymptomatic brain infarcts for this population, and we investigated age- and sex-related differences in brain volumes and silent brain infarcts. Total brain volume was 78.8% of intracranial (ICV) in men and 79.9% in women, and decreased with 0.18% per year. Cortical gray matter volume also decreased with age, but stronger in men than in women. Ventricular volume was 2.16% of ICV in men and 1.83% in women and increased with age, similarly in men and women. WML volume also increased with older age, but more strongly in men than in women. Silent brain infarcts were present in 14% of men and women, and increased to 24% of subjects aged 65 years or older. The decrease in total brain volume and cortical gray matter volume with increasing age was comparable with findings from the general population. However, subcortical brain atrophy and vascular pathology on MRI, as indicated by white matter lesions and silent brain infarcts, were more common in patients with arterial disease.

White matter lesions and brain atrophy are often found simultaneously on MRI in the elderly. Shared vascular risk factors may be an explanation for their concomitance. However, disturbances of white matter integrity could also be involved in the pathogenesis of brain atrophy. In **chapter 3.1**, we systematically reviewed the literature for studies that investigated the association of white matter lesions (WML) with measures of global brain atrophy and measures of medial temporal lobe atrophy. We also investigated whether there was sufficient evidence that this relation is independent of shared risk factors. We searched PubMed for studies published between 1980 and October 2007, combining search terms for WML with search terms for brain atrophy. For every study we recorded type and age of the study population, type and assessment of WML and brain atrophy, and the variables for which adjustments were made in the analyses. We identified 48 relevant studies. A significant relation between WML and brain atrophy was found in 37 studies, but only 10 out of 48 studies adjusted for shared risk factors. The majority of studies found an association between WML and brain atrophy, but it is not yet clear if this association is independent of shared risk factors.

In **chapter 3.2**, we investigated the independent association of white matter lesions (WML) and lacunar infarcts (LI) with global, cortical and subcortical brain atrophy in 840 patients with manifest arterial disease. Brain segmentation was used to quantify volumes of brain tissue, cerebrospinal fluid and WML. Total brain volume, ventricular volume and cortical gray matter volume were divided by intracranial volume to obtain brain parenchymal fraction (BPF), ventricular fraction (VF) and cortical gray matter fraction (GMF). Location and number of infarcts were rated visually. Mean ± SD BPF was 79.3 ± 2.8%, mean ± SD VF was 2.01 ± 0.95%, and mean ± SD GMF was 36.6 ± 3.3%. Linear regression analyses, adjusted for age, sex, vascular risk factors, intima-media thickness and LI showed that in patients with moderate to severe WML (upper quartile) BPF was lower (-0.51%; 95%CI -0.93 to -0.08), VF was higher (0.48%; 95%CI 0.31 to 0.65) and GMF was lower (-1.48%; 95%CI -2.07 to -0.88) than in patients with few WML (lower quartile). Presence of LI was associated with lower BPF (-0.52%; 95%CI -0.96 to -0.07) and higher VF (0.25%; 95%CI 0.07 to 0.42), but not with GMF, independent of WML and other potential confounders. WML are associated with global, subcortical and cortical brain atrophy, whereas LI are associated with global and subcortical atrophy, but not with cortical atrophy, suggesting an independent role for WML and LI in the pathogenesis of brain atrophy.

In **chapter 3.3**, we investigated whether white matter lesions (WML) and lacunar infarcts (LI) are independently associated with executive functioning and memory performance, and assessed whether these associations are mediated by global, cortical or subcortical brain atrophy. Neuropsychological tests assessing executive functioning (EXEC) and memory (MEM) were performed in 522 patients with arterial disease and raw scores were transformed into z-scores. We used linear regression analyses, adjusted for age, sex, education, intelligence, and vascular risk factors to investigate the association between WML, number of LI and cognitive performance.

One standard deviation higher WML volume was associated with worse EXEC (ß = -0.12; 95%CI -0.20 to -0.04), independent of LI and other covariates. Subcortical, but not global or cortical brain atrophy, attenuated this association (ß for EXEC = -0.09; 95%CI -0.18 to 0.02). Presence of two or more LI was associated with worse EXEC (ß = -0.48; 95%CI -0.87 to -0.09), independent of WML, other covariates and measures of brain atrophy. WML and LI were not associated with worse memory performance. Our findings not only suggest that WML and LI are associated with brain atrophy, but also show that they are both associated with worse executive functioning, independent of brain atrophy.

Brain perfusion can be assessed with several imaging techniques, but the majority of perfusion measurement techniques are impractical in large populations due to their complexity and their use of ionizing radiation or contrast agents. Therefore, large scale epidemiologic studies assessing cerebral blood flow are scarce. In **chapter 4.1** we investigated whether age, sex, vascular risk factors and location of arterial disease were associated with total cerebral blood flow (tCBF). We also compared total cerebral blood flow values from our study population with those from another study that were obtained in a sample of the general population. A higher total cerebral blood flow was found in patients with symptomatic vascular disease, but this association was statistically significant in only those patients in the 7th decade of life. The mean tCBF decreased with increasing age (-3.4 mL/min per year; 95% CI: -4.3 to -2.5). Diabetes (-27.6 mL/min; 95% CI: -52.6 to -2.6) and increasing body mass index (BMI) (-2.8 mL/min per BMI unit; 95% CI: -5.3 to -0.2) were associated with lower tCBF. Patients with vascular disease in a cerebral location had lower tCBF values (-39.7 mL/min; 95% CI: -65.1 to -14.3) than did patients with symptomatic vascular disease elsewhere in the vascular tree.

Possibly, cerebral hypoperfusion contributes to the pathogenesis of brain atrophy. In **chapter 4.2** we examined the relationship between cerebral blood flow (CBF) and global and subcortical brain atrophy, and investigated whether white matter lesions (WML) modified the relation between cerebral blood flow and brain atrophy. Total CBF was measured with MR angiography in 828 patients with arterial disease and was expressed per 100 ml brain volume. Linear regression analyses showed that lower CBF was associated with more subcortical brain atrophy, after adjusting for age, sex, vascular risk factors, intima-media thickness and lacunar infarcts, but only in patients with moderate to severe WML (upper quartile of WML): change in VF per SD decrease in CBF 0.18%, 95% CI 0.02 to 0.34. Our findings suggests that cerebral hypoperfusion in the presence of WML may cause subcortical brain atrophy, but longitudinal studies are needed to determine whether a lower CBF precedes the development of brain atrophy or is the result.

In **chapter 4.3**, we investigated whether cerebral blood flow (CBF) was associated with executive functioning and memory performance, and also investigated whether white matter lesions (WML) modified the relation between cerebral blood flow

and cognitive performance. We used linear regression analyses, adjusted for age, sex, education and intelligence, to investigate the association between CBF and cognitive performance and the effect of WML on the association between CBF and cognition. We found that WML modified the association between CBF and executive functioning (P for interaction < 0.001). Therefore, we repeated the analysis for patients with WML volumes above increasing cut-off points. The association between lower CBF and worse performance on executive functioning became stronger with increasing volumes of WML. Additional adjustment for vascular risk factors, intima-media thickness and lacunar infarcts did not materially change the association between CBF and executive functioning. Lower CBF was not associated with worse memory performance, and WML did not modify this association. In this population, lower cerebral blood flow is associated with worse executive performance, but only in the presence of WML. These findings suggest that patients with WML are more vulnerable to hypoperfusion-related cognitive impairment. Longitudinal studies are needed to determine whether a decrease in cerebral blood flow increases the risk for cognitive decline and dementia.

The studies described in this thesis showed that white matter lesions, lacunar infarcts, and subcortical brain atrophy were highly prevalent in our study population. White matter lesions seem to contribute to the development of brain atrophy, but they also appear to be an independent risk factor for impairment in executive functioning. Furthermore, white matter lesions may make persons more vulnerable to hypoperfusion.

The finding of a high prevalence of subcortical vascular pathology suggests that patients with arterial disease may be at increased risk of developing subcortical ischemic vascular disease. Global and cortical brain atrophy appeared to be a less prominent feature of our population. We speculate that subcortical ischemic vascular disease is a contributing factor in the pathogenesis of cognitive decline and dementia at a stage before brain atrophy contributes to this process. Our results are not only interesting from an etiologic point of view, but may also be clinically relevant. First, the vascular origin of subcortical ischemic brain damage makes it a potentially treatable target to prevent or slow down the development of brain atrophy and cognitive decline. Second, as our patients were relatively young their brain atrophy and cognitive impairment are probably at an early stage. If interventions become available to prevent progression of brain atrophy and cognitive decline, they are more likely to be effective in early stages of the disease. Third, patients with manifest arterial disease are probably at high risk of developing vascular cognitive impairment, and therefore these patients are the most likely to benefit from intervening therapies.

Starting in January 2006 all patients from the SMART-MR study have been invited for a second visit to our medical center after a mean follow-up period of 4 years. This follow-up study will allow us to further investigate causes and consequences of progression of vascular brain pathology on MRI, and may thereby help to better understand the underlying mechanisms of vascular cognitive impairment.

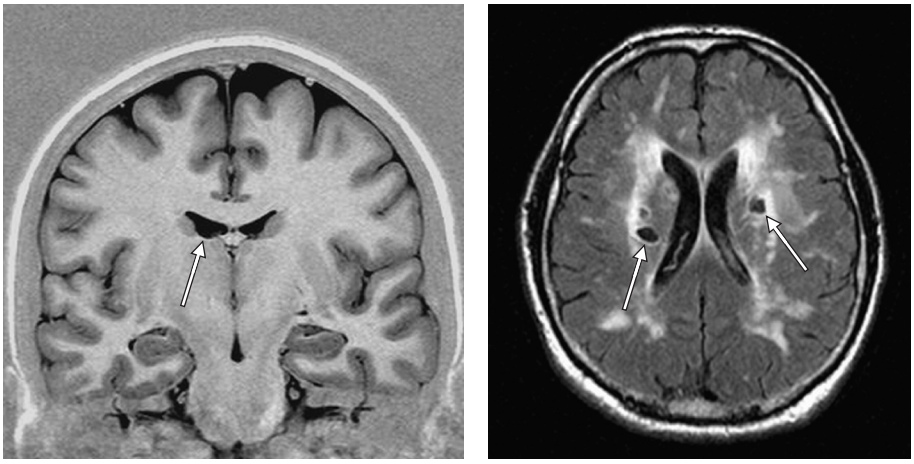
6

Summary
Nederlandse samenvatting
Dankwoord
Curriculum Vitae
Appendix

Hersenatrofie (het kleiner worden van de hersenen) wordt regelmatig gezien bij ouderen tijdens beeldvormend onderzoek met MRI van de hersenen. Een afname in het volume van de grijze stof aan de buitenkant van de hersenen is een teken van corticale atrofie en een toename van het volume van de ventrikels is een teken van subcorticale atrofie (atrofie van het binnenste gedeelte van de hersenen). Onderzoek naar hersenatrofie is belangrijk, omdat de mate en de snelheid van verergering van hersenatrofie geassocieerd zijn met het ontstaan van dementie. De afgelopen jaren hebben verschillende onderzoeken laten zien, dat het aandeel van vaatziekten in het ontstaan van cognitieve achteruitgang belangrijker is dan vroeger werd gedacht. Cognitieve achteruitgang door neurodegeneratie en door vaatziekten werden van oudsher beschouwd als twee aparte ziektenbeelden. Tegenwoordig wordt erkend, dat er een overlap is in de rol van beide processen in het ontstaan van achteruitgang van cognitieve functies. Er is echter nog meer onderzoek nodig om de rol van vaatziekten in het onstaan van hersenatrofie en cognitieve achteruitgang beter te begrijpen.

Het algemene doel van dit proefschrift was om een beter inzicht te krijgen in de bijdrage van vaatziekten in de ontwikkeling van hersenatrofie en cognitieve achteruitgang. Daarvoor hebben we eerst bij een grote groep patiënten met vaatziekten de hersenvolumes en uitingen van vaatschade in de hersenen onderzocht (**hoofdstuk 2**). Daarna hebben we onderzocht of witte stof afwijkingen en lacunaire infarcten (uitingen van schade aan de kleine bloedvaten in de hersenen) geassocieerd zijn met hersenatrofie. Ook hebben we onderzocht of witte stof afwijkingen en lacunaire infarcten geassocieerd zijn met cognitieve achteruitgang (**hoofdstuk 3**). Ten slotte hebben we het verband tussen hersendoorbloeding, hersenatrofie en cognitief functioneren onderzocht (**hoofdstuk 4**).

Tegenwoordig is het mogelijk om het volume van de hersenen en de omvang van witte stof afwijkingen nauwkeurig te meten. Recent zijn er meerdere studies gepubliceerd, die hersenvolumes en volumes van witte stof afwijkingen hebben beschreven van de algemene bevolking. In **hoofdstuk 2** hebben we het totale hersenvolume, het volume van de corticale grijze stof, het volume van de ventrikels en het volume van witte stof afwijkingen onderzocht bij 1044 patiënten (gemiddelde leeftijd 58 ± 10 jaar, 79% man) met vaatziekten. Daarnaast hebben we onderzocht hoe vaak deze patiënten een stil infarct (d.w.z. zonder duidelijke klachten) in de hersenen hebben. Het totale hersenvolume was gemiddeld 78,8% (als percentage van de schedelinhoud) bij mannen en 79,9% bij vrouwen en dit volume nam af met 0,18% per jaar. Het volume van de grijze stof in de cortex nam ook af met toenemende leeftijd. Deze afname was sterker bij mannen. Het volume van de ventrikels was 2,2% bij mannen en 1,8% bij vrouwen en dit volume nam toe met de leeftijd. Het volume van witte stof afwijkingen nam ook toe met hogere leeftijd en sneller bij mannen dan bij vrouwen. Stille herseninfarcten kwamen gemiddeld bij 14% van de patiënten voor en bij patiënten ouder dan 65 jaar was dat 24%. De afname in totaal hersenvolume en grijze stof volume was vergelijkbaar met studies in de algemene bevolking. Echter, ventrikelvergroting en tekenen van vaatschade in de hersenen, zoals witte stof afwijkingen en stille infarcten, kwamen vaker voor bij patiënten met vaatlijden.



Links: Afbeelding met MRI van de hersenen bij een gezond persoon, van voren gezien. Een afname in volume van de grijze stof aan de buitenkant van de hersenen is een uiting van corticale atrofie. Een toename in grootte van de ventrikels (pijl) is een uiting van subcorticale atrofie.
Rechts: Afbeelding met MRI van de hersenen bij een patiënt met uitgebreide witte stofafwijkingen en lacunaire infarcten (pijlen), van boven gezien.

Witte stof afwijkingen en hersenatrofie worden vaak tegelijkertijd gezien op MRI bij ouderen. Een verklaring hiervoor zou kunnen zijn, dat zowel witte stof afwijkingen als hersenatrofie worden veroorzaakt door vasculaire risicofactoren. Echter, het is ook mogelijk, dat schade aan de witte stof bijdraagt aan het ontstaan van hersenatrofie. In **hoofdstuk 3.1** hebben we een overzicht gemaakt van alle studies, die het verband tussen witte stof afwijkingen en hersenatrofie hebben onderzocht tussen januari 1980 en oktober 2007. Van elke studie noteerden we het soort en de gemiddelde leeftijd van de studiepopulatie, het type en de meetmethode van witte stof afwijkingen en hersenatrofie, en of er in het onderzoek rekening was gehouden met vasculaire risicofactoren. Uiteindelijk hebben we 48 studies gevonden en in 37 van deze studies werd een verband gevonden tussen witte stof afwijkingen en hersenatrofie. In slechts 10 van de 48 studies werd in de analyse rekening gehouden met de aanwezigheid van vasculaire risicofactoren. Uit de resultaten van deze studies kan nog niet met zekerheid worden geconcludeerd, dat er een verband is tussen witte stof afwijkingen en hersenatrofie, onafhankelijk van vasculaire risicofactoren.

In **hoofdstuk 3.2** hebben we zelf bij patiënten met vaatlijden onderzocht of er een verband is tussen witte stof afwijkingen, lacunaire infarcten en hersenatrofie. Hiervoor hebben we bij 840 patiënten nauwkeurig het totale hersenvolume, het volume van de corticale grijze stof, het volume van de ventrikels en het volume van witte stof afwijkingen gemeten. Een neuroradioloog beoordeelde in samenwerking met een onderzoeker of een patiënt één of meer infarcten had. In deze studie vonden we, dat patiënten met meer witte stof afwijkingen kleinere hersenen, minder corticale

grijze stof en grotere ventrikels hadden. Daarnaast vonden we, dat de aanwezigheid van één of meer lacunaire infarcten geassocieerd was met kleinere hersenen en grotere ventrikels. Deze verbanden werden niet verklaard door de aanwezigheid van vasculaire risicofactoren. We vonden geen verband tussen lacunaire infarcten en minder corticale grijze stof. Onze bevindingen ondersteunen de hypothese, dat witte stof afwijkingen en lacunaire infarcten beide bijdragen aan het ontstaan van hersenatrofie.

Uit andere studies is gebleken, dat witte stof afwijkingen zijn geassocieerd met een slechter cognitief functioneren. Het is echter nog niet duidelijk of witte stof afwijkingen en lacunaire infarcten allebei zijn geassocieerd met een slechter cognitief functioneren en of dit onafhankelijk is van hersenatrofie. In **hoofdstuk 3.3** hebben we onderzocht of er een verband is tussen witte stof afwijkingen, lacunaire infarcten en cognitief functioneren en of een eventueel verband kon worden verklaard door hersenatrofie. Voor deze studie onderzochten we bij 522 patiënten het cognitief funcionteren met een aantal neuropsychologische testen. Met een deel van deze testen werd onderzocht hoe goed het geheugen was en met een ander deel van de testen werd onderzocht hoe goed de executieve functies waren. Executieve functies zijn de hersenfuncties, die nodig zijn om gedrag te organiseren in nieuwe, onbekende situaties en omvatten processen, zoals wisselende aandacht, besluitvorming en planning. We vonden, dat witte stof afwijkingen geassocieerd waren met een slechter executief functioneren. Hoewel dit verband deels verklaard werd door subcorticale hersenatrofie, waren witte stof afwijkingen nog steeds geassocieerd met een slechter executief functioneren als er rekening werd gehouden met gelijktijdig aanwezige hersenatrofie. Daarnaast vonden we ook een verband tussen de aanwezigheid van meerdere lacunaire infarcten en een slechter executief functioneren. Witte stof afwijkingen en lacunaire infarcten waren niet geassocieerd met een slechter geheugen.

Een goede doorbloeding is belangrijk voor de levensvatbaarheid van de hersenen. De doorbloeding van de hersenen kan op verschillende manieren worden onderzocht. Omdat de meeste van deze manieren ingewikkeld zijn en er vaak röntgenstralen of contrastmiddelen nodig zijn voor deze onderzoeken, zijn er weinig grote studies die de hersendoorbloeding onderzoeken. Tegenwoordig is het mogelijk om met MR onderzoek de hoeveelheid bloed die door een bloedvat stroomt te meten. In **hoofdstuk 4.1** hebben we met deze methode bij 636 patiënten met vaatlijden de totale hoeveelheid bloed die naar de hersenen gaat gemeten. In vergelijking met een eerdere studie in een groep personen uit de algemene bevolking was deze hoeveelheid iets hoger bij patiënten met vaatziekten. Vervolgens hebben we onderzocht welke factoren bij patiënten met vaatlijden geassocieerd waren met de hoeveelheid bloed die naar de hersenen gaat. We vonden, dat een hogere leeftijd, diabetes (suikerziekte) en een hogere body mass index (een maat voor overgewicht) geassocieerd waren met een lagere bloedtoevoer naar de hersenen. Ten slotte vonden we, dat er bij patiënten met vaatziekte in de hersenen minder bloed naar de hersenen ging in vergelijking met patiënten met vaatziekte op een andere plek in het lichaam.

Een slechtere hersendoorbloeding zou mogelijk een rol kunnen spelen in het ontstaan van hersenatrofie. In **hoofdstuk 4.2** hebben we onderzocht of er een verband is tussen een lagere hersendoorbloeding en atrofie van de hersenen. Gezonde hersenen kunnen een lagere doorbloeding deels opvangen door de weerstand in de bloedvaten te verlagen. Er zijn aanwijzingen, dat de hersenen van personen met witte stof afwijkingen dit minder goed kunnen. We hebben daarom ook onderzocht of het verband tussen hersendoorbloeding en hersenatrofie sterker is in de aanwezigheid van witte stof afwijkingen. Om een maat te krijgen voor gemiddelde hersendoorbloeding hebben we bij 828 patiënten met vaatlijden de hoeveelheid bloed die naar de hersenen stroomt gemeten en deze hoeveelheid gedeeld door het totale hersenvolume. We vonden dat een lagere hersendoorbloeding geassocieerd was met meer subcorticale atrofie. Dit verband was inderdaad alleen aanwezig bij patiënten die grotere witte stof afwijkingen hadden. We vonden geen verband tussen een lagere hersendoorbloeding en globale hersenatrofie (afname van het totale hersenvolume). Dit zou er op kunnen wijzen, dat een lagere hersendoorbloeding, in combinatie met grotere witte stof afwijkingen, een oorzaak is van subcorticale hersenatrofie. Omdat we niet kunnen uitsluiten dat een verminderde hersendoorbloeding het resultaat is van hersenatrofie, is meer onderzoek nodig om dit te onderzoeken.

In **hoofdstuk 4.3** onderzochten we in een groep van 472 patiënten met vaatlijden of een verminderde hersendoorbloeding ook geassocieerd was met slechter cognitief functioneren en of dit verband sterker was in de aanwezigheid van witte stof afwijkingen. We vonden, dat patiënten met een lagere hersendoorbloeding slechter scoorden op de testen, die executief functioneren meten. Net als het verband tussen hersendoorbloeding en subcorticale hersenatrofie, hing dit af van de aanwezigheid van witte stof afwijkingen. Bij patiënten met grotere witte stof afwijkingen was het verband tussen een verminderde hersendoorbloeding en slechter executief functioneren sterker. We vonden echter geen verband tussen hersendoorbloeding en geheugen. Deze bevindingen wijzen er op, dat een lagere hersendoorbloeding dus niet alleen geassocieerd is met meer subcorticale hersenatrofie, maar ook met slechter executief functioneren. Tevens vormen deze bevindingen een aanwijzing, dat patiënten met witte stof afwijkingen gevoeliger zijn voor de schadelijke effecten van een verminderde hersendoorbloeding.

De onderzoeken in dit proefschrift laten zien, dat witte stof afwijkingen, lacunaire infarcten en subcorticale hersenatrofie vaak voorkwamen bij patiënten met vaatlijden. Witte stof afwijkingen en lacunaire infarcten lijken beide bij te dragen aan het ontstaan van hersenatrofie, maar zijn ook geassocieerd met slechter executief funcioneren, onafhankelijk van hersenatrofie. Verder zijn personen met witte stof afwijkingen mogelijk gevoeliger voor de schadelijke effecten van een lagere hersendoorbloeding. Het vaak voorkomen van tekenen van subcorticale schade suggereert dat patiënten met vaatlijden een verhoogd risico hebben op het ontstaan van subcorticale vaatziekte. Globale en corticale hersenatrofie lijken in deze populatie een mindere belangrijke rol te spelen. Wij denken daarom, dat subcorticale vaatschade in een eerder stadium bijdraagt aan het ontstaan van cognitieve achteruitgang en dementie dan hersenatrofie.

Onze bevindingen geven niet alleen inzicht in de mogelijke mechanismen die zorgen voor hersenatrofie en cognitieve achteruitgang, maar zijn mogelijk ook belangrijk voor de praktijk. Ten eerste: de rol van vaatziekte in het ontstaan van subcorticale schade suggereert, dat behandeling van vasculaire risicofactoren de ontwikkeling van hersenatrofie en cognitieve achteruitgang tegen kan gaan of kan afremmen. Ten tweede: aangezien de patiënten in onze studie met een gemiddelde leeftijd van 58 jaar relatief jong waren, bevinden de hersenatrofie en de cognitieve beperking zich waarschijnlijk nog in een vroeg stadium. Behandeling van vasculaire risicofactoren, die de verergering van hersenatrofie en cognitieve achteruitgang moet voorkomen, is waarschijnlijk het meest effectief als de schade nog in een vroeg stadium is. Ten derde; patiënten met vaatlijden hebben een verhoogd risico op het ontwikkelen van cognitieve achteruitgang en daarom zijn het juist deze patiënten, die het meeste baat kunnen hebben bij een behandeling die dit moet voorkomen.

Vanaf januari 2006 zijn alle deelnemers aan de SMART-MR studie uitgenodigd voor een vervolgonderzoek in het Universitair Medisch Centrum Utrecht. Naast het MR onderzoek van de hersenen zullen ook alle andere metingen worden herhaald. Het vergelijken van de resultaten van dit vervolgonderzoek met de resultaten van het eerste bezoek zal het mogelijk maken om de oorzaken en gevolgen van verergering van vasculaire schade in de hersenen verder te onderzoeken. We hopen hiermee uiteindelijk een beter inzicht te krijgen in de onderliggende oorzaken van cognitieve achteruitgang door vaatziekte.

6

Summary
Nederlandse samenvatting
Dankwoord
Curriculum Vitae
Appendix

Dat een van mijn begeleiders het op tijd afronden van dit proefschrift een godswonder noemde laat wel zien dat niet alles zonder slag of stoot is verlopen. Velen hebben direct of indirect bijgedragen aan dit wonder. Graag wil ik daarom de volgende personen bedanken voor het tot stand komen van dit proefschrift.

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6

Summary
Nederlandse samenvatting
Dankwoord
Curriculum Vitae
Appendix

Auke Peter Adriaan Appelman was born on April 9th, 1976, in Utrecht, the Netherlands. In 1994, after graduation from secondary school at the Sint Bonifatius College in Utrecht, he started his medical training at Utrecht University. During this period he participated in an investigation concerning the determinants of total cerebral blood flow under supervision of prof. dr. W.P.Th.M. Mali (department of Radiology) and prof. dr. Y. van der Graaf (Julius Center for Health Sciences and Primary Care). After obtaining his medical degree in August 2004, he started working on the research described in this thesis at the department of Radiology of University Medical Center Utrecht and the Julius Center for Health Sciences and Primary Care, again under supervision of prof. dr. W.P.Th.M. Mali and prof. dr. Y. van der Graaf and under supervision of dr. M.I. Geerlings and dr. ir. K.L. Vincken. In August 2007 he obtained a Master of Science degree in Clinical Epidemiology from Utrecht University. As of December 2007 he started as a resident in Radiology at Meander Medical Center, in Amersfoort, the Netherlands (dr. H.J. Baarslag). He will finish his residency at University Medical Center Utrecht (prof. dr. J.P.J. van Schaik).

6

Summary
Nederlandse samenvatting
Dankwoord
Curriculum Vitae
Appendix

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- W.P.Th.M. Mali, MD, PhD, department of Radiology
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