THE AGING BRAIN

IN PATIENTS WITH MANIFEST ARTERIAL DISEASE

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THE AGING BRAIN

IN PATIENTS WITH MANIFEST ARTERIAL DISEASE

Veroudering van de hersenen bij patiënten met manifest vaatlijden
(met een samenvatting in het Nederlands)

Proefschrift

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door

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CONTENTS

Chapter 1 General introduction 7

PART I LONGITUDINAL BRAIN CHANGES

Chapter 2 Longitudinal changes in brain volumes and cerebrovascular lesions on MRI in patients with manifest arterial disease: the SMART-MR study 19
Journal of the Neurological Sciences 2014;337(1-2):112-118

Chapter 3 Lower cerebral blood flow and progression of brain atrophy: the SMART-MR study 45
Submitted

Chapter 4 Longitudinal relation between cerebral small-vessel disease and cerebral blood flow: the SMART-MR study 59
Submitted

PART II RISK FACTORS FOR BRAIN AGING

Chapter 5 Hypertensive target organ damage and longitudinal changes in brain structure and function: the SMART-MR study 75
Submitted

Chapter 6 Hemoglobin, hematocrit and longitudinal changes in cerebral blood flow: the SMART-MR study 93
Submitted

PART III CONSEQUENCES OF BRAIN AGING

Chapter 7 Brain volumes and risk of cardiovascular events and mortality: the SMART-MR study 113
Neurobiology of Aging 2014;35(7):1624-1631

Chapter 8 General discussion 131

Summary 141
Nederlandse samenvatting 145
Dankwoord 151
Curriculum Vitae 155
Appendix 157

CHAPTER 1

GENERAL INTRODUCTION

Cardiovascular disease is a major health problem worldwide, with coronary heart disease and stroke as leading causes of morbidity and mortality.[1] Although the mortality from cardiovascular disease in Europe decreased during the past 30 years, the increasing incidence of cardiovascular disease and its risk factors, such as hypertension, obesity, and diabetes mellitus, are still a true concern.[2] Improved treatment has resulted in a more chronic pattern of disease with higher rates of long-term impairment of cognitive and physical functioning, hindering healthy aging.[3] Accumulating evidence suggests that cardiovascular disease and a high cardiovascular risk play an important role in the etiology of cognitive decline and dementia.[4] As intermediates in this relation between cardiovascular disease and functional decline in the aging population, structural brain changes have been proposed.

Brain atrophy and features of cerebral small-vessel disease, such as white matter lesions and lacunar infarcts, are frequently observed on brain magnetic resonance imaging (MRI) and are increasingly present with older age.[5] The pathophysiology of brain atrophy and cerebral small-vessel disease is still not completely understood, but atherosclerosis, endothelial dysfunction, and arterial stiffening are likely involved. Previous studies have reported evidence for the role of these atherosclerotic markers and cardiovascular risk factors and disease in brain atrophy and cerebral small-vessel disease (Figure 1).[6-14] For example, hypertension causes chronic hemodynamic stress, which leads to atherosclerotic changes of the cerebrovascular wall, namely hardening and thickening of the cerebrovascular wall and narrowing of the lumen. In combination with endothelial dysfunction, hypertension has therefore been recognized to have an important role in structural brain changes.[15-20] More direct measures of intracranial and extracranial atherosclerosis have also been associated with brain atrophy and cerebral small-vessel disease.[21] Carotid stenosis and carotid intima-media thickness may represent past exposure to cardiovascular risk factors and are thought to reflect different stages of the atherosclerotic process. Several cross-sectional studies[22-24] and at least one prospective study[25] have associated increased intima-media thickness and stenosis of the carotid arteries with brain atrophy and more cerebral small-vessel disease. In addition, burden of coronary atherosclerosis, represented by the coronary artery calcium score, has also been related to brain function and structure. Subjects with higher coronary artery calcium scores showed more brain atrophy and were more likely to have dementia and lower cognitive functions than subjects with less severe coronary atherosclerosis.[26,27]

The role of atherosclerosis in brain aging could also be explained by hemodynamic changes in the cerebral circulation. Under normal conditions, the brain is able to control its perfusion through cerebral autoregulation, depending on metabolic demand and perfusion pressure.[28] However, when the vascular wall is damaged due to atherosclerosis, cerebral autoregulation becomes impaired, resulting in cerebral hypoperfusion.[28] Diffuse and more focal brain tissue ischemia can develop and will result in brain atrophy and cerebral small-vessel disease (Figure 1 – blue arrows).[5] However, it can also be

hypothesized that brain atrophy and cerebral small-vessel disease are both caused by other processes, such as neurodegeneration and genetic predisposition,[5] and subsequently lead to cerebral hypoperfusion as there is less viable brain tissue with less need for oxygen and nutrients (Figure 1 – red arrows). A third possibility is that cerebral small-vessel disease modifies the relation between brain atrophy and cerebral perfusion (Figure 1 – green arrows). The possible causal relations between brain atrophy, cerebral small-vessel disease, and cerebral perfusion have been studied in the general and vascular population, but these studies were mostly cross-sectional, which limits conclusions on direction of associations.[29-36]

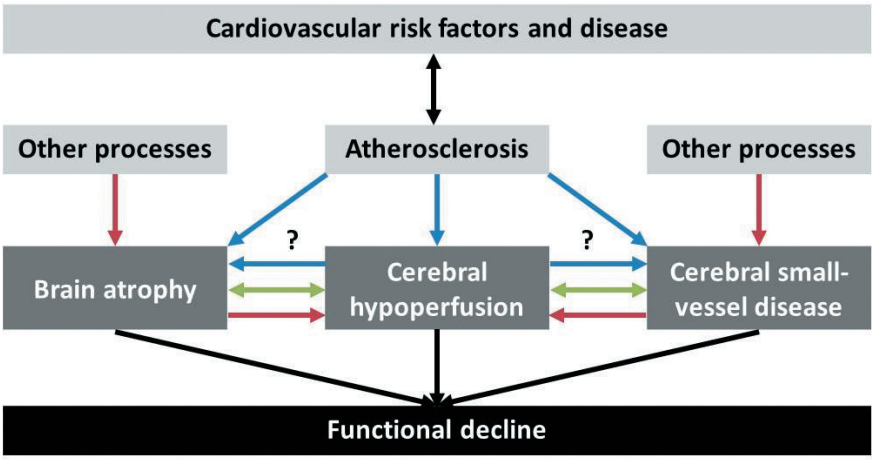

Figure 1 Possible relations between cardiovascular risk factors and disease, structural brain changes, and functional decline in a high-risk population

Because patients with cardiovascular disease or cardiovascular risk factors are more prone for structural brain changes, longitudinal studies are necessary to gain more insight in the etiology of brain aging as a possible contributor to future functional decline to guide potential targets for treatment and prevention.

SMART-MR STUDY

The research described in this thesis is performed using data from the SMART-MR study, which is a prospective cohort study within the Second Manifestations of ARTerial disease (SMART) study.[12,37] In the SMART study, patients with manifest arterial disease or risk factors for atherosclerosis are screened for additional risk factors and severity of atherosclerosis. Between May 2001 and December 2005, the vascular screening was extended with an MRI of the brain and from January 2003 onwards with additional neuropsychological tests. At baseline, 1309 patients were included in the SMART-MR study and of these patients, 754 participated in the follow-up examination of the SMART-MR study between January 2006 and May 2009. The extensive information on cardiovascular disease and its risk factors, cardiovascular events, mortality, and measurements of brain volumes, cerebral small-vessel disease, and cerebral

blood flow at two time points allow us to further elucidate the relation between cardiovascular disease and risk factors, longitudinal brain changes, and functional decline over time.

AIM AND THESIS OBJECTIVES

The overall aim of this thesis is to gain further insight in the aging brain and its causes and consequences in patients with manifest arterial disease. The first objective is to estimate longitudinal changes in brain volumes, cerebral small-vessel disease, and cerebral blood flow on MRI in a large cohort of patients with manifest arterial disease and to investigate their interrelations.

In this high-risk population, early aging of the cardiovascular system is known to interfere in the long-term effects of blood pressure.[15] Therefore, hypertensive target organ damage, as a marker for more chronic exposure to high blood pressure, might better represent hypertension as a possible cardiovascular risk factor for brain changes. Apart from cardiovascular risk factors, hemorheological variables, such as hemoglobin and hematocrit, are also known to result in brain changes through their effect on cerebral perfusion.[38-42] Several small-scale studies have been performed in populations with either high or low hemoglobin and hematocrit, but the effect of these variables within normal range in a vascular population has not been studied yet. Finally, when brain changes are considered as possible contributors to functional impairment, which, in turn, is also associated with an increased risk of morbidity and mortality in the general population, it can also be suggested that smaller brain volumes may result in more cardiovascular events and an increased risk of mortality. Therefore, the second objective is to study these possible risk factors and the consequences of the aging brain on morbidity and mortality in the SMART-MR study sample.

OUTLINE

In **part I**, brain changes in patients with manifest arterial disease will be described over a period of four years of follow-up. In **chapter 2**, we estimate longitudinal changes in brain volumes and cerebrovascular lesions in patients with manifest arterial disease. We determine if these changes accelerate over time and whether these brain changes are different for men and women and for the cardiovascular disease categories. **Chapters 3** and **4** describe the longitudinal interrelations between brain atrophy, cerebral small-vessel disease, and cerebral blood flow over four years of follow-up.

In **part II**, the possible risk factors for brain changes in patients with manifest arterial disease will be assessed. We study the combination of signs of hypertensive target organ damage in association with longitudinal changes in brain volumes, cerebral small-vessel disease, and cognitive performance in **chapter 5**. In **chapter 6**, the association of hemoglobin and hematocrit with longitudinal changes in cerebral blood flow over time will be described.

In **part III**, we investigate whether the different types of brain volumes are associated with an increased risk of cardiovascular events and mortality in patients with manifest arterial disease (**chapter 7**).

Finally, in **chapter 8**, we will discuss our findings in light of our overall aim.

1

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PART I
LONGITUDINAL BRAIN CHANGES

CHAPTER 2

LONGITUDINAL CHANGES IN BRAIN VOLUMES AND CEREBROVASCULAR LESIONS ON MRI IN PATIENTS WITH MANIFEST ARTERIAL DISEASE: THE SMART-MR STUDY

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ABSTRACT

INTRODUCTION

We estimated the progression of brain atrophy and cerebrovascular lesions on MRI in a prospective cohort of patients with various manifestations of arterial disease.

METHODS

Within the SMART-MR study, using brain MRI data from baseline and after on average 3.9 years of follow-up, intracranial volume (ICV), total brain, cortical gray matter, ventricular, white matter lesion volumes and visually rated infarcts were obtained from 663 patients (mean age 57 ± 9 years, 81% men).

RESULTS

Global and cortical atrophy increased quadratically with age. Men showed more progression of global and cortical atrophy than women (mean difference in change (95% CI): -0.25 (-0.44 to -0.06) and -0.94 (-1.35 to -0.52) %ICV) and had an increased risk of new brain infarcts (OR=2.7, 95% CI 1.2-6.1). Compared with coronary artery disease patients, cerebrovascular disease patients showed more progression of cortical and subcortical atrophy and an increased risk of new brain infarcts, and peripheral arterial disease patients showed more progression of cortical atrophy. These results were independent of cerebrovascular lesions and cardiovascular risk factors.

CONCLUSION

In patients with manifest arterial disease, brain atrophy tended to accelerate with older age and men had more progression of brain atrophy and cerebrovascular lesions than women. Additionally, patients with cerebrovascular and peripheral arterial disease showed the most prominent progression of atrophy and lesions.

INTRODUCTION

Cardiovascular disease is a major health problem worldwide, with coronary artery disease and stroke as the leading causes of morbidity and mortality. Improved possibilities to treat cardiovascular disease have led to increased survival rates of patients with cardiovascular disease. However, this has resulted in reduced health status and higher rates of functional disabilities in these aging patients.[1]

In patients with cardiovascular disease, cerebrovascular lesions - such as brain infarcts and white matter lesions - and brain volume loss are common findings on magnetic resonance imaging (MRI).[2] This is of clinical importance since it has been shown that the extent and rate of progression of these brain abnormalities increase the risk of cognitive and functional decline.[3-5]

Also, evidence has accumulated that cardiovascular risk factors and disease play an important role in the etiology of cerebrovascular lesions and brain atrophy[6-8] and it can therefore be suggested that persons with high vascular risk are more prone for accelerated brain aging. However, the effect of vascular disease present at various locations is not well studied.

In the general population, longitudinal studies have shown that loss of brain volume and accumulation of cerebrovascular lesions start at a slow rate in early adulthood and accelerate in older age.[5,6,9-11] However, to the best of our knowledge, no longitudinal studies have been performed in a population with vascular disease.

We estimated the rate of progression of brain atrophy and cerebrovascular lesions on MRI over on average 3.9 years of follow-up in a large cohort of patients with manifest arterial disease at various locations.

METHODS

SMART-MR STUDY

Data were used from the Second Manifestations of ARTerial disease-Magnetic Resonance (SMART-MR) study, a prospective cohort study aimed at investigating brain changes on MRI in independently living patients with manifest arterial disease. Details of the design and participants have been described elsewhere.[2,12] In brief, between May 2001 and December 2005, all patients, from both hospitalized inpatients and outpatient clinics, newly referred to the University Medical Center Utrecht with manifest arterial disease, such as coronary artery disease (CAD), cerebrovascular disease (CVD), peripheral arterial disease (PAD), and/or an abdominal aortic aneurysm (AAA), and without MR contraindications, were invited to participate. CAD 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 or stroke at inclusion and patients who reported stroke in the past were considered to have CVD. PAD was defined as surgery or angioplasty of the arteries supplying the lower extremities in the past or intermittent

claudication or rest pain at inclusion. AAA was defined as present abdominal aortic aneurysm (aortic diameter ≥3 cm) or previous AAA surgery. Multiple vascular diseases were defined as having more than one vascular disease, possible in any combination. Information on surgical interventions related to the inclusion criteria was documented. During a one-day visit to our medical center, all patients underwent an MRI of the brain, physical examination, ultrasonography of the carotid arteries, and blood sampling. Risk factors, medical history, and medication use were assessed using questionnaires. Between January 2006 and May 2009, follow-up measurements took place including MRI of the brain. In the period between baseline and follow-up measurements, patients received a questionnaire every six months to provide information on hospitalization and outpatient clinic visits. If a cardiovascular event or surgical intervention was reported, original source documents were retrieved and reviewed to determine the occurrence of cardiovascular disease and related surgical interventions. All possible events and surgical interventions were audited independently by three physicians.

The SMART-MR study was approved by the ethics committee of our institution and written informed consent was obtained from all participants.[2]

MAGNETIC RESONANCE PROTOCOL AND BRAIN SEGMENTATION

At baseline and follow-up, 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 transversal T1-weighted (repetition time [TR]/echo time [TE], 235/2 ms), T2-weighted (TR/TE, 2200/11 ms and 2200/100 ms), fast fluid-attenuated inversion recovery (FLAIR; TR/TE/inversion time [TI], 6000/100/2000 ms), and inversion recovery (IR; TR/TE/TI, 2900/22/410 ms) sequences (field of view, 230 x 230 mm; matrix size, 180 x 256; slice thickness, 4.0 mm; no gap; 38 slices).[2]

The T1-weighted gradient-echo, inversion recovery and FLAIR sequence were used for brain segmentation. The probabilistic segmentation technique has been described previously.[13] The segmentation program distinguishes cortical gray matter, white matter, sulcal and ventricular cerebrospinal fluid (CSF), and lesions. The results of the segmentation analysis were visually checked for the presence of infarcts and adapted if necessary to make a distinction between white matter lesions (WMLs) and infarct volumes.

BRAIN ATROPHY

At baseline and follow-up, total brain volume was calculated by summing the volumes of gray and white matter and, if present, the volumes of WML and infarcts. All volumes cranial to the foramen magnum were included. As a result, the total brain volume includes both the hemispheres, brainstem, and cerebellum. Total intracranial volume (ICV) was calculated by summing the total brain volume and the volumes of the sulcal and ventricular CSF. Total brain, cortical gray matter, and ventricular volumes were normalized for ICV and expressed as brain parenchymal fraction (BPF), cortical gray matter fraction (GMF), and ventricular fraction (VF).[2] Change in BPF, GMF, and VF were used as indicators of progression of global, cortical, and subcortical brain atrophy.

WHITE MATTER LESIONS AND INFARCTS

At baseline and follow-up, the whole brain was visually searched for infarcts by a trained investigator and a neuroradiologist, blinded to 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 ≥3 mm in diameter. Infarcts located in the white matter also had to be hypointense on T1-weighted and FLAIR images in order to distinguish them from WML. Dilated perivascular spaces were distinguished from infarcts on the basis of their location, form, and the absence of gliosis.[14] The location, affected flow territory and type were scored for every infarct. Brain infarcts were categorized as cortical infarcts, large subcortical infarcts, lacunar infarcts, and infratentorial infarcts. We defined large subcortical infarcts as infarcts sized >15 mm and not confluent with cortical infarcts. Lacunar infarcts were defined as infarcts sized 3-15 mm in diameter and located in the subcortical white matter, thalamus, or basal ganglia. Infratentorial infarcts were located in the brainstem or cerebellum. Volumes of periventricular and deep WML were summed to obtain the total volume of WML. Periventricular lesions were defined as WML adjacent to or within 1 cm of the lateral ventricles in both hemispheres and deep lesions were located in the deep white matter tracts and may or may not have adjoined periventricular lesions.[2] WML volumes were natural log transformed at baseline and follow-up, because they were not normally distributed.

VASCULAR SCREENING

During the patient's visit to the medical center, an overnight fasting blood sample was taken to determine glucose and lipid levels. Height and weight were measured without shoes and heavy clothing, and 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. Diabetes mellitus at baseline was defined as a referral diagnosis of diabetes mellitus, self-reported diabetes mellitus (use of glucose-lowering agents), glucose ≥11.1 mmol/L, or a known history of diabetes mellitus. Among subjects without a history of diabetes mellitus, but with a fasting plasma glucose ≥7.0 mmol/L at baseline, those receiving treatment with glucose-lowering agents within one year after baseline were considered as having diabetes mellitus at baseline. Hyperlipidemia was defined as total cholesterol >5.0 mmol/L, low-density lipoprotein cholesterol >3.2 mmol/L, or self-reported use of lipid-lowering drugs. Smoking habits and alcohol intake were assessed using questionnaires. Pack years of smoking were calculated and alcohol intake was categorized as never, former, or current. Patients who had quit drinking during the past year were assigned to the category current alcohol use. Ultrasonography was performed to measure the intima-media thickness (mm) in the left and right common carotid arteries, represented by the mean value of six measurements.[7]

STUDY SAMPLE

At baseline, 1309 patients participated in the baseline examination. The median interval between index cardiovascular event and MRI scan was 3.3 months (10th-90th percentile:

1.4-9.8 months). For patients who were recruited after an acute stroke (n=40), median interval between event and participation was 3.6 months, with a 10th-90th percentile of 1.1 and 10.1 months. Of these 1309 patients, 19 had no MRI, and 14 had no FLAIR sequence. In addition, in 44 patients brain volume data were missing due to motion or artefacts. As a result, baseline MRI data were available in 1232 patients.

At follow-up, all patients were invited in the same order as they were included at baseline, so that every patient had a follow-up examination after a median (interquartile range) follow-up period of 3.8 (3.7-4.1) years. Due to logistics and MRI capacity, it was not possible to have an exact follow-up period of four years for every patient. Of 1232 patients, 718 patients participated in the follow-up examination. Of these, 38 had no MRI, and in 17 patients brain volume data were missing due to motion or artefacts. Due to a temporary change in the MRI protocol, the IR sequence was missing in 116 patients and not adequate in 20 patients. This resulted in missing data on cortical gray matter in these 136 patients, as the IR sequence was obligatory for cortical gray matter segmentation. For segmentation of the other brain volumes, it was possible to only use the FLAIR sequence.[7,13] As a result, the analyses on the prospective data were performed in 663 patients.

Our sample (n=663) was younger, more often male, had more CAD and less PAD and multiple vascular diseases, had lower blood pressure levels, less often diabetes mellitus, and had less cerebrovascular lesions on MRI than the 569 patients who did not participate in the follow-up examination.

DATA ANALYSES

We used multiple imputation (10 datasets) using the statistical program R (aregImpute; version 2.10.0) to address missing values in the study sample of 663 patients. Data were analyzed using SPSS version 20.0 (Chicago, IL, USA), by pooling the 10 imputed datasets.[15]

First, baseline characteristics were calculated for the total study population and according to three age groups (<55 years, 55-64 years, and ≥65 years) and sex. Second, linear regression analysis was used to estimate the association of age (continuous and in three groups) and sex with change in BPF, GMF, VF, and WML volume. Logistic regression analysis was used to estimate the association of age and sex with any new brain infarct (all, cortical, and lacunar). New brain infarcts were calculated by subtracting the number of infarcts at baseline from the number of infarcts at follow-up. This number of infarcts was dichotomized for analysis as no new infarcts (≤0) and ≥1 new infarcts at follow-up. The respective brain MRI measures at follow-up were entered as dependent variable and age, sex, duration of follow-up period, and baseline brain MRI measures were entered as independent variables (model 1). When progression of brain atrophy was the outcome, additional adjustments were made for baseline brain infarcts and WML volume; when progression of WML was the outcome, additional adjustments were made for baseline brain infarcts and BPF; when new brain infarcts were the outcome, additional adjustments were made for baseline WML and BPF (model 2). Furthermore, in model 3 additional adjustments were made for cardiovascular risk factors (BMI, smoking, alcohol use, systolic and diastolic blood pressure, hyperlipidemia, diabetes mellitus, intima-media thickness).

To assess whether progression of brain atrophy or cerebrovascular lesions accelerated with age, quadratic age-terms were entered to model 1. To investigate whether the rate of progression of brain MRI outcomes associated with age differed for men and women, analyses were repeated across strata of sex and interaction terms between age and sex were added to the regression models (model 1).

Finally, to investigate the association of vascular disease categories (CAD, CVD, PAD, AAA, or multiple vascular diseases) with progression of brain atrophy or cerebrovascular lesions, these categories were added to model 1. Patients with CAD were used as the reference category.

RESULTS

The mean (SD, range) age of the population was 57 (9, 28-79) years and 81% were men. Table 1 shows baseline characteristics across 3 age groups and for men and women, see Supplemental Table 1.

After a median (interquartile range) follow-up period of 3.8 (3.7-4.1) years, the mean (SD) decrease in BPF and GMF was 0.98 (1.08) %ICV and 1.72 (2.17) %ICV, and the mean (SD) increase in VF was 0.19 (0.28) %ICV (Supplemental Table 2). The mean WML volume increase was 0.06 %ICV, ranging from 0.01 %ICV in the youngest age group to 0.13 %ICV in the oldest age group, and 82 patients (12% of 663) had one or more new brain infarcts; 3% was due to new cortical infarcts, 9% to new lacunar infarcts, and 1% to new infratentorial infarcts. One new large subcortical infarct was observed (Supplemental Table 2). Forty-four percent of the new brain infarcts were silent.

AGE AND SEX

Older age was associated with more progression of brain atrophy and cerebrovascular lesions during follow-up (Table 2). The mean differences in change (95% CI) in BPF, GMF, and VF between older (≥65 years) and younger patients (<55 years) were -1.20 (-1.43 to -0.97) %ICV, -1.58 (-2.07 to -1.10) %ICV, and 0.19 (0.14 to 0.25) %ICV. In younger patients, change in natural log transformed WML volume was negative: mean difference in change (95% CI) was -0.16 (-0.25 to -0.08) %ICV. However, with increasing age, natural log transformed WML volume increased, irrespective of other baseline brain MRI measures and cardiovascular risk factors (Table 2, models 2 and 3).

Older patients also showed a fourfold increased risk of new brain infarcts (Table 2, model 1), but this could partly be explained by WML volume, total brain volume, and cardiovascular risk factors (models 2 and 3). Furthermore, age-squared was associated with decreases in BPF (p=0.08) and GMF (p=0.03) over time, indicating that the atrophy tended to accelerate with older age.

Compared with women, men showed a larger age-adjusted decrease in BPF and GMF over time: mean differences in change (95% CI) between men and women were -0.25 (-0.44 to -0.06) %ICV and -0.94 (-1.35 to -0.52) %ICV (Table 2, model 1). These differences

Table 1 Baseline characteristics of the study sample (n=663) per age category

	Age categories		
	<55 years	55-64 years	≥65 years
	n=257	n=244	n=162
Age, years	48 ± 5	59 ± 3	70 ± 4
Sex, % men	81	82	81
Vascular disease location, %			
Coronary artery disease	56	52	44
Cerebrovascular disease	14	15	19
Peripheral arterial disease	14	14	7
Abdominal aortic aneurysm	2	4	4
Multiple vascular diseases	8	13	19
Cardiovascular risk factors			
BMI, kg/m^2	27 ± 4	27 ± 4	27 ± 4
Smoking, pack years[†]	22 (0, 46)	21 (0, 54)	14 (0, 55)
Alcohol intake, % current	77	82	76
Systolic blood pressure, mmHg	134 ± 18	142 ± 19	147 ± 22
Diastolic blood pressure, mmHg	82 ± 10	82 ± 10	80 ± 11
Hyperlipidemia, %	82	75	76
Diabetes mellitus, %	9	14	20
Intima-media thickness, mm	0.8 ± 0.2	1.0 ± 0.4	1.0 ± 0.3
Brain volumes, %ICV			
Brain parenchymal fraction	80.8 ± 2.0	79.2 ± 2.3	77.2 ± 2.4
Cortical gray matter fraction	37.7 ± 3.1	35.8 ± 3.0	34.7 ± 3.1
Ventricular fraction	1.7 ± 0.7	2.0 ± 0.7	2.6 ± 0.9
Total WML volume fraction[†]	0.07 (0.02, 0.22)	0.11 (0.03, 0.33)	0.18 (0.04, 0.90)
Cerebrovascular pathology, %			
Any infarct	20	28	37
Cortical	9	13	13
Large subcortical	1	0	1
Lacunar	11	17	27
Infratentorial	5	5	8
Cerebellum	4	3	6
Brainstem	2	3	3

Characteristics presented as mean ± standard deviation, unless stated otherwise.

BMI, body mass index; WML, white matter lesion.

[†] Median (10th-90th percentile).

Percentage of missing values before imputation: BMI 0.2%, smoking 0.3%, alcohol intake 0.5%, hyperlipidemia 1.8%, intima-media thickness 2.0%, cortical gray matter fraction 17.5%, all other variables 0.0%.

in BPF and GMF were irrespective of other baseline brain MRI measures (model 2: -0.27 (-0.47 to -0.08) %ICV and -1.02 (-1.44 to -0.60) %ICV) and cardiovascular risk factors (model 3: -0.32 (-0.52 to -0.12) %ICV and -1.10 (-1.53 to -0.67) %ICV). Men also showed more new brain infarcts, particularly new lacunar infarcts (Table 2 and Supplemental Table 3). Similar risk estimates were shown in model 2 and 3 (model 2: OR=3.03, 95% CI 1.28-7.14 and OR=3.60, 95% CI 1.20-10.80, respectively and model 3: OR=3.24, 95% CI 1.33-7.92 and OR=3.29, 95% CI 1.84-5.86, respectively). No differences between men and women were observed with respect to change in VF or WML volume over time (Table 2).

A significant interaction between age and sex was present for change in GMF (p=0.02). Stratified analyses showed that with older age, men had a larger decrease in GMF than women (Figure 1B). This interaction was not observed for BPF, VF, or WML (Figure 1A, C, D).

VASCULAR DISEASE

Compared with patients with CAD, patients with CVD showed larger decrease in GMF and larger increase in VF during follow-up after adjusting for age and sex: mean differences in change (95% CI) were -0.67 (-1.13 to -0.20) %ICV and 0.11 (0.05 to 0.16) %ICV (Table 2, model 1). The relation between CVD and VF showed similar effect estimates in models 2 and 3 (model 3: 0.09 (0.03 to 0.15) %ICV), but the effect estimates for the relation between CVD and GMF diminished and were no longer significant after further adjustments (model 3: -0.18 (-0.70 to 0.33) %ICV). CVD patients showed more new brain infarcts and in particular new cortical infarcts (Table 2 and Supplemental Table 3). In models 2 and 3, these risk estimates only marginally changed.

Patients with PAD showed a larger age and sex-adjusted decrease in GMF over time than patients with CAD (mean difference in change (95% CI): -0.63 (-1.15 to -0.12) %ICV (Table 2, model 1), and this was irrespective of baseline cerebrovascular lesions and cardiovascular risk factors (model 3: -0.63 (-1.16 to -0.10) %ICV).

Furthermore, AAA patients showed a significantly larger increase in VF than patients with CAD after adjusting for age and sex: mean difference in change (95% CI) was 0.13 (0.02 to 0.24) %ICV (Table 2, model 1). However, this was partly explained by cardiovascular risk factors (model 3). Patients with AAA also showed more new brain infarcts and in particular new cortical infarcts in model 1 (Table 2 and Supplemental Table 3). However, these findings were partly explained by WML volume, total brain volume, and cardiovascular risk factors (model 3: OR=3.05, 95% CI 0.94-9.90 and OR=6.98, 95% CI 1.03-47.52).

Patients with multiple vascular diseases showed no difference in change in brain volumes or progression of any infarct when compared with CAD patients.

Additional analyses were performed to study whether the accrual of new brain infarcts or WML volume change altered the associations, whether new vascular events during follow-up were of influence, and whether treatment effect was an issue in this study. First, progression of brain infarcts and WML volume was added to model 1. Results showed that the observed associations somewhat attenuated, but remained statistically significant (Supplemental Table 4, model 4).

Second, new vascular events during follow-up were added to model 3. Results showed

Table 2 Progression of measures of brain atrophy and cerebrovascular lesions on MRI after on average 3.9 years of follow-up in 663 patients with manifest arterial disease according to categories of age, sex, and vascular disease location

	Model	N	Change in BPF,	Change in GMF,	Change in VF,	Change in WML,	New brain infarcts,
			B (95% CI)[a]	B (95% CI)[a]	B (95% CI)[a]	B (95% CI)[a]	OR (95% CI)[b]
Age							
<55 years, reference[c]		257	0	0	0	0	1.0
55-64 years	1	244	-0.50 (-0.68; -0.32)**	-0.74 (-1.16; -0.33)**	0.11 (0.07; 0.16)**	0.30 (0.19; 0.42)**	1.67 (0.86-3.23)
	2		-0.48 (-0.66; -0.30)**	-0.65 (-1.05; -0.25)**	0.10 (0.05; 0.14)**	0.30 (0.18; 0.42)**	1.23 (0.61-2.49)
	3		-0.47 (-0.66; -0.28)**	-0.72 (-1.16; -0.28)**	0.09 (0.04; 0.14)**	0.28 (0.16; 0.41)**	1.08 (0.51-2.28)
≥65 years	1	162	-1.20 (-1.43; -0.97)**	-1.58 (-2.07; -1.10)**	0.19 (0.14; 0.25)**	0.59 (0.45; 0.73)**	3.99 (2.09-7.61)**
	2		-1.15 (-1.38; -0.92)**	-1.35 (-1.83; -0.88)**	0.16 (0.11; 0.22)**	0.59 (0.43; 0.74)**	1.70 (0.77-3.75)
	3		-1.13 (-1.37; -0.88)**	-1.39 (-1.94; -0.85)**	0.14 (0.08; 0.20)**	0.57 (0.40; 0.73)**	1.50 (0.69-3.27)
Sex							
Women, reference[c]		124	0	0	0	0	1.0
Men	1	539	-0.25 (-0.44; -0.06)*	-0.94 (-1.35; -0.52)**	0.02 (-0.03; 0.07)	-0.02 (-0.15; 0.11)	2.66 (1.17-6.06)*
	2		-0.27 (-0.47; -0.08)**	-1.02 (-1.44; -0.60)**	0.04 (-0.01; 0.09)	-0.02 (-0.16; 0.11)	3.03 (1.28-7.14)*
	3		-0.32 (-0.52; -0.12)**	-1.10 (-1.53; -0.67)**	0.04 (-0.01; 0.09)	-0.03 (-0.17; 0.11)	3.24 (1.33-7.92)*
Vascular disease location							
CAD, reference[c]		342	0	0	0	0	1.0
CVD	1	105	-0.20 (-0.41; 0.02)	-0.67 (-1.13; -0.20)*	0.11 (0.05; 0.16)**	0.09 (-0.06; 0.24)	2.40 (1.18-4.89)*
	2		0.00 (-0.24; 0.24)	-0.13 (-0.64; 0.39)	0.08 (0.02; 0.14)*	-0.03 (-0.19; 0.14)	2.15 (1.01-4.58)*
	3		-0.03 (-0.27; 0.21)	-0.18 (-0.70; 0.33)	0.09 (0.03; 0.15)**	-0.03 (-0.20; 0.13)	2.20 (1.00-4.82)
PAD	1	80	-0.18 (-0.42; 0.06)	-0.63 (-1.15; -0.12)*	0.04 (-0.02; 0.10)	-0.09 (-0.25; 0.08)	1.42 (0.59-3.42)
	2		-0.15 (-0.38; 0.09)	-0.53 (-1.04; -0.02)*	0.03 (-0.03; 0.09)	-0.11 (-0.27; 0.06)	1.40 (0.57-3.44)
	3		-0.14 (-0.38; 0.10)	-0.63 (-1.16; -0.10)*	0.03 (-0.03; 0.09)	-0.07 (-0.24; 0.09)	1.06 (0.40-2.78)

Table 2 (Continued) Progression of measures of brain atrophy and cerebrovascular lesions on MRI after on average 3.9 years of follow-up in 663 patients with manifest arterial disease according to categories of age, sex, and vascular disease location

	Model	N	Change in BPF, B (95% CI)[a]	Change in GMF, B (95% CI)[a]	Change in VF, B (95% CI)[a]	Change in WML, B (95% CI)[a]	New brain infarcts, OR (95% CI)[b]
Vascular disease location							
AAA	1	21	-0.02 (-0.45; 0.40)	0.26 (-0.67; 1.19)	0.13 (0.02; 0.24)*	0.07 (-0.22; 0.37)	3.99 (1.41-11.27)*
	2		0.03 (-0.40; 0.45)	0.40 (-0.52; 1.31)	0.11 (0.01; 0.22)*	0.05 (-0.24; 0.34)	2.99 (0.97-9.23)
	3		0.02 (-0.41; 0.45)	0.46 (-0.46; 1.39)	0.11 (-0.00; 0.21)	0.06 (-0.24; 0.36)	3.05 (0.94-9.90)
Multiple	1	84	-0.08 (-0.32; 0.15)	-0.24 (-0.75; 0.26)	0.02 (-0.04; 0.08)	0.10 (-0.06; 0.26)	0.89 (0.39-2.05)
	2		0.06 (-0.18; 0.31)	0.16 (-0.37; 0.68)	-0.00 (-0.07; 0.06)	0.02 (-0.15; 0.19)	0.73 (0.30-1.77)
	3		0.07 (-0.18; 0.32)	0.11 (-0.43; 0.64)	-0.02 (-0.08; 0.05)	0.03 (-0.15; 0.20)	0.63 (0.26-1.57)

Model 1: adjusted for age (not if age was independent variable), sex (not if sex was independent variable), baseline measurement of brain structure, and follow-up period; model 2: BPF, GMF, VF, model 1 additionally adjusted for lacunar and non-lacunar infarcts on MRI and WML; WML, model 1 additionally adjusted for lacunar and non-lacunar infarcts on MRI and BPF; brain infarcts, model 1 additionally adjusted for BPF and WML; model 3: model 2 additionally adjusted for systolic and diastolic blood pressure, diabetes mellitus, body mass index, alcohol use, pack years, hyperlipidemia, intima-media thickness.

BPF, brain parenchymal fraction; GMF, cortical gray matter fraction; VF, ventricular fraction; WML, natural log transformed white matter lesion volume (%ICV); CI, confidence interval; OR, odds ratio; CAD, coronary artery disease; CVD, cerebrovascular disease; PAD, peripheral arterial disease; AAA, abdominal aortic aneurysm.

[a] B represents % change in BPF, GMF, VF, and natural log transformed WML (as % of ICV) in comparison with the reference category after 3.9 ± 0.4 years of follow-up.

[b] OR for new brain infarcts, which were defined as change in number of infarcts of ≥1 infarct in comparison with the reference category after 3.9 ± 0.4 years of follow-up.

[c] Change (95% CI) in brain volumes (as % of ICV) for reference categories:
Age, <55 years: BPF -0.50 (-0.63 to -0.37), GMF -1.06 (-1.36 to -0.77), VF 0.10 (0.07 to 0.13), WML -0.16 (-0.25 to -0.08);
Sex, women: BPF -0.78 (-0.95 to -0.61), GMF -0.96 (-1.35 to -0.57), VF 0.17 (0.13 to 0.22), WML 0.11 (-0.01 to 0.23);
Vascular disease category, CAD: BPF -0.90 (-1.01 to -0.80), GMF -1.50 (-1.73 to -1.26), VF 0.16 (0.13 to 0.19), WML 0.08 (0.01 to 0.15).

* $p<0.05$.

** $p<0.005$.

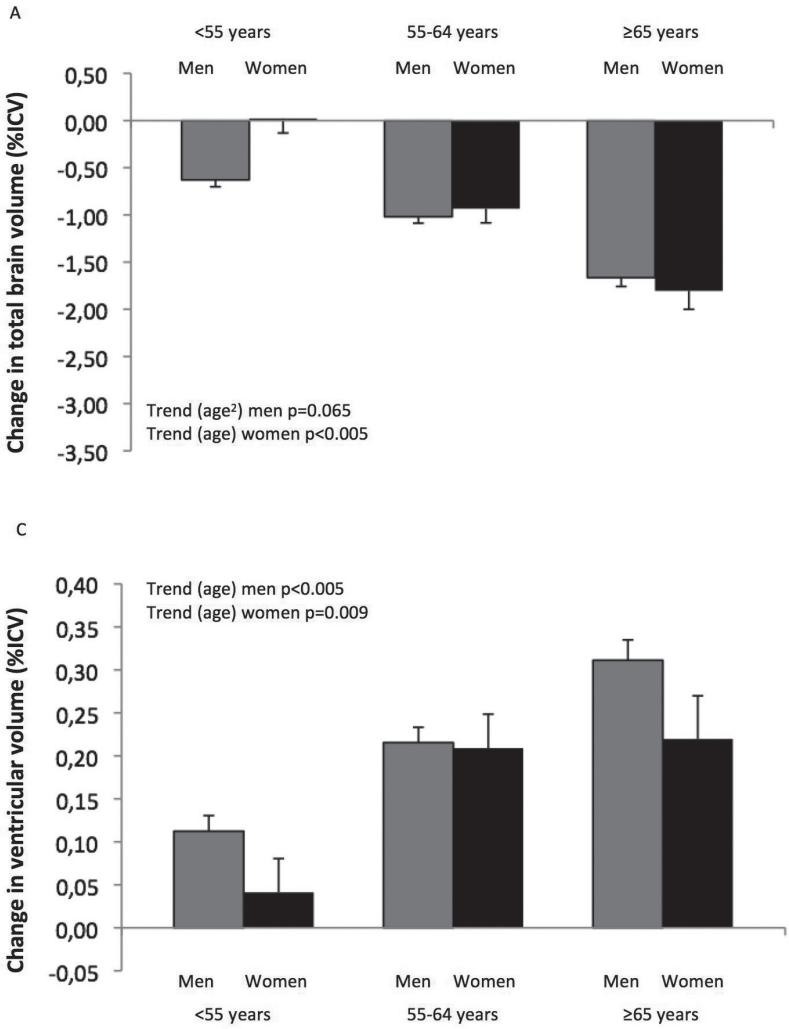
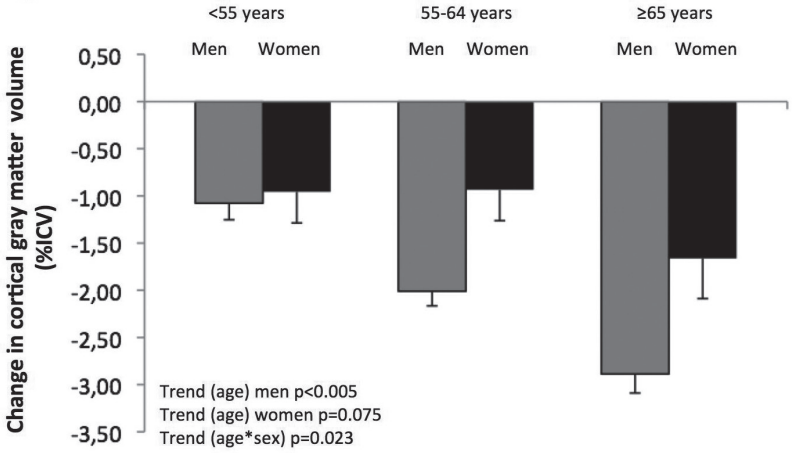
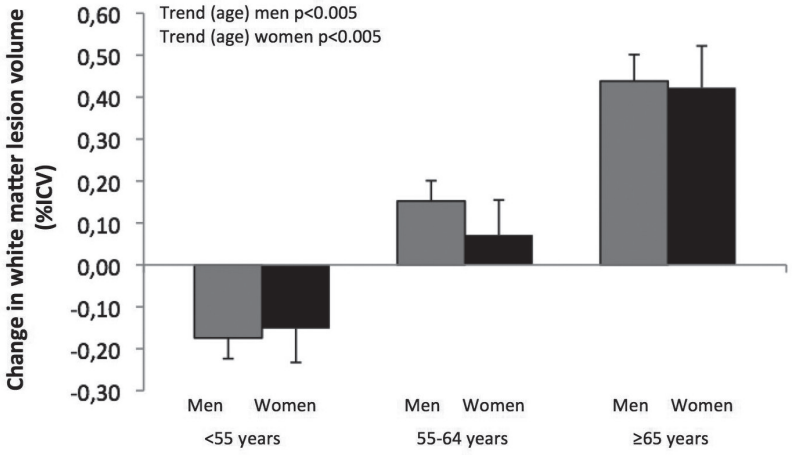


Figure 1 Longitudinal relation between age and change in total brain (A), cortical gray matter (B), ventricular (C), and white matter lesion volume (natural log transformed) (D) for men and women separately.
Bars represent the mean (standard error) adjusted change in brain volume after on average 3.9 years of follow-up. Adjusted for baseline measurement of brain structure and follow-up period.
ICV, intracranial volume.

B


<55 years
55-64 years
≥65 years
Men Women
Men Women
Men Women
Change in cortical gray matter volume (%ICV)
0,50
0,00
-0,50
-1,00
-1,50
-2,00
-2,50
-3,00
-3,50
Trend (age) men p<0.005
Trend (age) women p=0.075
Trend (age*sex) p=0.023

D


Trend (age) men p<0.005
Trend (age) women p<0.005
Change in white matter lesion volume (%ICV)
0,60
0,50
0,40
0,30
0,20
0,10
0,00
-0,10
-0,20
-0,30
Men Women
Men Women
Men Women
<55 years
55-64 years
≥65 years

no significant change in the effect estimates of the found associations (Supplemental Table 4, model 5).

Third, to study a possible treatment effect, analyses were repeated in a subset of the study population who had not undergone a surgical intervention prior to inclusion in the study (n=170, 26%) and the effect estimates of the associations of age and sex with changes in brain volumes and risk of new brain infarcts were similar (Supplemental Table 5). However, due to loss of power, we were not able to study a possible treatment effect on the associations of vascular disease categories with brain changes in this subset. Also, we additionally adjusted the analyses for surgical interventions during follow-up for the total study population (n=663) (Supplemental Table 4, model 6) and the effect estimates shown in Table 2 did not materially change.

Finally, since post-stroke focal brain atrophy can contribute to progression of brain atrophy in this population, we performed sensitivity analyses excluding 15 patients who were recruited within 90 days after an acute stroke, and the results did not materially change.

DISCUSSION

Within a large cohort of patients with various manifestations of arterial disease, we observed progression of global, cortical, and subcortical brain atrophy over 3.9 years of follow-up. With older age, progression of global and cortical atrophy tended to accelerate and was stronger in men than in women. Our results are in line with population-based longitudinal studies reporting annual gross brain volume decreases in the order of 0.2-0.6%, depending on the age of the cohort.[9-11,16] Compared with our cross-sectional findings,[2] changes in BPF and GMF are larger in our longitudinal analyses. This finding is in contrast with results in some earlier studies,[9,10,17] but confirmed in at least one other study.[11] Although there is no evident explanation for the difference in our cross-sectional and longitudinal findings, issues related to survival, non-response, or cohort effects might all have contributed to this difference.

Our finding that with older age, men showed more progression of cortical atrophy than women is in line with our cross-sectional findings[2] and other studies,[11,18,19] although not all.[17,20,21] This finding may be explained by the finding that compared with women, men more often had an unfavorable cardiovascular risk profile in our study population. However, adjusting for these cardiovascular risk factors did not explain the difference in progression of brain atrophy. In addition, it has also been suggested that sex hormones or differences in regional brain metabolism between men and women might play a role in brain atrophy, but the exact mechanisms are not entirely clear.[19]

In younger patients, we observed regression of WML volume, which was somewhat surprising. An explanation for the negative change in WML volume could be that WMLs are no longer measurable due to brain atrophy of the surrounding tissue or due to a nearby infarct. Other explanations are measurement error, since the WML volumes at baseline were already quite small, or resolution of WMLs. Earlier studies have suggested

that WMLs are not irreversible.[22,23] In addition, we observed progression of WML volume with age, which was similar for men and women. Other longitudinal studies who used volumetric assessment of change in WML volume reported similar[11] or somewhat larger changes in WML volume.[24,25] However, these studies were performed in smaller and older samples of the general population and the results may therefore be not fully comparable.

The risk of new brain infarcts also increased with age and was stronger in men than in women, particularly for new lacunar infarcts. This finding is in contrast with our cross-sectional findings and the results from a population-based longitudinal study on new brain infarcts, where there were no significant sex differences.[2,26] Additionally, for lacunar infarcts, a cross-sectional study in the general population showed a higher risk for women instead of men,[27] while another population-based study did not.[28] These inconsistent findings make it difficult to explain the association of new brain infarcts with male sex.

With respect to the different vascular disease locations, we found that, compared with CAD patients, patients with CVD had more progression of cortical and subcortical atrophy, and more new brain infarcts, in particular new cortical brain infarcts. However, the association with progression of cortical atrophy was partly explained by the presence of cerebrovascular lesions, such as brain infarcts and WMLs.

PAD patients had more progression of cortical atrophy compared with patients with CAD. These findings were independent of the presence of cardiovascular risk factors at baseline. Although we know that when manifest arterial disease is present in one vascular bed, there is an increased risk for developing manifest arterial disease in other vascular beds,[29] our findings suggest that manifest arterial disease at different locations might differentially influence progression of brain atrophy and cerebrovascular lesions. Earlier studies relating arterial disease to progression of brain pathology only evaluated patients with vascular disease present in one vascular bed. Presence of carotid atherosclerosis has been associated with increased risk of new brain infarcts, WMLs, and progression of brain atrophy.[7,23,30] Although there is little evidence on the relation between peripheral and abdominal atherosclerosis and progression of brain pathology, one cross-sectional study showed that aortic atherosclerosis was associated with more WMLs.[31] The possible differential effect of the location of arterial disease on progression of brain atrophy and/or cerebrovascular lesions could be explained by differences in cardiovascular risk factors or differences in treatment strategies between the vascular disease categories. We could only partly account for these possible differences by adjusting for baseline risk factors, but additional analyses suggested that our results could not be explained by treatment effects prior or during follow-up. Also, differences in hemodynamics, remodelling properties, and/or underlying arterial wall structure of the different vascular beds could play a role in the differential effect of the location of arterial disease on progression of brain atrophy and/or cerebrovascular lesions.[32]

To our knowledge, our study is the first to prospectively examine brain changes on MRI in a large cohort of well-defined patients with various manifestations of arterial disease. Other unique aspects of this study are the wide age range of the population and the availability of brain volumetric measurements on MRI at baseline and follow-up.

This made it possible to differentiate between the different brain tissues and to obtain relatively precise estimates of progression of brain atrophy and cerebrovascular lesions.

Limitations of this study are, first, that individuals who participated in the follow-up examination represented a relatively healthy group. This may have led to an underestimation of the progression of brain atrophy and cerebrovascular lesions. Second, the number of new brain infarcts was relatively small, especially new cortical and lacunar infarcts. This resulted in reduced power and the associations found in this study should therefore be interpreted with caution. Third, patients were classified in five distinct vascular disease categories according to patient history and inclusion criteria at baseline. Vascular events that took place in the follow-up period were, however, not included in the category definition. However, we did perform additional analyses adjusting for vascular events during follow-up and the results did not change.

In conclusion, in a large cohort of patients with various manifestations of arterial disease, we observed trends in accelerated progression of brain atrophy and progression of cerebrovascular lesions, especially in older men and patients with CVD or PAD.

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Supplemental Table 1 Baseline characteristics of the study sample (n=663) per sex category and in the total population

	Sex categories		All
	Men	Women	
	n=257	n=244	n=162
Age, years	58 ± 9	57 ± 10	57 ± 9
Vascular disease location, %			
Coronary artery disease	55	36	51
Cerebrovascular disease	14	23	16
Peripheral arterial disease	10	21	12
Abdominal aortic aneurysm	4	1	3
Multiple vascular diseases	13	11	13
Cardiovascular risk factors			
BMI, kg/m^2	27 ± 3	27 ± 4	27 ± 4
Smoking, pack years[†]	21 (0, 52)	14 (0, 43)	20 (0, 49)
Alcohol intake, % current	84	56	79
Systolic blood pressure, mmHg	140 ± 20	141 ± 20	140 ± 20
Diastolic blood pressure, mmHg	82 ± 10	80 ± 11	81 ± 10
Hyperlipidemia, %	80	71	78
Diabetes mellitus, %	13	15	13
Intima-media thickness, mm	0.9 ± 0.3	0.9 ± 0.2	0.9 ± 0.3
Brain volumes, %ICV			
Brain parenchymal fraction	79.1 ± 2.6	80.2 ± 2.3	79.3 ± 2.6
Cortical gray matter fraction	36.0 ± 3.3	37.5 ± 2.7	36.3 ± 3.3
Ventricular fraction	2.1 ± 0.9	1.8 ± 0.7	2.0 ± 0.8
Total WML volume fraction[†]	0.09 (0.03, 0.40)	0.12 (0.04, 0.44)	0.09 (0.03, 0.40)
Cerebrovascular pathology, %			
Any infarct	27	27	27
Cortical	11	11	11
Large subcortical	1	1	1
Lacunar	18	14	17
Infratentorial	6	7	6
Cerebellum	4	3	4
Brainstem	2	5	3

Characteristics presented as mean ± SD, unless stated otherwise.

BMI, body mass index; WML, white matter lesion.

[†] Median (10th-90th percentile).

Percentage of missing values before imputation: BMI 0.2%, smoking 0.3%, alcohol intake 0.5%, hyperlipidemia 1.8%, intima-media thickness 2.0%, cortical gray matter fraction 17.5%, all other variables 0.0%.

Supplemental Table 2 Brain volumes and number of infarcts at baseline, follow-up, and their changes in 663 patients with manifest arterial disease

	Baseline	Follow-up	Absolute change	Relative change to baseline brain measure
Brain volumes				
Intracranial volume (ICV), mL	1468 ± 129	1458 ± 128	-10.2 ± 27.8	-0.7 ± 1.9%
Total brain volume, mL	1165 ± 106	1142 ± 106	-22.3 ± 20.8	-1.9 ± 1.8%
Total brain volume, %ICV	79.3 ± 2.6	78.4 ± 3.0	-0.98 ± 1.08	-1.2 ± 1.4%
Cortical gray matter volume, mL	532 ± 57	504 ± 59	-29.0 ± 30.9	-5.4 ± 6.0%
Cortical gray matter volume, %ICV	36.3 ± 3.2	34.6 ± 3.6	-1.72 ± 2.17	-4.7 ± 6.2%
Ventricular volume, mL	30 ± 13	32 ± 15	2.6 ± 4.0	8.7 ± 12.9%
Ventricular volume, %ICV	2.0 ± 0.8	2.2 ± 1.0	0.19 ± 0.28	9.5 ± 13.2%
White matter lesion volume, mL	2.8 ± 5.2	3.7 ± 7.3	0.8 ± 3.4	38 ± 120%
White matter lesion volume, %ICV	0.19 ± 0.34	0.25 ± 0.49	0.06 ± 0.23	39 ± 120%

	Baseline	Follow-up	Absolute change
Cerebrovascular pathology, n			
Any infarct	399 in 179	534 in 212	135 in 82
Cortical	107 in 74	138 in 88	31 in 21
Large subcortical	7 in 6	8 in 7	1 in 1
Lacunar	237 in 114	331 in 138	94 in 58
Infratentorial	48 in 40	57 in 45	9 in 7
Cerebellum	28 in 26	32 in 29	4 in 4
Brainstem	20 in 17	25 in 21	5 in 5

Brain volumes and changes are represented as mean ± standard deviation and infarcts are presented as number of infarcts in number of patients.

ICV, intracranial volume; n, number of patients with brain infarct.

Supplemental Table 3 New cortical and lacunar infarcts on MRI in 663 patients with manifest arterial disease after on average 3.9 years of follow-up

	Model	n/N	New cortical infarcts, OR (95% CI)[a]	n/N	New lacunar infarcts, OR (95% CI)[a]
Age					
<55 years, reference		22/257	1.0	28/257	1.0
55-64 years	1	31/244	0.98 (0.34-2.89)	42/244	1.96 (0.85-4.51)
	2		0.69 (0.22-2.14)		1.58 (0.66-3.79)
	3		0.44 (0.13-1.55)		1.48 (0.58-3.77)
≥65 years	1	21/162	1.51 (0.51-4.45)	44/162	4.61 (2.06-10.33)**
	2		0.55 (0.14-2.16)		2.24 (0.86-5.86)
	3		0.33 (0.07-1.45)		2.28 (0.83-6.21)
Sex					
Women, reference		14/124	1.0	17/124	1.0
Men	1	60/539	2.12 (0.48-9.28)	97/539	3.05 (1.05-8.90)*
	2		2.03 (0.45-9.17)		3.60 (1.20-10.80)*
	3		3.02 (0.59-15.44)		3.29 (1.84-5.86)*
Vascular disease location					
CAD, reference		10/342	1.0	19/342	1.0
CVD	1	33/105	6.93 (2.12-22.62)**	47/105	1.26 (0.55-2.90)
	2		5.48 (1.63-18.40)*		1.01 (0.41-2.46)
	3		7.00 (1.96-25.04)**		0.91 (0.36-2.30)
PAD	1	5/80	0.95 (0.11-8.34)	11/80	0.98 (0.33-2.92)
	2		0.87 (0.10-7.69)		1.04 (0.34-3.13)
	3		0.64 (0.06-6.64)		0.83 (0.26-2.66)
AAA	1	1/21	6.16 (1.10-34.58)*	5/21	2.79 (0.85-9.19)
	2		4.77 (0.82-27.72)		1.99 (0.55-7.27)
	3		6.98 (1.03-47.52)*		1.56 (0.40-6.18)
Multiple	1	23/84	1.35 (0.24-7.50)	30/84	0.71 (0.27-1.83)
	2		1.05 (0.18-6.03)		0.61 (0.22-1.68)
	3		0.99 (0.17-5.96)		0.51 (0.18-1.43)

Model 1: adjusted for age (not if age was independent variable), sex (not if sex was independent variable), baseline measurement of brain structure, and follow-up period; model 2: model 1 + BPF and WML (%ICV); model 3: model 2 + systolic and diastolic blood pressure, diabetes mellitus, body mass index, alcohol use, pack years, hyperlipidemia, intima-media thickness.

n/N, number of patients with an infarct at baseline per category; OR, odds ratio; CI, confidence interval; CAD, coronary artery disease; CVD, cerebrovascular disease; PAD, peripheral arterial disease; AAA, abdominal aortic aneurysm; BPF, brain parenchymal fraction; WML, natural log transformed white matter lesions (%ICV); ICV, intracranial volume.

[a] OR for new brain infarcts in comparison with the reference category after 3.9 ± 0.4 years of follow-up. New brain infarcts were defined as change in number of infarcts of ≥1 infarct.

* $p<0.05$.

** $p<0.005$.

Supplemental Table 4 Progression of measures of brain atrophy and cerebrovascular lesions on MRI after on average 3.9 years of follow-up in 663 patients with manifest arterial disease according to categories of age, sex, and vascular disease location

	Model	N	Change in BPF, B (95% CI)[a]	Change in GMF, B (95% CI)[a]	Change in VF, B (95% CI)[a]	Change in WML, B (95% CI)[a]	New brain infarcts, OR (95% CI)[b]
Age							
<55 years, reference[c]		257	0	0	0	0	1.0
55-64 years	4	244	-0.50 (-0.68; -0.32)**	-0.65 (-1.08; -0.21)**	0.11 (0.06; 0.15)**	0.30 (0.18; 0.42)**	1.54 (0.78-3.02)
	5		-0.48 (-0.67; -0.29)**	-0.72 (-1.16; -0.29)**	0.10 (0.05; 0.14)**	0.29 (0.16; 0.41)**	1.08 (0.51-2.28)
	6		-0.47 (-0.66; -0.29)**	-0.71 (-1.15; -0.27)**	0.09 (0.04; 0.14)**	0.28 (0.16; 0.41)**	1.10 (0.52-2.32)
≥65 years	4	162	-1.16 (-1.39; -0.93)**	-1.36 (-1.87; -0.85)**	0.17 (0.12; 0.23)**	0.57 (0.42; 0.72)**	3.21 (1.60-6.44)**
	5		-1.13 (-1.37; -0.88)**	-1.41 (-1.95; -0.87)**	0.14 (0.09; 0.20)**	0.56 (0.40; 0.73)**	1.48 (0.68-3.24)
	6		-1.12 (-1.37; -0.88)**	-1.40 (-1.94; -0.86)**	0.14 (0.08; 0.20)**	0.57 (0.40; 0.73)**	1.55 (0.72-3.33)
Sex							
Women, reference[c]		124	0	0	0	0	1.0
Men	4	539	-0.22 (-0.41; -0.03)*	-0.89 (-1.31; -0.47)**	0.02 (-0.03; 0.07)	-0.04 (-0.17; 0.09)	2.53 (1.11-5.77)*
	5		-0.30 (-0.50; -0.10)**	-1.09 (-1.53; -0.66)**	0.04 (-0.01; 0.09)	-0.03 (-0.17; 0.11)	3.16 (1.29-7.72)*
	6		-0.31 (-0.51; -0.11)**	-1.11 (-1.54; -0.68)**	0.04 (-0.01; 0.09)	-0.03 (-0.17; 0.11)	3.36 (1.37-8.26)*
Vascular disease location							
CAD, reference[c]		342	0	0	0	0	1.0
CVD	4	105	-0.14 (-0.36; 0.07)	-0.54 (-1.02; -0.07)*	0.09 (0.04; 0.15)**	0.07 (-0.08; 0.23)	2.29 (1.12-4.69)*
	5		-0.07 (-0.31; 0.17)	-0.18 (-0.70; 0.34)	0.09 (0.03; 0.15)**	-0.04 (-0.20; 0.13)	2.32 (1.05-5.10)*
	6		-0.05 (-0.29; 0.19)	-0.15 (-0.67; 0.37)	0.09 (0.03; 0.15)**	-0.04 (-0.21; 0.13)	2.15 (0.98-4.76)

Supplemental Table 4 (Continued) Progression of measures of brain atrophy and cerebrovascular lesions on MRI after on average 3.9 years of follow-up in 663 patients with manifest arterial disease according to categories of age, sex, and vascular disease location

	Model	N	Change in BPF, B (95% CI)[a]	Change in GMF, B (95% CI)[a]	Change in VF, B (95% CI)[a]	Change in WML, B (95% CI)[a]	New brain infarcts, OR (95% CI)[b]
Vascular disease location							
PAD	4	80	-0.16 (-0.40; 0.07)	-0.60 (-1.11; -0.09)*	0.04 (-0.02; 0.10)	-0.09 (-0.26; 0.07)	1.34 (0.55-3.25)
	5		-0.12 (-0.36; 0.12)	-0.64 (-1.17; -0.11)*	0.02 (-0.04; 0.08)	-0.08 (-0.24; 0.09)	0.97 (0.36-2.60)
	6		-0.12 (-0.37; 0.12)	-0.66 (-1.19; -0.13)*	0.03 (-0.03; 0.09)	-0.07 (-0.24; 0.10)	1.09 (0.41-2.88)
AAA	4	21	0.04 (-0.38; 0.47)	0.41 (-0.53; 1.34)	0.11 (-0.00; 0.22)	0.06 (-0.24; 0.35)	3.99 (1.41-11.32)*
	5		0.05 (-0.37; 0.48)	0.44 (-0.49; 1.37)	0.10 (-0.01; 0.21)	0.06 (-0.24; 0.36)	2.79 (0.84-9.21)
	6		0.03 (-0.40; 0.46)	0.44 (-0.49; 1.36)	0.11 (-0.00; 0.21)	0.07 (-0.23; 0.36)	3.10 (0.95-10.18)
Multiple	4	84	-0.08 (-0.31; 0.16)	-0.21 (-0.71; 0.29)	0.01 (-0.04; 0.07)	0.10 (-0.06; 0.26)	0.89 (0.39-2.06)
	5		0.09 (-0.16; 0.33)	0.10 (-0.44; 0.63)	-0.02 (-0.08; 0.04)	0.02 (-0.15; 0.20)	0.58 (0.23-1.45)
	6		0.08 (-0.17; 0.33)	0.10 (-0.44; 0.64)	-0.02 (-0.08; 0.05)	0.03 (-0.15; 0.20)	0.65 (0.26-1.62)

Model 4: BPF, GMF, VF, model 1 + change in WML (natural log transformed) and new brain infarcts on MRI; WML, model 1 + change in BPF and new brain infarcts on MRI; brain infarcts, model 1 + change in BPF and change in WML; model 5: model 3 + new vascular events during follow-up; model 6: model 3 + new surgical interventions during follow-up.

BPF, brain parenchymal fraction; GMF, cortical gray matter fraction; VF, ventricular fraction; WML, natural log transformed white matter lesions (%ICV); CI, confidence interval; OR, odds ratio; CAD, coronary artery disease; CVD, cerebrovascular disease; PAD, peripheral arterial disease; AAA, abdominal aortic aneurysm.

[a] B represents % change in BPF, GMF, VF, and natural log transformed WML (as % of ICV) in comparison with the reference category after 3.9 ± 0.4 years of follow-up.

[b] OR for new brain infarcts, which were defined as change in number of infarcts of ≥1 infarct in comparison with the reference category after 3.9 ± 0.4 years of follow-up.

* p<0.05.

** p<0.005.

Supplemental Table 5 Progression of measures of brain atrophy and cerebrovascular lesions on MRI after on average 3.9 years of follow-up in 170 patients who had not undergone a surgical intervention prior to inclusion

	Model	N	Change in BPF, B (95% CI)[a]	Change in GMF, B (95% CI)[a]	Change in VF, B (95% CI)[a]	Change in WML, B (95% CI)[a]	New brain infarcts, OR (95% CI)[b]
Age							
<55 years, reference[c]		66	0	0	0	0	1.0
55-64 years	1	57	-0.72 (-1.12; -0.32)**	-0.56 (-1.35; 0.22)	0.11 (0.01; 0.20)*	0.47 (0.21; 0.72)**	0.96 (0.30-3.11)
≥65 years	1	47	-1.62 (-2.12; -1.11)**	-1.66 (-2.60; -0.73)**	0.17 (0.06; 0.28)**	0.51 (0.23; 0.79)**	3.42 (1.22-9.59)**
Sex							
Women, reference[c]		46	0	0	0	0	1.0
Men	1	124	-0.34 (-0.70; 0.02)	-0.88 (-1.60; -0.15)*	0.00 (-0.08; 0.09)	-0.04 (-0.28; 0.21)	2.58 (0.81-8.22)

Model 1: adjusted for age (not if age was independent variable), sex (not if sex was independent variable), baseline measurement of brain structure, and follow-up period.

BPF, brain parenchymal fraction; GMF, cortical gray matter fraction; VF, ventricular fraction; WML, natural log transformed white matter lesions (%ICV); CI, confidence interval; OR, odds ratio.

[a] B represents % change in BPF, GMF, VF, and natural log transformed WML (as % of ICV) in comparison with the reference category after 3.9 ± 0.4 years of follow-up.

[b] OR for new brain infarcts, which were defined as change in number of infarcts of ≥1 infarct in comparison with the reference category after 3.9 ± 0.4 years of follow-up.

[c] Change (95% CI) in brain volumes (as % of ICV) for reference categories:

Age, <55 years: BPF -0.29 (-0.58 to 0.00), GMF -0.93 (-1.49 to -0.36), VF 0.11 (0.05 to 0.18), WML -0.23 (-0.41 to -0.06);

Sex, women: BPF -0.73 (-1.03 to -0.43), GMF -0.94 (-1.57 to -0.30), VF 0.19 (0.12 to 0.27), WML 0.09 (-0.12 to 0.30).

* $p<0.05$.

** $p<0.005$.

CHAPTER 3

LOWER CEREBRAL BLOOD FLOW AND PROGRESSION OF BRAIN ATROPHY: THE SMART-MR STUDY

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Submitted

ABSTRACT

INTRODUCTION

Cerebral hypoperfusion may be involved in the etiology of brain atrophy. However, few studies examined the longitudinal association of cerebral blood flow (CBF) and progression of brain atrophy.

METHODS

We examined the longitudinal association of baseline CBF with change in brain volumes on 1.5 T MRI over 3.9 years of follow-up in 621 patients with manifest arterial disease (mean age 57 ± 10, 82% men) from the SMART-MR study. Total CBF was measured with MR angiography, expressed per 100 mL brain volume as parenchymal CBF. Brain segmentation was performed to quantify progression of global (decrease in brain parenchymal fraction (BPF)), cortical (decrease in cortical gray matter fraction (GMF)), and subcortical brain atrophy (increase in ventricular fraction (VF)).

RESULTS

Mean (standard deviation [SD]) parenchymal CBF at baseline was 51.9 (10.2) mL/min/100 mL brain volume. Regression analyses, adjusted for age, sex, and follow-up time showed that lower baseline parenchymal CBF was associated with progression of cortical, but not global or subcortical brain atrophy; mean differences in change (95% CI) in BPF, GMF, and VF per SD decrease in parenchymal CBF were -0.05 (-0.13 to 0.02), -0.35 (-0.55 to -0.15), and 0.01 (-0.01 to 0.03) %ICV, independent of cardiovascular risk factors and cerebrovascular lesions. However, excluding patients with carotid stenosis >70% reduced the effect estimate; mean difference in change (95% CI) in GMF per SD decrease in parenchymal CBF was -0.18 (-0.38 to 0.03) %ICV.

CONCLUSIONS

Baseline reduced parenchymal CBF is associated with progression of cortical, but not global or subcortical brain atrophy, but excluding patients with severe carotid stenosis reduced the strength of the association with almost 50%.

INTRODUCTION

Global and focal brain atrophy are common findings on magnetic resonance imaging (MRI) in older populations and in patients with high vascular risk. This is of clinical importance since it has been suggested that the extent and rate of progression of global and focal atrophy are associated with cognitive deterioration and conversion to dementia.[1,2]

It has been hypothesized that cerebral hemodynamic function plays an important role in the etiology of brain atrophy since cerebral perfusion and blood flow are important for adequate delivery of oxygen and nutrients to the brain.[3] In normal conditions, cerebral vessels have the ability to keep cerebral blood flow (CBF) within adequate values by means of myogenic, neurogenic, or metabolic mechanisms.[4,5] However, when these mechanisms fail, due to for example hypertension or vascular lesions in the brain feeding arteries,[6] cerebral hypoperfusion may occur.

Cross-sectional studies have shown that individuals with lower cerebral blood flow (CBF) have smaller brain volumes[7-9] and that this association may be mediated by cerebral small-vessel disease, which is characterized by white matter lesions (WML) and lacunar infarcts.[8] Also, patients with signs of systemic hypoperfusion, such as low blood pressure or reduced cardiac output, have more (progression of) brain atrophy.[10-12] In addition, carotid atherosclerosis has also been associated with brain atrophy which is possibly explained by reduced blood flow to the brain.[13-15] However, limited evidence exists on long-term effects of cerebral hypoperfusion on brain tissue as longitudinal studies are lacking.

We investigated the association between CBF at baseline and progression of global, cortical and subcortical brain atrophy on MRI over on average 4 years of follow-up in a large cohort of patients with manifest arterial disease.

METHODS

SMART-MR STUDY

Data were used from the Second Manifestations of ARTerial disease-Magnetic Resonance (SMART-MR) study, a prospective cohort study aimed at investigating brain changes on MRI in patients with manifest arterial disease. Details of the cohort have been described elsewhere.[16,17] In brief, between May 2001 and December 2005, all patients newly referred to the University Medical Center Utrecht with manifest arterial disease, such as coronary artery disease, cerebrovascular disease, peripheral arterial disease, and/or an abdominal aortic aneurysm, and without MR contraindications, were invited to participate. During a one-day visit to our medical center, all patients underwent an MRI of the brain, a physical examination, ultrasonography of the carotid arteries, and blood sampling. Risk factors, medical history, and medication use were assessed using questionnaires. Between January 2006 and May 2009, follow-up measurements took place, including MRI of the brain.

The SMART-MR study was approved by the ethics committee of the University Medical Center in Utrecht according to the principles of the Declaration of Helsinki and written informed consent was obtained from all participants.[16]

STUDY SAMPLE

Of the 1309 patients at baseline, all patients still alive (n=1238) were invited for follow-up measurements. In total, 754 patients of the surviving cohort participated, of whom 621 patients had adequate 2D MR angiography measurements at baseline and an MRI at both baseline and follow-up for adequate brain segmentation. Compared with the 617 patients who did not participate in the follow-up examinations, this sample (N=621) was younger (mean (standard deviation [SD]), 51.9 (10.2) vs. 59.4 (10.5) years), more often male (82 vs. 77%), more often current alcohol users (79 vs. 72%) and had more pack years of smoking (median (10^{th}-90^{th} percentile), 20 (0-49) vs. 16 (0-52)), but had less often diabetes (13 vs. 20%) and larger baseline brain parenchymal fraction (mean (SD), 79.3 (2.6) vs. 78.8 (3.0) %ICV).

MAGNETIC RESONANCE PROTOCOL AND BRAIN SEGMENTATION

At baseline and follow-up, 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 transversal T1-weighted (repetition time [TR]/echo time [TE], 235/2 ms), T2-weighted (TR/TE, 2200/11 ms and 2200/100 ms), fast fluid-attenuated inversion recovery (FLAIR; TR/TE/inversion time [TI], 6000/100/2000 ms), and inversion recovery (IR; TR/TE/TI, 2900/22/410 ms) sequences. Field of view (FOV) was 230 x 230 mm (matrix size, 180 x 256; slice thickness, 4.0 mm; no gap; 38 slices).[16]

On the basis of a localizer MR angiographic slab in the sagittal plane, a 2D 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) (TR/TE, 16/9 ms; flip angle, 7.5°; FOV, 250 x 250 mm; matrix size, 256 x 256; slice thickness, 5.0 mm; number of averaged signals, 8; velocity sensitivity, 100 cm/s).[18]

The T1-weighted, IR, and FLAIR sequence were used for the probabilistic segmentation technique, described previously.[19] The segmentation program distinguishes cortical gray matter, white matter, cerebrospinal fluid (CSF), and lesions. The results of the segmentation analysis were visually checked for the presence of infarcts and adapted if necessary to make a distinction between white matter lesions (WMLs) and infarct volumes.

CEREBRAL BLOOD FLOW

Image processing of the phase-contrast MRI flow measurements was performed by two investigators.[8,20] The flow through the left and right ICAs and BA were summed to calculate the total cerebral blood flow (tCBF, mL/min). Because tCBF strongly depends on the amount of brain tissue, we expressed tCBF per 100 mL brain volume to obtain parenchymal CBF.[20]

BRAIN ATROPHY

At baseline and follow-up, total brain volume was calculated by summing the volumes of gray and white matter and, if present, the volumes of WML and infarcts. All volumes cranial to the foramen magnum were included. Total intracranial volume (ICV) was calculated by summing the total brain volume and the volumes of the CSF. Total brain, cortical gray matter, and ventricular volumes were normalized for ICV and expressed as brain parenchymal fraction (BPF), cortical gray matter fraction (GMF), and ventricular fraction (VF).[16] Change in BPF, GMF, and VF were used as indicators of progression of global, cortical, and subcortical brain atrophy.

CEREBROVASCULAR LESIONS

At baseline and follow-up, the whole brain was visually searched for infarcts by a trained investigator and a neuroradiologist, blinded to all information of the patient. Discrepancies in rating were re-evaluated in a consensus meeting. Infarcts were defined as focal hyperintensities on T2-weighted images of ≥3 mm in diameter. Infarcts located in the white matter also had to be hypointense on T1-weighted and FLAIR images in order to distinguish them from WML. Dilated perivascular spaces were distinguished from infarcts on the basis of their location, form, and the absence of gliosis. Brain infarcts were categorized as lacunar infarcts and non-lacunar infarcts. Lacunar infarcts were defined as infarcts sized 3-15 mm in diameter and located in the subcortical white matter, thalamus, or basal ganglia. Volumes of periventricular and deep WML were summed to obtain the total volume of WML.[16] WML volumes were natural log transformed, because they were not normally distributed.

COVARIATES

Height and weight were measured and 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. Diabetes mellitus at baseline was defined as a referral diagnosis of diabetes mellitus, self-reported diabetes mellitus, use of glucose-lowering agents, glucose ≥11.1 mmol/L, or a known history of diabetes mellitus. Among subjects without a history of diabetes mellitus, but with a fasting plasma glucose ≥7.0 mmol/L at baseline, those receiving treatment with glucose-lowering agents within one year after baseline were considered as having diabetes mellitus at baseline. Hyperlipidemia was defined as total cholesterol >5.0 mmol/L, low-density lipoprotein cholesterol >3.2 mmol/L, or self-reported use of lipid-lowering drugs. Smoking habits and alcohol intake were assessed using questionnaires. Pack years of smoking were calculated and alcohol intake was categorized as never, former, or current users. Ultrasonography was performed to measure the intima-media thickness and carotid artery stenosis. Mean carotid intima-media thickness (in mm) was calculated for each patient based on 6 far-wall measurements of the left and right common carotid arteries as previously described.[21] The degree of the carotid artery stenosis at both sides was assessed with color Doppler-assisted duplex scanning. The severity of carotid artery stenosis was evaluated on the basis of blood flow velocity patterns.[22] The greatest stenosis

observed on the right or the left side of the common or internal carotid artery was taken to determine the severity of carotid artery disease. Carotid artery stenosis ≥50% was defined as peak systolic velocity >150 cm/s and a carotid artery stenosis ≥70% was defined as peak systolic velocity >210 cm/s.[22] We classified carotid stenosis into groups of 0 to 50% (no or limited), 50 to 70% (moderate), and >70% (severe).[13]

DATA ANALYSES

Missing data rarely occurs completely at random and complete case analyses leads to loss of statistical power and to biased results. We used multiple imputation (10 datasets) using the statistical program R (aregImpute; version 2.10.0) to address missing values in the study sample of 621 patients. Data were analyzed using SPSS version 20.0 (Chicago, IL, USA), by pooling the 10 imputed datasets.[23]

Baseline characteristics were calculated according to tertiles of parenchymal CBF for the total study population (n=621). Linear regression analysis was used to estimate the association of baseline parenchymal CBF with changes in BPF, GMF, or VF. The respective brain MRI measures at follow-up were entered as dependent variable and parenchymal CBF (per SD decrease) was entered as independent variable. All analyses were adjusted for age, sex, respective baseline brain MRI measure, and follow-up time (model 1). In model 2, additional adjustments were made for cardiovascular risk factors (systolic and diastolic blood pressure, diabetes mellitus, BMI, alcohol use, pack years, hyperlipidemia, carotid intima-media thickness). Furthermore, in model 3 additional adjustments were made for baseline WML volume and presence of lacunar and non-lacunar brain infarcts. To evaluate whether the associations were graded, the analyses were repeated for tertiles of parenchymal CBF. Next, as carotid stenosis could explain the association between parenchymal CBF and changes in brain volumes, the above-mentioned analyses were repeated after exclusion of patients with carotid stenosis of >70% (n=55). Finally, as presence of WML could modify the association between parenchymal CBF and brain volumes, as found in our cross-sectional analyses,[8] an interaction term was entered in the final model. If an interaction term was found significant ($p<0.05$), stratified analyses were performed according to tertiles of WML.

RESULTS

At baseline, the mean (SD) age of the study population (n=621) was 57 (10) years and the majority of the patients were men (n=509; 82%). Mean (SD) parenchymal CBF was 51.9 (10.2) mL/min/100 mL brain volume and during follow-up (mean 3.9, range 3.0-5.8 years), the mean (SD) decrease in BPF and GMF was 0.98 (1.09) and 1.68 (1.71) %ICV, and the mean (SD) increase in VF was 0.19 (0.28) %ICV. Patients with lower parenchymal CBF were older, more often men, had higher systolic blood pressure, more often cerebrovascular disease and carotid stenosis >70%, and had smaller brain volumes and more brain infarcts at baseline than patients with higher parenchymal CBF (Table 1).

Table 1 Baseline characteristics of the study sample according to tertiles of parenchymal cerebral blood flow (n=621)

	Tertiles of parenchymal CBF (mL/min/100 mL brain volume)		
	<47.1	47.1 – 55.0	>55.0
	n=207	n=207	n=207
Parenchymal CBF, mL/min/100 mL brain volume	41.4 ± 4.9	51.1 ± 2.4	63.2 ± 6.6
Age, years	59 ± 10	58 ± 10	56 ± 9
Sex, % men	88	82	75
Vascular disease location, %			
Coronary artery disease	61	61	64
Cerebrovascular disease	30	22	18
Peripheral arterial disease	15	20	19
Abdominal aortic aneurysm	5	6	6
Cardiovascular risk factors			
BMI, kg/m^2	27 ± 3	27 ± 4	27 ± 4
Smoking, pack years†	19 (0, 52)	18 (0, 52)	24 (0, 48)
Alcohol intake, % current	77	57	79
Systolic blood pressure, mmHg	142 ± 21	139 ± 10	138 ± 17
Diastolic blood pressure, mmHg	81 ± 11	82 ± 10	81 ± 10
Hyperlipidemia, %	76	80	78
Diabetes mellitus, %	15	11	14
Carotid atherosclerosis			
Intima media thickness, mm	1.0 ± 0.3	0.9 ± 0.3	0.9 ± 0.3
Carotid stenosis >70%, %	16	7	5
Brain volumes, %ICV			
Brain parenchymal fraction	78.9 ± 2.5	79.3 ± 2.6	79.8 ± 2.5
Cortical gray matter fraction	35.7 ± 3.2	36.3 ± 3.2	37.1 ± 3.3
Ventricular fraction	2.1 ± 0.9	2.0 ± 0.8	1.9 ± 0.8
Total WML volume fraction†	0.10 (0.03, 0.41)	0.09 (0.02, 0.53)	0.09 (0.03, 0.33)
Cerebrovascular pathology, %			
Any infarct	33	25	23
Lacunar infarct	19	18	14
Non-lacunar infarct	23	14	12

Characteristics presented as mean ± standard deviation, unless stated otherwise.

BMI, body mass index; ICV, intracranial volume; WML, white matter lesion.

† Median (10^{th}-90^{th} percentile).

Percentage of missing values before imputation: BMI 0.2%, smoking 0.3%, alcohol intake 0.5%, hyperlipidemia 1.8%, intima-media thickness 1.8%, carotid stenosis >50% 3.7%, cortical gray matter fraction 17.9%, all other variables 0.0%.

Table 2 Association between baseline parenchymal cerebral blood flow and change in brain volume over time (n=621)

	Model	Change in BPF	Change in GMF	Change in VF
		B (95% CI)[a]	B (95% CI)[a]	B (95% CI)[a]
Parenchymal CBF (per SD decrease)[b]	1	-0.05 (-0.13 to 0.02)	-0.35 (-0.55 to -0.15)*	0.01 (-0.01 to 0.03)
	2	-0.05 (-0.13 to 0.03)	-0.34 (-0.54 to -0.13)*	0.01 (-0.01 to 0.03)
	3	-0.04 (-0.12 to 0.04)	-0.28 (-0.48 to -0.08)*	0.01 (-0.01 to 0.03)

Model 1: adjusted for age, sex, follow-up time, and baseline brain volume; model 2: additionally adjusted for systolic and diastolic blood pressure, diabetes mellitus, body mass index, former and current alcohol use, pack years, hyperlipidemia, intima-media thickness; model 3: additionally adjusted for WML and lacunar and non-lacunar infarcts on MRI.

BPF, brain parenchymal fraction; GMF, cortical gray matter fraction; VF, ventricular fraction; CBF, cerebral blood flow; CI, confidence interval; SD, standard deviation.

[a] B represents % change (95% CI) in brain volumes (%ICV) per SD decrease in parenchymal CBF at baseline.

[b] SD parenchymal CBF=10.2 mL/min/100 mL brain volume.

* p<0.05.

Lower baseline parenchymal CBF was significantly associated with progression of cortical brain atrophy, but not with global or subcortical brain atrophy (Table 2). The mean differences in change (95% CI) in BPF, GMF, and VF per SD decrease in parenchymal CBF were -0.05 (-0.13 to 0.02) %ICV, -0.35 (-0.55 to -0.15) %ICV, and 0.01 (-0.01 to 0.03) %ICV. Adjusting for cardiovascular risk factors and cerebrovascular lesions on MRI (WML volume and brain infarcts) slightly attenuated the association between parenchymal CBF and change in GMF, but a statistically significant association remained (Table 2, models 2 and 3). Furthermore, excluding patients with carotid stenosis >70% (n=55) attenuated the relation between parenchymal CBF and progression of cortical brain atrophy; per SD decrease in parenchymal CBF the mean difference in change (95% CI) in GMF was -0.18 (-0.38 to 0.03) %ICV. Finally, WML did not modify the relation between parenchymal CBF and progression of brain atrophy; p-values for interaction were 0.15, 0.40, and 0.53 for BPF, GMF, and VF.

DISCUSSION

In this longitudinal study in a large cohort of patients with manifest arterial disease, lower parenchymal cerebral blood flow (parenchymal CBF) at baseline was associated with progression of cortical brain atrophy but not with global or subcortical brain atrophy on MRI over on average four years of follow-up. This association was independent of cardiovascular risk factors and cerebrovascular lesions. However, the association was largely explained by presence of severe carotid stenosis.

Previous cross-sectional studies have shown that individuals with lower parenchymal CBF had smaller brain volumes,[7-9,24] but no earlier studies have investigated the longitudinal relation between baseline CBF and progression of brain atrophy. One small-scaled study in 14 patients with ischemic cerebrovascular disease showed that a decline in CBF was associated with progression of brain atrophy, but as they investigated progression of brain atrophy and change in CBF simultaneously, it is also possible that another underlying process resulted in change in both measures.[24]

Our findings that lower baseline CBF was a contributor to progression of cortical brain atrophy over time, but not to progression of global or subcortical brain atrophy, suggests that the vulnerability of the brain to low perfusion values differs between brain regions.[4,5] A possible explanation for the observed relation between lower parenchymal CBF and progression of cortical brain atrophy may be found in higher metabolic demands of the cortical gray matter. Cortical gray matter contains the majority of cerebral neurons and has a higher blood flow than white matter.[5,25] It may therefore show more atrophy in response to lower cerebral perfusion than the brain as a whole as it no longer receives enough oxygen and nutrients. However, longitudinal studies using arterial-spin labelling MRI for regional CBF measures and cortical brain atrophy in healthy controls and patients with Alzheimer's disease showed that local CBF reductions were not necessarily accompanied by local cortical thinning over time.[26,27] As their study samples and MRI-techniques differed from ours, a comparison should be interpreted with caution.

Gray matter has also been associated with more reduction in cerebrovascular reactivity during aging than white matter.[28] Cerebrovascular reactivity, which represents the vascular ability to dilate on stimulation such as an increase in CO2, is an important feature of cerebral autoregulation. Decreased cerebrovascular reactivity results in a reduction in gray matter CBF as cerebral autoregulation is impaired, which consequently could have led to more cortical brain atrophy.

Another explanation for the relation between reduced CBF and subsequent cortical brain atrophy could be the presence of cerebrovascular lesions. Brain infarcts and WML have been associated with both cerebral hypoperfusion and more brain atrophy.[8,29-32] However, adjusting for cerebrovascular lesions in our analyses did only slightly change our effect estimates. Also, presence of WMLs did not significantly modify the relation between reduced CBF and progression of brain atrophy.

When we excluded patients with carotid stenosis >70%, the strength of the association between parenchymal CBF and cortical atrophy was reduced by almost 50% and became non-significant. The baseline characteristics of our study sample already showed that in the lowest tertile of parenchymal CBF, 26% of the patients had >70% carotid stenosis and that the mean brain volumes were smaller than in the upper two tertiles of parenchymal CBF. Patients with >70% carotid stenosis also showed more progression of global, cortical, and subcortical atrophy over time than patients with less or no carotid stenosis.[13] By excluding these patients, the extremes within the association between cerebral hypoperfusion and progression of cortical atrophy were removed, which may explain the almost 50% reduction of the effect estimate. Previous studies have also suggested that

carotid atherosclerosis is a likely marker of brain changes in terms of brain atrophy and presence of WML and silent cerebral infarcts.[13-15] As severe carotid stenosis may cause cerebral hypoperfusion, resulting in these brain changes, a good collateral circulation is necessary to restore cerebral hemodynamics in these patients.[33] One study was able to adjust for cerebral perfusion in the association between carotid stenosis and brain atrophy, which also resulted in a reduction of the effect estimates and suggested that cerebral perfusion could be a mediator in the observed association.[13]

Strengths of our study include the longitudinal design in a large well-defined cohort of patients with manifest arterial disease. The detailed information on cardiovascular risk factors and cerebrovascular lesions allowed us to adjust for these possible confounders in the association between baseline parenchymal CBF and changes in brain volumes over time.

Limitations of this study are, first, that we had an indirect measure of cerebral perfusion so that no information was available on regional differences in brain perfusion and their relation to global, cortical, and subcortical brain atrophy. Recent studies have demonstrated distinct spatial patterns of structural and functional brain alterations, which addresses the value of regional perfusion measures in future research.[26,27] Second, our study sample consisted of healthy survivors of the original SMART-MR study, which may have led to an underestimation of the progression of brain atrophy. Further, it is unknown to what extent our results are generalizable to the general older population, as our study sample consisted of patients with high vascular risk.

In conclusion, in a large cohort of patients with manifest arterial disease, baseline lower CBF was associated with progression of cortical brain atrophy, which was largely explained by presence of severe carotid stenosis. These results indicate that low CBF is involved in the process of neurodegeneration.

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CHAPTER 4

LONGITUDINAL RELATION BETWEEN CEREBRAL SMALL-VESSEL DISEASE AND CEREBRAL BLOOD FLOW: THE SMART-MR STUDY

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ABSTRACT

INTRODUCTION

It is known that cerebral small-vessel disease (CSVD) and reduced cerebral blood flow (CBF) are interrelated. However, the direction of the relation is unknown and longitudinal studies are scarce.

METHODS

Within the SMART-MR study, 1.5 T MRI of the brain, including an MR angiography, was obtained at baseline and after on average 3.9 years of follow-up in 575 patients with manifest arterial disease (mean age, 57 ± 10 years). Longitudinal associations between CSVD, characterized by white matter lesions (WML) and lacunar infarcts, and parenchymal CBF (per 100 mL brain volume) were estimated using regression analyses, adjusted for age, sex, follow-up time, and baseline brain measures.

RESULTS

Baseline parenchymal CBF was not associated with progression of CSVD over time. However, periventricular and deep WML at baseline were associated with decline in parenchymal CBF; per %ICV increase in periventricular and deep WML volume, mean decline (95% CI) in parenchymal CBF was -0.70 (-1.40 to -0.00) and -1.01 (-1.64 to -0.38) mL/min/100 mL brain volume, respectively. These associations were partly explained by cardiovascular risk factors, but remained statistically significant for deep WML (mean decline (95% CI) in parenchymal CBF per %ICV increase in periventricular and deep WML volume was -0.61 (-1.32 to 0.10) and -0.92 (-1.56 to -0.28) mL/min/100 mL brain volume). Lacunar infarcts at baseline were not associated with change in parenchymal CBF.

CONCLUSION

In patients with manifest arterial disease, periventricular and deep WML volumes at baseline were associated with decline in parenchymal CBF decline over time, but parenchymal CBF at baseline was not associated with CSVD progression over time.

INTRODUCTION

Cerebral small-vessel disease (CSVD), which is characterized by white matter lesions (WML) and lacunar infarcts on magnetic resonance imaging (MRI), is a leading cause of cognitive decline and functional loss, particularly in high risk populations.[1] Although the etiology of CSVD is not completely understood, there are indications that cerebral hemodynamic changes, including reduced cerebral blood flow (CBF), play a role.

Lower CBF has been related to more CSVD in the general population and in patients with cardiovascular disease.[2-6] In addition, patients with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) have significantly lower baseline CBF compared to controls.[7] However, most studies are cross-sectional, which limits conclusions on cause and effect. Reduced CBF could lead to CSVD, but it could also be hypothesized that existing CSVD has a secondary effect on underlying perfusion. To our knowledge, no studies exist that investigated the bidirectional longitudinal relation between CSVD and CBF.

We investigated whether baseline CBF was associated with progression of CSVD and whether baseline CSVD was associated with a decline of CBF during 4 years of follow-up in a large cohort of patients with manifest arterial disease.

METHODS

SMART-MR STUDY

Data were used from the Second Manifestations of ARTerial disease-Magnetic Resonance (SMART-MR) study, a prospective cohort study aimed at investigating brain changes on MRI in patients with manifest arterial disease. Details of the cohort have been described previously.[8,9] In brief, between May 2001 and December 2005, all patients newly referred to the University Medical Center Utrecht with manifest coronary artery disease, cerebrovascular disease, peripheral arterial disease, or an abdominal aortic aneurysm and without MR contraindications were invited to participate. During a one-day visit to our medical center, all patients underwent an MRI of the brain, physical examination, and ultrasonography of the carotid arteries. Blood and urine samples were collected after an overnight fast. Risk factors, medical history, and medication use were assessed using questionnaires. Between January 2006 and May 2009, follow-up measurements took place, including MRI of the brain.

The SMART-MR study was approved by the ethics committee of our institution and written informed consent was obtained from all patients.

STUDY SAMPLE

Of the 1309 patients at baseline, 1152 patients had adequate brain segmentation and 2D MR angiography measurements. Of these, all patients still alive (n=1076) were invited for follow-up measurements. In total, 674 of the surviving cohort participated, of whom 575

patients had an MRI at both baseline and follow-up, and adequate brain segmentation and 2D MR angiography measurements.

MAGNETIC RESONANCE IMAGING PROTOCOL AND BRAIN SEGMENTATION

At baseline and follow-up, 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 transversal T1-weighted (repetition time [TR]/echo time [TE], 235/2 ms), T2-weighted (TR/TE, 2200/11 ms and 2200/100 ms), fast fluid-attenuated inversion recovery (FLAIR; TR/TE/inversion time [TI], 6000/100/2000 ms), and inversion recovery (IR; TR/TE/TI, 2900/22/410 ms) sequences. Field of view (FOV) was 230 x 230 mm (matrix size, 180 x 256; slice thickness, 4.0 mm; no gap; 38 slices).[9]

On the basis of a localizer MR angiographic slab in the sagittal plane, a 2D 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) (TR/TE, 16/9 ms; flip angle, 7.5°; FOV, 250 x 250 mm; matrix size, 256 x 256; slice thickness, 5.0 mm; number of averaged signals, 8; velocity sensitivity, 100 cm/s).[10]

The T1-weighted, IR, and FLAIR sequences were used for the probabilistic segmentation technique, described previously.[11] The segmentation program distinguishes cortical gray matter, white matter, cerebrospinal fluid (CSF), and lesions. The results of the segmentation analysis were visually checked for the presence of infarcts and adapted if necessary to make a distinction between white matter lesions (WML) and infarct volumes. Total brain volume was calculated by summing the volumes of gray and white matter and, if present, the volumes of WMLs and infarcts. All volumes cranial to the foramen magnum were included. Total intracranial volume (ICV) was calculated by summing the total brain volume and the volume of the CSF.

CEREBRAL BLOOD FLOW

Image processing of the phase-contrast MR flow measurements was performed by two investigators.[12,13] The flow through the left and right ICAs and BA were summed to calculate the total cerebral blood flow (tCBF, mL/min). Because tCBF strongly depends on the amount of brain tissue, we expressed tCBF per 100 mL brain volume to obtain parenchymal CBF.[13]

CEREBRAL SMALL-VESSEL DISEASE

At baseline and follow-up, the whole brain was visually searched for infarcts by a trained investigator and a neuroradiologist, blinded to all information of the patient. Discrepancies in rating were re-evaluated in a consensus meeting. Infarcts were defined as focal hyperintensities on T2-weighted images of ≥3 mm in diameter. Infarcts located in the white matter also had to be hypointense on T1-weighted and FLAIR images in order to distinguish them from WML. Dilated perivascular spaces were distinguished from infarcts on the basis of their location, form, and the absence of gliosis. Brain infarcts were

categorized as lacunar infarcts (sized 3-15 mm in diameter and located in the subcortical white matter, thalamus, or basal ganglia) and non-lacunar infarcts (cortical infarcts, large subcortical infarcts, infratentorial infarcts). Progression of lacunar infarcts was defined as ≥1 new infarcts at follow-up versus no new lacunar infarcts. Periventricular WML were defined as adjacent to or within 1 cm of the lateral ventricles and deep WML were located in the deep white matter tracts and may or may not have adjoined periventricular lesions.[9] WML volumes were normalized for ICV and natural log transformed, because of non-normal distribution.

COVARIATES

Height and weight were measured and body mass index (BMI) was calculated (kg/m^2). Mean systolic and diastolic blood pressure (mmHg) were measured twice with a sphygmomanometer and the average of the two measures was calculated. Diabetes mellitus at baseline was defined as either a referral diagnosis of diabetes mellitus, self-reported diabetes mellitus, use of glucose-lowering agents, glucose ≥11.1 mmol/L, or a known history of diabetes mellitus. Among subjects without a history of diabetes mellitus, but with a fasting plasma glucose of ≥7.0 mmol/L at baseline, only those receiving treatment with glucose-lowering agents within one year after baseline were considered as having diabetes mellitus at baseline. Hyperlipidemia was defined as total cholesterol >5.0 mmol/L, low-density lipoprotein cholesterol >3.2 mmol/L, or self-reported use of lipid-lowering drugs. Smoking habits and alcohol intake were assessed using questionnaires. Pack years of smoking were calculated and alcohol intake was categorized as never, former, or current users. Ultrasonography was performed to measure the intima-media thickness (mm) and carotid artery stenosis.[14]

DATA ANALYSES

We used multiple imputation (10 datasets) using the statistical program R (aregImpute; version 2.10.0) to address missing values in the study sample of 575 patients. Data were analyzed using SPSS version 20.0 (Chicago, IL, USA) by pooling the 10 imputed datasets.

Baseline characteristics were calculated according to tertiles of total WML volume for the total study population (n=575). Linear regression analysis was used to estimate the prospective associations of baseline parenchymal CBF with progression of periventricular and deep WML volume over time. Logistic regression analysis was used to estimate the prospective association of baseline parenchymal CBF with risk of a new lacunar infarct over time. All analyses were adjusted for age, sex, follow-up time, and baseline periventricular or deep WML (for analyses with WML) or presence of lacunar infarcts at baseline (for analyses with infarcts) (model 1). We additionally adjusted for systolic and diastolic blood pressure, diabetes mellitus, BMI, alcohol use, pack years of smoking, hyperlipidemia, intima-media thickness, and carotid stenosis >50% in model 2. To examine whether the associations were independent of large brain infarcts, we additionally adjusted for non-lacunar infarcts in model 3. Finally, the prospective association of baseline periventricular and deep WML volume, and presence of lacunar infarcts with decline in parenchymal

CBF over time was estimated using linear regression analyses. In model 1, we adjusted for age, sex, baseline parenchymal CBF, and follow-up time. Further adjustments were made according to models 2 and 3 as mentioned above.

Table 1 Baseline characteristics of the study sample according to tertiles of total white matter lesion volume (n=575)

	Tertiles of WML at baseline		
	1	2	3
Total WML, %ICV	0-0.062	0.062-0.14	0.14-4.61
Age, years	54 ± 9	56 ± 9	62 ± 9
Sex, % men	86	80	78
Vascular disease location, %			
Coronary artery disease	70	66	54
Cerebrovascular disease	12	17	33
Peripheral arterial disease	17	18	19
Abdominal aortic aneurysm	3	6	10
Cardiovascular risk factors			
BMI, kg/m^2	27 ± 3	27 ± 4	27 ± 4
Smoking, pack years†	19 (0, 46)	20 (0, 46)	20 (0, 55)
Alcohol intake, % current	84	74	79
Systolic blood pressure, mmHg	138 ± 20	137 ± 19	143 ± 20
Diastolic blood pressure, mmHg	82 ± 11	81 ± 10	81 ± 10
Hyperlipidemia, %	78	81	74
Diabetes mellitus, %	9	10	18
Carotid atherosclerosis			
Intima media thickness, mm	0.9 ± 0.2	0.9 ± 0.2	1.0 ± 0.3
Carotid stenosis >50%, %	6	10	16
Brain volumes			
Intracranial volume, mL	1460 ± 122	1475 ± 122	1479 ± 141
Total brain volume, %ICV	80.0 ± 2.3	79.7 ± 2.3	78.5 ± 2.9
Cerebrovascular pathology			
Total WML volume, mL†	0.55 (0.19, 0.87)	1.33 (0.98, 1.78)	4.03 (2.15, 12.18)
Periventricular WML volume, mL†	0.26 (0.08, 0.52)	0.71 (0.34, 1.12)	2.29 (0.80, 7.71)
Deep WML volume, mL†	0.23 (0.05, 0.49)	0.61 (0.32, 0.98)	3.32 (0.72, 5.67)
Lacunar infarcts, %	8	10	31
Non-lacunar infarcts, %	10	11	20

Characteristics presented as mean ± standard deviation, unless stated otherwise.

WML, white matter lesion volume; BMI, body mass index; ICV, intracranial volume.

† Median (10^{th}-90^{th} percentile).

Percentage of missing values before imputation: BMI 0.2%, smoking 0.3%, alcohol intake 0.5%, hyperlipidemia 1.9%, intima-media thickness 1.4%, carotid stenosis >50% 3.1%, all other variables 0.0%.

RESULTS

At baseline, the mean (SD) age of the study population (n=575) was 57 (10) years and 81% were men. Baseline median (10^{th}-90^{th} percentile) periventricular and deep WML volume were 0.05 (0.01 to 0.22) %ICV and 0.04 (0.01 to 0.19) %ICV, respectively. Ninety-five patients had one of more lacunar infarct at baseline. Mean (SD) parenchymal CBF at baseline was 52.3 (9.8) mL/min/100 mL brain volume. Patients with larger WML volume were older, more often female, had higher systolic blood pressure, had more often cerebrovascular disease, diabetes mellitus and carotid stenosis >50%, and showed smaller brain volumes on MRI at baseline than patients with smaller WML volume (Table 1).

BASELINE PARENCHYMAL CBF AND PROGRESSION OF CSVD

After a mean (range) follow-up of 3.9 (3.0-5.8) years, the mean (SD) increases in periventricular and deep WML volume were 0.04 (0.11) %ICV and 0.02 (0.15) %ICV and 48 patients (8% of 575) developed one or more new lacunar infarcts. Baseline parenchymal CBF was not associated with progression of CSVD over 4 years of follow-up and adjusting for cardiovascular risk factors and carotid atherosclerosis did not change the effect estimates (Table 2).

Table 2 Association between baseline parenchymal CBF and progression of cerebral small-vessel disease over time (n=575)

		Change in periventricular WML (%ICV),	Change in deep WML (%ICV),	New lacunar infarcts,
	Model	B (95% CI)[a]	B (95% CI)[a]	OR (95% CI)[b]
Parenchymal CBF (per SD decrease) †	1	0.00 (-0.05 to 0.05)	0.03 (-0.04 to 0.11)	0.97 (0.69-1.35)
	2	-0.01 (-0.06 to 0.05)	0.03 (-0.05 to 0.11)	0.95 (0.67-1.33)
	3	0.00 (-0.06 to 0.05)	0.04 (-0.04 to 0.12)	0.93 (0.66-1.31)

Model 1: adjusted for age, sex, follow-up period, and baseline periventricular or deep WML – natural log transformed (for analyses with WML), or presence of lacunar infarcts at baseline (for analyses with infarcts); model 2: additionally adjusted for systolic and diastolic blood pressure, diabetes mellitus, body mass index, alcohol use, pack years, hyperlipidemia, intima-media thickness, and carotid stenosis >50%; model 3: additionally adjusted for non-lacunar infarcts.

WML, white matter lesions (natural log transformed); ICV, intracranial volume; CI, confidence interval; CBF, cerebral blood flow; SD, standard deviation.

† SD parenchymal CBF=9.8 mL/min/100 mL brain volume.

[a] B represents % change (95% CI) in periventricular or deep WML – natural log transformed (%ICV) per SD decrease in parenchymal CBF at baseline.

[b] OR represents odds ratio of new lacunar infarcts (yes/no) per SD decrease in parenchymal CBF at baseline.

Table 3 Association between baseline cerebral small-vessel disease and change in parenchymal CBF over time (n=575)

		Change in parenchymal CBF (mL/min/100 mL brain volume),
	Model	B (95% CI)[a]
Periventricular WML (per %ICV increase)	1	-0.70 (-1.40 to -0.00)*
	2	-0.70 (-1.40 to 0.01)
	3	-0.61 (-1.32 to 0.10)
Deep WML (per %ICV increase)	1	-1.01 (-1.64 to -0.38)*
	2	-1.00 (-1.63 to -0.37)*
	3	-0.92 (-1.56 to -0.28)*
Presence of lacunar infarct (yes/no)	1	-0.34 (-2.42 to 1.74)
	2	0.36 (-1.81 to 2.53)
	3	0.89 (-1.31 to 3.10)

Model 1: adjusted for age, sex, baseline parenchymal CBF, and follow-up period; model 2: additionally adjusted for systolic and diastolic blood pressure, diabetes mellitus, body mass index, alcohol use, pack years, hyperlipidemia, intima-media thickness, and carotid stenosis >50%; model 3: additionally adjusted for non-lacunar infarcts.

CBF, cerebral blood flow; CI, confidence interval; WML, white matter lesion volume (natural log transformed); ICV, intracranial volume.

* $p<0.05$.

[a] B represents absolute change (95% CI) in parenchymal CBF per %ICV WML (natural log transformed) or presence of lacunar infarcts at baseline.

BASELINE CSVD AND CHANGE IN PARENCHYMAL CBF

During follow-up, the mean (SD) decline in parenchymal CBF was -1.6 (11.0) mL/min/100 mL brain volume. Periventricular and deep WML volume at baseline were significantly associated with decline in parenchymal CBF in model 1: mean differences (95% CI) in parenchymal CBF decline per %ICV increase in periventricular and deep WML volume were -0.70 (-1.40 to -0.00) and -1.01 (-1.64 to -0.38) mL/min/100 mL brain volume (Table 3, model 1). Further adjusting for cardiovascular risk factors, carotid atherosclerosis, and non-lacunar infarcts slightly attenuated the effect estimates (Table 3, models 2 and 3). Presence of lacunar infarcts at baseline was not associated with change in parenchymal CBF (Table 3).

DISCUSSION

In a large cohort of patients with manifest arterial disease, periventricular and deep white matter lesion (WML) volumes at baseline were associated with a decline in parenchymal

cerebral blood flow (parenchymal CBF) during 4 years of follow-up. However, lower parenchymal CBF at baseline was not associated with progression of cerebral small-vessel disease (CSVD). These findings suggest that lower cerebral perfusion is a secondary effect of cerebral tissue damage rather than a contributor to CSVD.

Previous cross-sectional studies showed that patients with larger WML volume have lower CBF.[2-6] In addition, one longitudinal study showed that a decline in CBF was associated with progression of periventricular WML.[15] However, as they studied concurrent change in CBF and WML, it is possible that another underlying factor resulted in change in both measures.

In normal conditions, the brain is able to control for cerebral hypoperfusion through autoregulation by changing cerebrovascular resistance. However, in CSVD cerebrovascular resistance is impaired and the brain is no longer able to respond to decreases in cerebral perfusion pressure.[16] This may explain why we observed an association between baseline WML and decline in parenchymal CBF. Several other studies found evidence for impaired cerebrovascular resistance in areas with WML compared with normal appearing white matter.[6,17,18] Also, more WML in the brain will lead to relatively less normal cerebral tissue, with subsequent decrease in brain activity. As a result, less CBF is necessary to supply the brain with oxygen and nutrients. Indirect support for this hypothesis comes from studies in cognitively healthy individuals that showed that severity of WML is related to reduced glucose metabolism.[19,20] Another explanation could be that WML trigger endothelial dysfunction by impairing nitric oxide (NO) synthesis, which is normally responsible for relaxation of the vascular smooth muscle cells in response to decrease in perfusion pressure.[21]

A possible explanation for the lack of an association between baseline parenchymal CBF and CSVD progression could be that they represent different pathological processes. The development of CSVD could be the result of mechanisms unrelated to hemodynamic processes, such as neurodegeneration and genetic predisposition.[22,23] Circulating beta-amyloid peptides have been associated with more WML in patients with cognitive impairment[22] and in CADASIL patients, only cerebrovascular resistance and not CBF itself had prognostic value for the development of WML.[23] These studies suggest that other factors than cerebral hypoperfusion play a role in the pathogenesis of WML. Another possibility could be that our follow-up period was not long enough to show an association between baseline parenchymal CBF and progression of CSVD; cerebral perfusion may need to decrease below a certain threshold before it will result in ischemia-related injury.

Strengths of our study include the longitudinal design which allowed us to examine the bidirectional relations between parenchymal CBF and CSVD over time, and the available data on quantitative measurements of parenchymal CBF and CSVD in a large cohort of well-defined patients with manifest arterial disease. The extensive information on cardiovascular risk factors and other cerebrovascular lesions allowed us to investigate whether the associations between CSVD and parenchymal CBF over time were independent of these possible confounders.

Limitations of this study are, first, that participants in the follow-up examination

represent a relatively healthy group, which may have led to an underestimation of the progression of CSVD and decline in parenchymal CBF. Second, CBF divided by total brain volume (parenchymal CBF) is an indirect measure of brain tissue perfusion, and no information was available on regional differences in brain perfusion. The value of regional perfusion measures has been shown in studies where WML were more often present in regions with lower perfusion values.[6,24] Third, no information was available on other components that determine cerebral hemodynamics, such as cerebrovascular reactivity. Finally, as our study sample consisted of patients with manifest arterial disease, we do not know to what extent our results are generalizable to the general population.

In patients with manifest arterial disease, baseline periventricular and deep WML were associated with decline in parenchymal CBF, but baseline parenchymal CBF was not associated with progression of CSVD. Future longitudinal studies in both the general population and high-risk patients with longer follow-up periods could further elucidate the nature of the relationship between cerebral perfusion and CSVD.

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PART II
RISK FACTORS FOR BRAIN AGING

CHAPTER 5

HYPERTENSIVE TARGET ORGAN DAMAGE AND LONGITUDINAL CHANGES IN BRAIN STRUCTURE AND FUNCTION: THE SMART-MR STUDY

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Submitted

ABSTRACT

INTRODUCTION

High blood pressure has been related to structural and functional changes of the brain. In high-risk populations, hypertensive target organ damage might better represent exposure to high blood pressure than the blood pressure measurement itself. We examined the association of hypertensive target organ damage with longitudinal changes in brain structure and function within the SMART-MR study.

METHODS

Renal function, albuminuria, and left ventricular hypertrophy on electrocardiography were measured in 663 patients with manifest arterial disease (mean [SD] age 57 [9] years, 81% men). Automated brain segmentation was used to quantify progression of global brain atrophy and progression of cerebral small-vessel disease on 1.5 T MRI and memory and executive functioning were assessed, at baseline and after on average 3.9 years of follow-up.

RESULTS

Regression analyses showed that an increasing number of signs of target organ damage was associated with more progression of global brain atrophy and more rapid decline in memory performance. Compared to no target organ damage, mean differences in change in brain parenchymal fraction (95% CI) for 1 and ≥2 signs of organ damage were -0.12 (-0.30 to 0.06) and -0.41 (-0.77 to -0.05) %ICV and mean (95% CI) differences in change in memory performance (z-score) were -0.15 (-0.29 to -0.00) and -0.27 (-0.54 to -0.01). Results were independent of blood pressure, antihypertensive treatment, and other confounders. Hypertension target organ damage was not associated with progression of cerebral small-vessel disease or change in executive functioning.

CONCLUSION

Routinely assessed signs of hypertensive target organ damage could be used to identify patients at the highest risk of cognitive decline.

INTRODUCTION

Long-term observational studies have reported that hypertension during mid-life increases risk for brain atrophy, cerebrovascular lesions, and cognitive impairment later in life.[1,2] By contrast, the effects of late-life blood pressure levels on the brain are complex and less clear. In fact, several studies, particularly in very old individuals or in patients with high cardiovascular risk, suggest that lower blood pressure levels contribute to brain atrophy, cerebrovascular lesions, and more rapid cognitive decline.[2-5] Chronic exposure to high blood pressure levels leads to damage of target organs such as the heart, kidney, and brain. In populations with high cardiovascular risk, this damage of target organs might better represent exposure to high blood pressure than the blood pressure measurement itself, due to antihypertensive treatment effects and aging of the cardiovascular system.[6]

Guidelines recommend to routinely assess target organ damage by measuring renal function, albuminuria, and left ventricular hypertrophy on electrocardiography when evaluating vascular risk in patients.[7] These routinely assessed signs of hypertensive target organ damage, which are all inexpensive, straightforward, and easy to perform, have each been associated with increased risk for cardiovascular and cerebrovascular events and mortality.[8-10] Also, it has been shown that with an increasing number of target organs damaged, the risk of cardiovascular and cerebrovascular events increased.[11]

Although the individual signs of hypertensive target organ damage have been related to cognitive decline,[12-16] prospective studies relating these signs of target organ damage to other features of brain aging, such as progression of cerebral small-vessel disease and brain atrophy on magnetic resonance imaging (MRI) are, to our knowledge, non-existent. Also, the combined effect of these routinely assessed signs of damage on the brain has not been studied.

In the present study, our primary objectives were to evaluate the risk of the separate and combined presence of signs of hypertensive target organ damage on progression of brain atrophy and cerebral small-vessel disease on MRI and of cognitive decline in a large cohort of patients with manifest arterial disease and to assess whether these relations were independent of blood pressure levels and other cardiovascular risk factors.

METHODS

SMART-MR STUDY

Data were used from the Second Manifestations of ARTerial disease-Magnetic Resonance (SMART-MR) study, a prospective cohort study aimed at investigating brain changes on MRI in independently living patients with manifest arterial disease. Details of the design and participants have been described elsewhere.[17,18] In brief, between May 2001 and December 2005, all patients newly referred to the University Medical Center Utrecht with manifest coronary artery disease, cerebrovascular disease, peripheral arterial disease, or an abdominal aortic aneurysm and without MR contraindications

Table 1 Definitions of hypertensive target organ damage

Left ventricular hypertrophy on ECG	Sokolow–Lyon criterion: S in V1 + (R in V5 or V6, whichever is larger) ≥3.5 mV and/or Cornell voltage criterion: S in V3 + R in aVL >2.8 mV (in men), >2.0 mV (in women)
Impaired renal function	eGFR using the MDRD formula[a]: ≤60mL/min/1.73m^2
Albuminuria	Albumin (mg/L)/creatinine (mmol/L) ratio: ≥2.5 mg/mmol (in men), ≥3.5 mg/mmol (in women)

[a] MDRD=186 x (creatinine (umol)/88.4)$^{-1.154}$ x age$^{-0.203}$ (women x 0.742)

were invited to participate. During a one-day visit to our medical center, all patients underwent an MRI of the brain, physical examination, ultrasonography of the carotid arteries, electrocardiography (ECG), and blood and urine sampling. Risk factors, medical history, and medication use were assessed using questionnaires. Between January 2006 and May 2009, follow-up measurements took place, including MRI of the brain. The ethics committee of our institution approved the SMART-MR study and written informed consent was obtained from all participants.

5

HYPERTENSIVE TARGET ORGAN DAMAGE

Manifestations of hypertensive target organ damage, which were included in this study, were left ventricular hypertrophy (LVH), impaired renal function, and albuminuria as suggested by the guidelines.[7] Definitions are shown in Table 1. Creatinine was obtained after an overnight fast and the four-variable Modification of Diet in Renal Disease (MDRD) equation was used to calculate the estimated glomerular filtration rate (eGFR). Urine albumin/creatinine ratio was determined in a first morning-void urine sample. At baseline, a 12-lead 10 s ECG was obtained after 5 min rest with the patient in supine position. Measurements of R- and S-amplitudes and QRS timing were performed using the Marquette 12SL analysis program (General Electric Healthcare, Hoevelaken, the Netherlands).

CARDIOVASCULAR RISK FACTORS

Height and weight were measured and body mass index (BMI) was calculated (kg/m^2). Systolic and diastolic blood pressure (mmHg) were measured with a sphygmomanometer and the average of two measurements was calculated. Diabetes mellitus at baseline was defined as a referral diagnosis of diabetes mellitus, self-reported diabetes mellitus, use of glucose-lowering agents, glucose ≥11.1 mmol/L, or a known history of diabetes mellitus. Among subjects without a history of diabetes mellitus, but with fasting plasma glucose

of ≥7.0 mmol/L at baseline, only those receiving treatment with glucose-lowering agents within 1 year after baseline were considered as having diabetes mellitus at baseline. Hyperlipidemia was defined as total cholesterol >5.0 mmol/L, low-density lipoprotein cholesterol >3.2 mmol/L, or self-reported use of lipid-lowering drugs. Smoking habits and alcohol intake were assessed using questionnaires. Pack-years of smoking were calculated and alcohol intake was categorized as never, former, or current users. Ultrasonography was performed to measure the intima-media thickness (mm) and carotid stenosis.

MAGNETIC RESONANCE PROTOCOL AND BRAIN SEGMENTATION

At baseline and follow-up, 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 transversal T1-weighted (repetition time [TR]/echo time [TE], 235/2 ms), T2-weighted (TR/TE, 2200/11 ms and 2200/100 ms), fast fluid-attenuated inversion recovery (FLAIR; TR/TE/inversion time [TI], 6000/100/2000 ms), and inversion recovery (TR/TE/TI, 2900/22/410 ms) sequences. Field of view was 230 x 230 mm (matrix size, 180 x 256; slice thickness, 4.0 mm; no gap; 38 slices).[17] The T1-weighted gradient-echo, inversion recovery, and FLAIR sequences were used for the probabilistic brain segmentation technique.[19] The segmentation program distinguishes cortical gray matter, white matter, cerebrospinal fluid, and lesions. The results of the segmentation analysis were visually checked for the presence of infarcts and adapted if necessary to make a distinction between white matter lesions (WMLs) and infarct volumes.

BRAIN ATROPHY

At baseline and follow-up, total brain volume was calculated by summing the volumes of gray and white matter and, if present, the volumes of WML and infarcts. All volumes cranial to the foramen magnum were included. Total intracranial volume (ICV) was calculated by summing the total brain volume and the volumes of the cerebrospinal fluid. Total brain volume was normalized for ICV and expressed as brain parenchymal fraction.[17,20] Change in brain parenchymal fraction was used as an indicator of global brain atrophy.

CEREBRAL SMALL-VESSEL DISEASE

At baseline and follow-up, the whole brain was visually searched for infarcts by a trained investigator and a neuroradiologist, blinded to all information of the patient. Discrepancies in rating were re-evaluated in a consensus meeting. Infarcts were defined as focal hyperintensities on T2-weighted images of ≥3 mm in diameter. Infarcts located in the white matter also had to be hypointense on T1-weighted and FLAIR images in order to distinguish them from WML. Dilated perivascular spaces were distinguished from infarcts on the basis of their location, form, and the absence of gliosis. Brain infarcts were categorized as lacunar infarcts (sized 3-15 mm in diameter and located in the subcortical white matter, thalamus, or basal ganglia) and non-lacunar infarcts (cortical and large subcortical infarcts). Progression of lacunar infarcts was defined as ≥1 new infarct at follow-up versus no new infarcts. Periventricular WML were defined as adjacent to or within 1 cm of the lateral

ventricles and deep WML were located in the deep white matter tracts and may or may not have adjoined periventricular lesions.[17] Volumes of periventricular and deep WML were summed to obtain the total volume of WML. WML volumes were normalized for ICV and natural log transformed, because of non-normal distribution.

COGNITIVE TESTS

At baseline and follow-up, memory and executive functioning were assessed with neuropsychological tests, sensitive to mild impairments as described earlier.[21,22] Composite z-scores for the domains memory and executive functioning were calculated. The composite score for memory performance included immediate and delayed recall and the retention score of the 15-word learning test (a modification of the Rey Auditory Verbal Learning test) and the delayed recall of the Rey-Osterrieth Complex Figure test. The composite score for executive functioning included the Visual Elevator test, the Brixton Spatial Anticipation test, and the Verbal Fluency test (letter N for baseline and letter A for follow-up, one-minute time frame). Composite z-scores were computed by converting raw scores to standardized z-scores and averaging them across all subtests per domain. Before calculating z-scores, the scores of the Visual Elevator test and the Brixton Spatial Anticipation test were multiplied by -1 so that lower scores represented poorer performance. The composite z-scores at follow-up were calculated using the means and standard deviations of the baseline test scores. Premorbid intellectual functioning was assessed using the Dutch version of the National Adult Reading Test (DART).[23] Educational level was divided into seven categories, graded from primary school to academic degree, according to the Dutch educational system.

STUDY SAMPLE

Of the 1309 patients at baseline, MR segmentation data were not available in 77 patients. Of the remaining 1232 patients, 718 patients participated in the follow-up examination. In 55 patients, MR segmentation data were not available, so as a result, associations between number of damaged target organs and changes in brain parenchymal fraction, WML, and lacunar infarcts were studied in 663 patients.

Since neuropsychological assessment was introduced later in the study, analyses relating presence of hypertensive target organ damage to change in memory and executive functioning could be performed in 473 patients with available data on neuropsychological tests at baseline and follow-up. The patients participating in the follow-up examination (N=663 and N=473) were on average younger, more often men, had more often coronary artery disease, but less often had diabetes, hypertension, and signs of hypertensive target organ damage than those who were lost to follow-up (N=569 and N=759).

DATA ANALYSES

We used multiple imputation (10 datasets) using the statistical program R (aregImpute; version 2.10.0) to address missing values in the study sample of 663 patients. Data were analyzed using SPSS version 20.0 (Chicago, IL, USA), by pooling the 10 imputed datasets.[24]

Baseline characteristics were calculated across number of damaged target organs (0, 1, ≥2) for the total study population (n=663). Linear regression analysis and analysis of covariance were used to estimate the association of number of damaged target organs with change in brain parenchymal fraction, WML, and memory and executive functioning. Logistic regression analysis was used to estimate the association of number of damaged target organs with risk of new lacunar infarcts. All analyses were adjusted for age, sex, baseline brain measure or presence of lacunar infarcts, and follow-up time (model 1). When investigating change in cognitive functioning, additional adjustments were made in model 1 for DART-score and educational level. Systolic and diastolic blood pressure and antihypertensive medication were considered as confounders, but could be in the causal pathway as well and were therefore entered in model 2. To examine if the longitudinal association between number of damaged target organs and brain structure and function were explained by cardiovascular risk factors, further adjustments were made for BMI, alcohol use, smoking, hyperlipidemia, and diabetes mellitus (model 3). Finally, analyses were adjusted for intima-media thickness and non-lacunar brain infarcts in model 4.

RESULTS

At baseline, mean (SD, range) age of the study population (n=663) was 57 (9, 28-79) years and 81% were men. Of the total study population, 167 patients (30%) had one sign of hypertensive target organ damage and 32 patients (5%) had two or three signs of hypertensive target organ damage (Table 2). Albuminuria was most prevalent (20%), followed by LVH (10%), and impaired renal function (8%).

Patients with more than one sign of hypertensive target organ damage had less often coronary artery disease, but more often cerebrovascular disease, used less often alcohol, had higher blood pressure levels, and used more antihypertensive medication than patients with no signs of organ damage. In addition, patients with multiple signs of hypertensive target organ damage had smaller brain volumes and more cerebrovascular lesions at baseline (Table 2).

PROGRESSION OF BRAIN ATROPHY

After a mean (range) of 3.9 (3.0-5.8) years of follow-up, the mean decrease in brain parenchymal fraction was 0.98 (SD 1.08) %ICV. Increase in number of hypertensive target organ damage was associated with more progression of global brain atrophy (Table 3; p-trend was 0.02). Adjusting for blood pressure, use of antihypertensives, and other cardiovascular risk factors did not materially change the effect estimates (Table 3, models 2 and 3). Further adjusting the analyses for intima-media thickness and non-lacunar brain infarcts slightly attenuated the effect estimates, but a significant association between hypertensive target organ damage and change in brain parenchymal fraction remained; compared to no hypertensive target organ damage, mean differences in change in brain parenchymal fraction (95% CI) for 1 and ≥2 signs of hypertensive target organ damage

Table 2 Baseline characteristics of the study population according to number of hypertensive target organ damage

	Total population	Number of hypertensive target organ damage		
		0	1	≥2
	N=663	N=464	N=167	N=32
Demographics				
Age, years	57 ± 9	56 ± 9	61 ± 8	65 ± 11
Sex, % men	81	82	81	75
Vascular disease, %				
Coronary artery disease	62	64	59	53
Cerebrovascular disease	23	20	29	31
Peripheral arterial disease	18	17	20	28
Abdominal aortic aneurysm	6	5	8	16
Blood pressure				
Systolic blood pressure, mmHg	140 ± 20	137 ± 18	145 ± 21	152 ± 22
Diastolic blood pressure, mmHg	81 ± 10	81 ± 10	82 ± 11	84 ± 11
Use of antihypertensives, %	61	58	66	72
Target organ damage, %				
Left ventricular hypertrophy	10	0	29	59
Impaired renal function	8	0	17	69
Albuminuria	20	0	61	93
Other cardiovascular risk factors				
BMI, kg/m^2	27 ± 4	27 ± 3	27 ± 4	26 ± 4
Smoking, pack years[†]	20 (0, 49)	20 (0, 48)	23 (0, 56)	15 (0, 50)
Alcohol intake, % current	78	80	77	56
Hyperlipidemia, %	78	78	77	78
Diabetes mellitus, %	13	11	21	16
Carotid intima-media thickness, mm	0.9 ± 0.3	0.9 ± 0.2	1.0 ± 0.4	1.0 ± 0.3
Brain structures				
Brain parenchymal fraction, %ICV	79.3 ± 2.6	79.7 ± 2.4	78.5 ± 2.6	77.7 ± 3.3
Total WML volume fraction, %ICV[†]	0.09 (0.03, 0.40)	0.08 (0.03, 0.37)	0.12 (0.03, 0.46)	0.19 (0.04, 1.05)
Lacunar infarcts, %	17	14	23	41
Non-lacunar infarcts, %	17	16	18	25
Dutch adult reading test[†a]	82 (58, 97)	82 (60, 97)	80 (56, 96)	86 (54, 99)

Characteristics presented as mean ± standard deviation, unless stated otherwise.

BMI, body mass index; ICV, intracranial volume; WML, white matter lesion.

[†] Median (10th-90th percentile).

[a] Based on N=469 for the total population, N=321 for 0, N=125 for 1, and N=23 for ≥2 number of hypertensive target organ damage.

Percentage of missing values before imputation: left ventricular hypertrophy 7.7%, impaired renal function 6.0%, albuminuria 7.7%, carotid intima-media thickness 2.0%, BMI 0.2%, smoking 0.3%, alcohol intake 0.5%, hyperlipidemia 1.8%, Dutch adult reading test 0.8%.

Table 3 Hypertensive target organ damage and progression of global brain atrophy and cerebral small-vessel disease during 4 years of follow-up in 663 patients

Number of hypertensive target organ damage		N	Change in BPF (%ICV) Mean (95% CI)[a]	Change in WML (%ICV) Mean (95% CI)[a]	Risk of lacunar infarcts OR (95% CI)[a]
Model 1	0	464	Reference	Reference	Reference
	1	167	-0.12 (-0.30 to 0.06)	0.03 (-0.10 to 0.15)	1.41 (0.72-2.75)
	≥2	32	-0.41 (-0.77 to -0.05)*	-0.04 (-0.28 to 0.20)	1.20 (0.39-3.70)
Model 2	0	464	Reference	Reference	Reference
	1	167	-0.11 (-0.29 to 0.08)	0.03 (-0.10 to 0.15)	1.42 (0.72-2.80)
	≥2	32	-0.40 (-0.76 to -0.04)*	-0.05 (-0.29 to 0.20)	1.20 (0.38-3.82)
Model 3	0	464	Reference	Reference	Reference
	1	167	-0.08 (-0.27 to 0.11)	0.02 (-0.10 to 0.15)	1.29 (0.65-2.58)
	≥2	32	-0.41 (-0.78 to -0.04)*	-0.03 (-0.27 to 0.22)	1.11 (0.34-3.60)

Model 1: adjusted for age, sex, baseline brain measure or presence of lacunar infarcts, and follow-up time; model 2: additionally adjusted for systolic and diastolic blood pressure and use of antihypertensive medication; model 3: additionally adjusted for cardiovascular risk factors (body mass index, alcohol use, smoking, hyperlipidemia, diabetes mellitus).

BPF, brain parenchymal fraction; ICV, intracranial volume; CI, confidence interval; WML, white matter lesions – natural log transformed; and OR, odds ratio.

[a] Values are mean change or odds ratio (95% CI).

* $p<0.05$.

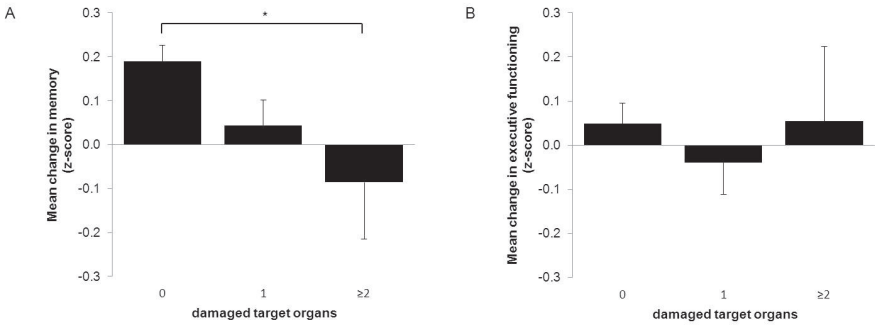

Figure 1 Number of hypertensive target organ damage and longitudinal changes in memory performance (A) and executive functioning (B) during 4 years of follow-up in 473 patients.

Bars represent mean change (standard error) in z-score for cognitive function adjusted for age, sex, educational level, Dutch version of the National Adult Reading test score, baseline z-score for memory performance or executive functioning, and follow-up time.

* p<0.05.

were -0.10 (-0.29 to 0.09) and -0.40 (-0.76 to -0.04) %ICV (model 4). Of the independent signs of hypertensive target organ damage, impaired renal function was most strongly related to progression of global brain atrophy (Supplemental Table 1).

5

PROGRESSION OF CEREBRAL SMALL-VESSEL DISEASE

At follow-up, the mean increase in WML volume was 0.06 (SD 0.23) %ICV and 9% of the patients had new lacunar brain infarcts. Presence of hypertensive target organ damage was not associated with progression of WML volume or an increased risk of new lacunar infarcts (Table 3). Adjusting for blood pressure, use of antihypertensives, other cardiovascular risk factors (Table 3, models 2 and 3), or intima-media thickness and non-lacunar infarcts (data not shown) did not materially change the effect estimates.

COGNITIVE DECLINE

Increase in hypertensive target organ damage was associated with greater decline in memory performance, but not with decline in executive functioning (Figure 1A and B). Compared to no hypertensive target organ damage, mean (95% CI) differences in change in z-score of memory performance for 1 and ≥2 signs of hypertensive target organ damage were -0.15 (-0.29 to -0.00) and -0.27 (-0.54 to -0.01) (model 1). Further adjustments for blood pressure, antihypertensive treatment, and other cardiovascular risk factors slightly attenuated the effect estimates; mean (95% CI) differences in change in z-score of memory performance were -0.14 (-0.28 to 0.00) and -0.28 (-0.55 to -0.01) (model 3). Also, adjusting for intima-media thickness and non-lacunar brain infarcts did not materially change the effect estimates (data not shown).

Of the independent signs of hypertensive target organ damage, albuminuria and

impaired renal function were most strongly related to decline in memory performance (Supplemental Table 2).

DISCUSSION

Within a large cohort of patients with manifest arterial disease, we observed that over 4 years of follow-up the combined presence of routinely measured signs of hypertensive target organ damage was associated with progression of global brain atrophy and greater decline in memory performance, but not with progression of markers of cerebral small-vessel disease or a decline in executive functioning. The observed associations were independent of blood pressure, antihypertensive medication, and other cardiovascular risk factors.

To the best of our knowledge, no studies exist that reported the relation between signs of hypertensive target organ damage and progression of brain atrophy. With respect to cognitive decline, our findings are in line with previous longitudinal studies that examined its relation with hypertensive target organ damage.[12-16,25] Impaired renal function, albuminuria, and left ventricular hypertrophy, all have been associated with a decline in global cognitive performance and memory performance.[12-14,16]

The association between hypertensive target organ damage and progression of global brain atrophy and the associated decline in memory performance might be explained by several mechanisms. Impaired renal function, albuminuria, and left ventricular hypertrophy are thought to reflect the long-term consequences of chronic exposure to hypertension. Albuminuria, for example, reflects a generalized and widespread endothelial dysfunction,[26] which is an important feature in hypertension. This might cause extravasation of blood proteins and reduced bioavailability of nitric oxide.[27,28] Chronic hypertension and endothelial dysfunction could lead to decreased vascular reactivity, which impairs autoregulation of cerebral perfusion. Subsequent hypoperfusion impairs adequate delivery of oxygen and nutrients to the brain and may therefore cause neurodegeneration resulting in brain atrophy and cognitive decline.[3,29] Furthermore, chronic hypertension has been associated with depositions of plaques and tangles in the brain which cause neurodegeneration and subsequently brain atrophy.[30,31] Chronic hypertension has also been associated with presence of microvascular brain lesions, such as microbleeds and microinfarcts in the brain[32] which could lead to brain atrophy and cognitive decline.[33] However, we were not able to measure these cerebral abnormalities on 1.5 T MRI. The use of ultra-high field MRI techniques would probably have resulted in more detailed information on these microvascular brain lesions and their possible role in the observed associations.

In our study, presence of hypertensive target organ damage was associated with more cerebral small-vessel disease at baseline, which is in line with previous cross-sectional studies.[27,34] However, increase in number of hypertensive target organ damage was not associated with progression of cerebral small-vessel disease and related decline in

executive functioning, which is in contrast with other studies.[15,25] The lack of a longitudinal association may be explained by selective survival. Patients with the most severe target organ damage most likely had the highest mortality or stroke risk and consequently were not represented in our follow-up analyses. This could have led to an underestimation of the effect of hypertensive target organ damage on progression of cerebral small-vessel disease. Further, these findings could be the result of antihypertensive treatment given during follow-up, which may have prevented the progression of cerebral small-vessel disease more than it could prevent brain atrophy.[35] Finally, it could also be that hypertensive target organ damage on the one hand and cerebral-small vessel disease on the other hand share common elements in the causal pathway, such as hypertension, but are independent processes.

Strengths of our study include the large cohort of well-defined patients with manifest arterial disease and the availability of brain volumetric measurements and cerebral small-vessel disease on MRI and data on cognitive performance at baseline and follow-up. Limitations of our study are that individuals who participated in the follow-up examination probably represent a relatively healthy group. This may have resulted in an underestimation of the effect of hypertensive target organ damage on progression of brain structure and function. Second, presence of hypertensive target organ damage was based on single measurements at baseline, which may have introduced inaccuracy or misclassification. Furthermore, the study cohort consisted of patients with manifest arterial disease, which limits the generalizability of the results. Finally, the use of antihypertensive medication may have influenced the presence of albuminuria and diagnosing LVH by use of electrocardiography is not optimal, as it has a low sensitivity to detect LVH.[36] However, all used measurements to detect impaired renal function, albuminuria, and LVH are straightforward, inexpensive, readily available, and routinely used in clinical practice.

Signs or multiple signs of hypertensive target organ damage, as measures of chronic hypertension, are associated with progression of global brain atrophy and greater decline in memory performance in patients with manifest arterial disease, independent of blood pressure, antihypertensive medication, and other cardiovascular risk factors. These signs of target organ damage, which are routinely assessed in clinical practice, could identify patients at the highest risk of future brain atrophy and associated cognitive decline.

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Supplemental Table 1 Individual hypertensive target organ damage and progression of global brain atrophy and cerebral small-vessel disease during 4 years of follow-up in 663 patients

Hypertensive target organ damage		Change in BPF (%ICV)	Change in WML (%ICV)	Risk of lacunar infarcts
	Model	Mean (95% CI)[a]	Mean (95% CI)[a]	OR (95% CI)[a]
Left ventricular hypertrophy	1	-0.03 (-0.28 to 0.21)	-0.03 (-0.20 to 0.14)	0.47 (0.16-1.34)
(N=64)	2	-0.02 (-0.26 to 0.23)	-0.03 (-0.20 to 0.14)	0.45 (0.15-1.33)
	3	-0.05 (-0.30 to 0.20)	-0.03 (-0.20 to 0.14)	0.47 (0.16-1.38)
Impaired renal function	1	-0.41 (-0.70 to -0.13)*	0.07 (-0.13 to 0.27)	1.83 (0.74-4.56)
(N=49)	2	-0.42 (-0.71 to -0.13)*	0.07 (-0.13 to 0.27)	1.86 (0.74-4.68)
	3	-0.41 (-0.70 to -0.12)*	0.09 (-0.11 to 0.29)	1.69 (0.66-4.31)
Albuminuria	1	-0.19 (-0.39 to 0.01)	-0.03 (-0.17 to 0.12)	1.68 (0.86-3.28)
(N=123)	2	-0.17 (-0.37 to 0.03)	-0.03 (-0.18 to 0.11)	1.72 (0.87-3.42)
	3	-0.14 (-0.35 to 0.06)	-0.03 (-0.18 to 0.12)	1.60 (0.79-3.23)

Model 1: adjusted for age, sex, baseline brain measure or presence of lacunar infarcts, and follow-up time; model 2: additionally adjusted for systolic and diastolic blood pressure and use of antihypertensive medication; model 3: additionally adjusted for cardiovascular risk factors (body mass index, alcohol use, smoking, hyperlipidemia, diabetes mellitus).

BPF, brain parenchymal fraction; ICV, intracranial volume; CI, confidence interval; WML, white matter lesions – natural log transformed; OR, odds ratio; N, number.

[a] Values are mean change or odds ratio (95% CI).

* $p<0.05$.

Supplemental Table 2 Individual hypertensive target organ damage and longitudinal changes in cognitive functioning during 4 years of follow-up in 473 patients

Hypertensive target organ damage		Change in memory	Change in executive functioning
	Model	Mean (95% CI)[a]	Mean (95% CI)[a]
Left ventricular hypertrophy (N=55)	1	-0.00 (-0.19 to 0.18)	0.03 (-0.20 to 0.26)
	2	-0.00 (-0.19 to 0.18)	0.02 (-0.21 to 0.25)
	3	-0.01 (-0.20 to 0.18)	0.00 (-0.23 to 0.23)
Impaired renal function (N=37)	1	-0.25 (-0.48 to -0.03)*	-0.07 (-0.37 to 0.23)
	2	-0.26 (-0.49 to -0.04)*	-0.07 (-0.37 to 0.23)
	3	-0.26 (-0.49 to -0.03)*	-0.08 (-0.39 to 0.22)
Albuminuria (N=82)	1	-0.18 (-0.33 to -0.02)*	-0.07 (-0.26 to 0.13)
	2	-0.18 (-0.34 to -0.03)*	-0.07 (-0.26 to 0.13)
	3	-0.17 (-0.33 to -0.01)*	-0.06 (-0.25 to 0.14)

Model 1: adjusted for age, sex, educational level, Dutch version of the National Adult Reading test score, baseline memory or executive functioning z-score, and follow-up time; model 2: additionally adjusted for systolic and diastolic blood pressure and use of antihypertensive medication; model 3: additionally adjusted for cardiovascular risk factors (body mass index, alcohol use, smoking, hyperlipidemia, diabetes mellitus).

CI, confidence interval; N, number.

[a] Values are mean (95% CI) change.

* $p<0.05$.

CHAPTER 6

HEMOGLOBIN, HEMATOCRIT AND LONGITUDINAL CHANGES IN CEREBRAL BLOOD FLOW: THE SMART-MR STUDY

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Submitted

ABSTRACT

INTRODUCTION

Hemoglobin and hematocrit are important determinants of blood viscosity and arterial oxygen content and may therefore influence cerebral blood flow (CBF).

METHODS

We examined cross-sectional and prospective associations of hemoglobin and hematocrit with CBF in 569 patients with manifest arterial disease (mean age 57 ± 10 years) with available data on magnetic resonance angiography to measure parenchymal CBF.

RESULTS

Mean (SD) parenchymal CBF at baseline was 52.3 (9.8) mL/min/100 mL and decreased with 1.5 (11.0) mL/min/100 mL after on average 3.9 years of follow-up. Linear regression analyses showed that higher hemoglobin and hematocrit were associated with lower baseline parenchymal CBF and more decline in parenchymal CBF over time, independent of cardiovascular risk factors and use of antiplatelet drugs, anticoagulants, or diuretics: adjusted mean differences (95% CI) in decline in parenchymal CBF between patients in the lower and upper quartiles of hemoglobin and hematocrit were -2.66 (-3.88 to -1.44) and -3.85 (-5.04 to -2.66) mL/min/100 mL.

CONCLUSION

Higher hemoglobin and hematocrit were associated with lower baseline parenchymal CBF and greater decline in parenchymal CBF over time, possibly as a result of physiological compensating mechanisms.

INTRODUCTION

For normal brain function, adequate cerebral perfusion is important to maintain a constant oxygen supply to prevent pathological brain changes and cognitive decline.[1] The regulation of cerebral blood flow (CBF) has been shown to depend both on blood viscosity and on arterial oxygen content.[2] Hemoglobin and hematocrit are important determinants of blood viscosity and arterial oxygen content. Hemoglobin mainly represents the oxygen carrying capacity of blood, where hematocrit, in combination with deformability of red blood cells, has the most dominant relation with blood viscosity.[3] This is reflected in the linear relation between hemoglobin and hematocrit through red blood cell volume; a decrease in red blood cell volume causes both a decrease in hemoglobin and in hematocrit.

Several small-scale studies in selected patient populations have shown changes in CBF in response to changes in hemoglobin and hematocrit.[4-8] In patients with anemia, lower hemoglobin and hematocrit were associated with higher levels of CBF while normalization of CBF was established after increasing hematocrit to normal levels.[5-7] In more pathological conditions, for instance in polycythemia which is characterized by elevated hematocrit as a result of raised red cell mass, lower cerebral perfusion levels have been observed, resulting in ischemia.[9] In addition, bloodletting in polycythemic patients, used to lower hematocrit, has been shown to improve CBF.[10] Overall, these studies show that very low and very high hemoglobin and hematocrit affects CBF. However, only few studies investigated the relation between blood rheology and CBF in subjects with hemoglobin and hematocrit within normal variation[8,11] and we are not aware of any studies that investigated the association of hemoglobin and hematocrit with longitudinal CBF changes.

We therefore investigated the association of hemoglobin and hematocrit with CBF and with changes in CBF over time in a large-scale cohort of patients with manifest arterial disease.

METHODS

SMART-MR STUDY

Data were used from the Second Manifestations of ARTerial disease-Magnetic Resonance (SMART-MR) study, a prospective cohort study aimed at investigating brain changes on magnetic resonance imaging (MRI) in independently living patients with manifest arterial disease. Details of the design and participants have been described previously.[12,13] In brief, between May 2001 and December 2005, all patients newly referred to the University Medical Center Utrecht with manifest coronary artery disease, cerebrovascular disease, peripheral arterial disease, or an abdominal aortic aneurysm and without MR contraindications were invited to participate. During a one-day visit to our medical center, all patients underwent an MRI of the brain, a physical examination, and ultrasonography

of the carotid arteries. Blood and urine samples were collected after an overnight fast. Risk factors, medical history, and medication use were assessed using questionnaires. Between January 2006 and May 2009, follow-up measurements took place, including MRI of the brain.

The SMART-MR study was approved by the ethics committee of the University Medical Center in Utrecht according to the principles of the Declaration of Helsinki and written informed consent was obtained from all patients.[13]

MAGNETIC RESONANCE IMAGING PROTOCOL

At baseline and follow-up, 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 transversal T1-weighted (repetition time [TR]/echo time [TE], 235/2 ms), T2-weighted (TR/TE, 2200/11 ms and 2200/100 ms), fast fluid-attenuated inversion recovery (FLAIR; TR/TE/inversion time [TI], 6000/100/2000 ms), and inversion recovery (TR/TE/TI, 2900/22/410 ms) sequences. Field of view (FOV) was 230 x 230 mm (matrix size, 180 x 256; slice thickness, 4.0 mm; no gap; 38 slices). On the basis of a localizer MR angiographic slab in the sagittal plane, a 2D 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). The 2D phase-contrast section was positioned through the ICAs and the BA (TR/TE, 16/9 ms; flip angle, 7.5°; FOV, 250 x 250 mm; matrix size, 256 x 256; slice thickness, 5.0 mm; number of signals averaged, 8; velocity sensitivity, 100 cm/s).[14]

BRAIN SEGMENTATION

The T1-weighted gradient-echo, inversion recovery, and FLAIR sequences were used for brain segmentation. The probabilistic segmentation technique has been described previously.[15] The segmentation program distinguishes cortical gray matter, white matter, sulcal and ventricular cerebrospinal fluid (CSF), and white matter lesions (WMLs). The results of the segmentation analysis were visually checked for the presence of infarcts and adapted if necessary to make a distinction between WML and infarct volumes. Total brain volume was calculated by summing the volumes of gray and white matter and, if present, the volumes of WMLs and infarcts. All cranial volumes to the foramen magnum were included. Infarcts were visually scored by an investigator and a neuroradiologist, blinded to clinical characteristics. Discrepancies in scoring were re-evaluated in a consensus meeting. Infarcts were defined as focal hyperintensities on T2-weighted images of >3 mm in diameter. Hyperintensities located in the white matter also had to be hypointense on T1-weighted and FLAIR images to distinguish them from WML. Dilated perivascular spaces were distinguished from infarcts on the basis of their location, form, and absence of gliosis. Infarcts were categorized as lacunar (sized 3-15 mm) and non-lacunar infarcts (cortical infarcts, large subcortical infarcts, infratentorial infarcts).[13]

CEREBRAL BLOOD FLOW

Post-processing of the flow measurements was performed by two researchers without

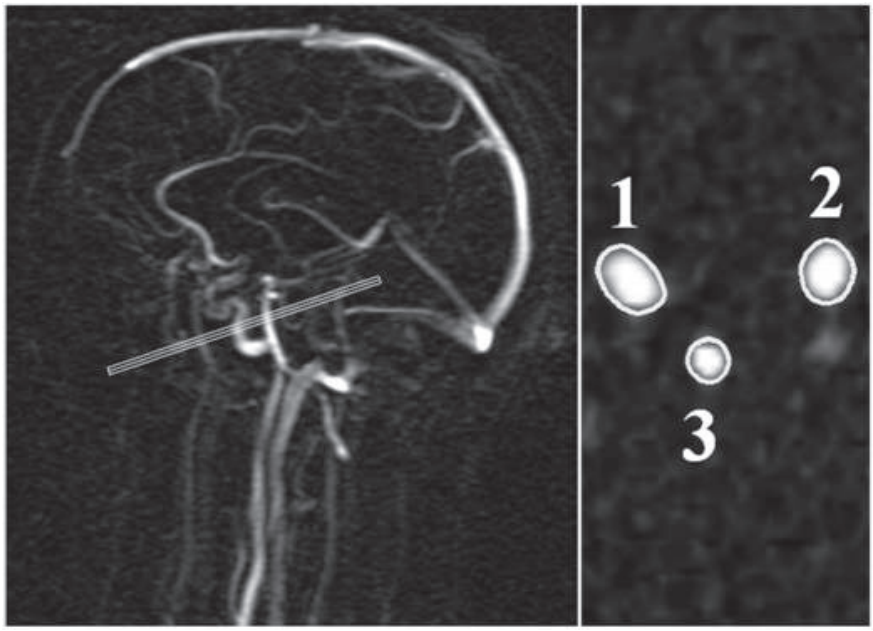

Figure 1 Sagittal localizer magnetic resonance angiography (MRA) image illustrating the positioning of a two-dimensional phase-contrast MRA slab used to measure the volume flow through the internal carotid arteries (1 and 3) and the basilar artery (2). Quantitative flow values were obtained by integrating across manually drawn regions of interest that enclose the vessels.

knowledge of clinical or brain characteristics of the 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. The average 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 post-processing procedures was shown in our research group, with a coefficient of variation of 5%.[14] The flow through the left and right ICAs and BA were summed to calculate the total cerebral blood flow (tCBF, mL/min) (Figure 1). Because tCBF strongly depends on the amount of brain tissue, we expressed tCBF per 100 mL brain volume to obtain parenchymal CBF.[16,17]

HEMOGLOBIN AND HEMATOCRIT

An overnight fasting venous whole blood sample was taken to measure hemoglobin, the iron-containing oxygen-transport protein in red blood cells, with a spectrophotometer and hematocrit, the volume of red blood cells compared to the total blood volume, using an impedance technique (Cell-Dyn 4000, Abbott, Abbott Park, Illinois, USA). Previous evaluation of this hematology analyzer showed good within- and between-batch reproducibility for hemoglobin and hematocrit with coefficients of variation of 0.5% and 1.8% for within-batch precision and 0.6% and 1.9% for between-batch

precision for hemoglobin and hematocrit, respectively.[18] The reference ranges from our laboratory for hemoglobin are 7.4-9.6 mmol/L for women and 8.6-10.7 mmol/L for men. The reference ranges for hematocrit are 0.36-0.46 L/L for women and 0.41-0.51 L/L for men. As hemoglobin and hematocrit are highly dependent on sex,[19] sex-pooled quartiles were calculated, i.e. quartiles were calculated in men and women separately and then combined to form sex-pooled quartiles of hemoglobin and hematocrit. This method assured that men and women, including the sex-related variations, were equally distributed in the categories. For hemoglobin, quartiles for women were divided in ≤8.0, 8.1-8.4, 8.5-8.7, and >8.7 mmol/L and for men in ≤8.7, 8.8-9.2, 9.3-9.6, and >9.6 mmol/L. For hematocrit, quartiles for women were divided in ≤0.37, 0.38-0.39, 0.40-0.41, and >0.41 L/L and for men in ≤0.40, 0.41-0.42, 0.43-0.44, and >0.44 L/L.

COVARIATES

Height and weight were measured without shoes and heavy clothing, and body mass index (BMI) was calculated (kg/m^2). Hypertension was defined as mean systolic blood pressure ≥140 mmHg and/or mean diastolic blood pressure ≥90 mmHg and/or use of antihypertensive medication. Medication use was assessed using questionnaires and categorized as users or non-users. Antihypertensive medication included β-blockers, diuretics, calcium channel blockers, α_1-receptor blockers, angiotensin-converting enzyme (ACE) inhibitors, and angiotensin receptor blockers. An overnight fasting venous blood sample was taken to determine glucose and lipid levels. Diabetes mellitus at baseline was defined as either a referral diagnosis of diabetes mellitus, self-reported diabetes mellitus, use of glucose-lowering agents, glucose ≥11.1 mmol/L, or a known history of diabetes mellitus. Among subjects without a history of diabetes mellitus, but with a fasting plasma glucose of ≥7.0 mmol/L at baseline, only those receiving treatment with glucose-lowering agents within one year after baseline were considered as having diabetes mellitus at baseline. Hyperlipidemia was defined as total cholesterol >5.0 mmol/L, low-density lipoprotein cholesterol >3.2 mmol/L, or self-reported use of lipid-lowering drugs. Smoking habits and alcohol intake were assessed using questionnaires. Pack years of smoking were calculated and alcohol intake was categorized as never, former, or current users. Patients who had quit drinking during the past year were assigned to the category current alcohol users. Ultrasonography was performed to measure the intima-media thickness (mm) in the left and right common carotid arteries, represented by the mean value of six measurements.

STUDY SAMPLE

Of the 1309 patients at baseline, 1152 patients had adequate brain segmentation and 2D MR angiography measurements as the necessary MR sequences were available and free from motion or other artefacts. Of these, all patients still alive (n=1076) were invited for follow-up measurements. In total, 674 of the surviving cohort gave written informed consent and participated, of whom 575 patients had an MRI at both baseline and follow-up and adequate 2D MR angiography measurements. Six patients had missing data on

hemoglobin and hematocrit, resulting in a sample of 569 patients for the cross-sectional and longitudinal analyses. In comparison with the 507 patients who did not participate in the follow-up examination, this sample (n=569) was younger, more often male, smoked less often, had less often diabetes and carotid atherosclerosis, had less cerebrovascular pathology on MRI, but comparable hemoglobin (mean (SD): 9.0 (0.7) vs. 9.0 (0.8) mmol/L) and hematocrit (mean (SD): 0.42 (0.03) vs. 0.42 (0.04) L/L).

DATA ANALYSES

Missing data rarely occurs completely at random and complete case analyses leads to loss of statistical power and to biased results.[20] We therefore used multiple imputation (10 datasets) using the statistical program R (aregImpute; version 2.10.0) to address missing values in the study sample of 569 patients. Data were analyzed using SPSS version 20.0 (Chicago, IL, USA), by pooling the 10 imputed datasets.

Baseline characteristics were calculated for the total population (n=569), across sex-pooled quartiles of hemoglobin and were expressed as mean with standard deviation or as median with the 10^{th}-90^{th} percentile. Categorical data were expressed as percentages. The correlation between hemoglobin and hematocrit at baseline was calculated with Pearson's correlation coefficient.

Multivariable linear regression analysis was used to investigate the association of hemoglobin and hematocrit with 1) cross-sectional measures of parenchymal CBF and 2) longitudinal change in parenchymal CBF by using baseline hemoglobin and hematocrit (in sex-pooled quartiles) as independent variables and parenchymal CBF at baseline (for cross-sectional data analyses) and parenchymal CBF at follow-up (for longitudinal data analyses) as the dependent variables. The lowest quartile was used as a reference, because a linear/gradual change in parenchymal CBF was expected with increasing hemoglobin and hematocrit, based on literature. The covariates were entered as blocks in the different models in multivariable linear regression analyses. All analyses were adjusted for age and current smoking and for the longitudinal data analyses, additional adjustments were made for baseline parenchymal CBF and follow-up time (model 1). In model 2, additional adjustments were made for use of antiplatelet drugs, anticoagulants, and diuretics (yes vs. no) since these drugs can influence both hemoglobin and hematocrit and change in CBF. To explore the role of atherosclerosis and cardiovascular risk, additional adjustments were made for vascular disease categories (presence vs. absence of coronary artery disease, presence vs. absence of cerebrovascular disease, presence vs. absence of peripheral artery disease, and presence vs. absence of abdominal aortic aneurysm), BMI (kg/m^2), current alcohol intake (yes vs. no), hypertension (yes vs. no), hyperlipidemia (yes vs. no), diabetes mellitus (yes vs. no), cholesterol levels (mmol/L), and intima-media thickness (mm) (model 3). Intima-media thickness was added to this model as an indicator of severity of atherosclerotic disease.

As carotid stenosis can modulate the association between hemoglobin, hematocrit and changes in CBF, the above-mentioned analyses were repeated after exclusion of patients with carotid stenosis of >70% (n=35).[21,22]

RESULTS

At baseline, the mean (SD) age of the study population (n=569) was 57 (10) years and the majority of the patients were men (n=466; 82%). Mean (SD) hemoglobin was 9.2 (0.7) mmol/L for men and 8.4 (0.6) mmol/L for women and mean (SD) hematocrit was 0.43 (0.03) L/L for men and 0.40 (0.03) L/L for women. For hemoglobin, 14% of men and 3% of women had levels below the reference value and 0.4% of men and 1% of women had levels above the reference value. For hematocrit, 20% of men and 6% of women had levels below the reference value and 0% of men and 1% of women had levels above the reference value. Baseline hemoglobin and hematocrit were highly correlated (Pearson's correlation coefficient r=0.90, p<0.05). Mean (SD) parenchymal CBF at the first scan was 52.3 (9.8) mL/min/100 mL in the study population of 569 persons, and at the follow-up scan after on average 3.9 years (SD=0.4), mean (SD) parenchymal CBF was 50.8 (10.3) mL/min/100 mL. Men and women with hemoglobin in the upper quartile were younger, more often current smokers, had less often diabetes and hypertension, used diuretics and antiplatelet drugs less often, but used more often anticoagulants than patients in the lower quartile of hemoglobin (Table 1). The correlations of baseline hemoglobin and hematocrit with baseline parenchymal CBF are visualized in Figures 2A and B.

At baseline, higher hemoglobin and hematocrit were cross-sectionally associated with lower levels of parenchymal CBF; mean differences in parenchymal CBF between lower and upper sex-pooled quartiles of hemoglobin and hematocrit were -3.39 (95% CI -5.73 to -1.05) and -4.15 (95% CI -5.31 to -2.99) mL/min/100 mL, respectively (Table 2, model 1). The results were independent of potential confounders and explanatory variables (Table 2, models 2 and 3).

In the prospective analyses, higher baseline hemoglobin and hematocrit were associated with more decline in parenchymal CBF at follow-up; mean differences in change in parenchymal CBF between lower and upper sex-pooled quartiles of hemoglobin and hematocrit were -2.69 (95% CI -3.86 to -1.52) and -3.91 (95% CI -5.05 to -2.77) mL/min/100 mL, respectively (model 1). The association between hemoglobin and decline in parenchymal CBF showed a linear trend, while the association between hematocrit and parenchymal CBF decline suggested a non-linear trend, with an abrupt decrease in decline in parenchymal CBF for the upper quartile (Figures 3A and B). The results were not explained by potential confounders or explanatory variables; mean differences in change

Characteristics presented as mean ± standard deviation, unless stated otherwise. ▶

BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; CBF, cerebral blood flow; ICV, intracranial volume.

† Median (10^{th}-90^{th} percentile).

Percentage of missing values: BMI 0.2%, alcohol intake 0.5%, smoking 0.4%, triglycerides 0.9%, HDL-cholesterol 0.7%, LDL-cholesterol 4.2%, hyperlipidemia 1.4%, intima-media thickness 1.4%, carotid stenosis >50% 3.2%.

Table 1 Baseline characteristics of the total population (n=569) according to sex-pooled quartiles of hemoglobin

	Sex-pooled quartiles of hemoglobin			
	Quartile 1	Quartile 2	Quartile 3	Quartile 4
	n=128	n=164	n=141	n=136
Age, years	60 ± 10	57 ± 9	56 ± 10	57 ± 9
Sex, % men	81	84	83	79
Hemoglobin, mmol/L	8.1 ± 0.5	8.9 ± 0.3	9.3 ± 0.3	9.8 ± 0.5
Hematocrit, L/L	0.38 ± 0.03	0.41 ± 0.01	0.43 ± 0.02	0.45 ± 0.02
Vascular disease categories, %				
Coronary artery disease	60	63	70	59
Cerebrovascular disease	22	25	16	20
Peripheral artery disease	22	15	16	18
Abdominal aortic aneurysm	7	5	4	9
Cardiovascular risk factors				
BMI, kg/m^2	27 ± 4	27 ± 3	27 ± 3	27 ± 3
Alcohol intake, % current	77	83	79	78
Smoking, % current	16	20	15	28
Systolic blood pressure, mmHg	142 ± 19	141 ± 20	136 ± 17	140 ± 22
Diastolic blood pressure, mmHg	81 ± 11	82 ± 9	80 ± 9	83 ± 12
Hypertension, %	88	80	79	82
Triglycerides, mmol/L	1.6 ± 1.1	1.7 ± 1.1	1.7 ± 1.0	1.9 ± 1.0
HDL-cholesterol, mmol/L	1.3 ± 0.5	1.3 ± 0.4	1.3 ± 0.4	1.3 ± 0.4
LDL-cholesterol, mmol/L	2.6 ± 0.9	2.9 ± 0.9	2.8 ± 0.8	2.8 ± 0.9
Hyperlipidemia, %	77	77	81	77
Diabetes mellitus, %	16	14	10	8
Carotid atherosclerosis				
Intima media thickness, mm	1.0 ± 0.2	0.9 ± 0.3	0.9 ± 0.2	0.9 ± 0.3
Carotid stenosis >50%, %	12	10	8	13
Medication, %				
Use of diuretics	18	10	7	9
Use of antiplatelet drugs	66	59	59	59
Use of anticoagulants	5	3	4	8
Brain measures				
Intracranial volume, mL	1464 ± 120	1472 ± 132	1482 ± 126	1472 ± 134
Total brain volume, mL	1151 ± 101	1170 ± 104	1179 ± 101	1172 ± 111
Total CBF, mL/min	609 ± 130	617 ± 116	622 ± 120	589 ± 124
Parenchymal CBF, mL/min/100 mL brain volume	52.9 ± 10.1	52.8 ± 9.1	52.9 ± 9.9	50.3 ± 10.1
White matter lesion fraction, %ICV[†]	0.10 (0.02-0.57)	0.09 (0.03-0.44)	0.09 (0.02-0.29)	0.09 (0.03-0.36)
Brain infarcts, %	26	30	18	24

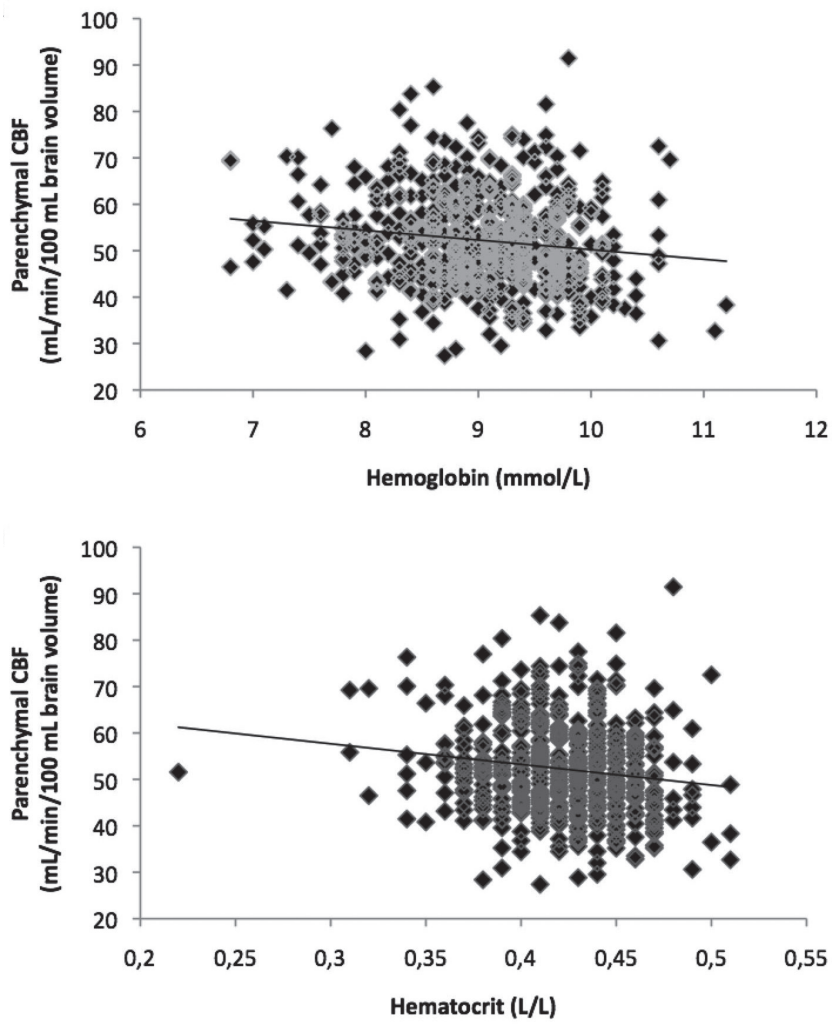

Figure 2 Scatterplots for the associations of hemoglobin (A) and hematocrit (B) with baseline parenchymal CBF.

in parenchymal CBF between lower and upper sex-pooled quartiles of hemoglobin and hematocrit were -2.66 (95% CI -3.88 to -1.44) and -3.85 (95% CI -5.04 to -2.66) mL/min/100 mL, respectively (model 3). However, presence of hypertension partly explained the association between hemoglobin and decline in parenchymal CBF at follow-up (data not shown).

Sensitivity analyses excluding patients with carotid stenosis >70% (n=35) showed no changes in the presented results; the mean differences in parenchymal CBF at baseline were -2.53 (95% CI -4.83 to -0.23) mL/min/100 mL and -3.77 (95% CI -6.12 to -1.43) mL/min/100 mL between the lower and upper sex-pooled quartiles for hemoglobin and

Table 2 Cross-sectional associations of baseline hemoglobin and hematocrit with parenchymal CBF

Difference in parenchymal CBF for hemoglobin					
		B (95% CI)[a]			
Model	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P-trend
1	0	-0.67 (-2.90 to 1.57)	-0.43 (-2.75 to 1.89)	-3.39 (-5.73 to -1.05)	0.008
2	0	-0.85 (-2.69 to 0.99)	-0.65 (-1.84 to 0.54)	-3.50 (-4.70 to -2.30)	0.006
3	0	-0.24 (-2.50 to 2.02)	-0.55 (-1.79 to 0.70)	-3.10 (-4.11 to -2.10)	0.014

Difference in parenchymal CBF for hematocrit					
		B (95% CI)[a]			
Model	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P-trend
1	0	-1.70 (-4.03 to 0.64)	-0.14 (-1.33 to 1.04)	-4.15 (-5.31 to -2.99)	0.002
2	0	-1.79 (-4.08 to 0.51)	-0.34 (-1.53 to 0.85)	-4.33 (-5.50 to -3.16)	0.001
3	0	-1.71 (-3.87 to 0.45)	-0.25 (-2.66 to 2.17)	-4.35 (-6.85 to -1.84)	0.002

Model 1: adjusted for age, current smoking; model 2: additionally adjusted for use of antiplatelet drugs, anticoagulants, and diuretics; model 3: additionally adjusted for vascular disease categories, body mass index, current alcohol intake, hypertension, hyperlipidemia, diabetes mellitus, cholesterol levels, and intima-media thickness.

Parenchymal CBF, parenchymal cerebral blood flow in mL/min/100 mL brain volume; CI, confidence interval.

[a] B represents mean (95% CI) difference in parenchymal CBF at baseline for sex-pooled quartiles of hemoglobin and hematocrit with quartile 1 as reference.

hematocrit, respectively (model 1). After follow-up, the mean differences in parenchymal CBF decline between the lower and upper sex-pooled quartile for hemoglobin and hematocrit were -2.57 (95% CI -4.78 to -0.35) mL/min/100 mL and -4.01 (95% CI -6.29 to -1.72) mL/min/100 mL, respectively (model 1). Further, excluding patients with hemoglobin and/or hematocrit levels below and above the reference values resulted in smaller effect estimates due to less power and a smaller distribution of hemoglobin and hematocrit, but a difference between the lower and upper sex-pooled quartiles was still present (data not shown).

DISCUSSION

In a population of patients with manifest arterial disease, we observed that higher hemoglobin and hematocrit were cross-sectionally associated with lower parenchymal cerebral blood flow (parenchymal CBF), and prospectively with more decline in parenchymal CBF over 4 years of follow-up. These results could not be explained by potential confounders or explanatory variables.

For normal cerebral function, an optimal CBF regulation is necessary to maintain

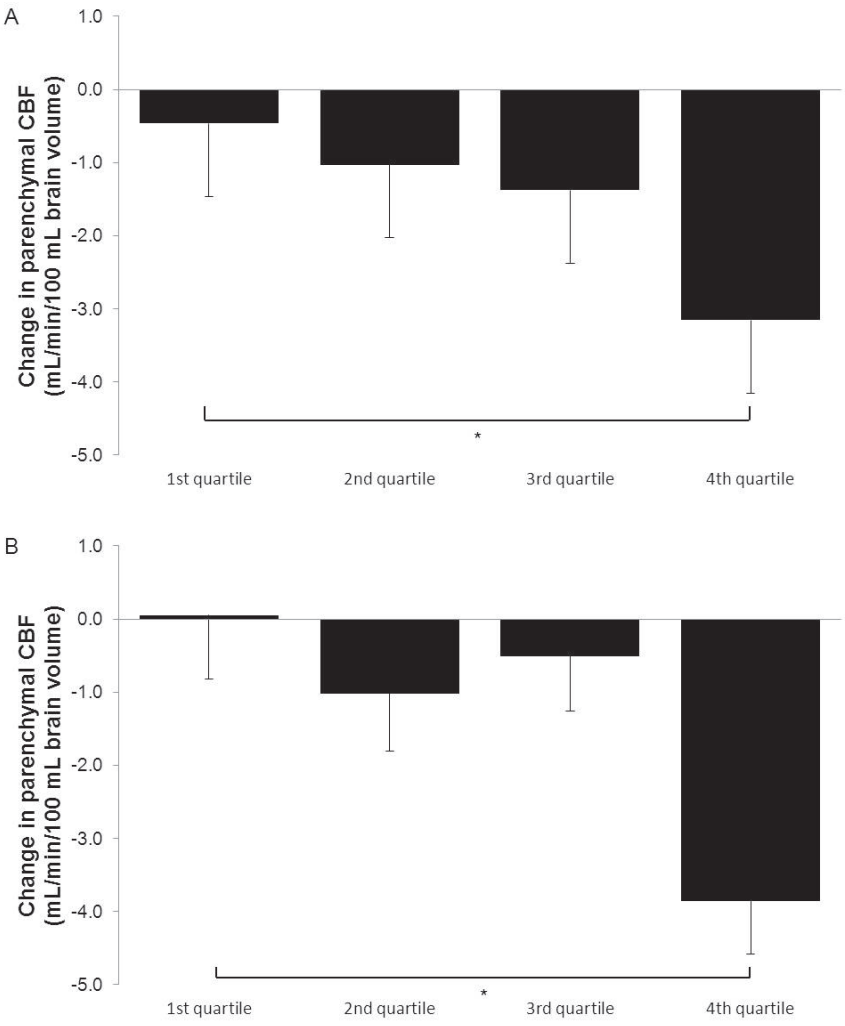

Figure 3 Prospective associations of sex-pooled quartiles of hemoglobin (A) and hematocrit (B) at baseline with change in parenchymal CBF over time.

Bars represent mean change (standard error) in parenchymal CBF after 3.9 years of follow-up, adjusted for age, current smoking, follow-up time, and baseline parenchymal CBF.

Hemoglobin and hematocrit quartiles were defined in a sex-pooled manner and rounded to the nearest tenth (for hemoglobin) or to the nearest hundredth (for hematocrit). Values for hemoglobin were ≤8.0, 8.1-8.4, 8.5-8.7, and >8.7 mmol/L for women across the quartiles and ≤8.7, 8.8-9.2, 9.3-9.6, and >9.6 mmol/L for men. Values for hematocrit were ≤0.37, 0.38-0.39, 0.40-0.41, and >0.41 L/L for women and ≤0.40, 0.41-0.42, 0.43-0.44, and >0.44 L/L for men across the quartiles.

CBF, cerebral blood flow.

* p<0.05.

constant oxygen supply. Cerebral blood flow regulation is primarily based on changes in cerebrovascular resistance; cerebral arteries and arterioles change in diameter in response to changes in metabolic activity and blood pressure.[2] However, cerebrovascular resistance is also influenced by blood viscosity and arterial oxygen content.[2] Elevated blood viscosity, in combination with rigid deformability of red blood cells, increases cerebrovascular resistance and thereby reduces CBF.[3] Blood viscosity is mainly determined by hematocrit and studies in patients with abnormally high or low hematocrit have shown that normalization of hematocrit leads to CBF values within the normal range.[6,10] Arterial oxygen content is determined by both hematocrit and hemoglobin and higher levels of CBF have been observed in anemic patients compared to healthy controls.[5,6] Low arterial oxygen content will increase CBF by vasodilation to maintain adequate transport of oxygen.[2]

Since hemoglobin, as an indicator of arterial oxygen content, and hematocrit, as an indicator of blood viscosity, are highly correlated, the individual role of these components on changes in CBF is difficult to extract. However, animal studies have reported a differential effect of blood viscosity and arterial oxygen content on CBF, depending on brain ischemia. In normal brain, CBF increases with reductions in blood viscosity and arterial oxygen content, whereas in ischemic brain, it is thought that only viscosity and not arterial oxygen content influences CBF, because vascular function may be impaired.[23]

Our cross-sectional results are in line with earlier cross-sectional and interventional studies in different patient populations and healthy individuals that found inverse associations between hemoglobin and hematocrit with CBF.[4-8,11] Here, we show that hemoglobin and hematocrit within normal ranges are also inversely associated with parenchymal CBF. Our prospective results show that higher hemoglobin and hematocrit were associated with greater decline in parenchymal CBF. The cross-sectional findings could be explained by a physiological response of the CBF to higher hemoglobin and hematocrit.[3] This physiological compensatory mechanism could also lead to more rapid decline in parenchymal CBF over time.

Apart from the contribution of blood viscosity and arterial oxygen content to cerebrovascular resistance, the ability to compensate for hypoperfusion can also be impaired due to rigidity of the vascular wall as a possible consequence of atherosclerosis.[6] In patients with manifest arterial disease and in the older population, vascular brain lesions, such as white matter lesions and brain infarcts, are common findings on brain MRI as a possible result of atherosclerosis.[16,24] It can therefore be hypothesized that our longitudinal results of more decline in parenchymal CBF over time with higher hemoglobin and hematocrit are the consequence of inadequate compensation due to these vascular brain lesions, but this possible explanation should be further studied.

The clinical consequences of high or low hemoglobin and hematocrit have been studied in different patient groups and in the general population. In non-demented older adults with hemoglobin within normal ranges, no association was found between hemoglobin and cognitive tests.[11] Very high hematocrit, such as in patients with polycythemia, have been found to increase the risk of thrombotic events and ischemia,[9,25]

and reducing hematocrit by blood donation or bloodletting has been associated with a decreased cardiovascular risk in men.[26] However, only few patients had very high hematocrit. The actual differences and decreases in parenchymal CBF in our study are rather small and within this time interval the clinical impact may be minimal. However, our results may give more insight in the mechanisms involved in lower CBF and the possible associated changes in brain structure and function in patients with cardiovascular disease. In addition, higher hemoglobin and hematocrit have been found to increase the risk of cardiovascular events and mortality in the general population.[27-29] However, these relationships may be U-shaped, because lower hemoglobin and hematocrit have also been associated with more atherosclerosis and an increased risk of cardiovascular disease.[27-31] The results of these different studies are therefore not conclusive and the possible clinical consequences of higher hemoglobin and hematocrit should be further investigated.

Strengths of this study are, first, the large number of patients with available data on CBF at baseline and follow-up. Second, the extensive information on medication use and cardiovascular risk factors, which made it possible to investigate whether the association of hemoglobin and hematocrit with changes in CBF were independent of these possible confounders or mediators.

A limitation of our study is that the use of phase-contrast MRI only resulted in an indirect measure of cerebral perfusion, which does not give information on regional changes in cerebral perfusion. Studies using arterial spin-labeling MRI, which is a noninvasive technique for perfusion territory measurements, provide a more direct measure of brain tissue perfusion and could gain more insight in the observed associations. Further, hemoglobin and hematocrit were assessed at baseline but not at follow-up. It was therefore not possible to examine whether CBF contributed to changes in hemoglobin and hematocrit. Also, vascular disease and cardiovascular risk factors, such as hypertension and intima-media thickness, are both related to hematocrit[21,32] and CBF.[17,22] However, adjusting for these potential confounders only slightly changed the observed associations, which remained significant. As our population consisted of patients with various manifestations of arterial disease, we do not know to what extent our findings are generalizable to a population without arterial disease. Lastly, individuals who participated in the follow-up examination represented a relatively healthy group, which may have led to an underestimation of the progression of CBF.

In conclusion, in patients with manifest arterial disease, higher hemoglobin and hematocrit were cross-sectionally associated with lower parenchymal cerebral blood flow, and prospectively with more decline in parenchymal cerebral blood flow. Whether these associations are the result of physiological compensating mechanisms and whether they have clinical consequences should be the focus for future studies.

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PART III
CONSEQUENCES OF BRAIN AGING

CHAPTER 7

BRAIN VOLUMES AND RISK OF CARDIOVASCULAR EVENTS AND MORTALITY: THE SMART-MR STUDY

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ABSTRACT

INTRODUCTION

Brain atrophy is a strong predictor for cognitive decline and dementia, and these are, in turn, associated with increased mortality in the general population. Patients with cardiovascular disease have more brain atrophy and a higher morbidity and mortality.

METHODS

We investigated if brain volumes on MRI were associated with the risk of cardiovascular events and mortality in patients with manifest arterial disease (n=1215, mean age 58 years). Automated brain segmentation was used to quantify intracranial volume, and volumes of total brain, sulcal cerebrospinal fluid, and ventricles.

RESULTS

After a median follow-up of 8.3 years, 184 patients died, 49 patients had an ischemic stroke, and 100 patients had an ischemic cardiac complication. Smaller relative brain volumes increased the risk of all-cause death (hazard ratio (HR) per standard deviation decrease in total brain volume: 1.58, 95% CI: 1.33-1.88), vascular death (HR 1.69, 95% CI: 1.35-2.13), and ischemic stroke (HR 1.96, 95% CI: 1.43-2.69), independent of cardiovascular risk factors.

CONCLUSION

These results suggest that brain volumes are an important determinant of poor outcome in patients with high cardiovascular risk.

INTRODUCTION

Brain atrophy is a strong predictor for cognitive decline, dementia, and functional disabilities,[1-3] and these are, in turn, associated with an increased risk of mortality in the general population.[4,5] Evidence has accumulated that cardiovascular risk factors and cardiovascular disease play an important role in the etiology of global and focal brain atrophy.[6,7] Because patients with cardiovascular disease also have an increased risk of new cardiovascular events and mortality,[8] it would be of interest to assess whether brain atrophy in patients with cardiovascular disease increase the risk of new vascular events and mortality.

Earlier studies in the general older population and in patients with established vascular disease already showed that vascular brain lesions are directly associated with an increased risk of stroke and all-cause death, irrespective of risk factors.[9-17] The pathophysiology of these particular brain lesions is still not completely clear, but they have been associated with atherosclerosis, hypertension, endothelial dysfunction, and failure of the blood-brain barrier. These underlying mechanisms result in impaired cerebral autoregulation and prothrombotic changes, and subsequent hypoperfusion and ischemia.[18,19] It is hypothesized that vascular brain lesions represent a continuous disease process with increasing vulnerability, thereby increasing the risk for vascular events and other disabilities, eventually leading to death.[10,15]

As both vascular brain lesions and cerebral hypoperfusion are associated with global and focal brain atrophy,[6,7,20] it can be suggested that brain atrophy also is a risk factor for new cardiovascular events and mortality in patients with cardiovascular disease. Some studies in older individuals from the general population have shown that markers of brain atrophy were directly related to an increased mortality risk, independent of risk factors.[14,16,21,22] However, no study has investigated this relation in a younger population with high cardiovascular risk or has differentiated between cardiovascular events, while it is thought that especially this population is at high risk of disability and mortality.

We investigated whether measures of brain volumes on magnetic resonance imaging (MRI) were related to all-cause mortality, including vascular and non-vascular death, and future cardiovascular events in a cohort of patients with manifest arterial disease.

METHODS

SMART-MR STUDY

Data were used from the Second Manifestations of ARTerial disease-Magnetic Resonance (SMART-MR) study, a prospective cohort study aimed at investigating brain changes on MRI in independently living patients with manifest arterial disease. Details of the design and participants have been described previously.[6,23] In brief, between May 2001 and December 2005, all patients newly referred to the University Medical Center Utrecht with manifest coronary artery disease, cerebrovascular disease, peripheral arterial disease, or an

abdominal aortic aneurysm and without MR contraindications were invited to participate. Coronary artery disease was defined as myocardial infarction, coronary artery bypass graft surgery, or percutaneous transluminal coronary angioplasty in the past or at inclusion. Cerebrovascular disease was defined as a transient ischemic attack or stroke at inclusion diagnosed by a neurologist or self-reported stroke in the past. Peripheral arterial disease was defined as surgery or angioplasty of the arteries supplying the lower extremities in the history or intermittent claudication or rest pain at inclusion. Aneurysm of the abdominal aorta (AAA) was defined as present AAA (distal aortic diameter ≥3 cm) or previous AAA surgery. During a one-day visit to our medical center, all patients underwent an MRI of the brain, a physical examination, ultrasonography of the carotid arteries, and blood sampling. Risk factors, medical history, and medication use were assessed using questionnaires. The SMART-MR study was approved by the ethics committee of our institution and written informed consent was obtained from all patients.[6]

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 transversal T1-weighted (repetition time [TR]/echo time [TE] 235/2 ms; field of view (FOV), 230 x 230 mm; matrix size, 224x179), T2-weighted (TR/TE 2200/11 ms and 2200/100 ms; FOV, 230x172.5 mm; matrix size, 256x168), fast fluid-attenuated inversion recovery (FLAIR; TR/TE/inversion time [TI] 6000/100/2000 ms; FOV, 230 x 172.5 mm; matrix size, 256 x 135), and inversion recovery (TR/TE/TI 2900/22/410 ms; FOV, 230 x 183.3 mm; matrix size, 256 x 150) sequences. The purpose of a short TI of 410 ms for the inversion recovery sequence was to obtain optimal T1-weighted gray-white matter contrast. Slice thickness was 4.0 mm, no gap, and 38 slices. No parallel imaging methods were used. The total duration of this protocol was 19 minutes.

BRAIN SEGMENTATION AND INFARCTS

The T1-weighted gradient-echo, inversion recovery, and FLAIR sequence were used for brain segmentation. The probabilistic segmentation technique has been described previously.[24] The segmentation program distinguishes cortical gray matter, white matter including deep gray matter, sulcal and ventricular cerebrospinal fluid (CSF), and white matter lesions (WML). The results of the segmentation analysis were visually checked for the presence of infarcts and adapted if necessary to make a distinction between WML and infarct volumes. Infarcts were visually scored by an investigator and a neuroradiologist, blinded to clinical characteristics. Discrepancies in rating were re-evaluated in a consensus meeting. Infarcts were defined as focal hyperintensities on T2-weighted images of >3 mm in diameter. Hyperintensities located in the white matter also had to be hypointense on T1-weighted and FLAIR images to distinguish them from WML. Dilated perivascular spaces were distinguished from infarcts on the basis of their location, form, and absence of gliosis. Infarcts were categorized as lacunar (sized 3-15 mm) and non-lacunar infarcts (cortical infarcts, large subcortical infarcts, infratentorial infarcts).[6]

BRAIN VOLUMES

Total brain volume was calculated by summing up the probabilistic volumes of gray and white matter and, if present, the volumes of WMLs and infarcts. All volumes cranial to the foramen magnum were included. Total intracranial volume (ICV) was calculated by summing up total brain and CSF volume. Total brain, cerebrospinal fluid, and ventricular volumes were normalized for ICV and expressed as brain parenchymal fraction (BPF), sulcal cerebrospinal fluid fraction (CSFF), and ventricular fraction (VF) (%ICV). WMLs were also normalized for intracranial volume (%ICV) and natural log transformed. Global brain atrophy was represented by smaller BPF and larger CSFF, while subcortical atrophy was represented by larger VF.

CARDIOVASCULAR RISK FACTORS

During the patient's visit to the medical center, an overnight fasting blood sample was taken to determine glucose and lipid levels. Height and weight were measured without shoes and heavy clothing, and 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. Diabetes mellitus at baseline was defined as either a referral diagnosis of diabetes mellitus, self-reported diabetes mellitus (use of glucose-lowering agents), glucose ≥11.1 mmol/L, or a known history of diabetes mellitus. Among subjects without a history of diabetes mellitus, but with a fasting plasma glucose of ≥7.0 mmol/L at baseline, only those receiving treatment with glucose-lowering agents within one year after baseline were considered as having diabetes mellitus at baseline. Hyperlipidemia was defined as total cholesterol >5.0 mmol/L, low-density lipoprotein cholesterol >3.2 mmol/L, or self-reported use of lipid-lowering drugs. Smoking habits and alcohol intake were assessed using questionnaires. Pack years of smoking were calculated and alcohol intake was categorized as never, former, or current users. Patients who had quit drinking during the past year were assigned to the category current alcohol users. Ultrasonography was performed to measure the intima-media thickness (mm) in the left and right common carotid arteries, represented by the mean value of six measurements.

STUDY SAMPLE

Of the 1309 patients participating in the SMART-MR study, MR data were irretrievable for 19 patients and 14 patients had no FLAIR sequence. In addition, in 44 patients brain volume data were unreliable because of motion or artefacts. Seventeen patients were lost to follow-up. As a result, the analyses were performed in 1215 patients.

FOLLOW-UP

Patients received a questionnaire every six months to provide information on hospitalization and outpatient clinic visits. If a cardiovascular event was reported, original source documents were retrieved and reviewed to determine the occurrence of cardiovascular disease. Three physicians of the Endpoint Committee audited all possible events independently. Patients were followed until death or refusal of further

Table 1 Definition of outcomes

All-cause death	Death of a vascular or non-vascular cause.
Death of a vascular cause	Death caused by myocardial infarction, stroke, sudden death (unexpected cardiac death occurring within one hour after onset of symptoms, or within 24 hours given convincing circumstantial evidence), congestive heart failure, rupture of abdominal aortic aneurysm, or death from another vascular cause.
Death of a non-vascular cause	Death caused by infection, cancer, unnatural death, or death from another non-vascular cause.
Ischemic stroke	Relevant clinical features that caused an increase in impairment of at least one grade on the modified Rankin scale, with or without a new relevant ischemic lesion at brain imaging, or death caused by stroke.
Ischemic cardiac complications	Myocardial infarction or sudden death.

participation. The outcomes used in this study were death (all-cause, vascular cause, non-vascular cause), ischemic stroke, and ischemic cardiac complications (e.g., myocardial infarction). Definitions of outcomes are given in Table 1.

DATA ANALYSES

We used multiple imputation (10 datasets) using the statistical program R (aregImpute; version 2.10.0) to address missing values in the study sample of 1215 patients. Data were analyzed using SPSS version 20.0 (Chicago, IL, USA), by pooling the 10 imputed datasets.

Patients were followed from the date of MRI scan until the first cardiovascular event, death from any cause, loss to follow-up, or end of follow-up (March 2012), whichever came first. Baseline characteristics were calculated for the total population and according to tertiles of brain parenchymal fraction. Pearson correlation coefficients were calculated to study the interrelationships between the individual brain volumes BPF, VF, and CSFF and the relation between the brain volumes and age. The brain volumes were highly correlated with each other and with age; the Pearson correlation coefficient between BPF and VF was -0.63, between BPF and CSFF was -0.95, and between VF and CSFF was 0.34 (all $p<0.05$). The relation between brain volumes and age was the highest for BPF; the correlation coefficient between BPF and age was -0.65, between VF and age was 0.47, and between CSFF and age was 0.59 (all $p<0.05$). Cox proportional hazards regression analysis was used to estimate the relation between brain volumes and occurrence of death (all-cause, vascular, and non-vascular), ischemic stroke, or ischemic cardiac complications, adjusted for age and sex (model 1). In model 2, additional adjustments were made for systolic blood pressure, diastolic blood pressure, diabetes mellitus, body mass index,

alcohol consumption (former and current), smoking (pack years), hyperlipidemia, and intima-media thickness; in model 3, we additionally adjusted for vascular brain lesions on MRI (presence of lacunar infarcts, non-lacunar infarcts, and WML volume). Brain volume measures were entered as a continuous variable (per standard deviation (SD) decrease for BPF or per SD increase for CSFF and VF) and in tertiles. Additional adjustments were made for the other brain volumes to study whether the associations of brain volume measures with mortality and cardiovascular events were independent from the other brain volumes. However, because BPF and CSFF were highly correlated (r=-0.95), we excluded CSFF from this model; for BPF and CSFF as determinants additional adjustments were made for VF, and for VF as a determinant additional adjustments were made for BPF.

To study effect modification, interaction terms for brain volumes and natural log transformed WML volume were added to model 1. Because the effects of brain volumes and WML load on mortality and cardiovascular events are expected to lead to an effect in a similar direction, a liberal cut-off p-value for interaction of $p<0.10$ was chosen.25 If an interaction term was found significant, further analyses were performed by creating joint exposure categories of measures of brain volumes (upper tertile of BPF, CSFF, or VF vs. lower two tertiles of BPF, CSFF, or VF) and categories of WML load (upper tertile of WML vs. lower two tertiles of WML). The reference category consisted of patients with the upper tertile of BPF or lower two tertiles of CSFF or VF and the lower two tertiles of WML. Cox proportional hazards regression analysis was used to calculate the relation between the different categories and the outcomes, according to model 1.

The proportional hazard assumptions were checked by examining the Schoenfeld residuals of the brain volumes against follow-up time for all outcomes. The plots of the residuals were horizontal and close to 0, so the proportional hazard assumptions were met.

RESULTS

In the study sample of 1215 patients, mean (SD, range) age was 58 years (10, 25-82 years), 80% were men, and approximately 97% was Caucasian. The mean (SD) baseline BPF, CSFF, and VF were 79.0 (2.9), 18.9 (2.4), and 2.1 (1.0) %ICV, respectively.

Participants with smaller BPF were older, more often men, had higher systolic blood pressure, more diabetes mellitus, larger WML volume, and more brain infarcts on MRI at baseline than participants with larger BPF (Table 2).

The average number (range) of follow-ups was 15 (0-21). In total, 184 patients died during a median follow-up period of 8.3 years (range 0.2-11.1 years), of whom 102 died because of a vascular cause and 82 because of a non-vascular cause. Forty-nine patients suffered from an ischemic stroke and 100 patients had an ischemic cardiac complication.

MORTALITY

Cox regression analyses showed that smaller BPF, larger CSFF, and larger VF were associated with an increased risk of all-cause mortality (Table 3, model 1 and Figure 1).

Table 2 Baseline characteristics of the study sample according to tertiles of brain parenchymal fraction

	BPF			All
	Lowest tertile	Middle tertile	Highest tertile	
	n=405	n=405	n=405	n=1215
Age, years	66 ± 8	58 ± 8	51 ± 8	58 ± 10
Sex, % men	85	81	73	80
Cardiovascular risk factors				
BMI, kg/m^2	27 ± 4	27 ± 4	27 ± 4	27 ± 4
Smoking, pack years†	21 (0, 62)	21 (0, 49)	15 (0, 43)	19 (0, 50)
Alcohol intake, % current	75	76	74	75
Systolic blood pressure, mmHg	146 ± 22	142 ± 22	137 ± 19	142 ± 21
Diastolic blood pressure, mmHg	82 ± 11	82 ± 11	82 ± 11	82 ± 11
Hyperlipidemia, %	78	77	81	79
Diabetes mellitus, %	26	15	8	16
Intima-media thickness, mm	1.0 ± 0.3	0.9 ± 0.3	0.8 ± 0.3	0.9 ± 0.3
Brain volumes (%ICV)				
Brain parenchymal fraction	75.8 ± 1.9	79.2 ± 0.7	82.0 ± 1.2	79.0 ± 2.9
Sulcal cerebrospinal fluid fraction	21.4 ± 1.7	18.8 ± 0.9	16.5 ± 1.1	18.9 ± 2.4
Ventricular fraction	2.8 ± 1.1	2.0 ± 0.7	1.5 ± 0.6	2.1 ± 1.0
Cortical gray matter fraction	33.0 ± 2.8	36.1 ± 2.1	38.9 ± 2.6	36.1 ± 3.5
Total WML volume fraction†	0.19 (0.05,1.02)	0.10 (0.03,0.39)	0.08 (0.03,0.22)	0.11 (0.03,0.53)
Infarcts, % of total[a]				
All	44	23	16	28
Lacunar	31	14	9	18
Cortical	19	11	5	12
Large subcortical	1	1	0	1
Infratentorial	11	5	3	7

Tertiles of BPF ranged from 65.4 to 77.9, 77.9 to 80.4, and 80.4 to 86.9 %ICV.

Characteristics presented as mean ± standard deviation, unless stated otherwise.

BPF, brain parenchymal fraction; BMI, body mass index; ICV, intracranial volume; WML, white matter lesion.

† Median (10th-90th percentile).

[a] % of participants per category with an infarct.

Percentage of missing values before imputation: BMI 0.1%, smoking 0.6%, alcohol intake 0.7%, systolic blood pressure 0.1%, diastolic blood pressure 0.1%, hyperlipidemia 1.6%, intima-media thickness 2.0%, cortical gray matter fraction 15.2%.

When making a distinction between causes of death, smaller BPF, larger CSFF, and larger VF were mainly associated with an increased risk of vascular death (Table 3 and Figure 1).

Adjusting for cardiovascular risk factors and vascular brain lesions on MRI (brain infarcts and WML volume) attenuated the associations between brain volumes and mortality risk, in particular for VF, but remained statistically significant for BPF and CSFF

Table 3 Relationship between brain volumes and risk of mortality and cardiovascular events in 1215 patients

		All-cause death	Vascular death	Non-vascular death	Ischemic stroke	Ischemic cardiac complications
Number of cases		184	102	82	49	100
Sum person years		9599.7	9599.7	9599.7	9399.3	9332.0
Number per 1000 person years		19.2	10.6	8.5	5.2	10.7
	Model	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)
BPF, %ICV (per SD decrease)	1	1.58 (1.33-1.88)*	1.69 (1.35-2.13)*	1.45 (1.11-1.89)*	1.96 (1.43-2.69)*	1.28 (1.00-1.65)
	2	1.46 (1.22-1.76)*	1.56 (1.23-1.98)*	1.31 (0.99-1.74)	1.83 (1.33-2.51)*	1.22 (0.94-1.58)
	3	1.36 (1.13-1.63)*	1.40 (1.10-1.78)*	1.27 (0.95-1.69)	1.51 (1.09-2.09)*	1.18 (0.91-1.53)
CSFF, %ICV (per SD increase)	1	1.45 (1.22-1.72)*	1.50 (1.20-1.87)*	1.39 (1.07-1.79)*	1.77 (1.30-2.41)*	1.23 (0.97-1.56)
	2	1.33 (1.12-1.59)*	1.37 (1.09-1.72)*	1.26 (0.96-1.66)	1.65 (1.21-2.24)*	1.19 (0.93-1.51)
	3	1.29 (1.08-1.53)*	1.30 (1.03-1.62)*	1.25 (0.95-1.63)	1.46 (1.09-1.97)*	1.17 (0.92-1.49)
VF, %ICV (per SD increase)	1	1.26 (1.09-1.44)*	1.35 (1.13-1.60)*	1.14 (0.92-1.42)	1.37 (1.05-1.78)*	1.12 (0.91-1.38)
	2	1.24 (1.08-1.44)*	1.33 (1.11-1.59)*	1.12 (0.88-1.41)	1.37 (1.05-1.79)*	1.08 (0.88-1.34)
	3	1.14 (0.98-1.33)	1.17 (0.96-1.43)	1.07 (0.84-1.37)	1.09 (0.80-1.49)	1.03 (0.82-1.29)

Model 1: adjusted for age and sex; model 2: additionally adjusted for systolic blood pressure, diastolic blood pressure, diabetes mellitus, body mass index, alcohol consumption (former and current), smoking (pack years), hyperlipidemia, and intima-media thickness; model 3: additionally adjusted for lacunar infarcts, non-lacunar infarcts, and white matter lesion volume (%ICV) (natural log transformed).

HR, hazard ratio; CI, confidence interval; BPF, brain parenchymal fraction; CSFF, sulcal cerebrospinal fluid fraction; VF, ventricular fraction; ICV, intracranial volume; SD, standard deviation.

* $p<0.05$.

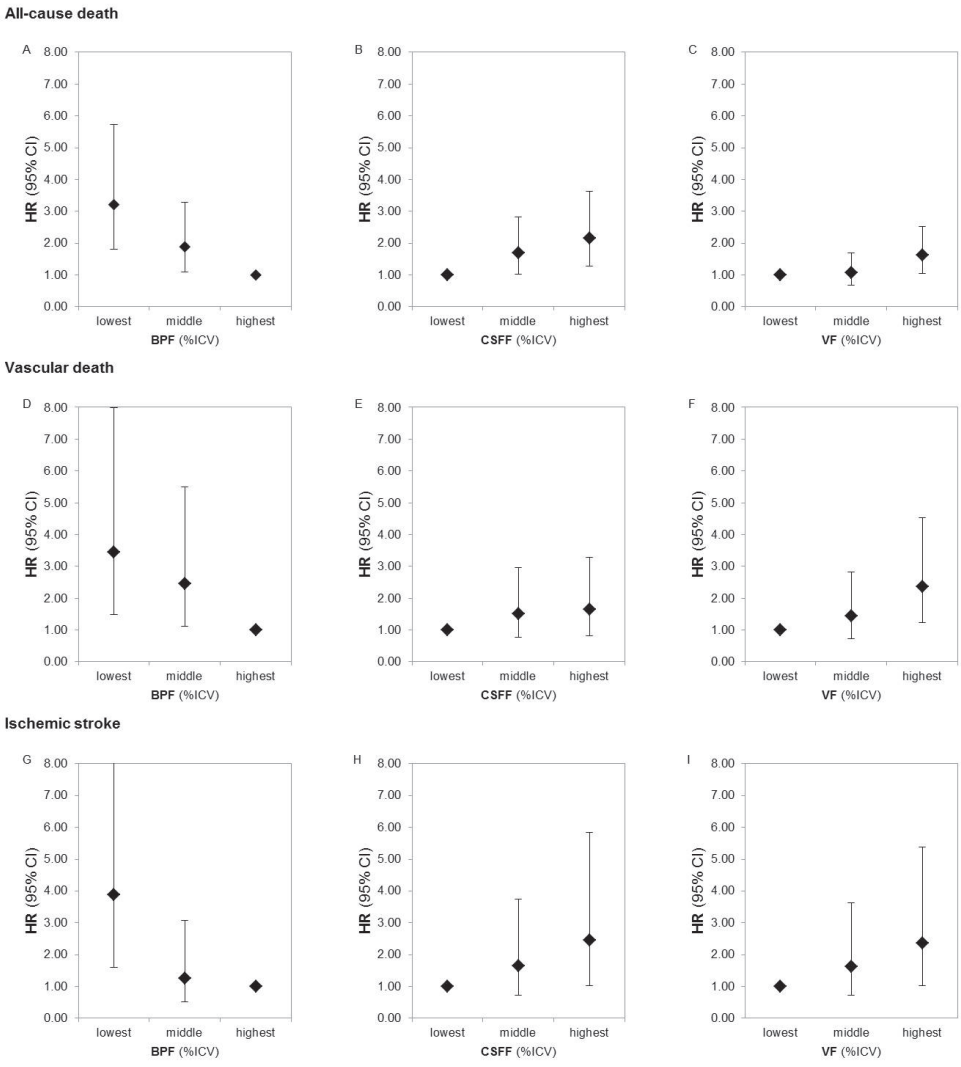

Figure 1 Relations between tertiles of BPF, CSFF, and VF and risk of all-cause death, vascular death, and ischemic stroke.

Hazard ratios (95% CI) for lowest BPF tertile, highest CSFF tertile, highest VF tertile vs. highest BPF tertile, lowest CSFF tertile, lowest VF tertile, according to model 1:

All-cause death: 3.21 (1.80-5.73), 2.15 (1.27-3.62), and 1.62 (1.04-2.52) (1a, b, and c);

Vascular death: 3.45 (1.49-7.99), 1.65 (0.83-3.29), and 2.36 (1.23-4.54) (1d, e, and f);

Ischemic stroke: 3.87 (1.60-9.39), 2.45 (1.03-5.85), and 2.36 (1.03-5.37) (1g, h, and i).

Tertiles of BPF ranged from 65.4 to 77.9, 77.9 to 80.4, and 80.4 to 86.9 %ICV; tertiles of CSFF ranged from 12.5 to 17.7, 17.7 to 19.8, and 19.8 to 31.0 %ICV; tertiles of VF ranged from 0.3 to 1.6, 1.6 to 2.3, and 2.3 to 7.9 %ICV. HR, hazard ratio; CI, confidence interval; BPF, brain parenchymal fraction; CSFF, sulcal cerebrospinal fluid fraction; VF, ventricular fraction; ICV, intracranial volume.

Table 4 Relationship between ventricular fraction and WML load and risk of mortality and ischemic stroke in 1215 patients

		All-cause death	Vascular death	Ischemic stroke
Number of cases		184	102	49
	N	HR (95% CI)	HR (95% CI)	HR (95% CI)
Small VF and small WML, reference	632	1.00	1.00	1.00
		n=47	n=22	n=14
Large VF and small WML	178	1.67 (1.05-2.65)*	2.14 (1.14-4.01)*	0.84 (0.24-2.99)
		n=33	n=20	n=3
Small VF and large WML	179	1.85 (1.17-2.94)*	2.14 (1.12-4.10)*	3.35 (1.50-7.46)*
		n=32	n=17	n=12
Large VF and large WML	226	2.27 (1.48-3.46)*	2.88 (1.60-5.19)*	5.10 (2.30-11.32)*
		n=72	n=43	n=20

Model 1: adjusted for age and sex.

N, number of patients per category; n, number of cases per category; HR, hazard ratio; CI, confidence interval; VF, ventricular fraction; WML, white matter lesion volume.

* p<0.05.

(Table 3, model 3). Additional adjustments for VF did not change the effect estimates and the associations of BPF and CSFF with all-cause mortality and vascular death remained significant (hazard ratios (HRs) for BPF, 95% CI were 1.34, 1.09-1.65 and 1.36,1.04-1.78, respectively; HRs for CSFF, 95% CI were 1.28, 1.07-1.52 and 1.29, 1.03-1.61).

Additional analyses were performed to study the combined effect of brain volumes and WML load; for all-cause mortality and vascular death, a significant interaction was found between VF and WML load (p=0.07 and p=0.03, respectively). Patients with large VF and large WML load showed a significantly higher risk for all-cause mortality and vascular death when compared with the reference category; HRs, 95% CI were 2.27, 1.48-3.46 and 2.88, 1.60-5.19, respectively (Table 4).

CARDIOVASCULAR EVENTS

Smaller brain volumes were also associated with an increased risk of ischemic stroke after adjusting for age and sex (Table 3, model 1 and Figure 1). Adjusting for cardiovascular risk factors and vascular brain lesions on MRI attenuated these associations, in particular for VF, although they remained statistically significant for BPF and CSFF (Table 3, model 3). Additional adjustments for VF did not change the effect estimates and the associations of BPF and CSFF with ischemic stroke remained significant (HRs, 95% CI were 1.57, 1.10-2.26 and 1.46, 1.08-1.96). Also, the relation between brain volumes and risk for ischemic stroke was similar in those with and without a history of stroke and/or brain infarct on MRI (Table 5).

Table 5 Relationship between brain volumes and risk of ischemic stroke in 1215 patients

HR for ischemic stroke		History of stroke and/or infarct on MRI (n=423)	No history of stroke and no infarcts on MRI (n=792)
Number of cases		33	13
Sum person years		3037.7	6361.6
Number per 1000 person years		10.9	2.0
	Model	HR (95% CI)	HR (95% CI)
BPF, %ICV (per SD decrease)	1	1.61 (1.11-2.33)*	1.70 (0.85-3.40)
CSFF, %ICV (per SD increase)	1	1.51 (1.06-2.14)*	1.61 (0.84-3.09)
VF, %ICV (per SD increase)	1	1.19 (0.88-1.62)	1.20 (0.64-2.23)

Model 1: adjusted for age and sex.

HR, hazard ratio; MRI, magnetic resonance imaging; CI, confidence interval; BPF, brain parenchymal fraction; CSFF, sulcal cerebrospinal fluid fraction; VF, ventricular fraction; ICV, intracranial volume; SD, standard deviation.

* p<0.05.

Additionally, the combined effect of brain volumes and WML load on risk of cardiovascular events was studied. A significant interaction was found between VF and WML load (p=0.09) for risk of ischemic stroke. Patients with large VF and large WML load showed a significantly higher risk for ischemic stroke when compared with the reference category; HR, 95% CI was 5.10, 2.30-11.32 (Table 4).

Finally, BPF, CSFF, and VF were not significantly associated with the risk of ischemic cardiac complications (Table 3).

DISCUSSION

In this large prospective cohort of patients with manifest arterial disease, we observed that smaller total brain, larger sulcal cerebrospinal fluid, and larger ventricular volume increased the risk of all-cause death. When making a distinction between vascular and non-vascular causes of death, smaller brain volumes were associated with an increased risk of vascular death, but showed no relation with the risk of non-vascular death, after adjusting for cardiovascular risk factors. Smaller brain volumes were also related to an increased risk of ischemic stroke, but this was largely explained by patients with a history of stroke or brain infarct on MRI. When the combined effect of brain volumes and white matter lesion load was studied, patients with larger ventricular volume in combination with large white matter lesion volume showed even higher risks for all-cause mortality, vascular death, and ischemic stroke, when compared with patients with smaller ventricular volume and smaller white matter lesion volume. We observed no association between brain volumes and the risk of ischemic cardiac complications. Although all presented

associations attenuated after adjusting for cardiovascular risk factors and vascular brain lesions on MRI; they remained significant for smaller total brain and larger sulcal cerebrospinal fluid volumes. This finding suggests that global atrophy, represented by smaller total brain and larger sulcal cerebrospinal fluid volume, independently contributes to a higher mortality risk, especially when death is because of a vascular cause, in this high risk population.

To our knowledge, this is the first study that prospectively examined the relation between brain volumes and the risk of cardiovascular events and mortality in a large population with high vascular risk. Our finding that smaller brain volumes were associated with an increased risk of all-cause death in patients with manifest arterial disease is in line with results from earlier studies in the general population. Both larger ventricular size in the population-based Cardiovascular Health Study[16] and larger CSF volumes in the Rotterdam Scan Study[14] were related to an increased risk of mortality. Additionally, studies in smaller and older samples of the general population also showed an increased mortality risk with smaller brain volumes.[21,22] Our finding of a relation between brain volumes and vascular death is in line with one study,[16] but in contrast with two others who found no significant relation between brain volumes and vascular death.[14,21] However, the definition of vascular death was not completely comparable within these studies as different combinations of International Classification of Diseases-codes were used[14,16] or the causes of vascular death were not further specified.[21] This comparison should therefore be interpreted with caution.

There are several possible explanations for the relation between smaller brain volumes and the increased risk of mortality and ischemic strokes. A population-based study in 85-year-old individuals proposed the explanation that the brain could be a marker for general health and thereby influence morbidity and mortality.[21] As the brain regulates several important functions for survival, such as blood pressure, fluid intake and secretion, electrolyte homeostasis, appetite, and body temperature,[26,27] poor regulation because of brain atrophy could result in poor general health and higher mortality. However, our population is relatively young and after taking some of these important functions (blood pressure and body mass index) and other possible confounders into account, we still found that smaller brain volumes are related to high mortality risk and occurrence of more cardiovascular events. The possibility of residual confounding cannot be fully ruled out, but this suggests that smaller brain volumes as 'poor general health markers' can only partly explain our results.

The relation between smaller brain volumes and increased risk of mortality and cardiovascular events could also be explained by shared underlying cardiovascular risk factors. Previous epidemiological studies have shown that increased cardiovascular risk, such as hypertension, diabetes, and atherosclerosis, increases the risk of brain atrophy.[6,7,28-30] Brain atrophy may therefore be a representative of chronic vascular damage.

Furthermore, the relation between smaller brain volumes and increased mortality risk could be explained by vascular brain lesions, such as WML or infarcts. Presence of these lesions showed an increased mortality risk in several populations, including our

own vascular population,[9-17] but excluding patients with brain infarcts and adjusting for vascular brain lesions on MRI only partly explained the brain volume-mortality relation. However, we did not measure other microvascular brain lesions in our study, such as microbleeds and microinfarcts, which could also explain the brain volume-mortality risk relation. In previous studies, microbleeds have been related to an increased mortality risk[31,32] and microinfarcts are commonly observed in autopsy studies and are associated with brain weight, cerebrovascular disease, and cognitive function.[33,34] The use of high-field and other MRI techniques such as diffusion tensor imaging may result in more detailed information on these microvascular lesions and functional abnormalities.

Apart from the vascular pathway, neurodegenerative diseases, such as Alzheimer's disease, are also known to influence brain atrophy and mortality.[4,5] In our analyses, we were not able to adjust for cognitive performance, because data on cognition were only available in a subset of our population. In this subset of 700 patients (58%), the median Mini-Mental State Examination score was 29 and only 4% scored below 26. So we expect that poor cognitive functioning will not have influenced the observed associations. In addition, results from the population-based studies already showed that additional adjustment for cognitive impairment did not change the association between smaller brain volumes and increased mortality risk.[14,16,21,22]

Major strengths of this study include the prospective design, the long follow-up period, the large number of MRI scans, and inclusion of several different vascular outcomes, which were studiously allocated. Further, the volumetric segmentation made it possible to differentiate between different brain volumes in relation to mortality and cardiovascular events. Also, the extensive information on cardiovascular risk factors, presence of atherosclerosis, and vascular brain lesions on MRI made it possible to assess the independent relation between brain volumes and cardiovascular event and mortality risk.

Limitations of our study are, first, that brain volumes were only assessed at baseline and while brain volume adjusted for intracranial volume is a good index of brain atrophy, it is not brain atrophy in the true sense of the term. Second, some events were misclassified. If an event was reported, original source documents were retrieved and reviewed by a committee to determine the occurrence of death and cardiovascular events and cause of death. However, because the physicians of the Endpoint Committee were blinded for brain volumes, any misclassification is likely to be non-differential and would lead to an underestimation of the effect of brain volumes on mortality and cardiovascular event risk. Also, we studied a population of patients with manifest arterial disease, which limits comparison with the general population.

In conclusion, in a large population of patients with manifest arterial disease, smaller brain volumes were related to an increased risk of mortality, especially when death was because of a vascular cause, and ischemic stroke, independent of cardiovascular risk factors. Additional research is needed to study whether brain volumes have added value in models to predict prognosis in this high-risk population.

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CHAPTER 8

GENERAL DISCUSSION

VASCULAR AGING

Normal aging is associated with changes of the vascular structure and function, characterized by decreased arterial compliance and increased arterial stiffness. This is the result of an age-dependent structural and biochemical process.[1] This process is called arteriosclerosis and is mainly located in the medial layer of the arterial wall. This process is very different from atherosclerosis, where atheromata are formed in the intima, leading to acute vascular events. Up till now, the stiffening of arteries is mainly explained by fibrosis and degeneration of elastin material in the arterial wall by advanced glycation end-products and calcifications. Calcifications have mainly been neglected as a cause of this stiffening, even though their presence is ubiquitous. This can be explained by the fact that calcifications in general are considered an end-stage in cardiovascular disease and not a cause of cardiovascular disease.[2] However, in the last decade, it has become increasingly clear that medial arterial calcifications actually reflect an active process involving the transformation of smooth muscle cells in the vessel wall leading to vascular stiffening and thus being a cause of cardiovascular disease.[3-5] This stiffening of the vessel wall probably gives rise to upstream, downstream and local, chronic, long-term vascular effects. The rate at which this stiffening occurs, however, differs widely between individuals. In some, it occurs relatively early, while others preserve an elastic arterial wall until very high age.[6] As early stiffening of central arteries is associated with increased risk of developing premature cardiovascular disease, arterial stiffening at younger age is a condition with increased cardiovascular risk, whereas arterial stiffening at higher age remarkably frequently is considered as normal aging. Yet, these older patients have a similar increased cardiovascular risk as younger patients. This divergent rate of stiffening has led to the concept of early vascular aging (Figure 1).[7,8] Early vascular aging can be "spontaneous" without a known cause, but it is also increasingly linked to the metabolic syndrome and especially to type 2 diabetes mellitus and chronic kidney disease.[9,10] Atherosclerosis and arteriosclerosis could share some risk factors, but probably have risk factors of their own too (Figure 2). Furthermore, it is likely that both conditions can occur sequentially or simultaneously. Anatomically, arterial stiffening and medial arterial calcifications may also involve the microcirculation, which could be related to tissue damage.

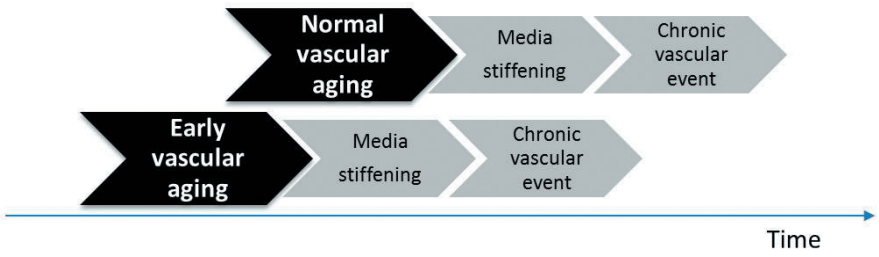

Figure 1 Divergent rate of arterial stiffening and the role of early vascular aging

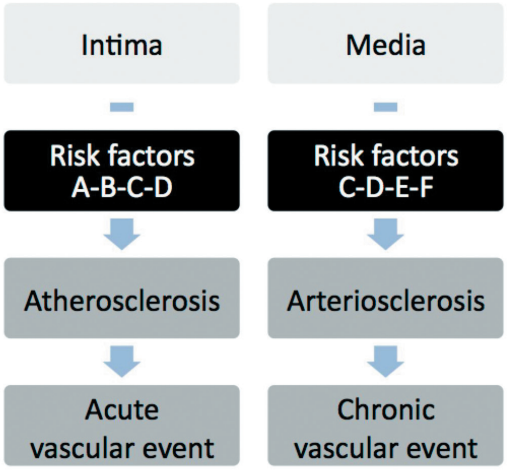

Figure 2 Risk factors in atherosclerotic changes in the intima and arteriosclerotic changes in the media and its consequences

VASCULAR AGING AND THE AGING BRAIN

We studied the effect of aging by looking at the progression of brain atrophy and cerebrovascular lesions (white matter lesions and brain infarcts) after on average four years of follow-up in 663 patients with manifest arterial disease within the SMART-MR study (**chapter 2**). Participants of the SMART-MR study present relatively young with cardiovascular disease, which may suggest that they also suffer from early vascular aging.

Within this population, we observed that global and cortical brain atrophy tended to accelerate with older age and that they were more pronounced in men than in women. We showed progression of white matter lesion volume with increasing age, similar for men and women. Compared to results from population-based studies in similar age groups, we showed that in patients with manifest arterial disease, the progression of brain atrophy and white matter lesions was not more pronounced than in the general population; the measures were either comparable or somewhat lower.[11-13] The incidence of new brain infarcts increased with age and was stronger in men than in women. These results were confirmed by other studies although the differences between men and women were different in several studies, which could be explained by different populations and methodological differences.[14-17] Another remarkable finding was that compared to patients with coronary artery disease, patients with peripheral arterial disease or abdominal atherosclerosis had more progression of brain atrophy. One explanation could be that patients with peripheral arterial disease and abdominal aortic aneurysm have more stiffening of their central and peripheral arteries, which could lead to pulse wave changes in the arteries leading to the brain, damaging brain tissue, and that such pulse wave changes do not occur when only the coronary arteries are diseased. It has been suggested that particularly the small arteries of the brain are vulnerable

to high pulsatile pressure and flow, caused by increased arterial stiffness.[18] This may contribute to brain atrophy and cerebral small-vessel disease. Overall, our results showed a consistent role for age and sex on longitudinal brain changes in patients with manifest arterial disease.

CARDIOVASCULAR RISK FACTORS AND THE AGING BRAIN

The traditional cardiovascular risk factors, such as hyperlipidemia and hypertension, do no longer have a large impact on longitudinal brain changes in patients with manifest arterial disease. In fact, with respect to blood pressure, lower (and not higher) blood pressure levels were related to more progression of brain atrophy, where it was suggested that low systolic and diastolic blood pressure could be a marker of reduced cardiac output due to heart failure and low diastolic blood pressure could be a marker of increased arterial stiffness.[19-21] Traditional cardiovascular risk factors no longer seem to be as important as they were. There can be several explanations for this. First, it could be that they play an important role in atherosclerosis, disease of the intima, and in acute events, but not in arteriosclerosis and aging. Therefore, we should look for other non-traditional possibly age-related and arteriosclerosis-related risk factors as targets for treatment and prevention of longitudinal brain changes. Second, the lack of impact of traditional cardiovascular risk factors could be due to the fact that a single abnormal risk factor value does not inform about the duration of the action of the risk factor or about the sensitivity of the patient for this particular risk factor, while this information is possibly more important in chronic vascular damage than it is in acute events. Therefore, it could be useful to search for better measures of exposure to the traditional cardiovascular risk factors and look for parameters disclosing the impact of the risk factor on a particular patient. In **chapter 5**, we showed that the combined presence of signs of hypertensive target organ damage was associated with progression of global brain atrophy and more decline in memory performance, but not with progression of cerebral small-vessel disease or a decline in executive functioning. Apparently, global brain atrophy and cerebral small-vessel disease are not in the same pathophysiological pathway. In general, target organ damage can be seen as either the sum of chronic exposure to certain cardiovascular risk factors that causes the damage and/or it shows the sensitivity to a certain cardiovascular risk factor in a particular patient, but this also means that not all patients with this cardiovascular risk factor show the same target organ damage as their sensitivity may differ. In both cases, visualizing target organ damage as an intermediate endpoint is possibly a good way to select the true exposure to certain cardiovascular risk factors and its real consequences for a certain patient. Recently, a similar approach was proposed by looking at telomere length in leucocytes. Measurement of this length could be a marker of acquired damage superimposed on pre-existent genetic predisposition.[22] More research will be necessary to explore this concept further.

In **chapter 7**, we showed an example of the value of organ damage in the prediction of cardiovascular events and mortality in patients with manifest arterial disease, where we showed that smaller total brain volume and larger cerebrospinal fluid and ventricular volumes increased the risk of death by any cause and in particular by a vascular cause

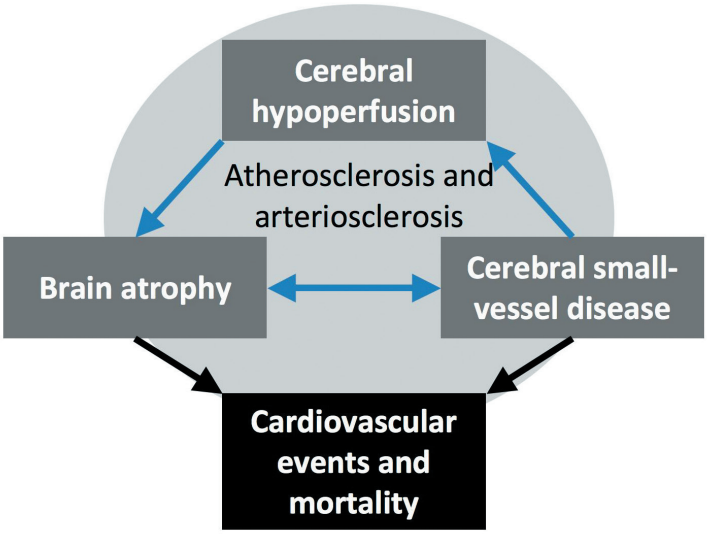

Figure 3 Possible circular pathway for the role of cerebral perfusion in the etiology of structural brain changes and its possible consequences in a high-risk population

(Figure 3). It can therefore be suggested that brain atrophy on its own already is such a sensitive marker of organ damage and is caused by a process that increases the risk of all-cause mortality in patients with manifest arterial disease.

CEREBRAL PERFUSION IN THE AGING BRAIN

To gain more insight in the etiology of structural brain changes over time, we studied the role of cerebral perfusion in aging of the brain. Adequate cerebral perfusion is necessary for delivery of oxygen and nutrients to the brain and this mechanism is usually controlled by cerebral autoregulation.[23,24] However, when this mechanism fails, cerebral hypoperfusion may occur, resulting in global or more focal ischemia. In our study population, it is likely that both atherosclerosis and arteriosclerosis might influence autoregulatory mechanisms, which can lead to cerebral hypoperfusion.[25-29] Our findings showed that cerebral hypoperfusion was related to brain atrophy (**chapter 3**) and that cerebral small-vessel disease was related to decline of cerebral blood flow (**chapter 4**). Previous research already showed that brain atrophy was associated with more cerebral small-vessel disease,[30] which suggests a circular pathway for the role of cerebral perfusion in the etiology of structural brain changes (Figure 3). However, it is also possible that in our study population, arterial stiffening at baseline already caused cerebral hypoperfusion, brain atrophy, and cerebral small-vessel disease, and that over four years of follow-up, this process was also responsible for progression of hypoperfusion, brain atrophy, and cerebral small-vessel disease. Therefore, it is not completely clear where this pathway starts and whether cerebral hypoperfusion is the cause or the consequence of brain

atrophy and progression of cerebral small-vessel disease. In addition, our previous results in **chapter 5** also contradict the fact that brain atrophy and cerebral small-vessel disease would be in the same pathophysiological pathway. On the other hand, interventions targeted on increasing cerebral perfusion might still prevent further progression of brain atrophy and cerebral small-vessel disease and its clinical consequences.

Although the role of cerebral perfusion in the etiology of structural brain changes remains uncertain (**chapters 3** and **4**), determinants of the regulation of cerebral perfusion could also be possible modifiable risk factors. Hemoglobin and hematocrit are important determinants of blood viscosity and arterial oxygen content in the regulation of cerebral blood flow.[24] We showed that high hemoglobin and hematocrit, still within normal range, resulted in lower levels of baseline cerebral blood flow and a decline in cerebral perfusion over time in patients with manifest arterial disease (**chapter 6**). Although the actual differences in our study were rather small and the follow-up period was probably not long enough to show possible clinical consequences of high hemoglobin and hematocrit, their effect on cerebral perfusion in patients with manifest arterial disease may offer new insight in the mechanisms of brain aging and possible targets for treatment and prevention of structural brain changes.

SUMMARY

Key findings

- Age and sex play a consistent role in structural brain changes over time, even in the presence of other traditional risk factors
- Cerebral hypoperfusion has a role in longitudinal brain changes, but whether it is the cause or the consequence is not clear
- In patients with manifest arterial disease, traditional (cardiovascular) risk factors no longer have a large impact on aging of the brain
- Parameters of exposure to traditional risk factors and indicators for sensitivity of patients for these risk factors, such as hypertensive target organ damage, are strong predictors of structural and functional brain changes over time
- Smaller brain volumes increase the risk of poor clinical outcome in patients with manifest arterial disease

Future directions

- Large population-based studies with a long follow-up period and multiple measurements of brain measures are needed and should focus on organ damage more than on traditional risk factor assessment to gain further insight in the etiology of structural brain changes during aging
- Future studies should focus on finding (cardiovascular) risk factors for arterial stiffening as this process is involved in research on aging and may provide new targets for future treatment and prevention

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SUMMARY

Cardiovascular disease is a major health problem worldwide and although the mortality from cardiovascular disease in Europe decreased, the increasing incidence of cardiovascular disease and its risk factors are still a true concern. Improved treatment has resulted in a more chronic pattern of disease with higher rates of long-term impairment of cognitive and physical functioning. Accumulating evidence suggests that cardiovascular disease and a high cardiovascular risk play an important role in the etiology of cognitive decline and dementia. As intermediates in this relation between cardiovascular disease and functional decline in the aging population, structural brain changes have been proposed.

The research described in this thesis was performed using data from the SMART-MR study, which is a prospective cohort study within the Second Manifestations of ARTerial disease (SMART) study. In the SMART study, patients with manifest arterial disease or risk factors for atherosclerosis are screened for additional risk factors and severity of atherosclerosis. Between May 2001 and December 2005, the vascular screening was extended with magnetic resonance imaging (MRI) of the brain and from January 2003 onwards with additional neuropsychological tests. At baseline, 1309 patients were included in the SMART-MR study and of these patients, 754 participated in the follow-up examination of the SMART-MR study between January 2006 and May 2009. The extensive information on cardiovascular disease and its risk factors, cardiovascular events, mortality, and measurements of brain volumes, cerebral small-vessel disease, and cerebral blood flow at two time points allowed us to further elucidate the relation between cardiovascular disease and risk factors and longitudinal brain changes.

The overall aim of this thesis was to gain further insight in the aging brain and its causes and consequences in patients with manifest arterial disease. The first objective was to estimate longitudinal changes in brain volumes, cerebral small-vessel disease, and cerebral blood flow on MRI and to investigate their interrelations. In **chapter 2**, we observed that global and cortical brain atrophy tended to accelerate with older age and that they were more pronounced in men than in women. We also showed regression of white matter lesion volume in younger patients, but progression of white matter lesion volume with increasing age, similar for men and women. The incidence of new brain infarcts also increased with age and was stronger in men than in women. Across the different cardiovascular disease categories we showed that brain atrophy and progression of cerebrovascular lesions differed. Compared with patients with coronary artery disease, patients with cerebrovascular disease showed more progression of cortical and subcortical brain atrophy and an increased risk for new brain infarcts, while patients with peripheral arterial disease showed more progression of cortical brain atrophy.

To study the longitudinal relations between brain atrophy and cerebral small-vessel disease, we studied how cerebral blood flow, as an indicator of cerebral perfusion,

was associated with these features of the aging brain. We found that lower cerebral blood flow at baseline resulted in more progression of cortical brain atrophy after four years of follow-up (**chapter 3**). However, the magnitude of progression of cortical brain atrophy was reduced with 50% after excluding patients with a severe carotid stenosis. Lower cerebral blood flow was not associated with progression of global or subcortical brain atrophy. In **chapter 4**, we showed that lower cerebral blood flow was also not associated with progression of cerebral small-vessel disease, but vice versa, baseline white matter lesions were associated with more decline in cerebral blood flow after four years of follow-up, particularly when located deep in the white matter as opposed to periventricular white matter lesions. The absence of an association between low cerebral blood flow at baseline and progression of white matter lesions might suggest that cerebral perfusion is part of a circular pathway in the etiology of structural brain changes; cerebral hypoperfusion leads to brain atrophy (**chapter 3**) – brain atrophy is associated with more cerebral small-vessel disease – cerebral small-vessel disease leads to decline of cerebral blood flow (**chapter 4**) and so on. However, it is also possible that in our study population, other processes at baseline already caused cerebral hypoperfusion, brain atrophy, and cerebral small-vessel disease, and that over four years of follow-up, these processes were also responsible for progression of hypoperfusion, brain atrophy, and cerebral small-vessel disease. Therefore, it is not completely clear where this pathway starts and whether cerebral hypoperfusion is the cause or the consequence of brain atrophy and progression of cerebral small-vessel disease.

Our second objective was to investigate two possible modifiable risk factors for brain aging and to study the long-term effect of brain aging on cardiovascular morbidity and mortality in patients with manifest arterial disease. In **chapter 5**, we studied the association between hypertensive target organ damage, as a measure of chronic hypertension, and structural and functional brain changes over four years of follow-up. We observed that the combined presence of signs of hypertensive target organ damage was associated with progression of global brain atrophy and more decline in memory performance, but not with progression of cerebral small-vessel disease or a decline in executive functioning. Apparently, global brain atrophy and cerebral small-vessel disease are not in the same pathophysiological pathway.

As cerebral perfusion was of interest in the etiology of structural brain changes over time in this thesis, we studied hemoglobin and hematocrit as two hemorheological variables, which are of influence on the regulation of cerebral blood flow (**chapter 6**). We showed that high hemoglobin and hematocrit, still within normal range, resulted in lower levels of baseline cerebral blood flow and a decline in cerebral blood flow over time in patients with manifest arterial disease. The actual differences in our study were rather small and the follow-up period may not be long enough to show an effect of hemoglobin and hematocrit on functional decline, but in our population of patients with manifest arterial disease it can be anticipated that the effect of hemoglobin and hematocrit is of importance as they are already more prone for functional decline.

The long-term effect of brain aging on cardiovascular morbidity and mortality in patients with manifest arterial disease was studied in **chapter 7**. We showed that smaller total brain volume and larger cerebrospinal fluid and ventricular volumes increased the risk of death by any cause and in particular by a vascular cause. For the risk of a cardiovascular event, smaller brain volumes increased the risk of ischemic stroke, but this association was explained by the presence of a history of stroke or a brain infarct on MRI. We observed no association between brain volumes and the risk of non-vascular death or an ischemic cardiac complication. It can therefore be suggested that brain atrophy on its own already is a sensitive marker of organ damage and is caused by a process that increases the risk of all-cause mortality in patients with manifest arterial disease.

In **chapter 8**, we discussed the main findings of this thesis in a broader context. We discussed the concept of early vascular aging, where early stiffening of central arteries, due to calcification of the medial layer of the arterial wall, is associated with increased risk of developing premature cardiovascular disease. Arterial stiffening and medial arterial calcifications may also involve the microcirculation, which could be related to tissue damage. We also discussed that traditional cardiovascular risk factors, such as hyperlipidemia and hypertension, do no longer have a large impact on longitudinal brain changes in patients with manifest arterial disease. There can be several explanations for this. First, they could play an important role in atherosclerosis, disease of the intima, and in acute events, but not in medial arterial calcifications and aging. Second, the lack of impact of traditional cardiovascular risk factors could be due to the fact that a single abnormal risk factor value does not inform about the duration of the action of the risk factor or about the sensitivity of the patient for this particular risk factor, while this information is possibly more important in chronic vascular damage than it is in acute vascular events. Therefore, we proposed organ damage as a better parameter of exposure to traditional risk factors and a better indicator of sensitivity of patients for these risk factors, which could offer a new possible target for treatment and prevention of longitudinal brain changes.

In the future, large population-based studies with a long follow-up period and multiple measurements of brain measures are needed, which should focus on organ damage associated with vascular aging more than on traditional risk factor assessment. Future studies should also focus on finding novel therapeutic targets for vascular aging, including arterial stiffening, as this is most likely a central process in the development of structural brain changes and associated cognitive decline.

NEDERLANDSE SAMENVATTING

Hart- en vaatziekten zijn een wereldwijd probleem voor de gezondheid. Hoewel de kans op sterfte als gevolg van hart- en vaatziekten in Europa de afgelopen jaren is afgenomen, baren de toegenomen aanwezigheid van hart- en vaatziekten en gerelateerde risicofactoren wel zorgen. Verbeterde behandeling heeft dan wel geresulteerd in minder sterfte, maar als gevolg daarvan zijn meer mensen chronisch ziek. Daardoor zijn er ook meer problemen in cognitief en lichamelijk functioneren op de lange termijn. Er is inmiddels steeds meer bewijs dat hart- en vaatziekten en gerelateerde risicofactoren een belangrijke rol spelen in het ontstaan van cognitieve achteruitgang en dementie. Structurele veranderingen van de hersenen zijn daarbij genoemd als mogelijke verklaring voor deze relatie tussen hart- en vaatziekten en functionele achteruitgang in de ouder wordende populatie.

Het doel van dit proefschrift was om meer inzicht te krijgen in veroudering van de hersenen en de oorzaken en gevolgen hiervan voor patiënten met vaatziekten. Hiervoor hebben we gebruik gemaakt van gegevens van de Second Manifestations of ARTerial disease-Magnetic Resonance studie, kortom de SMART-MR studie. In deze langlopende studie worden structurele veranderingen van de hersenen van patiënten met vaatziekten onderzocht met behulp van Magnetic Resonance Imaging (MRI) beelden. Tussen mei 2001 en december 2005 werden 1309 patiënten, die in verband met vaatziekten verwezen werden naar het Universitair Medisch Centrum Utrecht, uitgenodigd om mee te doen aan een uitgebreide screening. Hierbij werden de bloedvaten onderzocht met behulp van echografie, werd bloed- en urineonderzoek uitgevoerd en ondergingen de deelnemers een MRI van de hersenen. Ook werd informatie verzameld over de aanwezigheid van risicofactoren voor hart- en vaatziekten, de medische voorgeschiedenis en het medicijngebruik. Bij een deel van deze groep patiënten is dit complete onderzoek na ongeveer vier jaar herhaald. Tot slot werd de sterfte onder de deelnemers bijgehouden. Met deze uitgebreide informatie konden we meer te weten te komen over structurele veranderingen van de hersenen op MRI bij een grote groep patiënten met vaatziekten.

Als eerste hebben we onderzocht hoe hersenatrofie (afname in hersenvolume), vaatschade in de hersenen (witte stofafwijkingen en infarcten) en doorbloeding van de hersenen veranderden over tijd en hoe die aan elkaar gerelateerd waren. In **hoofdstuk 2** laten we zien dat totale hersenatrofie en atrofie van de grijze stof (als maat voor corticale atrofie) sneller verliep op hoge leeftijd en dat dit effect sterker was bij mannen dan bij vrouwen. We vonden ook dat bij jonge patiënten het volume van de witte stofafwijkingen afnam, terwijl dat juist bij oude patiënten toenam. Geslacht speelde hierbij geen rol. Bij oudere patiënten nam ook het aantal nieuwe herseninfarcten toe, bij mannen meer dan bij vrouwen. Verder hebben we bekeken hoe hersenatrofie en vaatschade in de hersenen zich ontwikkelden bij de verschillende groepen patiënten met vaatziekten. In vergelijking met patiënten met een hartziekte hadden patiënten na een beroerte of

een TIA meer corticale atrofie en meer toename van het volume van de hersenkamers (als maat voor subcorticale atrofie). Ook hadden zij een groter risico op een nieuw herseninfarct. Patiënten met vaatziekten in de benen lieten ook meer corticale atrofie zien dan patiënten met een hartziekte. Deze resultaten geven aan dat de onderliggende mechanismen die leiden tot hersenatrofie en vaatschade in de hersenen wellicht anders werken in de verschillende groepen patiënten met vaatziekten.

Om meer inzicht te krijgen in de relatie tussen hersenatrofie en vaatschade in de hersenen over tijd hebben we onderzocht of de doorbloeding van de hersenen gerelateerd was aan deze maten van veroudering van de hersenen. We vonden dat verminderde doorbloeding van de hersenen aan het begin van de studie na vier jaar leidde tot meer corticale atrofie, maar niet tot meer totale of subcorticale atrofie (**hoofdstuk 3**). Verminderde doorbloeding van de hersenen leidde ook niet tot meer vaatschade in de hersenen (**hoofdstuk 4**). Deze bevindingen suggereren dat vaatschade in de hersenen wordt veroorzaakt door andere processen dan verminderde doorbloeding van de hersenen, maar dat verminderde doorbloeding van de hersenen wel leidt tot corticale atrofie. We moeten echter wel toevoegen dat dit effect grotendeels bepaald werd door de patiënten met ernstige vernauwing van de halsslagaderen. Bij hen was het effect van verminderde doorbloeding van de hersenen op corticale atrofie namelijk veel sterker dan bij de andere patiënten. Echter ook in de groep patiënten zonder ernstig vernauwing van de halsslagaderen leidde verminderde doorbloeding van de hersenen tot corticale atrofie, echter wel in mindere mate.

In **hoofdstuk 4** zien we ook dat de aanwezigheid van witte stofafwijkingen aan het begin van de studie leidde tot meer afname van de doorbloeding van de hersenen over vier jaar tijd en dan vooral wanneer deze afwijkingen in de diepe structuren van de hersenen waren gelegen. Onze resultaten lieten geen relatie zien tussen verminderde doorbloeding van de hersenen aan het begin van de studie en toename van witte stofafwijkingen over tijd.

Samenvattend denken we dat de doorbloeding van de hersenen een onderdeel is in een kringloop van het ontstaan van structurele veranderingen van de hersenen: verminderde doorbloeding van de hersenen leidt tot hersenatrofie (**hoofdstuk 3**) – hersenatrofie is geassocieerd met meer vaatschade in de hersenen (resultaat van eerder SMART-onderzoek) – vaatschade in de hersenen leidt tot verslechtering van de doorbloeding van de hersenen (**hoofdstuk 4**) enzovoort. We weten alleen niet goed waar deze kringloop begint; is verminderde doorbloeding van de hersenen de oorzaak of het gevolg van hersenatrofie en vaatschade in de hersenen? Om deze vraag te kunnen beantwoorden is meer uitgebreid onderzoek nodig.

Het tweede doel van dit proefschrift was om meer inzicht te krijgen in de oorzaken en gevolgen van veroudering van de hersenen bij patiënten met vaatziekten. Hiervoor hebben we twee risicofactoren voor hersenveroudering onderzocht en hebben we naar de langetermijneffecten van hersenveroudering op het optreden van hart- en vaatziekten en sterfte gekeken. In **hoofdstuk 5** zien we hoe orgaanschade door hypertensie, als maat

voor chronische blootstelling aan een hoge bloeddruk, gerelateerd is aan structurele en functionele veranderingen van de hersenen na vier jaar follow-up. We vonden dat wanneer meerdere organen beschadigd waren door hypertensie, er sprake was van meer totale hersenatrofie en achteruitgang van het geheugen, maar dat er geen sprake was van meer vaatschade in de hersenen of achteruitgang van executief functioneren. Aangezien we al hadden gevonden dat doorbloeding van de hersenen belangrijk was voor structurele veranderingen van de hersenen over tijd, hebben we vervolgens twee factoren in het bloed onderzocht die van belang zijn voor de doorbloeding van de hersenen, namelijk hemoglobine en hematocriet (**hoofdstuk 6**). Hemoglobine is een eiwit in de rode bloedcellen dat verantwoordelijk is voor transport van zuurstof in het bloed. Hematocriet geeft aan hoeveel rode bloedcellen er in het bloed zitten en zegt daarmee iets over de 'vloeibaarheid' van het bloed. Uit ons onderzoek bleek dat hoge hemoglobine- en hematocrietwaarden, binnen de normale grenzen, gerelateerd waren aan verminderde doorbloeding van de hersenen aan het begin van de studie en meer achteruitgang van de doorbloeding van de hersenen na vier jaar follow-up. De verschillen in onze studie waren erg klein en de follow-up periode was wellicht niet lang genoeg voor concrete effecten van hemoglobine en hematocriet op functionele achteruitgang. Toch kunnen we ervanuit gaan dat het effect van hemoglobine en hematocriet bij onze groep van patiënten met vaatziekten van belang is, aangezien deze patiënten al gevoelig zijn voor functionele achteruitgang.

De langetermijneffecten van veroudering van de hersenen op hart- en vaatziekten en gerelateerde sterfte bij patiënten met vaatziekten beschrijven we in **hoofdstuk 7**. We vonden dat een klein totaal hersenvolume aan het begin van de studie gerelateerd was aan een verhoogd risico op algemene sterfte en zelfs specifiek op sterfte door een vasculaire oorzaak, zoals door een dodelijk hartinfarct. Een klein hersenvolume gaf ook een verhoogd risico op een herseninfarct, maar die relatie werd verklaard door het eerder doormaken van een herseninfarct. We zagen geen relatie tussen hersenvolume aan het begin van de studie en het risico op het doormaken van een hartaanval. Al deze resultaten waren onafhankelijk van de aanwezigheid van risicofactoren voor hart- en vaatziekten. We denken dat hersenatrofie een maat is voor chronische vaatschade en dat bij deze patiënten met een verhoogd risico op hart- en vaatziekten, hersenatrofie op zich al een verhoogd risico geeft op sterfte.

In **hoofdstuk 8** beschrijven we de belangrijkste bevindingen van dit proefschrift en plaatsen we deze in een breder kader. We hebben het concept 'vervroegde vaatveroudering' uitgelegd, waarbij op jonge leeftijd vaatverstijving van de grote bloedvaten optreedt. Dit kan tot gevolg hebben dat mensen ook op jonge leeftijd al een verhoogde kans hebben op hart- en vaatziekten. Deze vaatverstijving heeft wellicht ook effect op de kleine bloedvaten en kan dus leiden tot weefselschade van bijvoorbeeld de hersenen. Verder hebben we aandacht besteed aan het feit dat de klassieke risicofactoren voor hart- en vaatziekten, zoals hypertensie en verhoogd cholesterol, tegenwoordig geen grote rol meer spelen in structurele hersenveranderingen over tijd bij patiënten met vaatziekten.

Dit kan mogelijk verklaard worden doordat deze risicofactoren geen belangrijke rol spelen in de vaatverstijving en bij veroudering, maar meer van invloed zijn bij het acuut optreden van hart- en vaatziekten, zoals bij een hersenbloeding. Het kan echter ook zo zijn dat een eenmalige meting van een risicofactor te weinig informatie geeft over de duur van de blootstelling aan deze risicofactor of over de gevoeligheid van de patiënt voor deze risicofactor, terwijl juist deze informatie belangrijk is voor vaatschade op de lange termijn. We stelden daarom voor om orgaanschade als nieuwe maat voor risicoschatting van hart- en vaatziekten te gebruiken, omdat deze beter weergeeft hoe de blootstelling en gevoeligheid voor een bepaalde risicofactor is dan het meten van de risicofactor op zich. Mogelijk biedt dit nieuwe aangrijpingspunten voor toekomstig behandelen en voorkomen van hersenveranderingen over tijd bij patiënten met vaatziekten.

De belangrijkste bevindingen van dit proefschrift waren dat 1) leeftijd en geslacht een consistente rol spelen in structurele hersenveranderingen over tijd, 2) doorbloeding van de hersenen een rol speelt in structurele hersenveranderingen over tijd, maar het is niet duidelijk of het de oorzaak is of juist het gevolg van de veranderingen, 3) de klassieke risicofactoren voor hart- en vaatziekten, zoals hypertensie en verhoogd cholesterol, bij patiënten met vaatziekten geen grote invloed hebben op structurele hersenveranderingen, 4) maten die de duur van blootstelling of de gevoeligheid voor klassieke risicofactoren aangeven sterke voorspellers zijn voor structurele en functionele hersenveranderingen, en 5) een klein hersenvolume het risico verhoogt op sterfte bij patiënten met vaatziekten.

In de toekomst is het belangrijk dat grote studies worden opgezet die gebaseerd zijn op de algemene populatie die voor langere tijd gevolgd kan worden met meerdere metingen van bijvoorbeeld hersenveranderingen met behulp van MRI. Ook is het belangrijk dat dan niet alleen naar de klassieke risicofactoren voor hart- en vaatziekten wordt gekeken, maar juist meer naar het gevolg van deze risicofactoren, zoals orgaanschade, omdat zij een betere voorspeller kunnen zijn voor functionele achteruitgang en een potentieel doel kunnen zijn voor behandeling en preventie.

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CURRICULUM VITAE

Pieternella Hendrika (Erika) van der Veen was born on the 3rd of April 1984 in Leiderdorp, the Netherlands. After finishing secondary school at the Bonaventuracollege in Leiden in 2002, she studied Biomedical Sciences at the Leiden University, where she obtained her bachelor degree in 2005. In combination with studying for her master degree in Biomedical Sciences, she started her medical training, also at the Leiden University in 2005. After obtaining both her Master of Science in Biomedical Sciences and her medical degree in 2010, she worked as a resident in Surgery at the Hagaziekenhuis in The Hague. In September 2011, she started the work described in this thesis at the Julius Center for Health Sciences and Primary Care and the department of Radiology at the University Medical Center Utrecht, under supervision of prof. dr. Y. van der Graaf, prof. dr. W.P.T.M. Mali, dr. M.I. Geerlings, and dr. M. Muller. In September 2014, she started her residency in Radiology at the University Medical Center Utrecht, under supervision of dr. R.A.J. Nievelstein. Erika van der Veen is married to Peter Batenburg.

APPENDIX

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