Population Imaging of Cerebral Small Vessel Disease

Mariëlle M.F. Poels
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ACKNOWLEDGEMENTS

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Population Imaging of Cerebral Small Vessel Disease

Beeldvorming van cerebrale microangiopathie in de algemene bevolking

Proefschrift
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en volgens besluit van het College van Promoties.

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door

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geboren te Venray
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Chapter 1

General Introduction
As we grow older, our brain tends to shrink. We become increasingly vulnerable to dementia and stroke, two common disorders in the elderly that have great impact on brain functioning and the way people live their lives. The burden of these diseases will rapidly grow over the coming years as a result of aging populations worldwide.\(^1\)\(^-\)\(^2\) Currently, dementia (11.2\%) and stroke (9.5\%) already contribute substantially to the number of years lived with disability of people aged 60 and older; this is considerably more than the years of disability caused by cardiovascular disease (5.0\%) or by all forms of cancer (2.4\%) together.\(^3\) Since few therapeutic possibilities exist for these neurological diseases in the elderly, effective prevention strategies are urgently needed.

In order to develop successful approaches to prevent dementia and stroke, it is crucial to explore the early presymptomatic phases of these diseases. Markers that enable us to detect disease in an early stage before the clinical syndromes of dementia and stroke become apparent are strongly needed to gain more insight in the causes of dementia and stroke, to identify persons who will develop these diseases, and to provide opportunities to alter or stop the disease process. Magnetic resonance imaging (MRI) has proven to be a very suitable technique for the investigation of these presymptomatic phases as it offers detailed information about presymptomatic pathology in the brain without exposure to radiation.\(^4\)

There is a growing body of evidence illustrating that dementia and stroke are not two entirely distinctive entities. These diseases share many risk factors; they commonly co-exist; and they may have synergistic or additive effects on brain function.\(^5\) However, exactly how these vascular and neurodegenerative pathologies are linked remains a critical question in understanding the mechanisms underlying both diseases. Cerebral small vessel disease – i.e., pathologic alterations in the small penetrating arteries and arterioles of the brain – occupies an important position within the spectrum of presymptomatic brain pathology as it has been associated with both dementia and stroke.\(^6\)\(^-\)\(^8\)

Lacunar infarcts and white matter lesions are widely recognized imaging markers of cerebral small vessel disease that have been extensively studied with MRI since the early 1990s.\(^9\) In more recent years, advances in MRI technology have led to the discovery of a new imaging marker of cerebral small vessel disease: cerebral micro-
bleeds, small foci of chronic blood products in normal (or near normal) brain tissue.\textsuperscript{10} Like lacunar infarcts and white matter lesions, cerebral microbleeds are frequently found on brain scans of elderly people, and they share common cardiovascular risk factors.\textsuperscript{11-13} Even more so than other markers of small vessel disease, cerebral microbleeds have the potential to represent the missing link between vascular and neurodegenerative pathologies as they are considered to reflect a combination of vascular damage and the pathology of Alzheimer’s disease.\textsuperscript{14} Exploring the determinants, distribution and clinical correlates of cerebral microbleeds may therefore contribute to a better understanding of the pathophysiological processes underlying cerebral small vessel disease and the ways through which these are linked to dementia and stroke.

Classic cardiovascular risk factors have been extensively studied in relation to established markers of cerebral small vessel disease, but do not fully explain its presence.\textsuperscript{9} Further progress in the understanding of the pathophysiology underlying cerebral small vessel disease may therefore also be made by studying potential new determinants of established markers of small vessel disease, and by defining how these markers are associated with clinical outcomes. Several markers of vascular pathophysiology including arterial stiffness and reduced cerebral blood flow have been proposed for further research.

The main objective of this thesis is to provide new insights into the pathophysiology of small vessel disease in the aging brain. This is accomplished by (i) examining cerebral microbleeds as new imaging marker of small vessel disease; (ii) identifying new vascular risk factors for cerebral small vessel disease; and (iii) exploring how these vascular risk factors and imaging markers of cerebral small vessel disease relate to clinical outcomes. All studies described in this thesis are conducted as part of the Rotterdam Scan Study, a large-scale prospective population-based imaging study that aims to investigate causes and consequences of age-related brain changes.\textsuperscript{15}

Chapter 2 focuses on cerebral microbleeds as new imaging marker of cerebral small vessel disease. A previous study from the Rotterdam Scan Study reported on the high prevalence of cerebral microbleeds in 1062 community-dwelling persons aged 60 years and older.\textsuperscript{13} The number of participants in the Rotterdam Scan Study has since almost quadrupled, and the study now also includes persons aged 45 to 60 years old. In paragraph 2.1, results are presented on the prevalence and determinants of
cerebral microbleeds in this larger and younger cohort from the general population. The distribution of microbleeds across the different lobes of the brain may provide additional insights in the presumed association of lobar microbleeds with underlying vasculopathy: This lobar distribution is described in paragraph 2.2. Microbleed detection is heavily dependent on MRI parameters, magnetic field strength and post-processing algorithms.\textsuperscript{16-18} However, it has been debated whether improved microbleed detection also leads to clinically relevant data. Whether participants who only depict microbleeds on a high-resolution MRI sequence differ with respect to risk profile and risk of new microbleeds from participants who depict microbleeds also on a conventional MRI sequence is examined in paragraph 2.3. Over recent years, serial MRI data on the presence of microbleeds have become available. Paragraph 2.4 addresses at what rate microbleeds occur with aging and whether microbleeds, once present, can disappear over time. Low serum total cholesterol levels are associated with an increased risk of symptomatic intracerebral hemorrhage and presence of cerebral microbleeds.\textsuperscript{13,19-20} The relative contribution of lipid fractions to these associations is unclear and requires further investigation. In paragraph 2.5, results are presented on the association of serum HDL-cholesterol, LDL-cholesterol and triglycerides with risk of intracerebral hemorrhage or presence of cerebral microbleeds. Vascular pathology plays a prominent role in impaired cognitive function.\textsuperscript{21-22} Cerebral microbleeds may reflect underlying vascular disease,\textsuperscript{13} but their role in cognitive function is largely unknown. This chapter concludes with a study on the association between cerebral microbleeds and cognitive function in paragraph 2.6.

Chapter 3 is dedicated to new vascular risk factors for cerebral small vessel disease and to how these vascular risk factors and imaging markers of cerebral small vessel disease are related to clinical outcomes. Arterial stiffness – a relatively novel marker of cardiovascular damage – is associated with an excess risk of stroke in the general population.\textsuperscript{23} Mechanisms for a possible association between arterial stiffness and stroke involve pathways that include cerebral small vessel disease. The relation of arterial stiffness with cerebral small vessel disease is discussed in paragraph 3.1. As vascular factors and stroke have been associated with cognitive function and dementia,\textsuperscript{21} an association between increased arterial stiffness and cognitive decline or dementia has been suggested. This association is explored in paragraph 3.2. Another pathway through which underlying vascular mechanisms may contribute to cognitive impairment is hypoperfusion of the brain.\textsuperscript{24} Paragraph 3.3 discusses how
cerebral blood flow relates to cognitive function and whether this association is independent of brain volume. Cerebral small vessel disease may be considered an intermediate phenotype between vascular risk factors on the one hand and cerebral end-organ damage and clinical outcomes on the other hand. Therefore, the assessment of cerebral small vessel disease may be relevant in predicting individual stroke risk. Whether the assessment of cerebral small vessel disease on brain MRI can improve the prediction of stroke beyond the prediction based on the classic stroke risk factors from the Framingham Stroke Risk Function is investigated in paragraph 3.4.

An unintended but inevitable consequence of the high resolution, state-of-the-art MRI techniques used in our study combined with the large number of scans acquired, is the detection of unexpected asymptomatic brain abnormalities unrelated to the purpose of our study. In chapter 4, the prevalence, determinants and clinical course of these incidental findings are assessed in the population under study.

This thesis concludes in chapter 5 with a review of the main findings in the context of current knowledge, the discussion of relevant methodological aspects, and suggestions for future research.
REFERENCES


Chapter 2

Cerebral Microbleeds
Prevalence and determinants of cerebral microbleeds: An update of the Rotterdam Scan Study

Stroke, 2010;41:S103-106

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ABSTRACT

Background – We previously reported on the high prevalence of cerebral microbleeds (CMBs) in community-dwelling people aged 60 years and older. Moreover, we found that their spatial distribution likely reflects differences in underlying etiology. We have since almost quadrupled the number of participants in our study and expanded it to include persons of 45 years and older. We examined the prevalence and determinants of microbleeds in this larger and younger cohort from the general population.

Methods – In 3979 persons (mean age, 60.3 years), we performed brain MRI at 1.5T, including a sequence optimized for visualization of CMBs. Associations between APOE genotype, cardiovascular risk factors, and markers of cerebrovascular disease with the presence and location of CMBs were assessed by multiple logistic regression adjusted for age, sex, and relevant confounders.

Results – Microbleed prevalence gradually increased with age, from 6.5% in persons aged 45 to 50 years to 35.7% in participants of 80 years and older. Overall, 15.3% of all subjects had at least 1 CMB. Cardiovascular risk factors and presence of lacunar infarcts and white matter lesions were associated with microbleeds in a deep or infratentorial region, whereas APOE ε4 and diastolic blood pressure were related to microbleeds in a strictly lobar location.

Conclusions – Findings in this larger population are in line with our previous results and, more importantly, extend these to a younger age group. CMBs are already present at middle age, and prevalence rises strongly with increasing age. We confirmed that determinants of the presence of cerebral microbleeds differ according to their location in the brain.
INTRODUCTION

Cerebral microbleeds (CMBs) can be imaged using MRI and are commonly found in patients admitted with stroke, as well as the general elderly population.\(^1\)\(^-\)\(^3\)

We have previously shown in a population of 1062 elderly (aged 60 years and older) from the Rotterdam Scan Study that microbleeds in deep or infratentorial regions were associated with known risk factors for hypertensive vasculopathy, whereas lobar microbleeds, rather, seemed indicative of underlying cerebral amyloid angiopathy.\(^3\) These findings are in line with the specific underlying vascular pathological changes that have been found in symptomatic intracerebral hemorrhage (ICH) and suggest a parallel between (asymptomatic) CMBs and symptomatic ICH.\(^4\)\(^-\)\(^5\) However, the clinical diagnosis of symptomatic ICH does not accurately reflect the actual disease processes of cerebral amyloid angiopathy and hypertensive vasculopathy, which may have begun several years before.\(^6\) CMBs may therefore be an early imaging biomarker of bleeding-prone vasculopathy in asymptomatic people. Because amyloid angiopathy, as well as hypertensive vasculopathy, is thought to accumulate progressively over time, CMBs are likely to be present already in the middle-aged.\(^6\)\(^-\)\(^7\) However, few data exist regarding the population prevalence of microbleeds in people younger than 60 years of age.\(^8\)\(^-\)\(^9\)

Recently, we expanded the Rotterdam Scan Study with persons of 45 years and older and have now a population-based cohort with information on CMBs of almost 4000 participants. This enabled us to evaluate whether we could corroborate and extend our previous findings regarding the prevalence and clinical correlates of microbleeds in the general population in a larger cohort and over a wider age range.

METHODS

Participants

The study is based on the Rotterdam Scan Study, an ongoing population-based cohort study investigating age-related brain changes on MRI. We previously reported on the first 1375 invited participants.\(^3\) We have since extended our cohort with participants of 45 years of age or older, and we currently have invited a total of 4898 parti-
We excluded individuals who were demented (N=30) or had MRI contraindications (N=389). Of 4479 eligible persons, 4082 (91%) participated. Because of physical inabilities, imaging could not be performed in 44 individuals. Of 4038 complete MRI examinations, 59 scans had to be excluded because of motion artifacts or susceptibility artifacts, leaving 3979 scans to be analyzed.

**Brain MRI**

We performed a multisequence MRI protocol on a 1.5-T scanner (GE Healthcare). A custom-made accelerated 3D T2*-weighted gradient-recalled echo (3D T2* GRE) sequence with high spatial resolution and long echo time was used for microbleed detection. The other sequences in the imaging protocol consisted of 3 high-resolution axial scans (i.e., a T1-weighted sequence, a proton density-weighted sequence, and a fluid-attenuated inversion recovery [FLAIR] sequence).

**Rating of Cerebral Microbleeds**

All 3D T2* GRE scans were reviewed by 1 of 5 trained raters (all with more than 1 year experience in microbleed rating) who recorded the presence, number, and location of cerebral microbleeds. CMBs were categorized into 1 of 3 locations: lobar (cortical gray and subcortical or periventricular white matter), deep (deep gray matter: basal ganglia and thalamus; and the white matter of the corpus callosum, internal, external, and extreme capsule), and infratentorial (brain stem and cerebellum).

**Cerebrovascular Disease on MRI**

Lacunar and cortical infarcts were rated on FLAIR, proton density-weighted, and T1-weighted sequences by the same raters who had scored cerebral microbleeds according to criteria described previously. White matter lesion volume (milliliters) was quantified with a validated fully automated tissue classification technique and was calculated by summing all voxels of the white matter lesion-class across the whole brain.

**APOE Genotyping**

APOE genotyping was performed on coded genomic DNA samples and was available in 3689 participants (93%). The distributions of APOE genotype and allele frequencies in this population were in Hardy-Weinberg equilibrium.
Cardiovascular Risk Factors

Cardiovascular risk factors were examined by interview and laboratory and physical examination as described previously. Risk factors included in our analyses were systolic and diastolic blood pressure, pulse pressure, hypertension (categorized into mild and severe according to World Health Organization criteria), smoking, diabetes mellitus, serum total cholesterol, and high-density lipoprotein. The use of lipid-lowering drugs and blood pressure-lowering medication was assessed by interview and house visits during which medication use was registered.

Data Analysis

We calculated the prevalence of cerebral microbleeds in 10-year age strata. We made separate categories for “strictly lobar microbleeds” (persons who had ≥1 microbleeds restricted to a lobar location) and “deep or infratentorial microbleeds” (persons with ≥1 microbleeds in a deep or infratentorial location with or without lobar microbleeds).

We assessed the relationship between prevalence of microbleeds and APOE allele status and cardiovascular risk factors with multiple logistic regressions. To examine whether cerebral microbleeds were more frequent in persons with brain infarcts or white matter lesions, we used multiple logistic regression models and adjusted for age and sex and additionally for cardiovascular risk factors. White matter lesion volume was natural log-transformed because of skewness of the untransformed measure. We computed interaction terms to see whether the effects varied with age. Finally, we excluded persons with cortical infarcts on MRI and repeated all analyses.

RESULTS

Table 1 shows the characteristics of the study population. Mean age was 60.3 years, and 2164 (54.4%) were women. A total of 609 of 3979 (15.3%) had 1 or more microbleeds on MRI. Of these, 214 (5.4%) had multiple microbleeds. Increasing age was associated with a higher prevalence of microbleeds, as well as presence of multiple microbleeds. In the age category 45 to 50 years, 6.5% had at least 1 microbleed, whereas this proportion was 35.7% in the participants ≥80 years of age (Table 2). There was no significant difference in microbleed prevalence between men and women in all age categories.
Strictly lobar microbleeds were significantly more often present in carriers of the APOE ε4 allele compared with carriers of the ε3/ε3 genotype (Table 3). This association appeared even stronger in persons with multiple strictly lobar microbleeds (age-adjusted odds ratio, 2.06; 95% confidence interval, 1.34 to 3.17). When we analyzed persons with deep or infratentorial microbleeds, excluding those with additional lobar microbleeds, the association with APOE ε4 strongly attenuated (odds ratio, 0.72; 95% confidence interval, 0.35 to 1.48). No associations were found between APOE ε2 allele carriers and presence of microbleeds in any location. However, we did confirm the previously found association between the ε2/ε2 genotype and strictly lobar microbleeds.

Table 4 shows the effect of cardiovascular determinants on the presence of microbleeds. Systolic blood pressure, pulse pressure, (severe) hypertension, and smoking were all related to presence of deep or infratentorial bleeds, whereas diastolic blood pressure was related to lobar microbleeds. In contrast with our previous results, we did not find an association of serum cholesterol level with the presence of strictly lobar microbleeds.

Lacunar infarcts were strongly associated with the presence of deep or infratentorial microbleeds but not with microbleeds in a lobar region. White matter lesion volume was associated with microbleeds in any location but strongest with those in a deep or infratentorial region. Presence of cortical infarcts was not related to microbleeds (Table 5). These results did not change after additional adjustment for cardiovascular risk factors (data not shown).

When we evaluated whether the effects varied with age, we found no significant interactions. All analyses were also performed after exclusion of participants with cortical infarcts on MRI (N=111). This did not alter any of the associations described above.
### Table 1. Characteristics of the study population (N=3979)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years, mean ± SD</td>
<td>60.3 ± 8.7</td>
</tr>
<tr>
<td>Women, N (%)</td>
<td>2164 (54.4)</td>
</tr>
<tr>
<td>Systolic blood pressure, mean ± SD</td>
<td>135.3 ± 19.5</td>
</tr>
<tr>
<td>Diastolic blood pressure, mean ± SD</td>
<td>81.7 ± 10.8</td>
</tr>
<tr>
<td>Mild hypertension, N (%)</td>
<td>1658 (42.0)</td>
</tr>
<tr>
<td>Severe hypertension, N (%)</td>
<td>516 (13.1)</td>
</tr>
<tr>
<td>Smoking (ever), N (%)</td>
<td>2795 (70.6)</td>
</tr>
<tr>
<td>Diabetes mellitus, N (%)</td>
<td>317 (8.1)</td>
</tr>
<tr>
<td>Serum total cholesterol (mmol/L), mean ± SD</td>
<td>5.60 ± 1.04</td>
</tr>
<tr>
<td>Serum HDL-cholesterol (mmol/L), mean ± SD</td>
<td>1.43 ± 0.42</td>
</tr>
<tr>
<td>APOE ε2 allele carrier, N (%)</td>
<td>548 (13.8)</td>
</tr>
<tr>
<td>APOE ε4 allele carrier, N (%)</td>
<td>1078 (27.1)</td>
</tr>
<tr>
<td>Cortical infarct on MRI, N (%)</td>
<td>111 (2.8)</td>
</tr>
<tr>
<td>Lacunar infarct on MRI, N (%)</td>
<td>213 (5.4)</td>
</tr>
<tr>
<td>Subcortical infarct on MRI, N (%)</td>
<td>4 (0.1)</td>
</tr>
<tr>
<td>White matter lesions on MRI (mL), median</td>
<td>2.5 (1.5-4.5)</td>
</tr>
</tbody>
</table>

Data are missing for blood pressure (N=20), hypertension (N=33), smoking (N=22), diabetes (N=81), serum cholesterol (N=53), APOE genotype (N=290), white matter lesions (N=45).

### Table 2. Age-specific prevalence of cerebral microbleeds (10-year strata)

<table>
<thead>
<tr>
<th>Age-range</th>
<th>No. of persons</th>
<th>Cerebral microbleeds</th>
<th>Multiple cerebral microbleeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>45-50 y</td>
<td>413</td>
<td>27 (6.5)</td>
<td>3 (0.7)</td>
</tr>
<tr>
<td>50-59 y</td>
<td>1696</td>
<td>195 (11.5)</td>
<td>57 (3.4)</td>
</tr>
<tr>
<td>60-69 y</td>
<td>1350</td>
<td>227 (16.8)</td>
<td>66 (4.9)</td>
</tr>
<tr>
<td>70-79 y</td>
<td>377</td>
<td>109 (28.9)</td>
<td>56 (14.9)</td>
</tr>
<tr>
<td>&gt;80 y</td>
<td>143</td>
<td>51 (35.7)</td>
<td>32 (22.4)</td>
</tr>
<tr>
<td>Total</td>
<td>3979</td>
<td>609 (15.3)</td>
<td>214 (5.4)</td>
</tr>
</tbody>
</table>

### Table 3. APOE allele status and the presence of cerebral microbleeds

<table>
<thead>
<tr>
<th></th>
<th>All microbleeds (N=609)</th>
<th>Strictly lobar microbleeds (N=413)</th>
<th>Deep or infratentorial microbleeds ‡ (N=196)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, per year*</td>
<td>1.06 (1.05-1.07)</td>
<td>1.05 (1.04-1.06)</td>
<td>1.08 (1.06-1.09)</td>
</tr>
<tr>
<td>Women, versus men†</td>
<td>0.99 (0.80-1.13)</td>
<td>0.97 (0.79-1.19)</td>
<td>0.90 (0.67-1.20)</td>
</tr>
<tr>
<td>APOE ε4, versus ε3/ε3‡</td>
<td>1.35 (1.10-1.65)</td>
<td>1.37 (1.08-1.74)</td>
<td>1.37 (0.97-1.93)</td>
</tr>
<tr>
<td>APOE ε2, versus ε3/ε3‡</td>
<td>1.05 (0.80-1.38)</td>
<td>1.07 (0.78-1.46)</td>
<td>1.02 (0.64-1.61)</td>
</tr>
<tr>
<td>APOE ε4/ε4, versus ε3/ε3‡</td>
<td>1.32 (0.76-2.31)</td>
<td>1.43 (0.76-2.69)</td>
<td>1.11 (0.40-3.12)</td>
</tr>
<tr>
<td>APOE ε2/ε2, versus ε3/ε3‡</td>
<td>2.44 (0.91-6.57)</td>
<td>3.09 (1.08-8.79)</td>
<td>1.28 (0.16-10.31)</td>
</tr>
</tbody>
</table>

* Adjusted for sex.
† Adjusted for age.
‡ With or without lobar microbleeds.
### Table 4. Cardiovascular determinants and the presence of cerebral microbleeds

<table>
<thead>
<tr>
<th></th>
<th>All microbleeds (N=609)</th>
<th>Strictly lobar microbleeds (N=413)</th>
<th>Deep or infratentorial microbleeds† (N=196)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Systolic BP</strong> per SD increase</td>
<td>1.13 (1.03-1.24)</td>
<td>1.11 (0.99-1.24)</td>
<td>1.17 (1.01-1.36)</td>
</tr>
<tr>
<td><strong>Diastolic BP</strong> per SD increase</td>
<td>1.10 (1.01-1.21)</td>
<td>1.14 (1.02-1.27)</td>
<td>1.05 (0.90-1.22)</td>
</tr>
<tr>
<td><strong>Pulse pressure</strong> per SD increase</td>
<td>1.09 (0.99-1.20)</td>
<td>1.04 (0.93-1.16)</td>
<td>1.19 (1.02-1.38)</td>
</tr>
<tr>
<td><strong>Hypertension,</strong> Mild, versus none</td>
<td>1.14 (0.93-1.39)</td>
<td>1.03 (0.81-1.30)</td>
<td>1.45 (1.03-2.06)</td>
</tr>
<tr>
<td><strong>Smoking,</strong> ever versus never</td>
<td>1.22 (1.00-1.50)</td>
<td>1.11 (0.87-1.40)</td>
<td>1.57 (1.09-2.27)</td>
</tr>
<tr>
<td><strong>Diabetes,</strong> yes versus no</td>
<td>0.98 (0.72-1.35)</td>
<td>0.89 (0.60-1.30)</td>
<td>1.18 (0.73-1.91)</td>
</tr>
<tr>
<td>Serum total cholesterol† per SD increase</td>
<td>0.97 (0.89-1.07)</td>
<td>1.00 (0.89-1.11)</td>
<td>0.93 (0.79-1.09)</td>
</tr>
<tr>
<td>Serum HDL-cholesterol† per SD increase</td>
<td>0.96 (0.87-1.06)</td>
<td>0.93 (0.82-1.04)</td>
<td>1.04 (0.88-1.22)</td>
</tr>
</tbody>
</table>

* All values are age and sex-adjusted.
† Additionally adjusted for the use of blood pressure-lowering medication.
‡ With or without lobar microbleeds.
BP: blood pressure, HDL: high-density lipoprotein.

### Table 5. Cerebral vascular disease and the presence of cerebral microbleeds

<table>
<thead>
<tr>
<th></th>
<th>All microbleeds (N=609)</th>
<th>Strictly lobar microbleeds (N=413)</th>
<th>Deep or infratentorial microbleeds† (N=196)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cortical infarcts,</strong> versus no infarct</td>
<td>1.06 (0.65-1.75)</td>
<td>1.02 (0.58-1.82)</td>
<td>1.17 (0.52-2.63)</td>
</tr>
<tr>
<td><strong>Lacunar infarcts,</strong> versus no infarct</td>
<td>2.37 (1.70-3.30)</td>
<td>1.20 (0.75-1.93)</td>
<td>5.16 (3.41-7.80)</td>
</tr>
<tr>
<td><strong>White matter lesion volume</strong>, per SD increase</td>
<td>1.36 (1.23-1.50)</td>
<td>1.17 (1.03-1.31)</td>
<td>1.83 (1.57-2.14)</td>
</tr>
</tbody>
</table>

* All values are age and sex-adjusted.
† Natural log-transformed.
‡ With or without lobar microbleeds.
DISCUSSION

In our population-based study, we found that microbleed prevalence gradually increased with age, from 6.5% in the age category of 45 to 50 years old to 35.7% in participants of 80 years and older. Furthermore, we confirmed our previous findings that determinants of the presence of cerebral microbleeds differed according to their location in the brain.

Strengths of our study are its population-based setting, the high response rate, and large sample size. Moreover, an important strength is our wide age range, which enabled us to investigate the presence and determinants of microbleeds not only in the general elderly population but also in the middle-aged population. Furthermore, we used a custom-made accelerated 3D T2*GRE sequence that has shown to have a higher sensitivity in detecting cerebral microbleeds when compared with conventional 2D T2*GRE sequences.\(^{11}\)

Several other studies have reported on the overall prevalence of microbleeds in the general population.\(^{2,3,8,9,14}\) Reported frequencies of microbleeds, however, varied largely among studies (3.1% to 23.5%). One explanation for the differences in reported prevalence is the difference in mean age between the studies (mean age, 53 to 76 years).\(^{2,3,8,9,14}\) Comparisons between studies are further hampered by the differences in MRI scanning protocols. As mentioned previously, especially the MRI sequence used is of major importance for the detection rate of microbleeds.\(^{15}\)

Consistent with the Age, Gene/Environment Susceptibility (AGES) Reykjavik Study,\(^{2}\) but not with the Framingham study,\(^{14}\) we again report a significant overrepresentation of APOE \(\varepsilon4\) carriers among people with presence of microbleeds. Previously, it was thought that this may be explained by the differences in mean age (AGES, Framingham; mean age of 76 and 64 years, respectively).\(^{15}\) However, we consistently find the association between APOE \(\varepsilon4\) genotype and lobar CMBs even in this much younger cohort with an average age of 60 years.

Furthermore, we robustly confirmed the association between cardiovascular risk factors, i.e., systolic blood pressure, hypertension, smoking, and microbleeds in a deep or infratentorial region. This is in contrast to results from the AGES\(^2\) and Framingham
study,\textsuperscript{14} which did not find clear associations of cardiovascular factors with CMBs, but is in line with data from most other studies.\textsuperscript{8-9} Again, a possible explanation for the discrepancy may be heterogeneity in study populations and MRI scanning protocols.

CMBs are thought to represent the asymptomatic counterpart of ICH, and it is hypothesized that they may precede symptomatic ICH.\textsuperscript{16} If indeed true, our results suggest that the vascular changes (either hypertensive or amyloid) leading to a symptomatic ICH are progressive in aging and are already present during midlife. This is in line with knowledge that cerebral amyloid angiopathy accumulates progressively over each decade of life.\textsuperscript{6} Furthermore, hypertension is known by its long, clinically asymptomatic period, in which arteriolosclerosis of small, deep-penetrating arteries can already be proven in autopsy tissue.\textsuperscript{7} Prevention strategies for both hypertensive and amyloid angiopathy should thus start early in life and may be aided by non-invasive imaging biomarkers that indicate early disease, such as CMBs.

In conclusion, our study shows that prevalence of microbleeds gradually increases with age and that CMBs are also present in the middle-aged population. Furthermore, the study confirms that microbleed location may relate to specific underlying vascular pathological changes.
REFERENCES


2.2

Lobar distribution of cerebral microbleeds

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ABSTRACT

Objective – To investigate the distribution of lobar microbleeds over the different lobes, taking into account lobar volume and clustering effects of multiple microbleeds.

Design – Population-based, cross-sectional analysis.

Setting – The Rotterdam Scan Study.

Participants – A total of 198 persons (age range, 61-95 years) with lobar microbleeds.

Main Outcome Measures – Distribution of microbleeds over different lobes.

Results – We found that lobar cerebral microbleeds occurred significantly more often in the temporal lobe, a region known to be more affected in cerebral amyloid angiopathy.

Conclusion – This study corroborates the presumed association of lobar microbleeds with cerebral amyloid angiopathy.
INTRODUCTION

Cerebral microbleeds (CMBs) can be detected with T2*-weighted gradient-echo magnetic resonance imaging (MRI) and are associated with presence and risk of intracerebral hemorrhage. Previous articles found that microbleeds in deep or infratentorial regions were associated with hypertension, whereas lobar microbleeds share risk factors with cerebral amyloid angiopathy (CAA). Little is known, however, about the spatial distribution of these lobar microbleeds. An imaging study of patients with CAA showed that microbleeds as well as intracerebral hemorrhage occurred more often in the temporal and occipital lobes, which fits autopsy studies describing a posterior predilection of vascular pathology in CAA.

Knowledge of the spatial distribution of lobar CMBs in the general population might contribute to our understanding of their pathophysiology and may corroborate the presumed association of lobar microbleeds with CAA.

To date, only one population-based study of elderly persons examined spatial distribution of lobar bleeds and found that microbleeds occur more often in the parietal lobe. However, merely counting the number of microbleeds per lobe might give a distorted interpretation because volumetric differences between lobes are not taken into account. Furthermore, in persons with multiple microbleeds, consecutive microbleeds tend to occur in proximity of a preceding bleed.

Therefore, in the population-based Rotterdam Scan Study, we investigated the spatial distribution of lobar microbleeds, taking into account the volume of the separate lobes and clustering effects of multiple microbleeds.

METHODS

Setting
This study is based on the Rotterdam Scan Study. We previously described the prevalence and risk factors of cerebral microbleeds in a population of 1062 persons without dementia. Of these, 250 had, in total, 1151 microbleeds. Microbleeds that were located in the deep or infratentorial brain region were discarded from our
present analysis, as we aimed to investigate the spatial distribution of lobar micro-
bleeds, leaving 838 lobar microbleeds in 198 persons for the analyses.

**Brain MRI**
We performed a multisequence MRI protocol on a 1.5-T scanner (GE Healthcare,
Milwaukee, Wisconsin). A custom-made, accelerated, 3-dimensional, T2*-weighted,
gradient-recalled echo sequence with high spatial resolution and long echo time was
used for microbleed detection. The other sequences in the imaging protocol consisted
of three high-resolution axial scans, i.e., a T1-weighted sequence, a proton density-
weighted sequence, and a fluid-attenuated inversion recovery sequence. Slice position
of the T1- and T2*-weighted gradient-recalled echo scans was matched.

**Rating of Cerebral Microbleeds**
Microbleeds were defined as focal areas of very low signal intensity. All scans
were reviewed by 1 of 2 trained raters, as described previously. In brief, they recorded
the presence, number, and slice location of all microbleeds. Intraobserver and inter-
observer agreement was good, with $\kappa$ values of 0.87 and 0.85 respectively. Subsequently,
all microbleeds were manually labeled by a single trained rater using the Montreal Neuro-
logical Institute tool Display.

**Assessment of Lobar Distribution of Microbleeds**
For assessment of lobar distribution of microbleeds, we first created a template
scan in which the lobes were labeled according to a slightly modified version of the
segmentation protocol as described by Bodke et al into left and right frontal, parietal,
temporal, and occipital lobes. Subsequently, we used validated non-rigid registration
to map this template to each scan, in which microbleeds were manually labeled. By
combining this lobar segmentation with the labeled microbleeds, we obtained the
microbleed distribution per lobe.

**Statistical Analysis**
We analyzed the distribution of lobar microbleeds in 4 groups: (1) participants
with lobar CMBs (with or without microbleeds located in a deep or infratentorial brain
region); (2) participants with multiple lobar CMBs (>1 lobar microbleeds with or
without microbleeds located in a deep or infratentorial brain region); (3) participants
with strictly lobar CMBs (without microbleeds located in a deep or infratentorial brain
region); and (4) multiple, strictly lobar CMBs (>1 lobar microbleeds without CMBs located in a deep or infratentorial brain region). These groups meet with varying degrees the criteria for the presumed underlying CAA pathology. 

Using the null hypothesis, the distribution of CMBs across the lobes would be the same as the volume percentages of each lobe based on the template scan. To test whether CMBs were equally distributed throughout the brain, we used the $\chi^2$ test. The binomial test was used to examine per lobe whether the microbleeds that occurred in each lobe were in proportion to the mean volume of that specific lobe. We accounted for clustering effects by adding random effects for within subject variation.

**RESULTS**

Table 1 presents the characteristics of the study population. The mean age was 72.5 years, and 96 (48.5%) were women. The Figure illustrates the spatial distribution of all lobar microbleeds. The CMBs were not uniformly distributed throughout the brain ($P=0.04$). Table 2 shows the distribution of lobar microbleeds in all participants ($N=198$) and in participants with multiple lobar ($N=81$), strictly lobar ($N=134$), and multiple strictly lobar microbleeds ($N=35$). Most microbleeds were located in the temporal lobe, i.e., 32.6% in participants with lobar microbleeds, 32.9% in participants with multiple lobar microbleeds, 29.9% in participants with strictly lobar microbleeds, and 31.4% in participants with multiple strictly lobar microbleeds (after correction for random effects).

Compared with the expected distribution based on the volume of the lobes, lobar cerebral microbleeds occurred significantly more often in the temporal ($P<0.001$) and parietal lobes ($P=0.04$). The CMBs occurred significantly less often than expected in the frontal lobe ($P<0.001$). Moreover, temporal and parietal CMBs (Figure) did not appear to be uniformly distributed in these lobes, but rather appear primarily in the posterior part of the temporal and parietal lobes. Similar results were found in participants with multiple lobar microbleeds and (multiple) strictly lobar microbleeds.
Table 1. Characteristics of the study population (N=198)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years, mean ± SD</td>
<td>72.5 ± 8.3</td>
</tr>
<tr>
<td>Women, N (%)</td>
<td>96 (48.5)</td>
</tr>
<tr>
<td>Systolic blood pressure, mean ± SD</td>
<td>146.1 ± 19.7</td>
</tr>
<tr>
<td>Diastolic blood pressure, mean ± SD</td>
<td>79.6 ± 11.0</td>
</tr>
<tr>
<td>Mild hypertension, N (%)</td>
<td>97 (49.7)</td>
</tr>
<tr>
<td>Severe hypertension, N (%)</td>
<td>48 (24.6)</td>
</tr>
<tr>
<td>Smoking (ever), N (%)</td>
<td>151 (77.0)</td>
</tr>
<tr>
<td>Diabetes mellitus, N (%)</td>
<td>17 (8.6)</td>
</tr>
<tr>
<td>Serum total cholesterol (mmol/L), mean ± SD</td>
<td>5.56 ± 0.98</td>
</tr>
<tr>
<td>Serum HDL-cholesterol (mmol/L), mean ± SD</td>
<td>1.41 ± 0.39</td>
</tr>
<tr>
<td>APOE ε2 allele carrier, N (%)</td>
<td>27 (14.3)</td>
</tr>
<tr>
<td>APOE ε4 allele carrier, N (%)</td>
<td>69 (36.5)</td>
</tr>
<tr>
<td>Cortical infarct on MRI, N (%)</td>
<td>9 (4.5)</td>
</tr>
<tr>
<td>Lacunar infarct on MRI, N (%)</td>
<td>36 (18.2)</td>
</tr>
<tr>
<td>White matter lesions on MRI, ml, median (interquartile range)</td>
<td>5.0 (2.4-10.8)</td>
</tr>
</tbody>
</table>

Data are missing for blood pressure (N=3), hypertension (N=3), smoking (N=2), serum cholesterol (N=5), APOE genotype (N=9), white matter lesions (N=1).

Figure. Sagittal (A) and axial (B) image of the distribution of all lobar microbleeds.

Each spot represents a single microbleed (uniform size representation)
### Table 2. Distribution of lobar cerebral microbleeds

<table>
<thead>
<tr>
<th>Lobe volume</th>
<th>Participants with lobar microbleeds (N=198)*</th>
<th>Participants with multiple lobar microbleeds (N=81)†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Expected %</td>
<td>Microbleeds</td>
</tr>
<tr>
<td>Frontal lobe</td>
<td>40.6</td>
<td>237</td>
</tr>
<tr>
<td>Temporal lobe</td>
<td>22.8</td>
<td>273</td>
</tr>
<tr>
<td>Parietal lobe</td>
<td>22.6</td>
<td>212</td>
</tr>
<tr>
<td>Occipital lobe</td>
<td>13.9</td>
<td>116</td>
</tr>
<tr>
<td>Total</td>
<td>838</td>
<td></td>
</tr>
</tbody>
</table>

| Lobe volume | Participants with strictly lobar microbleeds (N=134)‡,§ | Participants with multiple strictly lobar microbleeds (N=35)|| |
|-------------|----------------------------------------------------------|----------------------------------------------------------|
|             | Expected % | Microbleeds | Observed # | O/E | Microbleeds | Observed # | O/E |
| Frontal lobe | 40.6       | 78          | 28.5 ††    | 0.70 | 51          | 29.1 ††    | 0.72 |
| Temporal lobe| 22.8       | 82          | 29.9 ††    | 1.31 | 55          | 31.4 ††    | 1.38 |
| Parietal lobe| 22.6       | 77          | 28.1 ‡‡    | 1.24 | 46          | 26.3       | 1.16 |
| Occipital lobe| 13.9       | 37          | 13.5       | 0.97 | 23          | 13.1       | 0.94 |
| Total        | 274        |             |            |     | 175         |             |     |

O/E: observed divided by expected.

* Lobar microbleeds with or without microbleeds in a deep or infratentorial region (median, 1; interquartile range, 1-3).
† More than 1 lobar microbleed with or without microbleeds in a deep or infratentorial region (median, 4; interquartile range, 2-8).
‡ Lobar microbleeds without microbleeds in a deep or infratentorial region (median, 1; interquartile range, 1-2).
§ Small differences in the number of participants with strictly lobar microbleeds between this article and our previous article are caused by differences in labeling procedures (automatic labeling versus visual rating scale).
|| More than 1 lobar microbleed without microbleeds in a deep or infratentorial region (median, 2; interquartile range, 2-5).
# Percentages corrected for clustering effects by added random effects for within subject variation.
** P<0.05 for observed vs. expected distribution, based on lobar volume percentages.
†† P<0.01 for observed vs. expected distribution, based on lobar volume percentages.
DISCUSSION

We found in the general population that lobar microbleeds show a predilection for the posterior brain regions, particularly the temporal lobes.

Some strengths of our study are its population-based setting, high response rate, and large sample size. Moreover, an important strength of our article compared with previous articles is that we took into account lobar volume when analyzing spatial distribution of microbleeds, and thus different a priori probabilities for microbleed occurrence. Furthermore, we also took into consideration the tendency of microbleeds to cluster.

A possible limitation of our study is misclassification of cerebral microbleeds, as small blood vessels and calcification may resemble cerebral microbleeds. However, mimics of cerebral microbleeds can usually be disregarded based on location and shape. Furthermore, as the high spatial resolution of our MRI sequence enabled us to distinguish the linear shape of sulcal vessels from the typical round or ovoid, blind-ending shape of CMBs, we believe that we did not label any more structures than there are microbleeds.

We found lesions preferentially in the temporal and parietal lobe but not in the occipital lobe, as has been described in previous studies that investigated the distribution of CMBs in patients with CAA and AD. There may be three reasons for the absence of occipital predilection in our study. First, most studies that describe the distribution of CMBs or amyloid burden are done in patients with CAA or AD, whereas our study was done in the general elderly population. It may be that CMBs occur preferentially in occipital regions in patients with moderate to severe CAA, whereas persons with mild CAA do not share this predilection. Only one other population-based study assessed the lobar location of microbleeds and suggested that lobar microbleeds show a predilection for the parietal brain area; they also did not find an overrepresentation of CMBs in the occipital brain area. Second, although the evidence of the relationship between lobar CMBs and CAA is accumulating, we cannot exclude that some factor other than CAA might account for the distribution of CMBs in this general elderly population. Last, differences across studies in the definition of the border between the occipital, parietal, and temporal lobes may play a role. Because
there is no clear sulcal landmark between the three lobes except for the parieto-occipital sulcus, the definition between the occipital and parietal lobes is somewhat arbitrary.\(^8\) As we especially found a predilection to microbleeds in the surrounding areas of these borders, the setting of the border may influence lobar predilection.

Our finding that microbleeds are found preferentially in the posterior regions of the brain and are underrepresented in the frontal lobes is consistent with previous studies that described the lobar distribution of microbleeds.\(^3,6,18\) The only population-based study that studied the lobar location of microbleeds suggested that lobar microbleeds show a predilection for the parietal brain area.\(^6\) However, the authors did not correct for lobar volume and therefore their results may have been driven by volume differences between the lobes.\(^6\) Only one clinical study in patients with CAA took lobar volume into account in the same way as we did and found lesions preferentially in the temporal and occipital lobes.\(^3\)

The posterior predilection of microbleeds in CAA has been hypothesized to relate to pattern of $\beta$-amyloid accumulation. It is thought that decreased pulse pressure and interstitial fluid pumping may lead to lower clearance of vascular $\beta$-amyloid.\(^{19}\) In the posterior lobes, these processes may be most clearly reduced, resulting in more vascular pathology and, consequently, more microbleeds.

Cerebral amyloid angiopathy–related CMBs are thought to be multiple and to occur primarily in lobar brain regions.\(^2,10\) In our study, the distribution pattern of lobar CMBs in participants with multiple lobar CMBs was similar to the distribution in participants with multiple, strictly lobar CMBs. Moreover, we found, on average, more CMBs per person in the group with multiple lobar CMBs compared with the group with multiple, strictly lobar CMBs (Table 1). Taken together, this suggests that multiple lobar CMBs might be as indicative of CAA as multiple, strictly lobar CMBs.

In conclusion, our findings show that lobar microbleeds occur more often in the temporal lobe and are underrepresented in the frontal lobe. This corroborates the presumed association of lobar microbleeds with CAA in the general population.
REFERENCES


Improved MRI detection of cerebral microbleeds more accurately identifies persons with vasculopathy.

AJNR Am J Neuroradiol. In press

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2.4

Incidence of cerebral microbleeds in the general population

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ABSTRACT

Background – Cerebral microbleeds are frequently seen in the general elderly population, but it is unknown at what rate they occur with aging and whether once present can disappear over time.

Methods – As part of the Rotterdam Scan Study, 831 persons (mean age, 68.5 years) underwent repeated brain MRI with a mean interval of 3.4 years. We assessed determinants of incident microbleeds in relation to their location with multiple logistic regressions.

Results – Overall prevalence of microbleeds increased from 24.4% at baseline to 28.0% at follow-up. Eighty-five persons (10.2%) developed new microbleeds. Microbleeds at baseline predicted development of new microbleeds (OR, 5.38; 95% CI, 3.34 to 8.67). In only 6 persons with microbleeds at baseline, fewer microbleeds were present at the follow-up examination. Cardiovascular risk factors, presence of lacunar infarcts, and larger white matter lesion volume at baseline were all associated with incident deep or infratentorial microbleeds, whereas people with the apolipoprotein E ε4/ε4 genotype or larger white matter lesion volume had a higher risk of incident strictly lobar microbleeds.

Conclusions – Incidence of microbleeds in the general population over a 3-year interval was substantial and microbleeds rarely disappeared. Risk factors for incident microbleeds were similar to those for prevalent microbleeds and differed according to microbleed location. These results support the assessment of microbleeds on T2*-weighted MRI as a possible marker of both cerebral amyloid angiopathy and hypertensive vasculopathy progression.
INTRODUCTION

Cerebral microbleeds (CMBs) are hypointense lesions seen on T2*-weighted gradient echo MRI that may be indicative of past microhemorrhages. Although their prognosis is yet not completely understood, several clinical studies suggested that CMBs might predict future risk of (recurrent) stroke. Cross-sectional studies have reported on the prevalence and determinants of microbleeds in patients with stroke as well as in the general elderly population. Although numbers varied widely across studies due to differences in MRI technique used, overall, the prevalence of CMBs both in patients with stroke and in the elderly was high and increased with age. Most consistent risk factors were age and hypertension, but markers of cerebral small vessel disease were found to be related as well. In addition, accumulating evidence suggests that the spatial distribution of CMBs may reflect specific underlying vascular pathological changes, in particular cerebral amyloid angiopathy or hypertensive vasculopathy.

Progression of these underlying vascular pathological changes may be reflected by development of new CMBs. It is unknown, however, at what rate microbleeds occur with aging in community-dwelling elderly and whether once present can disappear over time. Few longitudinal data on CMBs development exist, and existing studies were all small and performed in specific subgroups such as memory clinic patients or patients with cerebral amyloid angiopathy. To date, there has been no longitudinal study exploring the incidence of microbleeds and its determinants in the general population.

In the population-based Rotterdam Scan Study, we sought to investigate the incidence of CMBs and the location of these new microbleeds. Furthermore, we studied the determinants of incident CMBs in relation to their location.

METHODS

Participants

From 2005 to 2006, 1062 non-demented people (at that time all ≥60 years) underwent a baseline examination that included a brain MRI scan as part of the Rotterdam Scan Study. From October 2008 to January 2010 these participants were reinvited for follow-up MRI. The Institutional Review Board approved the study.
Of the 1062 participants at baseline, 80 people were not eligible to participate in the second MRI examination (dead, N=54; new MRI contraindications [e.g., pacemaker], N=10; institutionalized, N=9; untraceable, N=7). Of 982 eligibles, 848 participated and gave written informed consent (response rate 85%). Due to physical problems (e.g., backache), imaging could not be completed in 14 individuals. Of 834 complete MRI examinations, 3 scans were excluded because of severe artifacts, leaving 831 persons with complete and reliable baseline and follow-up MRI examinations.

**Brain MRI**

We performed an identical MRI protocol on the same 1.5-T scanner (GE Healthcare, Milwaukee, WI) at both time points. A 3-dimensional T2*-weighted gradient-recalled echo sequence was used for microbleed detection. The other sequences in the protocol consisted of a T1-weighted sequence, a proton density-weighted sequence, and a fluid-attenuated inversion recovery sequence. No scanner software upgrades or hardware alterations were applied during the study period to ensure comparability of scan data over time.

**Rating of CMBs**

At both time points, all 3-dimensional T2*-weighted gradient-recalled echo scans were reviewed by 1 of 5 trained raters who recorded the presence, number, and location of microbleeds. All raters were blinded to the other MRI sequences, clinical data, and apolipoprotein E (APOE) genotyping; and the 3-dimensional T2*-weighted gradient-recalled echo scan did not reveal the presence of infarcts or white matter lesions. Microbleeds were defined as focal areas of very low signal intensity. Signal voids caused by sulcal vessels, symmetrical calcifications in the basal ganglia, choroid plexus and pineal calcifications, and signal averaging from bone were excluded. Intraobserver (N=500, 1 rater) and interobserver (N=300) reliabilities were \( \kappa = 0.87 \) and \( \kappa = 0.85 \), which corresponds to very good agreement. CMBs were categorized into 1 of 3 locations: lobar, deep, or infratentorial. Scans of subjects rated positive for CMBs at at least 1 of 2 time points were included in a side-by-side comparison (M.M.P. and M.W.V.) blinded to the time point of the scans to assess the final number and location of microbleeds in each scan.
Cerebrovascular Disease on MRI
Infarcts were rated on fluid-attenuated inversion recovery, proton density-weighted, and T1-weighted sequences at baseline by the same raters who had scored CMBs according to criteria described previously. White matter lesion volume was quantified with a validated tissue classification technique.

Cardiovascular Risk Factors and APOE Genotyping
Cardiovascular risk factors at baseline were examined by interview and laboratory and physical examination as previously described. APOE genotyping was performed on coded genomic DNA samples. The distributions of APOE genotype and allele frequencies in this population were in Hardy-Weinberg equilibrium.

Data Analysis
We tested differences in baseline characteristics between persons who participated in both examinations and persons who refused or were ineligible to participate in the second MRI using analysis of covariance adjusted for age and sex.

Incidence of CMBs was calculated in 10-year age strata and separately in strata of presence of microbleeds at baseline MRI. Subsequently, we made categories for “strictly lobar incident microbleeds” (persons with ≥1 new microbleeds restricted to a lobar location) and “deep or infratentorial incident microbleeds” (persons with ≥1 new microbleeds in a deep or infratentorial location with or without concomitant lobar microbleeds) and assessed whether the incidence differed according to microbleed location at baseline using multiple logistic regressions.

Next, we assessed the relation of vascular risk factors, APOE allele status, and cerebrovascular disease at baseline to CMB incidence with multiple logistic regressions. These analyses were also performed according to microbleed location. All regression analyses were adjusted for age, sex, and scan interval. To examine independency of risk factors, we used multivariable modeling, including lacunar infarcts, white matter lesion volume, and vascular risk factors. Analyses were performed using the statistical package SPSS 15.0 (SPSS Inc, Chicago, IL).
RESULTS

Table 1 shows the characteristics of all 1062 participants at baseline. Persons who participated in both MRI examinations were younger compared with persons who participated in the first examination only. Furthermore, participants refusing second MRI were more often APOE ε2 carriers, whereas ineligible persons had higher cholesterol, more lacunar infarcts, and a higher white matter lesion volume at baseline, even when differences in age and sex were taken into account.

Mean interval between the two MRI assessments was 3.4 years (range, 2.3 to 4.5 years). During this period, overall prevalence of microbleeds increased from 24.4% to 28.0%. Eighty-five of the 831 participants (10.2%) developed new microbleeds on MRI, of whom 38 (4.6%) had multiple new microbleeds (e.g., Figure). CMB incidence increased with age from 7.6% in persons aged 60 to 69 years to 18.6% in participants >80 years and no significant differences were observed between sexes. Among persons with new microbleeds, 60% had incident strictly lobar CMBs, whereas 40% had incident deep or infratentorial CMBs (Table 2). Significantly more participants with microbleeds at baseline developed new CMBs during the time interval compared with participants without CMBs at baseline (25.1% versus 5.1%, Table 2; OR, 5.38; 95% CI, 3.34 to 8.67; Table 3). This risk was even higher for persons with multiple CMBs at baseline (OR, 7.15; 95% CI, 4.11 to 12.44). Moreover, CMB location at baseline strongly predicted the location of new CMBs (Table 3).

Only in 6 persons (3% of participants with CMBs at baseline) we found less CMBs at follow-up compared with the baseline MRI scan; these persons were added to the group of no incident microbleeds. In 4 of these 6 persons, 1 CMB was scored at baseline, whereas we assessed no CMBs at follow-up; in 1 person CMB count decreased from 2 to 1; in 1 person 11 CMBs were scored on the baseline scan and only 6 at follow-up. Furthermore, there were another 6 participants in whom not all CMBs that were seen at baseline were recognized at the follow-up scan, yet the total number of CMBs did not decrease over time. Except for 1, all of these had >5 CMBs at baseline and showed an increase in total CMB count at follow-up. In the overall population, we scored 258 new microbleeds, whereas only 18 microbleeds seemed to disappear over time.
Older age, high systolic blood pressure, high pulse pressure, and hypertension at baseline were all associated with development of new microbleeds (Table 4). When stratified according to location, we found an association of high systolic blood pressure, high pulse pressure, and hypertension with incident deep or infratentorial microbleeds but not with new lobar CMBs. With increasing serum total cholesterol, the incidence of microbleeds in a deep or infratentorial location decreased (Table 4).

There were no significant differences in microbleed incidence in either location for carriers of either the APOE ε2 allele or the APOE ε4 allele when compared with persons with the ε3/ε3 genotype (Table 4). However, when we restricted our analyses to APOE ε4/ε4, we did find an association with development of new microbleeds (OR, 4.43; 95% CI, 1.44 to 13.64). This was especially true for new strictly lobar incident CMBs (OR, 6.60; 95% CI, 1.90 to 22.89), whereas there was no significant association between APOE ε4/ε4 and deep or infratentorial CMBs. Only 5 participants carried the APOE ε2/ε2 genotype; none of them developed new microbleeds during the study period.

Cortical infarcts at baseline were not associated with microbleed incidence (Table 5). Lacunar infarcts were strongly related to incident CMBs in participants with new deep or infratentorial microbleeds (OR, 4.46; 95% CI, 1.79 to 11.10), whereas this association in persons with strictly lobar CMBs was less strong and not significant. White matter lesion volume at baseline MRI increased the risk of incident microbleeds in either location (Table 5).

Additional multivariable modeling to examine independency of risk factors did not change the results (data not shown).
Table 1. Baseline characteristics of participants who had a second MRI assessment and for those who refused or were ineligible

<table>
<thead>
<tr>
<th></th>
<th>Participants with a second MRI N=831</th>
<th>Participants who refused a second MRI† N=148</th>
<th>Participants ineligible for a second MRI‡ N=83</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years, mean ± SD</td>
<td>68.5 ± 6.3</td>
<td>72.5 ± 8.5*</td>
<td>76.1 (8.4)*</td>
</tr>
<tr>
<td>Women, N (%)</td>
<td>418 (50.3)</td>
<td>83 (56.1)</td>
<td>42 (50.6)</td>
</tr>
<tr>
<td>Systolic blood pressure, mean ± SD</td>
<td>143.8 ± 18.1</td>
<td>146.1 ± 19.8</td>
<td>147.0 ± 22.0</td>
</tr>
<tr>
<td>Diastolic blood pressure, mean ± SD</td>
<td>80.7 ± 10.4</td>
<td>78.7 ± 10.1</td>
<td>77.6 ± 9.3</td>
</tr>
<tr>
<td>Pulse pressure</td>
<td>63.2 ± 15.6</td>
<td>67.5 ± 17.5</td>
<td>69.4 ± 21.1</td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild, N (%)</td>
<td>426 (51.6)</td>
<td>76 (52.1)</td>
<td>39 (49.4)</td>
</tr>
<tr>
<td>Severe, N (%)</td>
<td>159 (19.2)</td>
<td>32 (21.9)</td>
<td>23 (29.1)</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never, N (%)</td>
<td>241 (29.3)</td>
<td>34 (23.8)</td>
<td>15 (19.0)</td>
</tr>
<tr>
<td>Past, N (%)</td>
<td>343 (41.7)</td>
<td>63 (44.1)</td>
<td>39 (49.4)</td>
</tr>
<tr>
<td>Current, N (%)</td>
<td>239 (29.0)</td>
<td>46 (32.1)</td>
<td>25 (31.6)</td>
</tr>
<tr>
<td>Diabetes mellitus, N (%)</td>
<td>68 (8.3)</td>
<td>19 (13.4)</td>
<td>8 (10.4)</td>
</tr>
<tr>
<td>Serum total cholesterol, mmol/L, mean ± SD</td>
<td>5.69 ± 0.97</td>
<td>5.70 ± 0.94</td>
<td>5.43 ± 0.87*</td>
</tr>
<tr>
<td>APOE ε2 allele carrier, N (%)</td>
<td>115 (14.6)</td>
<td>29 (20.7)*</td>
<td>11 (15.3)</td>
</tr>
<tr>
<td>APOE ε4 allele carrier, N (%)</td>
<td>206 (26.1)</td>
<td>44 (31.4)</td>
<td>23 (31.9)</td>
</tr>
<tr>
<td>Cortical infarct on baseline MRI, N (%)</td>
<td>21 (2.5)</td>
<td>9 (6.1)</td>
<td>7 (8.4)</td>
</tr>
<tr>
<td>Lacunar infarct on baseline MRI, N (%)</td>
<td>64 (7.7)</td>
<td>12 (8.1)</td>
<td>17 (20.5)*</td>
</tr>
<tr>
<td>Subcortical infarct on baseline MRI, N (%)</td>
<td>1 (0.1)</td>
<td>1 (0.7)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>White matter lesions on baseline MRI, ml, median (interquartile range)</td>
<td>3.8 (2.2-7.4)</td>
<td>5.2 (2.5-13.3)</td>
<td>8.8 (4.6-18.3)*</td>
</tr>
</tbody>
</table>

* Age and sex adjusted mean, median or percentage is significantly different (P<0.05) from participants with a second MRI.
† Participants refusing a second MRI (N=134) and participants with no (complete) examination (N=14).
‡ Ineligible participants (N=80) and participants with ungradable MRI (N=3).

Data are missing for blood pressure/hypertension (N=11), smoking (N=17), diabetes mellitus (N=24), serum cholesterol (N=17), APOE genotype (N=62), and white matter lesions (N=18).
Figure. Example of incident microbleeds on 3-dimensional T2*-weighted gradient-recalled echo MRI during a scan interval of 3 years. Arrows indicate new microbleeds on the follow-up scan. A, Baseline scan 2006. B, Follow-up scan 2009.

Table 2. Incidence of CMBs in 10-year age groups, in strata of presence of CMBs on baseline MRI

<table>
<thead>
<tr>
<th>Age range (years)</th>
<th>Overall</th>
<th>No prevalent CMBs on baseline MRI</th>
<th>Prevalent CMBs on baseline MRI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of persons</td>
<td>Incident CMBs N (%)</td>
<td>Multiple incident CMBs, N (%)</td>
</tr>
<tr>
<td>60-69</td>
<td>580</td>
<td>44 (7.6)</td>
<td>21 (3.6)</td>
</tr>
<tr>
<td>70-79</td>
<td>192</td>
<td>30 (15.6)</td>
<td>11 (5.7)</td>
</tr>
<tr>
<td>80-97</td>
<td>59</td>
<td>11 (18.6)</td>
<td>6 (10.2)</td>
</tr>
<tr>
<td>Total</td>
<td>831</td>
<td>85 (10.2)</td>
<td>38 (4.6)</td>
</tr>
</tbody>
</table>
### Table 3. Presence of CMBs at baseline and incidence of CMBs

<table>
<thead>
<tr>
<th>Risk of incident CMBs</th>
<th>OR (95% CI)*</th>
<th>Deep or infratentorial incident CMBs†</th>
</tr>
</thead>
<tbody>
<tr>
<td>CM Bs at baseline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multiple CM Bs at baseline</td>
<td>5.38 (3.34-8.67)</td>
<td>4.99 (2.75-9.06)</td>
</tr>
<tr>
<td>One strictly lobar CM B at baseline</td>
<td>7.15 (4.11-12.44)</td>
<td>5.69 (2.85-11.36)</td>
</tr>
<tr>
<td>Multiple strictly lobar CM Bs at baseline</td>
<td>4.32 (2.05-9.11)</td>
<td>6.53 (2.86-14.90)</td>
</tr>
<tr>
<td>One deep or infratentorial CM B at baseline</td>
<td>2.76 (1.13-6.76)</td>
<td>3.24 (1.15-9.12)</td>
</tr>
<tr>
<td>Multiple deep or infratentorial CM Bs at baseline†</td>
<td>7.25 (3.62-14.51)</td>
<td>3.02 (1.06-8.56)</td>
</tr>
</tbody>
</table>

* All values are odds ratios (OR) with 95% confidence intervals (95% CI), adjusted for age, sex and scan interval.
† With or without concomitant new lobar CM Bs.

### Table 4. Cardiovascular risk factors, APOE allele status and incidence of CMBs

<table>
<thead>
<tr>
<th>Risk of incident CMBs</th>
<th>OR (95% CI)*</th>
<th>Deep or infratentorial incident CMBs‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>CM Bs at baseline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, per year</td>
<td>1.06 (1.03-1.10)</td>
<td>1.07 (1.03-1.11)</td>
</tr>
<tr>
<td>Women, versus men</td>
<td>0.69 (0.43-1.09)</td>
<td>0.69 (0.39-1.24)</td>
</tr>
<tr>
<td>Systolic BP† per SD increase</td>
<td>1.29 (1.03-1.61)</td>
<td>1.18 (0.89-1.56)</td>
</tr>
<tr>
<td>Diastolic BP† per SD increase</td>
<td>1.04 (0.83-1.32)</td>
<td>0.95 (0.70-1.27)</td>
</tr>
<tr>
<td>Pulse pressure† per SD increase</td>
<td>1.33 (1.06-1.67)</td>
<td>1.27 (0.95-1.70)</td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild, versus none</td>
<td>1.82 (0.97-3.40)</td>
<td>1.68 (0.80-3.53)</td>
</tr>
<tr>
<td>Severe, versus none</td>
<td>2.57 (1.27-5.17)</td>
<td>1.44 (0.58-3.60)</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Past, versus never</td>
<td>0.98 (0.56-1.72)</td>
<td>0.97 (0.47-2.02)</td>
</tr>
<tr>
<td>Current, versus never</td>
<td>0.78 (0.41-1.51)</td>
<td>0.92 (0.40-2.10)</td>
</tr>
<tr>
<td>Diabetes mellitus, yes versus no</td>
<td>1.22 (0.56-2.69)</td>
<td>0.76 (0.23-2.53)</td>
</tr>
<tr>
<td>Serum total cholesterol‡ per SD increase</td>
<td>0.89 (0.69-1.15)</td>
<td>1.08 (0.79-1.48)</td>
</tr>
<tr>
<td>APOE ε4, versus ε3/ε3</td>
<td>1.19 (0.69-2.05)</td>
<td>1.58 (0.82-3.07)</td>
</tr>
<tr>
<td>APOE ε4/ε4, versus ε3/ε3</td>
<td>4.43 (1.44-13.64)</td>
<td>6.60 (1.90-22.89)</td>
</tr>
<tr>
<td>APOE ε2, versus ε3/ε3</td>
<td>1.20 (0.60-2.37)</td>
<td>1.11 (0.44-2.80)</td>
</tr>
</tbody>
</table>

* All values are odds ratios (OR) with 95% confidence intervals (95% CI), adjusted for age, sex and scan interval (when applicable).
† Additionally adjusted for the use of blood pressure-lowering medication.
‡ Additionally adjusted for the use of lipid-lowering drugs.
§ With or without concomitant new lobar CM Bs.
BP: blood pressure.
Table 5. Cerebrovascular disease and incidence of CMBs

<table>
<thead>
<tr>
<th>Risk of incident CMBs</th>
<th>OR (95% CI)*</th>
<th>Deep or infratentorial incident CMBs‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>All incident CMBs</td>
<td>N=85</td>
<td>N=51</td>
</tr>
<tr>
<td>Cortical infarcts, versus no infarct</td>
<td>1.88 (0.60-5.90)</td>
<td>1.46 (0.32-6.70)</td>
</tr>
<tr>
<td>Lacunar infarcts, versus no infarct</td>
<td>3.04 (1.58-5.87)</td>
<td>2.26 (0.96-5.34)</td>
</tr>
<tr>
<td>White matter lesion volume† per SD increase</td>
<td>1.89 (1.47-2.44)</td>
<td>1.86 (1.36-2.55)</td>
</tr>
</tbody>
</table>

* All values are odds ratios (OR) with 95% confidence intervals (95% CI), adjusted for age, sex and scan interval.
† Natural log-transformed.
‡ With or without concomitant new lobar CMBs.

**DISCUSSION**

Incidence of microbleeds in this population-based study over a 3-year interval was approximately 10% and microbleeds rarely disappeared. Participants with CMBs at baseline had an almost 5-fold increased risk of developing new microbleeds during the follow-up period compared with persons without CMBs at baseline. This was especially true for persons with multiple microbleeds at baseline. Risk factors for incident microbleeds were similar to those for prevalent microbleeds and differed according to microbleed location.

Strengths of this study are its population-based setting and its large number of participants with repeated MRI using a 3-dimensional T2*-weighted gradient-recalled echo sequence with proven high sensitivity for microbleed detection. Moreover, we performed an identical MRI protocol on the same 1.5-T scanner at both time points without software or hardware alterations to optimize comparability between scans over time. Another strength is that the raters were blinded to the time point of the scans. This approach prevented overestimation of incident microbleeds and allowed us to assess potential vanishing of CMBs over time. A possible limitation of the study is that selective dropout may have influenced our results. People who participated were younger and healthier compared with those who refused a second MRI scan or, in particular, were ineligible. If at all, this may have led us to underestimate the true incidence of microbleeds in the population at large. Due to this selection, associations
between risk factors and CMB incidence may also have been underestimated. However, when we repeated the cross-sectional analyses we previously reported on at baseline\textsuperscript{5} in the 831 persons with a second MRI assessment, we found similar associations (data not shown), indicating limited selection bias.

In the previously mentioned cross-sectional analyses,\textsuperscript{5} we found an association between cardiovascular risk factors, presence of lacunar infarcts, and white matter lesions and prevalent microbleeds in a deep or infratentorial region but not in a lobar location. Moreover, APOE ε4 carriers had significantly more often strictly lobar CMBs than non-carriers. In our current study, we observed similar associations between these risk factors and development of new CMBs in the specified locations, indicating that microbleed incidence, in line with microbleed prevalence, may result from different underlying vascular pathology, that is, cerebral amyloid angiopathy and hypertensive vasculopathy.

Few studies in selected subgroups, mainly among patients with stroke and patients with cerebral amyloid angiopathy, have reported on CMB incidence.\textsuperscript{9–14} These studies report a wide range of incidence rates (12% to 50%), which may largely be explained by differences in study populations, scan interval time, and MRI protocols. Of note is that the use of an optimized high-resolution sequence like in our study likely results in a higher incidence of microbleeds compared with less sensitive sequences.\textsuperscript{16} Overall, studies have consistently shown that microbleeds at baseline predict development of new microbleeds.\textsuperscript{9–13} Moreover, vascular risk factors have been associated with incident CMBs in previous studies.\textsuperscript{9–10} However, only one study made separate categories according to CMB location and they did not find a relation of vascular risk factors and incidence of deep or infratentorial CMBs.\textsuperscript{9} In our study, the association between vascular risk factors and incident deep or infratentorial microbleeds remained significant after adjustment for other markers of small vessel disease. This suggests that deep or infratentorial microbleeds are an independent indicator of hypertensive vasculopathy.

Previous studies in memory clinic patients and patients with cerebral amyloid angiopathy found an association between APOE ε4 or ε2 carriership and incident CMBs.\textsuperscript{9,13} Although we could not reproduce this in the general population, we did find that the APOE ε4/ε4 genotype was related to incident CMBs, in particular incident
strictly lobar microbleeds. An explanation for the lack of an association with APOE ε4 allele in our study compared with others may be the higher prevalence of both determinant and outcome in previous clinical studies compared with our community-dwelling elderly population. In contrast to the baseline cross-sectional study, we now also found an association between white matter lesion volume at baseline and incidence of strictly lobar microbleeds, which is in line with other studies. It may be hypothesized that vascular amyloid deposition alters white matter perfusion and thus causes white matter lesions through vessel stenosis, vasoactive effects of β-amyloid, and smooth muscle cell necrosis that results in loss of vasoreactivity.

In our study, in 12 persons (1.4% of overall study population; 5.9% of persons with CMBs at baseline), some CMBs that were present at baseline seemed to disappear over time. In only 6 of these, this led to a decrease in overall microbleed count at the follow-up examination. This low percentage is comparable with other studies that also found a very small percentage of microbleeds to disappear over time. In contrast, some studies suggested that there may be more widespread dynamism and resolution of CMBs, but these studies were based on very few cases. Furthermore, lower quality of imaging will not only influence the incidence rate of microbleeds, but will also result in microbleeds not being detected on either baseline or follow-up MRI scan, which may falsely suggest resolution of microbleeds. We cannot, however, rule out the possibility that some CMBs both occurred and disappeared in the time interval between both scans, although this number is likely low based on our available data.

Our results support the assessment of microbleeds on T2*-weighted MRI as a possible marker of both cerebral amyloid angiopathy and hypertensive vasculopathy progression. Strict control of vascular risk factors, especially in persons who already have microbleeds, may potentially slow down progression of pathology and may perhaps prevent symptomatic intracerebral hemorrhage in the long run. Further studies are warranted to investigate this hypothesis.
REFERENCES


2.5

Serum lipid levels, presence of cerebral microbleeds and risk of intracerebral hemorrhage

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Aad van der Lugt
Monique M.B. Breteler
M. Arfan Ikram
ABSTRACT

Objective – Low serum total cholesterol levels are associated with an increased risk of symptomatic intracerebral hemorrhage and with presence of asymptomatic cerebral microbleeds. The relative contribution of lipid fractions to these associations is unclear and requires investigation. We determined whether serum HDL-cholesterol, LDL-cholesterol, and triglycerides are associated with risk of intracerebral hemorrhage and presence of cerebral microbleeds.

Methods and Results – Nine thousand sixty-eight stroke-free community-dwelling persons aged ≥55 were followed from baseline (1990–2001) up to January 1, 2009, of whom 85 suffered from intracerebral hemorrhage during follow-up. Brain MRI was carried out in 789 healthy participants, of whom 162 had cerebral microbleeds. Triglycerides were strongly and inversely associated with intracerebral hemorrhage, independently of HDL-cholesterol, LDL-cholesterol, and potential confounders (HR for highest versus lowest quartile: 0.20 [0.06–0.69]). Triglycerides were also associated with deep or infratentorial microbleeds (odds ratio for highest versus lowest quartile: 0.37 [0.14–0.96]), but not with strictly lobar microbleeds. No associations were found for HDL-cholesterol or LDL-cholesterol.

Conclusion – Low serum triglyceride levels were associated with an increased risk of intracerebral hemorrhage and with the presence of deep or infratentorial cerebral microbleeds. This provides novel insights into the role of lipid fractions, particularly triglycerides, in the etiology of intracerebral hemorrhage.
INTRODUCTION

Intracerebral hemorrhage accounts for about 10% to 15% of strokes and is a devastating disease for which there are currently no curative treatment options.\(^1-2\) Therefore, identification of modifiable risk factors is highly important. Low levels of serum total cholesterol have long been recognized as a possible risk factor for intracerebral hemorrhage.\(^3\) The exact role of cholesterol in the pathogenesis of intracerebral hemorrhage is unclear, although some studies suggest that low cholesterol levels make the cerebrovascular endothelium fragile and vulnerable for leakage and rupture.\(^4-6\) Low total cholesterol levels also relate to the presence of cerebral microbleeds,\(^7-8\) which are thought to be asymptomatic precursors of symptomatic intracerebral hemorrhage.\(^9-10\) Establishing overlap in risk factors for intracerebral hemorrhage and cerebral microbleeds may thus aid in the early detection of persons at an increased risk of intracerebral hemorrhage.

Although various cohort studies show that serum total cholesterol levels are inversely related with intracerebral hemorrhage,\(^11-22\) it is unclear how various serum lipid fractions associate with intracerebral hemorrhage. Studies investigating lipid-fractions, i.e., low-density lipoprotein (LDL-) cholesterol, high-density lipoprotein (HDL-) cholesterol and triglycerides, have reported inconsistent results.\(^13-17,19,23-25\) However, recent evidence suggests that the association between total cholesterol levels and risk of intracerebral hemorrhage is mainly driven by low triglyceride levels.\(^19,23\) Still, further confirmation of these results is needed. Moreover, it is unclear whether similar patterns of lipid fractions also underlie the association of cholesterol with cerebral microbleeds.

Therefore, we investigated in a large population-based cohort of community-dwelling elderly people whether serum total cholesterol and in particular the levels of LDL-cholesterol, HDL-cholesterol and triglycerides are associated with the risk of intracerebral hemorrhage. Because we also aimed to investigate the potential of these lipid fractions as risk factors for preclinical disease, we studied the associations between lipid fractions and the presence of cerebral microbleeds.
METHODS

Source Population

The Rotterdam Study is an ongoing prospective population-based cohort study that focuses on causes and consequences of diseases that are frequent in the elderly. The rationale and design of the study have been described extensively elsewhere.\textsuperscript{26} Briefly, the cohort started in 1990 and included 7983 participants who were aged ≥55 years and living in Ommoord, a district in Rotterdam in the Netherlands (Rotterdam Study I). In 2000, the cohort was expanded with 3011 participants who had reached the age of 55 or had moved into the district since the start of the study (Rotterdam Study II). All participants underwent a comprehensive set of baseline examinations which were repeated during regular follow-up visits, approximately every 3 to 5 years. In 2005, a random subset of Rotterdam Study II underwent brain MRI. The study was approved by the Medical Ethics Committee of Erasmus MC University Medical Center Rotterdam and all participants gave written informed consent to participate in the study.

Measurement of Serum Lipid Levels

Venous blood samples were obtained from all participants at each visit (Figure). In 1990, non-fasting serum total cholesterol and HDL-cholesterol levels were measured, using enzymatic colorimetric methods (Kone Specific Analyzer, Kone Instruments). From 1997 onwards, fasting total cholesterol and HDL-cholesterol as well as fasting triglyceride levels were determined using comparable enzymatic procedures (Hitachi Analyzer, Roche Diagnostics). Lipid measurements were carried out at Erasmus Medical Center in two laboratories (Department of Epidemiology laboratory and Clinical Chemistry laboratory), which both participated in the Dutch National Cholesterol Standardization Program, analogous to the Center for Disease Control and Prevention quality assurance and standardization programs (Atlanta, GA). All measurements fulfilled the WHO criteria for precision and accuracy of lipid measurements. Non-high density lipoprotein cholesterol (non-HDL-cholesterol) was calculated by subtracting HDL-cholesterol from total cholesterol. The Friedewald equation was used to estimate LDL-cholesterol.\textsuperscript{27} Pearson’s correlation coefficients for the various lipid fractions were weak to modest: HDL-cholesterol and triglycerides, \( r = 0.50 \); HDL-cholesterol and LDL-cholesterol, \( r = -0.07 \); and LDL-cholesterol and triglycerides, \( r = 0.20 \). Triglyceride levels were natural log-transformed because their distribution was severely skewed to the right.
Population for Analysis
Because in different examination visits either non-fasting or fasting blood samples were drawn, we performed our analyses based on the following three combined sets of participants. This was done in order to obtain the largest numbers of intracerebral hemorrhage (figure):

- **Set 1:** participants of the first examination of Rotterdam Study I (baseline 1990-1993) and the first examination of Rotterdam Study II (baseline 2000-2001);
- **Set 2:** persons who took part in the third examination of Rotterdam Study I (1997-1999) and the first examination of Rotterdam Study II (baseline 2000-2001); and
- **Set 3:** a random subset of participants of the second examination of Rotterdam Study II (2004-2005) who underwent brain MRI.

Of the total of 10994 Rotterdam Study participants, we excluded persons who had had a stroke before their first examination (N=363), participants who had not given consent for the collection of follow-up data from general practitioners (N=195), participants who had not visited the research center for blood sampling due to death, refusal or physical inability (N=929), and participants of whom blood draw or storage had failed (N=412). Because the Friedewald equation is not valid if triglyceride levels exceed 4.52 mmol/L (400 mg/dL), we further excluded participants with triglyceride levels above this value (N=31). This resulted in a total of 9068 participants for Set 1 (Rotterdam Study I, N=6753; Rotterdam Study II, N=2315). Of the 4797 persons who took part in the third examination of Rotterdam Study I, we excluded 243 participants who had a prevalent stroke at that examination, 5 participants who had not given informed consent for the collection of follow-up data, 1072 participants of whom lipid levels were not available, and 24 participants with triglyceride levels >4.52 mmol/L. Combining these 3458 persons with 2315 from Rotterdam Study II resulted in 5773 participants in Set 2. Set 3 comprised a random subset of Rotterdam Study II participants who underwent brain MRI and of whom we had lipid measurements available (N=789).

Assessment of Stroke
Stroke was defined according to WHO criteria as a syndrome of rapidly developing clinical signs of focal (or global) disturbance of cerebral function, with symptoms lasting 24 hours or longer or leading to death, with no apparent cause other than of
vascular origin. History of stroke at baseline was assessed during the baseline interview and verified by reviewing medical records. After enrollment, participants were continuously monitored for incident stroke through automated linkage of the study database with files from general practitioners. Nursing home physicians’ files and files from general practitioners of participants who moved out of the district were checked on a regular basis as well. Additional information was obtained from hospital records. Potential strokes were reviewed by research physicians, and verified by an experienced stroke neurologist (P.J.K.). Strokes were further classified as cerebral infarction or intracerebral hemorrhage on the basis of neuroimaging reports. If neuroimaging was lacking, a stroke was classified as unspecified. Subarachnoid hemorrhages due to ruptured aneurysms were not considered stroke events.

Participants were followed from study entry to stroke, death, last health status update when they were known to be stroke-free, or January 1, 2009, whichever came first. Follow-up was complete up to January 1, 2009 for 98.4% of potential person years.

Brain MRI and Rating of Cerebral Microbleeds

A multisequence MRI protocol was carried out on a 1.5-T scanner (GE Healthcare, Milwaukee, WI). A custom-made accelerated 3-dimensional T2*-weighted gradient-recalled echo (3D T2* GRE) sequence with high spatial resolution and long echo time was used for microbleed detection. All 3D T2* GRE scans were reviewed by 1 of 2 trained raters who recorded the presence, number, and location of cerebral microbleeds. Cerebral microbleeds were categorized into 1 of 3 locations: lobar (cortical gray and subcortical or periventricular white matter), deep (deep gray matter: basal ganglia and thalamus, and the white matter of the corpus callosum, internal, external, and extreme capsule), and infratentorial (brain stem and cerebellum).

Other Measurements

Trained research physicians visited all participants at home for standardized questionnaires about their health status and medical history, including questions about current medication use, cigarette smoking behavior and average amount of alcohol intake. Subsequently, all participants visited the research center twice for physical examination and blood sampling. Blood pressure was calculated as the average of two measurements at the right brachial artery with a random-zero sphygmomanometer after 5 minutes of rest while the subject was in a sitting position. Hypertension was
defined as a diastolic blood pressure of \( \geq 90 \) mmHg and/or a systolic blood pressure of \( \geq 140 \) mmHg, and/or the use of blood pressure-lowering medication.\(^{31}\) Diabetes mellitus was defined as a fasting serum glucose level \( \geq 7.0 \) mmol/L, a non-fasting or postload serum glucose level \( \geq 11.1 \) mmol/L and/or the use of blood glucose-lowering drugs. Fasting serum insulin level was determined by metric assay (Biosource Diagnostics, Camarillo, CA).\(^{32}\) Body mass index was calculated as weight (in kilograms) divided by the square of height (in meters).

**Statistical Analysis**

Cox regression was used to calculate hazard ratios and 95% confidence intervals for the associations between lipid levels and risk of intracerebral hemorrhage, expressed per standard deviation increase in serum lipid level. Associations between total cholesterol, HDL-cholesterol and non-HDL-cholesterol, and intracerebral hemorrhage were calculated in Set 1 (Figure); associations between LDL-cholesterol and triglycerides, and intracerebral hemorrhage were calculated in Set 2 (Figure). For both sets, we constructed 2 models. In model 1 we adjusted for age, sex, lipid-lowering medication use (yes/no) and Rotterdam Study subcohort (RS-I/RS-II). In model 2, we adjusted for age, sex, and a propensity score that included the following potential confounders: lipid-lowering medication use, systolic blood pressure (continuous), blood pressure-lowering medication use (yes/no), diabetes mellitus (yes/no), serum glucose level (continuous), current cigarette smoking (yes/no), body mass index (continuous), antithrombotic medication use (yes/no), alcohol intake (continuous) and subcohort. We adjusted for a propensity score instead of individual confounders because the number of intracerebral hemorrhages was small compared to the large number of potential confounders.\(^{33-34}\)
**Figure. Schematic overview of the Rotterdam Study population**

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**RS: Rotterdam Study; ICH: intracerebral hemorrhage.**

Lipid measurements in Set 1: total cholesterol, HDL-cholesterol, and non-HDL-cholesterol (non-fasting).

Lipid measurements in Set 2 and Set 3: total cholesterol, HDL-cholesterol, non-HDL-cholesterol, LDL-cholesterol, and triglycerides (fasting).

Statin treatment might modify the association between lipid levels and intracerebral hemorrhage. Therefore, we investigated whether the associations between lipid levels and intracerebral hemorrhage differed across strata of lipid-lowering medication use and we tested the presence of interaction. These analyses were performed in Set 2. Hazard ratios were expressed per standard deviation increase in lipid level, and were adjusted for age, sex, and a propensity score of potential confounders (systolic blood pressure, blood pressure-lowering medication use, diabetes mellitus, serum glucose level, serum insulin level, current cigarette smoking, body mass index, antithrombotic medication use, alcohol intake and subcohort).
Subsequently, we investigated whether the associations between HDL-cholesterol, LDL-cholesterol, triglycerides, and risk of intracerebral hemorrhage were independent of each other, by entering these lipid fractions simultaneously in the Cox regression models. The analyses were performed in Set 2. We adjusted for age, sex, a propensity score of potential confounders (lipid-lowering medication use, systolic blood pressure, blood pressure-lowering medication use, diabetes mellitus, serum glucose level, serum insulin level, current cigarette smoking, body mass index, antithrombotic medication use, alcohol intake and subcohort) and for the other lipid fractions. To verify the log-linearity of associations, we also categorized lipid levels in quartiles using the lowest quartile as the reference category.

Finally, we determined the associations between HDL-cholesterol, LDL-cholesterol and triglycerides, and the presence of deep or infratentorial (with or without lobar microbleeds) versus strictly lobar cerebral microbleeds, using logistic regression models. These analyses were carried out in Set 3. Associations were adjusted for age, sex, a propensity score (lipid-lowering medication use, systolic blood pressure, blood pressure-lowering medication use, diabetes mellitus, serum glucose level, serum insulin level, current cigarette smoking, body mass index, antithrombotic medication use, and alcohol intake), and the complementary lipid fractions. Odds ratios were expressed per standard deviation increase and in quartile categories.

In each set, covariate adjustments were based on data that were collected during the same visit as the blood sample in which the lipid levels were determined. We did not have complete data on all covariates. Alcohol intake was missing in 15.4%; other covariates were missing in less than 4% of participants. Missing values in covariates were imputed with a linear regression model based on age and sex. All analyses were performed using SPSS for Windows, version 16.0 (SPSS Inc., Chicago, Illinois).

RESULTS

During 97956 person years of follow-up (median 9.7 years), 1005 participants developed a first-ever stroke, which was classified in 85 as intracerebral hemorrhage, in 561 as cerebral infarction and in 359 as unspecified. Of the 85 intracerebral hemorrhages, 73 had occurred in Rotterdam Study I (33 after the start of the third visit), and
Cerebral microbleeds were present in 162 (20.5%) of the 789 participants who underwent brain MRI; microbleeds were localized in deep or infratentorial brain regions in 65 participants and were strictly lobar in location in 97. Baseline characteristics of the Rotterdam Study population are shown in the Figure and in Table 1. Alcohol intake and antithrombotic medication use were higher in 1997 and 2000 than in 1990, whereas median total cholesterol and non-HDL-cholesterol levels were much higher in 1990 than in 1997 and 2000. Lipid-lowering medication use was more common 1997 and 2000 than in 1990 (13.1% and 12.0% versus 2.4%).

Table 2 shows the associations between serum lipid levels and risk of intracerebral hemorrhage. As expected, decreasing levels of total serum cholesterol were associated with an increasing risk of intracerebral hemorrhage. This was particularly due to an inverse association between the non-HDL-cholesterol fraction and intracerebral hemorrhage. LDL-cholesterol was not associated with risk of intracerebral hemorrhage. Triglyceride levels showed a strong inverse association with risk of intracerebral hemorrhage, independently of age, sex, lipid-lowering medication use and multiple potential confounders. Stratification by lipid-lowering medication use did not reveal differences across strata, although numbers were small. Consequently, probability values for interaction were all non-significant (Table 3).

In Table 4 we present the independent effects of each of the lipid fractions on risk of intracerebral hemorrhage. The borderline association between HDL-cholesterol and intracerebral hemorrhage was strongly attenuated after adjustment for triglyceride levels. In contrast, the strong association between increasing triglyceride levels and decreasing risk of intracerebral hemorrhage was not affected by adjustments for HDL-cholesterol or LDL-cholesterol levels.

Associations between HDL-cholesterol, LDL-cholesterol, triglycerides, and presence of cerebral microbleeds are shown in Table 5. Serum triglyceride levels were strongly and inversely associated with the presence of deep or infratentorial microbleeds, but not with strictly lobar microbleeds. There was also a trend towards an inverse relationship between HDL-cholesterol and LDL-cholesterol and presence of deep or infratentorial microbleeds, but these associations were far from significant.
Table 1. Baseline characteristics of the Rotterdam Study population

<table>
<thead>
<tr>
<th></th>
<th>Rotterdam Study I</th>
<th>Rotterdam Study II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First Visit</td>
<td>Third Visit</td>
</tr>
<tr>
<td>(1990-1993)</td>
<td>N=6753</td>
<td>N=3458</td>
</tr>
<tr>
<td></td>
<td>First Visit</td>
<td>First Visit</td>
</tr>
<tr>
<td>(2000-2001)</td>
<td>N=2315</td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>68.1 (62.0-75.5)</td>
<td>71.0 (66.5-76.6)</td>
</tr>
<tr>
<td>Female sex, %</td>
<td>60.0</td>
<td>58.5</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>138 (123-153)</td>
<td>141 (128-156)</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>73 (66-81)</td>
<td>75 (68-82)</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>55.5</td>
<td>70.6</td>
</tr>
<tr>
<td>Blood pressure-lowering medication, %</td>
<td>31.5</td>
<td>39.5</td>
</tr>
<tr>
<td>Glucose level, mmol/L</td>
<td>6.2 (5.5-7.4)</td>
<td>5.6 (5.2-6.1)*</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>10.6</td>
<td>13.2</td>
</tr>
<tr>
<td>Insulin level, pmol/L</td>
<td>ND</td>
<td>66 (46-94)*</td>
</tr>
<tr>
<td>Current cigarette smoking, %</td>
<td>20.8</td>
<td>15.8</td>
</tr>
<tr>
<td>Alcohol intake, gram/day</td>
<td>3.4 (0.2-14.8)</td>
<td>4.3 (0.7-15.7)</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>25.9 (23.8-28.4)</td>
<td>26.4 (24.2-29.0)</td>
</tr>
<tr>
<td>Antithrombotic medication, %</td>
<td>4.6</td>
<td>22.6</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>6.6 (5.8-7.4)</td>
<td>5.8 (5.2-6.5)*</td>
</tr>
<tr>
<td>HDL-cholesterol, mmol/L</td>
<td>1.3 (1.1-1.6)</td>
<td>1.3 (1.1-1.6)*</td>
</tr>
<tr>
<td>Non-HDL-cholesterol, mmol/L</td>
<td>5.2 (4.4-6.0)</td>
<td>4.4 (3.8-5.1)*</td>
</tr>
<tr>
<td>LDL-cholesterol, mmol/L</td>
<td>ND</td>
<td>3.7 (3.2-4.3)*</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>ND</td>
<td>1.3 (1.0-1.8)*</td>
</tr>
<tr>
<td>Lipid-lowering medication, %</td>
<td>2.4</td>
<td>13.1</td>
</tr>
</tbody>
</table>

Values are medians (interquartile range) or percentages.
ND: not determined.
* Fasting serum levels.

Table 2. Associations between serum lipid levels and risk of intracerebral hemorrhage (Set 1 or Set 2)

<table>
<thead>
<tr>
<th></th>
<th>Population for analysis</th>
<th>At risk, N</th>
<th>Events, N</th>
<th>Model I HR (95% CI)</th>
<th>Model II HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>Set 1</td>
<td>9068</td>
<td>85</td>
<td>0.74 (0.58-0.94)</td>
<td>0.75 (0.59-0.95)</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>Set 1</td>
<td>9068</td>
<td>85</td>
<td>1.15 (0.96-1.38)</td>
<td>1.17 (0.98-1.39)</td>
</tr>
<tr>
<td>Non-HDL-cholesterol</td>
<td>Set 1</td>
<td>9068</td>
<td>85</td>
<td>0.71 (0.56-0.90)</td>
<td>0.71 (0.56-0.90)</td>
</tr>
<tr>
<td>LDL-cholesterol</td>
<td>Set 2</td>
<td>5773</td>
<td>45</td>
<td>0.96 (0.71-1.29)</td>
<td>0.96 (0.71-1.30)</td>
</tr>
<tr>
<td>Triglycerides*</td>
<td>Set 2</td>
<td>5773</td>
<td>45</td>
<td>0.69 (0.50-0.94)</td>
<td>0.62 (0.44-0.86)</td>
</tr>
</tbody>
</table>

Analyses based on Set 1 or Set 2 (Figure).
Hazard ratios are expressed per SD increase in lipid level.
* Triglyceride levels are natural log-transformed.
Model I: Adjusted for age, sex, lipid-lowering medication use and subcohort.
Model II: Adjusted for age, sex, and a propensity score of potential confounders (lipid-lowering medication use, systolic blood pressure, blood pressure-lowering medication use, diabetes mellitus, serum glucose level, current cigarette smoking, body mass index, antithrombotic medication use, alcohol intake and subcohort).
Note: HRs from Set 1 and Set 2 are not directly comparable because of different datasets and log-transformation of triglycerides.
Table 3. Associations between serum lipid levels and risk of intracerebral hemorrhage stratified by lipid-lowering medication use at baseline (Set 2)

<table>
<thead>
<tr>
<th></th>
<th>No lipid-lowering medication use</th>
<th>Lipid-lowering medication use</th>
<th>P interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>At risk, N</td>
<td>Events, N</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>5042</td>
<td>40</td>
<td>0.92 (0.67-1.28)</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>5042</td>
<td>40</td>
<td>1.21 (0.94-1.55)</td>
</tr>
<tr>
<td>Non-HDL-cholesterol</td>
<td>5042</td>
<td>40</td>
<td>0.85 (0.62-1.17)</td>
</tr>
<tr>
<td>LDL-cholesterol</td>
<td>5042</td>
<td>40</td>
<td>0.94 (0.68-1.28)</td>
</tr>
<tr>
<td>Triglycerides*</td>
<td>5042</td>
<td>40</td>
<td>0.65 (0.46-0.92)</td>
</tr>
</tbody>
</table>

Analyses are based on Set 2 (Figure).
Hazard ratios are expressed per SD increase in lipid level and are adjusted for age, sex, and a propensity score of potential confounders (systolic blood pressure, blood pressure-lowering medication use, diabetes mellitus, serum glucose level, serum insulin level, current cigarette smoking, body mass index, antithrombotic medication use, alcohol intake and subcohort).

* Triglyceride levels are natural log-transformed.
### Table 4. Associations between lipid levels and intracerebral hemorrhage, adjusted for the complementary lipid fractions (Set 2)

<table>
<thead>
<tr>
<th>Lipid Level</th>
<th>N (At risk)</th>
<th>N (Events)</th>
<th>HR (95% CI) Basic model</th>
<th>HR (95% CI) + LDL-C</th>
<th>HR (95% CI) + TG</th>
<th>HR (95% CI) + LDL-C &amp; TG</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HDL-cholesterol per SD</strong></td>
<td>5773</td>
<td>45</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quartile 1</td>
<td>1473</td>
<td>9</td>
<td>1.00 (ref)</td>
<td>1.00 (ref)</td>
<td>1.00 (ref)</td>
<td>1.00 (ref)</td>
</tr>
<tr>
<td>Quartile 2</td>
<td>1417</td>
<td>8</td>
<td>0.91 (0.35-2.39)</td>
<td>0.91 (0.35-2.38)</td>
<td>0.76 (0.29-2.03)</td>
<td>0.76 (0.28-2.01)</td>
</tr>
<tr>
<td>Quartile 3</td>
<td>1454</td>
<td>9</td>
<td>1.05 (0.40-2.73)</td>
<td>1.05 (0.40-2.73)</td>
<td>0.76 (0.28-2.07)</td>
<td>0.74 (0.27-2.03)</td>
</tr>
<tr>
<td>Quartile 4</td>
<td>1429</td>
<td>19</td>
<td>2.13 (0.89-5.11)</td>
<td>2.14 (0.89-5.15)</td>
<td>1.30 (0.49-3.46)</td>
<td>1.29 (0.48-3.45)</td>
</tr>
<tr>
<td><strong>LDL-cholesterol per SD</strong></td>
<td>5773</td>
<td>45</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.07 (0.78-1.46)</td>
<td></td>
<td></td>
<td>0.96 (0.71-1.30)</td>
<td>1.01 (0.74-1.37)</td>
<td>1.07 (0.78-1.46)</td>
<td></td>
</tr>
<tr>
<td>Quartile 1</td>
<td>1443</td>
<td>11</td>
<td>1.00 (ref)</td>
<td>1.00 (ref)</td>
<td>1.00 (ref)</td>
<td>1.00 (ref)</td>
</tr>
<tr>
<td>Quartile 2</td>
<td>1444</td>
<td>11</td>
<td>0.93 (0.40-2.16)</td>
<td>0.96 (0.41-2.24)</td>
<td>1.03 (0.44-2.44)</td>
<td>1.03 (0.44-2.41)</td>
</tr>
<tr>
<td>Quartile 3</td>
<td>1443</td>
<td>14</td>
<td>1.21 (0.54-2.71)</td>
<td>1.30 (0.58-2.93)</td>
<td>1.47 (0.64-3.33)</td>
<td>1.47 (0.65-3.33)</td>
</tr>
<tr>
<td>Quartile 4</td>
<td>1443</td>
<td>9</td>
<td>0.76 (0.31-1.89)</td>
<td>0.82 (0.33-2.04)</td>
<td>0.99 (0.39-2.52)</td>
<td>0.98 (0.39-2.50)</td>
</tr>
<tr>
<td><em><em>Triglycerides</em> per SD</em>*</td>
<td>5773</td>
<td>45</td>
<td>0.63 (0.46-0.88)</td>
<td>0.62 (0.44-0.87)</td>
<td>0.66 (0.46-0.95)</td>
<td>0.64 (0.44-0.94)</td>
</tr>
<tr>
<td>Quartile 1</td>
<td>1457</td>
<td>18</td>
<td>1.00 (ref)</td>
<td>1.00 (ref)</td>
<td>1.00 (ref)</td>
<td>1.00 (ref)</td>
</tr>
<tr>
<td>Quartile 2</td>
<td>1426</td>
<td>10</td>
<td>0.52 (0.24-1.14)</td>
<td>0.51 (0.23-1.12)</td>
<td>0.54 (0.25-1.20)</td>
<td>0.53 (0.24-1.17)</td>
</tr>
<tr>
<td>Quartile 3</td>
<td>1442</td>
<td>13</td>
<td>0.65 (0.31-1.33)</td>
<td>0.62 (0.29-1.31)</td>
<td>0.69 (0.32-1.50)</td>
<td>0.66 (0.30-1.47)</td>
</tr>
<tr>
<td>Quartile 4</td>
<td>1448</td>
<td>4</td>
<td>0.18 (0.06-0.57)</td>
<td>0.18 (0.06-0.56)</td>
<td>0.21 (0.06-0.71)</td>
<td>0.20 (0.06-0.69)</td>
</tr>
</tbody>
</table>

Analyses based on Set 2 (Figure).
* Triglyceride levels are natural log-transformed.

Basic model: adjusted for age, sex, and a propensity score of potential confounders (lipid-lowering medication use, systolic blood pressure, blood pressure-lowering medication use, diabetes mellitus, serum glucose level, serum insulin level, current cigarette smoking, body mass index, antithrombotic medication use, alcohol intake and subcohort).

HDL-C indicates HDL-cholesterol; LDL-C indicates LDL-cholesterol; TG indicates triglycerides.
Table 5. Lipid levels and cerebral microbleeds (Set 3)

<table>
<thead>
<tr>
<th>Lipid Level</th>
<th>Quartile 1</th>
<th>Quartile 2</th>
<th>Quartile 3</th>
<th>Quartile 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL-cholesterol per SD</td>
<td>724 97</td>
<td>724 97</td>
<td>724 97</td>
<td>724 97</td>
</tr>
<tr>
<td>Quartile 1</td>
<td>[0.4-1.1]</td>
<td>181 28</td>
<td>178 26</td>
<td>187 21</td>
</tr>
<tr>
<td>Quartile 2</td>
<td>[1.1-1.3]</td>
<td>180 23</td>
<td>172 20</td>
<td>182 16</td>
</tr>
<tr>
<td>Quartile 3</td>
<td>[1.3-1.6]</td>
<td>187 27</td>
<td>182 16</td>
<td>182 16</td>
</tr>
<tr>
<td>Quartile 4</td>
<td>[1.6-5.5]</td>
<td>185 19</td>
<td>181 19</td>
<td>182 16</td>
</tr>
</tbody>
</table>

| LDL-cholesterol per SD | 724 97 | 724 97 | 724 97 | 724 97 |
| Quartile 1 | [0.1-3.2] | 172 21 | 170 20 | 176 25 | 176 25 |
| Quartile 2 | [3.2-3.7] | 180 30 | 172 20 | 172 20 | 172 20 |
| Quartile 3 | [3.7-4.3] | 187 27 | 180 27 | 182 27 | 182 27 |
| Quartile 4 | [4.3-7.9] | 185 19 | 180 19 | 182 16 | 178 12 |

| Triglycerides* per SD | 724 97 | 724 97 | 724 97 | 724 97 |
| Quartile 1 | [0.4-1.0] | 174 22 | 174 22 | 174 22 | 174 22 |
| Quartile 2 | [1.0-1.3] | 181 23 | 173 23 | 177 23 | 177 23 |
| Quartile 3 | [1.3-1.8] | 182 27 | 180 27 | 182 27 | 182 27 |
| Quartile 4 | [1.8-4.3] | 187 25 | 181 25 | 182 25 | 182 25 |

Analyses based on Set 3 (Figure).
* Triglyceride levels are natural log transformed.
† With or without lobar microbleeds.
‡ Presence of strictly lobar microbleeds vs. absence of microbleeds.
§ Presence of deep or infratentorial microbleeds vs. absence of microbleeds.
Odds ratios are adjusted for age, sex, a propensity score (lipid-lowering medication use, systolic blood pressure, blood pressure-lowering medication use, diabetes mellitus, serum glucose level, serum insulin level, current cigarette smoking, body mass index, antithrombotic medication use and alcohol intake), and HDL-cholesterol, LDL-cholesterol and triglyceride levels when applicable.
DISCUSSION

In this prospective population-based cohort study among people aged 55 years or older who were free from stroke at baseline, we confirmed that serum total cholesterol levels were inversely associated with the risk of intracerebral hemorrhage. When investigating the various lipid fractions, we found that the association was due to a strong inverse relationship between triglyceride levels and risk of intracerebral hemorrhage, and not due to HDL-cholesterol or LDL-cholesterol levels. Similarly, we found an inverse association between triglyceride levels and the presence of cerebral microbleeds in the deep or infratentorial brain regions.

Strengths of this study include the prospective and population-based design, the large number of participants, and the long duration and completeness of follow-up. Furthermore, we were able to study lipid levels in association with both asymptomatic microbleeds and symptomatic intracerebral hemorrhage. However, the study also has limitations. We did not include 1368 participants in the analysis because of incomplete data on lipid levels. These participants were older (median age, 73 versus 66 years), more often female (66% versus 59%), and more likely to have cardiovascular risk factors. It is possible that exclusion of these participants introduced a selection bias that could have affected the estimates. Loss to follow-up, another potential source of selection bias, was only 1.6%. Another issue is that 36% of strokes were classified as “unspecified” because neuroimaging had not been performed, which is similar to unspecified stroke rates reported in other population-based or even hospital-based studies. Therefore it is likely that an unknown number of intracerebral hemorrhages were misclassified as unspecified. Apart from conventional stroke risk factors, major determinants of unspecified stroke risk are older age, living in a nursing home and dementia prior to stroke. Although this misclassification was independent of exposure measurement and therefore non-differential, it may have resulted in an underestimation of the true associations between lipid levels and intracerebral hemorrhage. However, because we observed very similar patterns between lipid levels and cerebral microbleeds, we think that misclassification, if any, has not importantly influenced our results. In addition, differences were observed in cholesterol levels, use of lipid-lowering medication and some other covariates between the sets of 1990, 1997 and 2000. These differences are likely to be explained by time-effects between successive follow-up visits. Given that we adjusted for these variables
in the analyses, these differences probably did not have a strong impact on the results. MRI scans were carried out approximately 5 years after blood samples were drawn. However, previous studies have shown that, once present, cerebral microbleeds rarely disappear.38

We found that low triglyceride levels are associated with an increased risk of intracerebral hemorrhage, independently of lipid-lowering medication use, levels of LDL-cholesterol and HDL-cholesterol, and other potential confounders. This finding is in agreement with results from the Three-City Study, which reported a similar inverse association between low triglyceride levels and intracerebral hemorrhage,23 and with results from a pooled cohort study among ARIC and CHS participants.19 However, three other studies did not detect an association between triglyceride levels and intracerebral hemorrhage.14-16 Analyses of the Copenhagen Heart Study and Oslo Study were based on non-fasting triglyceride levels and included only few events.14-15 Furthermore, results of the Oslo Study were based on 21-years of follow-up, which may have diluted the effect.15 The lack of an association observed by the Japan Lipid Intervention Trial could be due to the fact that they only included hypercholesterolemic patients with relatively high triglyceride levels.16

Although the mechanism of the association between triglyceride levels and intracerebral hemorrhage is unknown, there are some possible explanations. Several studies have suggested that high triglyceride levels favor a prothrombotic state because they are positively correlated with the vitamin K-dependent coagulation factors VII and IX, and with plasminogen activator inhibitor and blood viscosity.39 Likewise, one could hypothesize that low triglyceride levels may result in a prohemorrhagic state. Another possible explanation is that low triglyceride levels may contribute to weakness of the vascular endothelium. Cholesterol and fatty acids are essential elements of all cell membranes. In vitro studies have shown that low cholesterol levels result in increased permeability of erythrocyte membranes,4 and animal studies reported that low cholesterol levels cause smooth muscle degeneration and endothelial weakness in small intracerebral arteries.18 Therefore it has been hypothesized that very low cholesterol levels may contribute to the development of a fragile endothelium, prone to leakage and rupture.5 However, whether any of these perspectives explain the observed association between low triglyceride levels and the risk of intracerebral hemorrhage remains uncertain and requires further investigation. We also cannot
exclude the possibility of residual confounding by unmeasured determinants, for example diet or physical activity, or due to the fact that lipid levels and confounders were measured only once. Therefore, studies using time-varying analyses are needed to explore whether intraindividual fluctuations in lipid levels and confounders influence the results.

We further found a comparable inverse association between triglyceride levels and presence of cerebral microbleeds, which provides accumulating support for a parallel between asymptomatic microbleeds and symptomatic intracerebral hemorrhage. However, although not significant, associations of HDL-cholesterol and LDL-cholesterol with cerebral microbleeds seemed somewhat different from the associations with intracerebral hemorrhage. This may indicate that intracerebral hemorrhage and cerebral microbleeds are reflections of a different stage of arteriolosclerosis. Moreover, we cannot fully rule out the possibility that intracerebral hemorrhage and microbleeds do not completely share the same underlying pathology.

Our finding that triglycerides are related to deep or infratentorial microbleeds rather than lobar microbleeds may provide etiologic clues for the association between triglycerides and intracerebral hemorrhage. In a previous study, we showed that lobar microbleeds are indicative of underlying amyloid angiopathy, whereas deep or infratentorial microbleeds are associated with known risk factors for arteriolosclerosis. The association between triglycerides and deep or infratentorial microbleeds but not lobar microbleeds underscores these differences in underlying pathology and is suggestive for a role of triglyceride levels through development of arteriolosclerotic microangiopathy.

To conclude, in this large population-based cohort study among elderly people we found that low serum triglyceride levels were associated both with an increased risk of intracerebral hemorrhage as well as with the presence of deep or infratentorial cerebral microbleeds. This finding provides novel insights into the role of lipid metabolism in the etiology of intracerebral hemorrhage. Though the exact mechanism of the association remains unclear, triglyceride levels may aid in the identification of people at risk for intracerebral hemorrhage.
REFERENCES


Cerebral microbleeds are associated with worse cognitive function

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ABSTRACT

Background – Cerebral microbleeds are frequently found in the general elderly population and may reflect underlying vascular disease, but their role in cognitive function is unknown.

Methods – We investigated the association between cerebral microbleeds and performance in multiple cognitive domains in 3979 non-demented persons (mean age, 60.3 years). MMSE-score and neuropsychological tests were used to assess global cognition and the following cognitive domains: memory, information processing speed, executive function and motor speed. We used number of microbleeds as continuous variable, and additionally distinguished between persons with no microbleeds, 1 microbleed, 2-4 microbleeds, and ≥5 microbleeds. The association of microbleeds with different cognitive domains was estimated using linear regression models. Additional adjustments were made for vascular risk factors, brain atrophy and other imaging markers of cerebral small vessel disease. We stratified analyses by location of microbleeds.

Results – A higher number of microbleeds was associated with lower MMSE-score and worse performance on tests of information processing speed and motor speed. When analyzed per category, presence of 5 or more microbleeds was associated with worse performance in all cognitive domains, except memory. These associations were most robust in participants with strictly lobar microbleeds, whereas after additional adjustments associations disappeared for deep or infratentorial microbleeds.

Conclusions – Presence of numerous microbleeds, especially in a strictly lobar location, is associated with worse performance on tests measuring cognitive function, even after adjustments for vascular risk factors and other imaging markers of small vessel disease. These results suggest an independent role for microbleed-associated vasculopathy in cognitive impairment.
INTRODUCTION

Vascular pathology plays a prominent role in cognitive decline and dementia.\(^1\)\(^-\)\(^2\) Lacunar infarcts and white matter lesions, both markers of cerebral small vessel disease, are important contributors to this relation.\(^3\) In recent years, cerebral microbleeds (CMBs), detected by susceptible MRI sequences, have been recognized as an additional marker of cerebral small vessel disease. CMBs are reported to be highly prevalent in memory clinic patients and patients with Alzheimer’s disease.\(^4\)\(^-\)\(^5\) Moreover, we have previously shown that microbleeds are also very common in the general elderly population.\(^6\)

Different hypotheses exist about how CMBs may influence cognitive function. Microbleeds may reflect focal damage of brain tissue and when located in strategic areas could interfere with cognitive processes.\(^7\) On the other hand, CMBs could also be a more general marker for underlying vascular disease, in particular cerebral amyloid angiopathy (CAA) or hypertensive arteriolosclerosis,\(^6\)\(^,\)\(^8\) and as such may influence cognition.\(^7\)

The majority of clinical studies did not demonstrate an association between the presence of microbleeds and cognitive function, but all were based on small sample sizes.\(^4\)\(^-\)\(^5\),\(^9\)\(^-\)\(^11\) Studies investigating this relation in community-dwelling elderly are scarce.\(^12\)\(^-\)\(^14\) Moreover, most studies did not distinguish between different cognitive domains, and did not differentiate between different underlying vascular pathologies.

In a large sample of non-demented persons from the general population, we examined how the presence and location of microbleeds related to various domains of cognitive function. In addition, we investigated whether these associations were independent of vascular risk factors, brain atrophy and markers of cerebral small vessel disease.
METHODS

Participants
The study is based on the Rotterdam Scan Study, an ongoing population-based cohort study investigating age-related brain changes on MRI. At the time of the present study, we had invited a total of 4898 participants. We excluded individuals who were demented (N=30) or had MRI contraindications (N=389). Of 4479 eligible persons, 4082 (91%) participated. Due to physical inabilities, imaging could not be performed in 44 individuals. Of 4038 persons with complete MRI examinations, 59 had to be excluded because of motion artifacts or susceptibility artefacts on their scans, leaving 3979 persons to be analyzed.

Standard Protocol Approvals, Registrations, and Patient Consents
The institutional review board approved the study, and written informed consent was obtained from all participants.

Brain MRI
We performed a multisequence MRI protocol on a 1.5-T scanner (GE Healthcare, Milwaukee, WI). A custom-made accelerated three-dimensional T2*-weighted gradient-recalled echo (3D T2* GRE) sequence with high spatial resolution and long echo time was used for microbleed detection. The other sequences in the imaging protocol consisted of 3 high-resolution axial scans, i.e., a T1-weighted sequence, a proton density-weighted sequence, and a fluid-attenuated inversion recovery (FLAIR) sequence.

Analyses of Brain MRI
All 3D T2* GRE scans were reviewed by 1 of 5 trained raters who recorded the presence, number, and location of microbleeds. All raters were blinded to the clinical data, and APOE genotyping. Microbleeds were defined as focal areas of very low signal intensity. Signal voids caused by sulcal vessels, symmetric calcifications in the basal ganglia, choroid plexus, and pineal calcifications, and signal averaging from bone were excluded. Intraobserver (N=500; 1 rater) and interobserver (N=300) kappa coefficients were κ=0.87 and κ=0.85, which corresponds to very good agreement. CMBs were categorized into 1 of 3 locations: lobar, deep, and infratentorial.
Lacunar and cortical infarcts were rated on FLAIR, proton density-weighted and T1-weighted sequences by the same raters who had scored cerebral microbleeds according to criteria described previously. Tissue classification into cerebrospinal fluid, grey matter, normal white matter, and white matter lesions was done with a validated fully-automated tissue classification technique. Brain tissue volumes were calculated by summing all voxels of a certain tissue across the whole brain. Brain atrophy was defined as total brain tissue volume expressed as percentage of intracranial volume.

**Cognitive Function**

Cognitive testing was performed at the preceding regular visit of study participants to the research center. Mean interval between cognitive testing and brain MRI was 4.0 months ± 5.7 (standard deviation). The neuropsychological test battery included the Mini Mental State Examination (MMSE), a 15-Word Verbal Learning Test (15-WLT) based on Rey’s recall of words, the Stroop test, the Letter-Digit Substitution Task (LDST), the Purdue Pegboard test, and a Word Fluency test. We generated Z-scores (individual test score minus mean test score divided by the standard deviation) for each cognitive test, except for MMSE. To obtain more robust measures, we constructed compound scores for information-processing speed, executive function, memory, global cognitive function and motor speed. The Z-scores for the Stroop tasks were inverted for use in these compound scores, as higher scores on the Stroop task indicate a worse performance whilst higher scores on all other tests indicate a better cognitive function. The compound score for memory was the average of the Z-scores for the immediate and delayed recall of the 15-WLT. Executive function was constructed by averaging the Z-scores for the Stroop interference subtask, the LDST and the Word Fluency Test. Information processing speed was the average of the Z-scores for the Stroop reading and Stroop color naming test and the LDST. For global cognitive function we used the average of the Z-scores of the Stroop task (average of all 3 subtasks), the LDST, the Word Fluency test, and the immediate and delayed recall of the 15-WLT. Motor speed was defined by the Z-score for the Purdue Pegboard test (both hands).

**Assessment of Covariates**

During the initial interview at study entry the attained level of education was assessed according to the standard classification of education. In our analysis, we used seven levels of education: 1) primary education; 2) low-level vocational training;
3) medium-level secondary education; 4) medium-level vocational education; 5) general secondary education; 6) higher-level vocational education; and 7) university-level education.

Cardiovascular risk factors were examined by interview, and laboratory and physical examination as previously described. Risk factors included in our analyses were systolic and diastolic blood pressure, smoking, diabetes, and serum total cholesterol. The use of lipid-lowering drugs and blood pressure-lowering medication was assessed by interview and house visits during which medication use was registered.

APOE genotyping was performed on coded genomic DNA samples, and was available for 3689 participants (93%). The distributions of APOE genotype and allele frequencies in this population were in Hardy-Weinberg equilibrium.

**Data Analysis**

As number of microbleeds may be a marker of the severity of the underlying disease, we investigated the association of number of microbleeds continuously per standard deviation (SD) increase with cognitive function. Furthermore, as microbleeds numbers were highly skewed to the left, we additionally categorized the numbers of microbleeds as: no microbleeds, 1 microbleed, 2-4 microbleeds and 5 or more microbleeds per person.

The association between microbleeds and cognitive function was assessed using linear regression models, with microbleeds as independent and cognitive compound scores as dependent variable. All analyses were adjusted for age, sex and level of education. Additional adjustments were made for vascular risk factors (i.e., systolic blood pressure, diastolic blood pressure, use of blood pressure-lowering medication, smoking, diabetes, serum total cholesterol, and use of lipid-lowering drugs). To elucidate whether the association of CMBs with cognitive performance is independent of brain atrophy and other imaging markers of small vessel disease, further analyses were also adjusted for brain atrophy, white matter lesion volume and presence of lacunar infarcts. White matter lesion volume was natural log transformed because of skewness of the untransformed measure.
Analyses were also performed by strata defined by microbleed location (i.e., strictly lobar microbleeds versus deep or infratentorial microbleeds [with or without additional lobar microbleeds]). Additionally, we performed analysis stratified according to APOE genotype to investigate whether the association between CMBs and cognition differed between APOE ε4-carriers and non-carriers. Finally, we repeated all analyses after exclusion of participants with cortical infarcts on MRI. All analyses were performed using the statistical package SPSS 17.0 for Windows.

**RESULTS**

Table 1 shows the characteristics of all participants. Mean age was 60.3 years, and 2164 (54.4%) were women. A total of 609 of 3979 (15.3%) had 1 or more microbleeds on MRI; 395 (64.9%) persons had 1 CMB, 143 (23.5%) had 2-4 CMBs and 71 (11.7%) had 5 or more CMBs. Of those with microbleeds, 413 (67.8%) had CMBs in a strictly lobar location. Mean MMSE score was 28.0 ± 1.8.

Table 2 shows results for the association between microbleeds and performance on cognitive tests. Per SD increase, a higher microbleed number was significantly associated with lower MMSE-score and worse performance on tests of information processing speed and motor speed. When analyzed per category, presence of numerous (≥5) CMBs was significantly associated with lower MMSE score and with worse performance on tests of information processing speed, executive function, global cognition and motor speed, but not with memory performance (model 1 in Table 2). Additional adjustment for vascular risk factors did not change these results (model 2 in Table 2). Statistical significance remained for the association between (numerous) microbleeds and worse performance on information processing speed and motor speed upon correcting for brain atrophy, white matter lesion volume and lacunar infarcts (model 3 in Table 2).

When participants were subdivided into those with strictly lobar CMBs and those with deep or infratentorial CMBs, we found strong associations between strictly lobar microbleeds and information processing speed, and additionally for MMSE-score and motor speed when numerous microbleeds were present (model 1 in Table 3). When additionally adjusting for vascular risk factors, brain atrophy and other imaging
markers of small vessel disease, the associations attenuated marginally, but number of strictly lobar CMBs per SD increase was still related to information processing speed, whereas presence of 5 or more lobar CMBs remained associated with information processing speed as well as motor speed (model 3 in Table 3). In persons with deep or infratentorial microbleeds (with or without additional lobar microbleeds), a higher number of microbleeds was associated with lower MMSE score and worse performance on tests of motor speed, whereas presence of 5 or more microbleeds was related to worse information processing speed, executive function, global cognition and motor speed, even after adjusting for vascular risk factors (model 1 and 2 in Table 4). However, these associations disappeared after additional adjustments for brain atrophy and other imaging markers of small vessel disease; only the association between number of microbleeds per SD increase and motor speed remained significant (model 3 in Table 4).

After stratifying the study population for the presence of an APOE ε4 allele, we found that presence of 5 or more microbleeds was associated with MMSE score, information processing speed, executive function and global cognitive function in APOE ε4 non-carriers, but we found no associations between microbleeds and cognitive function in APOE ε4 carriers. (Table 5)

Repeating all analyses after excluding persons with a cortical infarct on MRI did not change any of the associations (data not shown).
<table>
<thead>
<tr>
<th></th>
<th>N=3979</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Table 1. Characteristics of the study population</strong></td>
<td></td>
</tr>
<tr>
<td>Age, years, mean ± SD</td>
<td>60.3 ± 8.7</td>
</tr>
<tr>
<td>Women, N (%)</td>
<td>2164 (54.4)</td>
</tr>
<tr>
<td>Highest education obtained, N (%)</td>
<td></td>
</tr>
<tr>
<td>Primary education</td>
<td>373 (9.5)</td>
</tr>
<tr>
<td>Low level vocational training</td>
<td>709 (18.1)</td>
</tr>
<tr>
<td>Medium level secondary education</td>
<td>715 (18.2)</td>
</tr>
<tr>
<td>Medium level vocational education</td>
<td>937 (23.9)</td>
</tr>
<tr>
<td>General secondary education</td>
<td>768 (19.6)</td>
</tr>
<tr>
<td>Higher level vocational education</td>
<td>212 (5.4)</td>
</tr>
<tr>
<td>University level education</td>
<td>212 (5.4)</td>
</tr>
<tr>
<td>Cognitive test results*, mean ± SD</td>
<td></td>
</tr>
<tr>
<td>Mini mental state examination (score)</td>
<td>28.0 ± 1.8</td>
</tr>
<tr>
<td>Letter-digit substitution task (correct answers)</td>
<td>31.4 ± 6.9</td>
</tr>
<tr>
<td>Word fluency test (correct answers)</td>
<td>23.2 ± 6.0</td>
</tr>
<tr>
<td>Stroop reading subtask (time in seconds)</td>
<td>16.7 ± 3.9</td>
</tr>
<tr>
<td>Stroop color naming subtask (time in seconds)</td>
<td>22.7 ± 5.4</td>
</tr>
<tr>
<td>Stroop interference subtask (time in seconds)</td>
<td>45.2 ± 15.7</td>
</tr>
<tr>
<td>15-Word verbal learning test, immediate recall trial 1t/m3 (correct answers)</td>
<td>23.7 ± 10.1</td>
</tr>
<tr>
<td>15-Word verbal learning test, delayed recall (correct answers)</td>
<td>7.8 ± 3.0</td>
</tr>
<tr>
<td>Purdue pegboard test</td>
<td>10.7 ± 1.9</td>
</tr>
<tr>
<td>APOE ε2 allele carrier, N (%)</td>
<td>548 (13.8)</td>
</tr>
<tr>
<td>APOE ε4 allele carrier, N (%)</td>
<td>1078 (27.1)</td>
</tr>
<tr>
<td>Brain atrophy, total brain volume as % of intra-cranial volume, mean ± SD</td>
<td>83.9 ± 3.6</td>
</tr>
<tr>
<td>White matter lesions, mL, median (interquartile range)</td>
<td>2.5 (1.5-4.5)</td>
</tr>
<tr>
<td>Lacunar infarct on MRI, N (%)</td>
<td>212 (5.3)</td>
</tr>
<tr>
<td>Cortical infarct on MRI, N (%)</td>
<td>111 (2.8)</td>
</tr>
</tbody>
</table>

Data are missing for education (N=53), APOE genotype (N=290), brain atrophy (N=66), and white matter lesions (N=45).

* Cognitive test results were available for Mini mental state examination in N=3959, Letter-digit substitution task in N=3903, Word fluency test in N=3912, Stroop task in N=3673, 15-Word learning test immediate recall in N=3712, 15-Word learning test delayed recall in N=3705, and Purdue pegboard test in N=3846.
Table 2. Association of categories of microbleeds with cognitive function using linear regression models

<table>
<thead>
<tr>
<th></th>
<th>MMSE</th>
<th>Z-score Information</th>
<th>Z-score Processing Speed</th>
<th>Z-score Executive Function</th>
<th>Z-score Global Cognitive Function</th>
<th>Z-score Motor Function</th>
<th>Z-score Speed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of microbleeds, per SD increase, Model I</td>
<td>-0.06 (-0.12;-0.01)</td>
<td>-0.01 (-0.04;0.02)</td>
<td>-0.04 (-0.07;-0.01)</td>
<td>-0.02 (-0.04;0.01)</td>
<td>-0.02 (-0.04;0.00)</td>
<td>-0.07 (-0.10;-0.04)</td>
<td></td>
</tr>
<tr>
<td>1 microbleed (N=395), versus none, Model I</td>
<td>0.04 (-0.15;0.22)</td>
<td>0.02 (-0.07;0.11)</td>
<td>0.04 (-0.04;0.12)</td>
<td>0.02 (-0.06;0.09)</td>
<td>0.02 (-0.05;0.08)</td>
<td>0.11 (0.02;0.20)</td>
<td></td>
</tr>
<tr>
<td>2-4 microbleeds (N=143), versus none, Model I</td>
<td>-0.22 (-0.52;0.07)</td>
<td>-0.04 (-0.18;0.10)</td>
<td>-0.01 (-0.14;0.12)</td>
<td>0.01 (-0.11;0.12)</td>
<td>-0.02 (-0.12;0.08)</td>
<td>-0.05 (-0.20;0.09)</td>
<td></td>
</tr>
<tr>
<td>≥5 microbleeds (N=71), versus none, Model I</td>
<td>-0.54 (-0.95;-0.12)</td>
<td>-0.09 (-0.29;0.11)</td>
<td>-0.46 (-0.66;-0.27)</td>
<td>-0.26 (-0.43;-0.08)</td>
<td>-0.24 (-0.39;-0.08)</td>
<td>-0.42 (-0.63;-0.20)</td>
<td></td>
</tr>
</tbody>
</table>

| Number of microbleeds, per SD increase, Model II| -0.06 (-0.12;-0.01) | -0.01 (-0.04;0.02)  | -0.04 (-0.06;-0.01)     | -0.02 (-0.04;0.01)          | -0.02 (-0.04;0.01)               | -0.06 (-0.10;-0.03)    |
| 1 microbleed, versus none, Model II            | 0.03 (-0.15;0.22)  | 0.02 (-0.07;0.11)   | 0.04 (-0.04;0.12)        | 0.02 (-0.06;0.09)           | 0.02 (-0.05;0.08)               | 0.11 (0.02;0.20)       |
| 2-4 microbleeds, versus none, Model II         | -0.22 (-0.51;0.08) | -0.04 (-0.17;0.10)  | -0.01 (-0.14;0.12)       | 0.01 (-0.10;0.13)           | -0.02 (-0.12;0.09)               | -0.04 (-0.19;0.11)     |
| ≥5 microbleeds, versus none, Model II          | -0.51 (-0.92;-0.09) | -0.08 (-0.28;0.12)  | -0.43 (-0.63;-0.24)      | -0.23 (-0.40;-0.05)         | -0.22 (-0.37;-0.06)              | -0.37 (-0.58;-0.16)    |

| Number of microbleeds, per SD increase, Model III| -0.05 (-0.10;0.01) | -0.002 (-0.03;0.03)  | -0.03 (-0.06;-0.01)     | -0.01 (-0.04;0.01)          | -0.01 (-0.03;0.01)               | -0.05 (-0.09;-0.02)    |
| 1 microbleed, versus none, Model III           | 0.03 (-0.15;0.21)  | 0.03 (-0.06;0.11)   | 0.03 (-0.05;0.11)        | 0.02 (-0.06;0.09)           | 0.02 (-0.05;0.08)               | 0.11 (0.02;0.20)       |
| 2-4 microbleeds, versus none, Model III        | -0.19 (-0.49;0.11) | -0.02 (-0.16;0.12)  | 0.01 (-0.12;0.13)        | 0.03 (-0.08;0.15)           | 0.00 (-0.10;0.11)               | -0.02 (-0.16;0.13)     |
| ≥5 microbleeds, versus none, Model III         | -0.40 (-0.82;0.03) | -0.03 (-0.23;0.18)  | -0.38 (-0.58;-0.19)      | -0.16 (-0.34;0.02)          | -0.16 (-0.31;0.00)              | -0.29 (-0.50;-0.07)    |

Model I: adjusted for age, sex and level of education.
Model II: additionally adjusted for systolic blood pressure, diastolic blood pressure, use of blood pressure-lowering medication, smoking, diabetes, total cholesterol, and use of lipid-lowering drugs.
Model III: additionally adjusted for brain atrophy, white matter lesion volume and lacunar infarcts.

Values represent difference in Z-scores (or for MMSE, difference in absolute score) for number of microbleeds per standard deviation (SD) increase or for each category of microbleed number. Significant values are bolded.
Table 3. Association of categories of strictly lobar microbleeds with cognitive function using linear regression models

<table>
<thead>
<tr>
<th>Number of strictly lobar microbleeds, per SD increase, Model I</th>
<th>Difference in test scores (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 strictly lobar microbleed (N=308), versus none, Model I</td>
<td>-0.02 (-0.08;0.03)</td>
</tr>
<tr>
<td>2-4 strictly lobar microbleeds (N=83), versus none, Model I</td>
<td>-0.32 (-0.70;0.06)</td>
</tr>
<tr>
<td>≥5 strictly lobar microbleeds (N=22), versus none, Model I</td>
<td>-0.78 (-1.52;0.05)</td>
</tr>
<tr>
<td>Number of strictly lobar microbleeds, per SD increase, Model II</td>
<td></td>
</tr>
<tr>
<td>1 strictly lobar microbleed, versus none, Model II</td>
<td>-0.02 (-0.08;0.01)</td>
</tr>
<tr>
<td>2-4 strictly lobar microbleeds, versus none, Model II</td>
<td>-0.32 (-0.70;0.06)</td>
</tr>
<tr>
<td>≥5 strictly lobar microbleeds, versus none, Model II</td>
<td>-0.75 (-1.48;0.01)</td>
</tr>
<tr>
<td>Number of strictly lobar microbleeds, per SD increase, Model III</td>
<td></td>
</tr>
<tr>
<td>1 strictly lobar microbleed, versus none, Model III</td>
<td>-0.02 (-0.08;0.04)</td>
</tr>
<tr>
<td>2-4 strictly lobar microbleeds, versus none, Model III</td>
<td>-0.30 (-0.68;0.08)</td>
</tr>
<tr>
<td>≥5 strictly lobar microbleeds, versus none, Model III</td>
<td>-0.68 (-1.42;0.05)</td>
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</table>

<table>
<thead>
<tr>
<th>MMSE</th>
<th>Z-score Information</th>
<th>Z-score Executive</th>
<th>Z-score Global Cognitive</th>
<th>Z-score Motor Function</th>
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<tbody>
<tr>
<td>Memory</td>
<td>Processing Speed</td>
<td>Function</td>
<td>Function</td>
<td>Speed</td>
</tr>
<tr>
<td>-0.02 (-0.08;0.03)</td>
<td>-0.001 (-0.03;0.02)</td>
<td>-0.04 (-0.07;-0.02)</td>
<td>-0.01 (-0.03;0.02)</td>
<td>-0.01 (-0.03;0.01)</td>
</tr>
<tr>
<td>-0.02 (-0.23;0.18)</td>
<td>0.01 (-0.09;0.11)</td>
<td>0.01 (-0.08;0.09)</td>
<td>-0.01 (-0.09;0.08)</td>
<td>0.00 (-0.07;0.07)</td>
</tr>
<tr>
<td>-0.32 (-0.70;0.06)</td>
<td>-0.06 (-0.24;0.11)</td>
<td>-0.13 (-0.29;0.03)</td>
<td>-0.02 (-0.17;0.12)</td>
<td>-0.05 (-0.18;0.08)</td>
</tr>
<tr>
<td>-0.78 (-1.52;0.05)</td>
<td>0.04 (-0.31;0.38)</td>
<td>-0.80 (-1.15;-0.45)</td>
<td>-0.22 (-0.53;0.10)</td>
<td>-0.22 (-0.49;0.06)</td>
</tr>
</tbody>
</table>

Number of strictly lobar microbleeds, per SD increase, Model III

| 1 strictly lobar microbleed, versus none, Model III | -0.02 (-0.08;0.04) | 0.00 (-0.03;0.03) | -0.04 (-0.07;0.02) | -0.01 (-0.03;0.02) | -0.01 (-0.03;0.01) | -0.04 (-0.09;0.01) |
| 2-4 strictly lobar microbleeds, versus none, Model III | -0.04 (-0.24;0.17) | 0.01 (-0.08;0.11) | -0.01 (-0.09;0.08) | -0.01 (-0.09;0.07) | 0.00 (-0.08;0.07) | 0.06 (-0.04;0.16) |
| ≥5 strictly lobar microbleeds, versus none, Model III | -0.30 (-0.68;0.08) | -0.05 (-0.23;0.13) | -0.13 (-0.29;0.03) | -0.02 (-0.16;0.13) | -0.04 (-0.17;0.09) | -0.04 (-0.23;0.15) |
| ≥5 strictly lobar microbleeds, versus none, Model III | -0.68 (-1.42;0.05) | 0.08 (-0.26;0.43) | -0.79 (-1.13;-0.45) | -0.19 (-0.50;0.12) | -0.19 (-0.46;0.09) | -0.39 (-0.77;-0.01) |

Model I: adjusted for age, sex and level of education.
Model II: additionally adjusted for systolic blood pressure, diastolic blood pressure, use of blood pressure-lowering medication, smoking, diabetes, total cholesterol, and use of lipid-lowering drugs.
Model III: additionally adjusted for brain atrophy, white matter lesion volume and lacunar infarcts.
Values represent difference in Z-scores (or for MMSE, difference in absolute score) for number of microbleeds per standard deviation (SD) increase or for each category of microbleed number. Significant values are bolded.
Table 4. Association of categories of deep or infratentorial microbleeds with cognitive function using linear regression models

<table>
<thead>
<tr>
<th>Number of deep or infratentorial microbleeds, per SD increase, Model I</th>
<th>MMSE</th>
<th>Z-score Information</th>
<th>Z-score Processing Speed</th>
<th>Z-score Executive Function</th>
<th>Z-score Global Cognitive Function</th>
<th>Z-score Motor Function</th>
<th>Z-score Motor Speed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 deep or infratentorial microbleed (N=87), versus none, Model I</td>
<td>-0.07 (-0.13; -0.01)</td>
<td>-0.01 (-0.04; 0.02)</td>
<td>-0.02 (-0.05; 0.01)</td>
<td>-0.02 (-0.05; 0.01)</td>
<td>-0.02 (-0.04; 0.01)</td>
<td>-0.06 (-0.09; -0.03)</td>
<td></td>
</tr>
<tr>
<td>2-4 deep or infratentorial microbleeds (N=60), versus none, Model I</td>
<td>0.23 (-0.14; 0.61)</td>
<td>0.05 (-0.12; 0.23)</td>
<td>0.17 (0.01; 0.33)</td>
<td>0.11 (-0.04; 0.25)</td>
<td>0.09 (-0.05; 0.22)</td>
<td>0.29 (0.11; 0.48)</td>
<td></td>
</tr>
<tr>
<td>≥5 deep or infratentorial microbleeds (N=49), versus none, Model I</td>
<td>-0.11 (-0.56; 0.34)</td>
<td>-0.01 (-0.22; 0.20)</td>
<td>0.14 (-0.06; 0.34)</td>
<td>0.05 (-0.13; 0.23)</td>
<td>0.02 (-0.14; 0.18)</td>
<td>-0.07 (-0.29; 0.16)</td>
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<table>
<thead>
<tr>
<th>Number of deep or infratentorial microbleeds, per SD increase, Model II</th>
<th>MMSE</th>
<th>Z-score Information</th>
<th>Z-score Processing Speed</th>
<th>Z-score Executive Function</th>
<th>Z-score Global Cognitive Function</th>
<th>Z-score Motor Function</th>
<th>Z-score Motor Speed</th>
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<tbody>
<tr>
<td>1 deep or infratentorial microbleed, versus none, Model II</td>
<td>0.24 (-0.14; 0.61)</td>
<td>0.06 (-0.12; 0.23)</td>
<td>0.17 (0.00; 0.33)</td>
<td>0.11 (-0.04; 0.26)</td>
<td>0.09 (-0.04; 0.22)</td>
<td>0.31 (0.13; 0.50)</td>
<td></td>
</tr>
<tr>
<td>2-4 deep or infratentorial microbleeds, versus none, Model II</td>
<td>0.10 (-0.55; 0.35)</td>
<td>0.00 (-0.21; 0.21)</td>
<td>0.16 (-0.04; 0.36)</td>
<td>0.07 (-0.11; 0.25)</td>
<td>-0.03 (-0.13; 0.19)</td>
<td>-0.04 (-0.26; 0.18)</td>
<td></td>
</tr>
<tr>
<td>≥5 deep or infratentorial microbleeds, versus none, Model II</td>
<td>-0.40 (-0.89; 0.10)</td>
<td>-0.14 (-0.39; 0.10)</td>
<td>-0.27 (-0.50; -0.04)</td>
<td>-0.23 (-0.44; -0.02)</td>
<td>-0.22 (-0.40; -0.03)</td>
<td>-0.35 (-0.60; -0.10)</td>
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<table>
<thead>
<tr>
<th>Number of deep or infratentorial microbleeds, per SD increase, Model III</th>
<th>MMSE</th>
<th>Z-score Information</th>
<th>Z-score Processing Speed</th>
<th>Z-score Executive Function</th>
<th>Z-score Global Cognitive Function</th>
<th>Z-score Motor Function</th>
<th>Z-score Motor Speed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 deep or infratentorial microbleed, versus none, Model III</td>
<td>-0.05 (-0.11; 0.01)</td>
<td>-0.001 (-0.03; 0.03)</td>
<td>-0.01 (-0.04; 0.02)</td>
<td>-0.01 (-0.04; 0.02)</td>
<td>-0.01 (-0.03; 0.02)</td>
<td>-0.04 (-0.08; -0.01)</td>
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<tr>
<td>2-4 deep or infratentorial microbleeds, versus none, Model III</td>
<td>0.25 (-0.13; 0.62)</td>
<td>0.07 (-0.11; 0.25)</td>
<td>0.17 (0.01; 0.33)</td>
<td>0.12 (-0.03; 0.26)</td>
<td>0.10 (-0.03; 0.23)</td>
<td>0.32 (0.14; 0.51)</td>
<td></td>
</tr>
<tr>
<td>≥5 deep or infratentorial microbleeds, versus none, Model III</td>
<td>-0.05 (-0.50; 0.40)</td>
<td>0.02 (-0.18; 0.23)</td>
<td>0.20 (0.00; 0.39)</td>
<td>0.11 (-0.07; 0.28)</td>
<td>0.07 (-0.09; 0.22)</td>
<td>0.01 (-0.21; 0.23)</td>
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Model I: adjusted for age, sex and level of education.
Model II: additionally adjusted for systolic blood pressure, diastolic blood pressure, use of blood pressure-lowering medication, smoking, diabetes, total cholesterol, and use of lipid-lowering drugs.
Model III: additionally adjusted for brain atrophy, white matter lesion volume and lacunar infarcts.
Values represent difference in Z-scores (or for MMSE, difference in absolute score) for number of microbleeds per standard deviation (SD) increase or for each category of microbleed number. Significant values are bolded.
<table>
<thead>
<tr>
<th></th>
<th>MMSE</th>
<th>Z-score Information</th>
<th>Z-score Processing Speed</th>
<th>Z-score Executive Function</th>
<th>Z-score Global Cognitive Function</th>
<th>Z-score Motor Function</th>
<th>Z-score Speed</th>
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<tr>
<td><strong>APOE ε4 carriers (N=1078)</strong></td>
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<td>Number of microbleeds, per SD increase</td>
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<tr>
<td>1 microbleed (N=109), versus none</td>
<td>0.01 (-0.07;0.08)</td>
<td>0.003 (-0.03;0.04)</td>
<td>-0.01 (-0.04;0.02)</td>
<td>-0.001 (-0.03;0.03)</td>
<td>-0.003 (-0.03;0.02)</td>
<td>-0.04 (-0.08;0.01)</td>
<td></td>
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<tr>
<td>2-4 microbleeds (N=52), versus none</td>
<td>-0.14 (-0.47;0.20)</td>
<td>-0.07 (-0.23;0.09)</td>
<td>-0.02 (-0.17;0.13)</td>
<td>-0.04 (-0.18;0.10)</td>
<td>-0.05 (-0.17;0.07)</td>
<td>0.08 (-0.10;0.26)</td>
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<tr>
<td>≥5 microbleeds (N=27), versus none</td>
<td>-0.03 (-0.51;0.45)</td>
<td>-0.03 (-0.26;0.20)</td>
<td>0.04 (-0.18;0.25)</td>
<td>0.03 (-0.17;0.23)</td>
<td>0.00 (-0.17;0.17)</td>
<td>0.05 (-0.20;0.31)</td>
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<tr>
<td><strong>APOE ε4 non-carriers (N=2611)</strong></td>
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<td>Number of microbleeds, per SD increase</td>
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<tr>
<td>1 microbleed (N=257), versus none</td>
<td>0.10 (-0.13;0.33)</td>
<td>0.10 (-0.01;0.21)</td>
<td>0.06 (-0.04;0.16)</td>
<td>0.03 (-0.06;0.12)</td>
<td>0.06 (-0.02;0.14)</td>
<td>0.10 (-0.01;0.21)</td>
<td></td>
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<tr>
<td>2-4 microbleeds (N=82), versus none</td>
<td>-0.29 (-0.68;0.10)</td>
<td>0.02 (-0.16;0.20)</td>
<td>0.00 (-0.17;0.17)</td>
<td>0.07 (-0.08;0.22)</td>
<td>0.04 (-0.09;0.18)</td>
<td>-0.06 (-0.25;0.13)</td>
<td></td>
</tr>
<tr>
<td>≥5 microbleeds (N=36), versus none</td>
<td>-0.72 (-1.31;-0.13)</td>
<td>-0.01 (-0.31;0.28)</td>
<td>-0.67 (-0.95;-0.39)</td>
<td>-0.26 (-0.51;-0.01)</td>
<td>-0.22 (-0.45;0.00)</td>
<td>-0.28 (-0.57;0.02)</td>
<td></td>
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</tbody>
</table>

Adjusted for age, sex, level of education, systolic blood pressure, diastolic blood pressure, use of blood pressure-lowering medication, smoking, diabetes, total cholesterol, use of lipid-lowering drugs, brain atrophy, white matter lesion volume and lacunar infarcts.

Values represent difference in Z-scores (or for MMSE, difference in absolute score) for number of microbleeds per standard deviation (SD) increase or for each category of microbleed number. Significant values are bolded.
DISCUSSION

Our finding that microbleed number is associated with worse performance on all domains of cognitive tests except memory suggest that microbleeds reflect vascular pathology that, in addition to, and independent of other imaging markers of small vessel disease, contributes to cognitive impairment mainly by affecting non-memory-related cognitive function.

Strengths of our study are its population-based setting, the large sample size and use of our custom-made accelerated 3D T2*GRE sequence with proven high sensitivity for microbleed detection. We assessed a broad range of cognitive domains and made a distinction between different locations of microbleeds in the brain. In addition, we were able to investigate the role of microbleeds independent of vascular risk factors, brain atrophy and other imaging markers of small vessel disease. This is especially important as previous published results showed that white matter lesions and lacunar infarcts affect cognitive function. A potential limitation is the cross-sectional study design, which restricts our interpretation of cause and consequence, although it is biologically less plausible that cognitive deterioration leads to cerebral microbleeds instead of vice versa.

There are only few previous studies that assessed the relation between microbleeds and cognition in the general elderly population, and most of them only used MMSE as a marker of global cognition and did not investigate other cognitive domains. Recently, the population-based AGES-Reykjavik study reported on the association between microvascular damage and the association with multiple cognitive domains and dementia. Our findings are largely in line with theirs, as they also found an association between multiple CMBs and cognitive dysfunction, in particular slower processing speed and poorer executive function. This study, however, also included demented individuals, which makes it difficult to evaluate the role of CMBs on cognitive deterioration in non-demented individuals.

In a clinical setting, several studies examined in selected groups of patients the relation between microbleeds and cognitive dysfunction. Studies among memory clinic patients and AD patients, however, yielded conflicting results, and most studies again only used the MMSE score. In a small neurovascular clinic population, multiple
cognitive domains were investigated and a marked difference in the prevalence of executive dysfunction between patients with and without CMBs was found. This is in line with our study, though we additionally found associations with other cognitive domains.

We previously found that carriers of the APOE ε4 allele had cerebral microbleeds more often compared to non-carriers. Moreover, literature describes poorer performance on neuropsychological tests in non-demented individuals with 1 or 2 APOE ε4 alleles compared to APOE non-carriers. In the present study we found, however, associations between 5 or more microbleeds and several cognitive domains in APOE ε4 non-carriers, but a lack of associations in APOE ε4 carriers. Further elucidation is needed for these seemingly paradoxical findings.

The finding of a significant association between a single deep or infratentorial microbleed and better motor speed is seemingly counterintuitive and may be a chance finding in the view of multiple tests that we performed. Analyzed as a continuous variable, number of microbleeds was significantly associated with worse performance on tests of motor speed, underscoring this notion.

The observation that strictly lobar microbleeds and deep or infratentorial microbleeds are related to cognition in a different way may be explained in various ways. On the one hand, microbleeds could be a general marker for (the severity of) underlying vascular disease, in particular CAA or hypertensive arteriolosclerosis, and as such may influence cognition. We found that associations between deep or infratentorial microbleeds and cognition were not only weaker than those for strictly lobar microbleeds, but these were also not independent of brain atrophy and other markers of small vessel disease, whilst associations for lobar microbleeds and cognition were very robust. This is in line with our previous findings that the location of microbleeds in the brain likely reflects differences in underlying etiology. Thus, deep or infratentorial microbleeds are probably associated with cognition through related hypertensive vasculopathy. In contrast, strictly lobar microbleeds are thought to be a marker of pathologies associated with cerebral amyloid angiopathy, such as vascular deposition of β-amyloid or neuritic plaques, that in themselves impair cognition. 32-33 Along this line of thinking, it could be postulated that microbleeds in various locations differentially relate to specific cognitive domains due to different underlying pathology. Yet,
which cognitive domains are specifically involved in CAA and hypertensive arteriosclerosis, is, to our knowledge, largely unknown.

An alternative hypothesis relating microbleed location to cognition is that microbleeds reflect focal damage of brain tissue and when located in strategic areas could interfere with cognitive processes, by causing disconnections in functional pathways. For example, there may be more direct or indirect effects of strictly lobar microbleeds to surrounding brain tissue compared to deep or infratentorial microbleeds as the location of these lesions may lead to more disconnection of functionally important cortical and subcortical structures.\(^7,34\) Along this line of thinking, it has been suggested that disruption of frontal-basal ganglia connections may provide a plausible mechanism by which microbleeds in frontal and basal ganglia regions cause executive dysfunction.\(^30\) In this respect, microbleed location may be of less importance in tasks of speed and attention as these are thought to reflect more widely distributed cognitive skills.\(^30\) To further explore this hypothesis, more information on the exact location of microbleeds would be needed, such as in which lobe they occur or even more detailed using voxel-based analysis. Though we (visually) rated microbleed location as being lobar, deep or infratentorial, we as yet have not further information on their exact location. Advances in automated microbleed detection using computer algorithms will enable the examination of specific regional microbleed distributions in the brain with different cognitive domains in the near future.\(^35\)

Contrary to our findings, in the AGES-Reykjavik study associations between microbleeds and cognition were found to be strongest for microbleeds located in the deep hemispheric or infratentorial regions.\(^14\) Though difficult to explain, it may be that a higher mean age and subsequent more cardiovascular risk factors, brain infarcts, and a higher load of subcortical and periventricular white matter hyperintensities in their study compared to our study has influenced this. Although both their study and our study adjusted for these factors, there may be residual variation in underlying pathology causing these differences between the study samples.

We especially found numerous strictly lobar microbleeds to be related to worse cognitive function. Studies in AD patients found a mainly lobar distribution of microbleeds that corresponds with the distribution described in sporadic CAA cases.\(^10,36\) Therefore, it is suggested that CMBs in AD patients are more likely to be related to CAA.
rather than due to hypertensive vasculopathy.\textsuperscript{7} Both the Honolulu-Asia Aging Study (HAAS) and the MRC Cognitive Function and Ageing Study (CFAS) found associations of CAA with cognition even after controlling for age and Alzheimer disease pathology.\textsuperscript{37-38} More recently, the Religious Orders Study found moderate-to-very severe CAA, but not mild-to-moderate CAA, to be associated with lower performance in specific cognitive domains, most notably perceptual speed.\textsuperscript{39} Our results thus corroborate the suggestion that high numbers of strictly lobar microbleeds may be one of the manifestations of CAA in a certain stage. Furthermore, microbleeds were recently postulated as the potential “missing link” in the interaction between CAA and hypertensive arteriolosclerosis in the pathogenesis of AD.\textsuperscript{40} The robust associations we assessed between numerous strictly lobar microbleeds and cognitive function further support this notion.
REFERENCES


Chapter 3

Vascular Risk Factors and Clinical Outcomes
3.1

Arterial stiffness and cerebral small vessel disease
3.2

Arterial stiffness, cognitive decline and risk of dementia

Stroke. 2007;38:888-892

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Marieke van Oijen
Francesco U.S. Mattace-Raso
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Peter J. Koudstaal
Jacqueline C.M. Witteman
Monique M.B. Breteler
ABSTRACT

Background – Arterial stiffness is associated with an increased risk of myocardial infarction and stroke, independent of classical vascular risk factors. Vascular factors and stroke are associated with cognitive function and dementia. We examined whether arterial stiffness was independently associated with cognitive function and dementia.

Methods – The present study was based on the Rotterdam Study, a prospective population-based cohort study ongoing since 1990. During the third examination (1997-1999) arterial stiffness was measured by assessment of pulse wave velocity and carotid distensibility. Cognitive function was assessed during the third and fourth examination (2002-2004) with a neuropsychological test battery. We used linear and logistic regression to estimate the association of arterial stiffness with cognitive function and cognitive decline. From the third examination until January 1, 2005, we identified 156 incident dementia cases. Cox proportional hazard models were used to estimate the association between arterial stiffness and the risk of dementia.

Results – After adjustment for cardiovascular risk factors we found an association of increased pulse wave velocity with poorer performance on the Stroop test (adjusted β-coefficient [95% confidence interval] 1.13 [0.26 to 1.99] per standard deviation increase in pulse wave velocity) but not with performance on other cognitive tests. No associations were found between measures of arterial stiffness and cognitive decline or risk of dementia after adjustment for cardiovascular factors.

Conclusions – We did not identify arterial stiffness as an independent risk factor of cognitive decline or risk of dementia.
INTRODUCTION

Arterial stiffness is a predictor of cardiovascular disease and mortality. Recently, the aortic pulse wave velocity index, a measure of arterial stiffness, was found to be of added value above traditional cardiovascular risk factors in the prediction of coronary heart disease and stroke.\(^1\) Elevated arterial stiffness is a result of structural and functional changes of the vessel wall that occur with aging. Furthermore, higher arterial stiffness is associated with higher systolic blood pressure, increased pulse pressure, and atherosclerosis.\(^2\)

Many studies have demonstrated an association of vascular factors and cerebrovascular disease with dementia and cognitive decline.\(^3\) Recently, some studies reported an association between increased arterial stiffness, measured by pulse wave velocity (PWV), and poor cognitive function and suggested that arterial stiffness may be a determinant of cognitive decline and dementia.\(^4\)–\(^6\) However, these studies were all small, cross-sectional, and mostly performed in selected clinic-based samples. To date, no prospective studies have been reported that examined the association of arterial stiffness with cognitive decline or dementia.

In this study we set out to investigate the association between arterial stiffness and cognitive function, cognitive decline, and risk of dementia in the general population. Furthermore, to investigate whether arterial stiffness may be an independent risk factor of cognitive decline and dementia, we examined whether these associations were independent of cardiovascular factors.

METHODS

Study Sample

This study was based on the Rotterdam Study, a prospective population-based cohort study among 7983 elderly subjects aged 55 years and older.\(^7\) Baseline examinations were performed from 1990 through 1993. Participants were interviewed at their homes and subsequently examined at the research center. Follow-up examinations were conducted in 1993 to 1994, 1997 to 1999, and in 2002 to 2004. The Medical Ethics Committee of Erasmus Medical Center approved the study, and written informed
consent was obtained from all participants. Arterial stiffness was first measured during the third survey in 1997 to 1999 in 3779 of the 4024 persons who visited the research center. Missing information on PWV or carotid distensibility was almost entirely because of logistic reasons, particularly malfunctioning equipment or unavailability of technicians.

Cognitive function was assessed at the third examination in 1997 to 1999 and the fourth examination in 2002 to 2004. We excluded individuals with dementia at the time of the third examination, which left 3714 persons who had arterial stiffness measurements, underwent neuropsychological testing, and were not demented at the third examination to be included in our analyses. Follow-up for incident dementia was virtually complete until January 1, 2005. Of the 3714 persons, 947 persons did not visit the research center for the fourth examination. Of these, 527 persons had died and 420 persons refused to visit the center. As a result, the analyses regarding arterial stiffness and change in cognitive function were based on 2767 persons who underwent neuropsychological testing during 1997 to 1999 and 2002 to 2004.

Measures of Arterial Stiffness: PWV and Carotid Distensibility

Carotid–femoral PWV, a measure of aortic stiffness, was measured with persons in supine position. PWV was assessed with an automatic device (Complior; Artech Medica). The time delay between the rapid upstroke of the feet of simultaneously recorded pulse waves in the carotid artery and the femoral artery was recorded. The distance between recording sites in the carotid and the femoral arteries (the carotid artery and the groin) was measured with a tape over the surface of the body. The ratio between the foot-to-foot delay and the distance covered by the pulse wave is the PWV and is expressed in meters per second.

Carotid distensibility was measured at the right common carotid artery with the subjects in supine position and the head slightly tilted to the contralateral side. The vessel wall motion was assessed with a duplex scanner (ATL Ultra mark IV, operation frequency 7.5 MHz) connected to a vessel wall movement detector system. After 5 minutes of rest, a region at 1.5 cm proximal to the origin of the bulb of the artery was identified using B-mode ultrasound. The displacement of the arterial walls was obtained by processing the radio frequency signals originating from two selected sample volumes positioned over the anterior and posterior walls. The end-diastolic
diameter (D), the absolute stroke change in diameter during systole (ΔD), and the relative stroke change in diameter (ΔD/D) were computed as the mean of four cardiac cycles of three successive recordings. Blood pressure was measured twice with a Dinamap automatic blood pressure recorder during the measurement session. The mean was taken as the person’s reading. Pulse pressure (ΔP) was defined as the difference between systolic and diastolic blood pressure. The cross-sectional arterial wall distensibility coefficient was calculated according to the following equation: distensibility coefficient = \(2 \frac{\Delta D}{D} / \Delta P \times 10^{-3} \text{kPa}\). In a reproducibility study performed among 47 subjects, the intraclass correlation coefficient was 0.80 for both the PWV and the carotid distensibility coefficient.

Assessment of Cognitive Function

The Mini Mental State Examination (MMSE) is a widely used test for global cognitive function. Executive cognitive function was measured with the Letter-Digit Substitution Task, an abbreviated Stroop Test and the Word Fluency Test. The Letter-Digit Substitution Task is a modified version of the Symbol Digit Modalities Test and asks the participants to make as many letter-digit combinations as possible in 60 seconds. The abbreviated Stroop test consists of 3 subtasks in which the participant is shown a card with 40 items that have to be named. The first card contains color names, printed in black; the second card contains colored blocks; the third card contains color names, printed in a different color than the color name. As an outcome we used time needed for the third trial in which the participants are asked to name the color in which the color name is printed. In the Word Fluency Test, used to test verbal fluency, participants were asked to name as many animals as possible within 60 seconds.

Diagnosis of Dementia

The diagnosis of dementia was made after a 3-step protocol. Two brief tests of cognition (MMSE and Geriatric Mental State schedule organic level) were used to screen all subjects. Screen-positives (MMSE score <26 or Geriatric Mental State schedule organic level >0) underwent the Cambridge examination for mental disorders of the elderly (Camdex). Subjects who were suspected of having dementia were examined by a neuropsychologist if additional neuropsychological testing was required for diagnosis. When available, imaging data were used. In addition, the total cohort was continuously monitored for incident dementia through computerized linkage between the study database and digitalized medical records from general practitioners and the
Regional Institute for Outpatient Mental Health Care. The diagnosis of dementia and subtypes of dementia was made in accordance with internationally accepted criteria for dementia, Alzheimer disease (AD) (NINCDS-ADRDA), and vascular dementia (NINDS-AIREN) by a panel of a neurologist, a neuropsychologist, and a research physician.

**Covariates**

Level of education was obtained during the baseline interview and dichotomized into primary education or less and more than primary education. At the research center clinical measures were obtained. Systolic and diastolic blood pressures were measured twice on the right arm with a random-zero sphygmomanometer, after the participant had been seated for at least five minutes. The mean of the two blood pressure values was used in the analyses. Pulse pressure was defined as the difference between systolic and diastolic blood pressure. Mean arterial pressure was calculated as diastolic blood pressure plus one-third of the pulse pressure. The body mass index was calculated (weight [kg]/length [m²]). Fasting serum total and high-density lipoprotein cholesterol values were determined by an automated enzymatic procedure (Boehringer Mannheim System). Genotyping for APOE was performed on coded DNA specimens without knowledge of diagnosis. Persons were categorized on the basis of presence or absence of an APOE ε4 allele. Persons with the APOE ε2/ε4 genotype were excluded from the analyses. Furthermore, ultrasonography of both carotid arteries was performed. As an indicator of atherosclerosis of the carotid arteries we used intima-media thickness. Common carotid intima-media thickness was determined as the average of the maximum intima-media thickness of near-wall and far-wall measurements, and the average of left and right common carotid intima-media thickness was computed. Diabetes mellitus was defined as the use of blood glucose-lowering medication and/or fasting serum glucose level ≥7.0 mmol/L.

**Data Analysis**

First, we examined the association of PWV and carotid distensibility per standard deviation (SD) increase with cognitive function by means of linear regression models, adjusted for age, sex, and education. Further adjustment was made for mean arterial pressure and heart rate as these measures have a direct effect on arterial stiffness. Then, to examine whether associations were independent of other vascular factors, we adjusted for body mass index, smoking, intima-media thickness, total cholesterol, high-density lipoprotein cholesterol, and diabetes mellitus.
Next, we examined the association between arterial stiffness and cognitive decline with logistic regression models. Decline on cognitive tests was defined as a negative difference between the test scores from the third and fourth examination >1 SD of the mean difference. Analyses were adjusted as described for the cross-sectional analyses. Because stroke has been related to cognition and an association has been reported between arterial stiffness and risk of stroke, we adjusted for prevalent and incident strokes in additional analyses. To assess whether associations differed across age categories we repeated the analyses in strata of age (≤75 years and >75 years). We repeated the analyses adjusting for cognitive function at baseline. Also, analyses were repeated excluding 89 persons of the 2767 persons included in the population for analyses regarding change in cognitive function who had become demented during follow-up.

Finally, we used Cox proportional hazard models to examine the association between arterial stiffness and the risk of dementia and subtypes of dementia. Follow-up time was defined as the time of arterial stiffness measurement until dementia diagnosis, death, or end of study, whichever came first. We examined the association of PWV and carotid distensibility with dementia per SD increase of PWV and carotid distensibility. We adjusted for age, sex, and education, and subsequently for mean arterial pressure, heart rate, and cardiovascular risk factors. To investigate whether APOE genotype modified the association of PWV and carotid distensibility with dementia, we examined the association in strata of carriers and non-carriers of the APOE ε4 allele and computed interaction terms between measures of arterial stiffness and the APOE genotype.

RESULTS

Characteristics of persons who visited the center during the third and fourth examination are shown in Table 1. Persons who participated in both the third and the fourth examination had a better cardiovascular risk profile and performed better on all cognitive tests compared with persons who only participated in the third examination also when differences in age were taken into account. PWV and carotid distensibility were normally distributed and inversely correlated (Spearman correlation coefficient, -0.41; P<0.001).
After adjustment for age, sex, and education, statistically significant associations were found for increased PWV and worse performance on the MMSE, the Stroop Test, and the Word Fluency Test, and for decreased carotid distensibility and worse performance on the MMSE and the Stroop Test (model 1 in Table 2). Adjustments for mean arterial pressure and heart rate attenuated all associations. The associations were attenuated further after adjusting for cardiovascular factors and only the association between PWV and worse performance on the Stroop Test remained statistically significant (model 2 in Table 2).

No association was found between arterial stiffness and cognitive decline (models 1 and 2 in Table 3). The incidence rate of stroke in the Rotterdam Study was 9.4 per 1000 person-years. Additional adjustment for prevalent and incident stroke did not change the associations. The associations were similar for younger and older people (≤75 years, >75 years). Associations did not change after adjusting for baseline cognitive function and after exclusion of persons who had become demented.

The incidence rate of dementia in the Rotterdam Study was 9.8 per 1000 person-years. During a mean (SD) follow-up of 4.4 (0.9) years, we identified 156 persons with incident dementia (including 89 persons who visited the research center during the fourth survey and 67 patients who were identified through medical records), of whom 136 persons had AD diagnosed and 11 persons had vascular dementia diagnosed. Table 4 shows that PWV and carotid distensibility were not associated with risk of dementia. For AD, the hazard ratio (95% CI) was 0.90 (0.75 to 1.07) per SD increase in PWV. For vascular dementia, there seemed to be an association (hazard ratio [95% CI] 1.47 [0.94 to 2.30]) per SD increase in PWV, although this was not significant, possibly because of the low number of vascular dementia cases. Per SD increase in carotid distensibility, the hazard ratio (95% CI) for AD was 1.14 (0.91 to 1.43) and 0.58 (0.21 to 1.57) for vascular dementia. The associations did not differ between carriers and non-carriers of the APOE ε4 allele (probability value of the interaction term between PWV and APOE genotype was 0.89 and 0.94 between carotid distensibility and APOE genotype).
<table>
<thead>
<tr>
<th></th>
<th>Third examination</th>
<th>Third but not fourth examination</th>
<th>Third and fourth examination</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td>3714</td>
<td>947</td>
<td>2767</td>
</tr>
<tr>
<td><strong>Men (% )</strong></td>
<td>42.3</td>
<td>43.6</td>
<td>41.9</td>
</tr>
<tr>
<td><strong>Age (years) (SD )</strong></td>
<td>72.0 (6.7)</td>
<td>75.7 (7.3)</td>
<td>70.7 (6.0)</td>
</tr>
<tr>
<td><strong>Primary education (% )</strong></td>
<td>3.8</td>
<td>7.0 (6.3)</td>
<td>2.7</td>
</tr>
<tr>
<td><strong>Prevalent stroke cases (% )</strong></td>
<td>10.5 (4.0)</td>
<td>14.5 (4.3)</td>
<td>9.2 (4.2)</td>
</tr>
<tr>
<td><strong>Pulse wave velocity (m/s) (SD )</strong></td>
<td>106.7 (12.8)</td>
<td>108.2 (12.7)</td>
<td>106.1 (12.5)*</td>
</tr>
<tr>
<td><strong>Distensibility coefficient (10⁻³k Pa) (SD )</strong></td>
<td>3.8</td>
<td>34.9</td>
<td>27.0</td>
</tr>
<tr>
<td><strong>Mean arterial pressure (mmHg) (SD )</strong></td>
<td>106.7 (12.8)</td>
<td>108.2 (12.7)</td>
<td>106.1 (12.5)*</td>
</tr>
<tr>
<td><strong>Heart rate (bpm) (SD )</strong></td>
<td>75.2 (14.6)</td>
<td>77.2 (15.4)</td>
<td>74.5 (14.2)*</td>
</tr>
<tr>
<td><strong>Body mass index (kg/m²) (SD )</strong></td>
<td>26.8 (4.0)</td>
<td>26.7 (4.1)</td>
<td>26.9 (3.9)</td>
</tr>
<tr>
<td><strong>Diabetes mellitus (% )</strong></td>
<td>10.9</td>
<td>16.3</td>
<td>9.0*</td>
</tr>
<tr>
<td><strong>Total cholesterol (mmol/L) (SD )</strong></td>
<td>26.8 (4.0)</td>
<td>26.7 (4.1)</td>
<td>26.9 (3.9)</td>
</tr>
<tr>
<td><strong>HDL-cholesterol (mmol/L) (SD )</strong></td>
<td>26.8 (4.0)</td>
<td>26.7 (4.1)</td>
<td>26.9 (3.9)</td>
</tr>
<tr>
<td><strong>Intima media thickness (mm) (SD )</strong></td>
<td>26.8 (4.0)</td>
<td>26.7 (4.1)</td>
<td>26.9 (3.9)</td>
</tr>
<tr>
<td><strong>Mini mental state examination (SD )</strong></td>
<td>26.8 (4.0)</td>
<td>26.7 (4.1)</td>
<td>26.9 (3.9)</td>
</tr>
<tr>
<td><strong>Word fluency test (number of correct answers) (SD )</strong></td>
<td>26.8 (4.0)</td>
<td>26.7 (4.1)</td>
<td>26.9 (3.9)</td>
</tr>
<tr>
<td><strong>Stroop test (seconds) (SD )</strong></td>
<td>26.8 (4.0)</td>
<td>26.7 (4.1)</td>
<td>26.9 (3.9)</td>
</tr>
<tr>
<td><strong>Letter-digit substitution task (number of correct answers) (SD )</strong></td>
<td>26.8 (4.0)</td>
<td>26.7 (4.1)</td>
<td>26.9 (3.9)</td>
</tr>
</tbody>
</table>

* Age and sex adjusted difference between the groups P<0.05.
Table 2. Association of arterial stiffness with cognitive function using linear regression models

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th></th>
<th>Model 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Difference in test scores (95% CI)</td>
<td></td>
<td>Difference in test scores (95% CI)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MMSE</td>
<td>Letter-digit substitution task</td>
<td>Stroop test</td>
<td>Word fluency test</td>
</tr>
<tr>
<td>PWV per SD increase</td>
<td>-0.08 (-0.15; -0.01)</td>
<td>-0.19 (-0.42; 0.05)</td>
<td>1.39 (0.65; 2.13)</td>
<td>-0.31 (-0.50; -0.12)</td>
</tr>
<tr>
<td>CD per SD increase</td>
<td>0.09 (0.01; 0.16)</td>
<td>0.12 (-0.13; 0.36)</td>
<td>-0.82 (-1.56; -0.08)</td>
<td>0.14 (-0.06; 0.35)</td>
</tr>
</tbody>
</table>

Model 1: adjusted for age, sex and education.
Model 2: additionally adjusted for mean arterial pressure, heart rate, current smoking, diabetes mellitus, body mass index, total cholesterol, high density lipid cholesterol and intima media thickness.

PWV: pulse wave velocity, CD: carotid distensibility.

Table 3. Association between arterial stiffness and cognitive decline using logistic regression models

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th></th>
<th>Model 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odds ratio for decline (95% CI)</td>
<td></td>
<td>Odds ratio for decline (95% CI)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MMSE</td>
<td>Letter-digit substitution task</td>
<td>Stroop test</td>
<td>Word fluency test</td>
</tr>
<tr>
<td>PWV per SD increase</td>
<td>0.98 (0.86-1.12)</td>
<td>1.14 (1.00-1.31)</td>
<td>1.00 (0.85-1.17)</td>
<td>1.07 (0.95-1.21)</td>
</tr>
<tr>
<td>CD per SD increase</td>
<td>0.94 (0.81-1.09)</td>
<td>0.90 (0.78-1.04)</td>
<td>0.99 (0.83-1.18)</td>
<td>0.91 (0.80-1.03)</td>
</tr>
</tbody>
</table>

Model 1: adjusted for age, sex and education.
Model 2: additionally adjusted for mean arterial pressure, heart rate, current smoking, diabetes mellitus, body mass index, total cholesterol, high density lipid cholesterol and intima media thickness.

PWV: pulse wave velocity, CD: carotid distensibility.
Table 4. Association between arterial stiffness and risk of dementia using Cox proportional hazard models

<table>
<thead>
<tr>
<th></th>
<th>PWV Hazard ratio (95% CI)</th>
<th>CD Hazard ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Per SD increase</td>
<td>Model 1 0.97 (0.82-1.15)</td>
<td>Model 2 0.91 (0.75-1.10)</td>
</tr>
<tr>
<td></td>
<td>Model 1 1.08 (0.88-1.34)</td>
<td>Model 2 1.05 (0.81-1.35)</td>
</tr>
</tbody>
</table>

Model 1: adjusted for age, sex and education.
Model 2: additionally adjusted for mean arterial pressure, heart rate, current smoking, diabetes mellitus, body mass index, total cholesterol, high density lipid cholesterol and intima media thickness.

PWV: pulse wave velocity, CD: carotid distensibility.

DISCUSSION

We did not find an association between arterial stiffness and cognitive decline or the risk of dementia. Although we found associations between arterial stiffness and several domains of cognitive function in cross-sectional analyses, these associations were small and after adjustment for mean arterial pressure, heart rate, and cardiovascular risk factors, only the association between increased PWV and poor performance on the Stroop Test remained.

Some aspects of the present study need to be discussed. Strengths of the Rotterdam Study are its population-based setting, its large number of persons, and its virtually complete follow-up. A limitation of the study is that, because information on cognitive decline was only available for persons who participated in both the third and the fourth examination, selective attrition may have affected the results of our analyses regarding change in cognitive function. Persons included in these analyses were younger and had a better cardiovascular risk profile (including measures of arterial stiffness) than persons who did not participate in the fourth examination. Because age and cardiovascular factors are associated with cognitive function, this may have affected our power to find an association with cognitive decline. Another limitation is that the results of the analyses regarding cognitive decline may have been affected by regression to the mean. Regression to the mean may result in an underestimation of the association. However, follow-up was complete for the outcome dementia and we also found no relation between arterial stiffness and risk of dementia.
Few studies have examined the association between arterial stiffness and cognition. Recently, an association, independent of cardiovascular factors, between increased PWV and impaired cognitive function, defined by MMSE score, was found in patients who were referred to a memory clinic and in community-dwelling elderly. Our finding of an association independent of cardiovascular factors between increased PWV and worse performance on the Stroop Test is in line with the notion that arterial stiffness affects cognitive function. However, we did not find independent associations between increased arterial stiffness and other cognitive tests. This may be explained by more extensive adjustments in our study compared with previous studies, including adjustments for carotid intima-media thickness, an indicator of atherosclerosis, body mass index, pulse rate, and mean arterial pressure.

Few studies examined the association between arterial stiffness and dementia. In one study 308 elderly with symptoms of memory loss were evaluated and classified in four groups (AD, vascular dementia, mild cognitive impairment, and normal cognitive function). Persons with vascular dementia, AD, and mild cognitive impairment had a higher PWV than those without cognitive impairment after adjustments for age, sex, systolic blood pressure, antihypertensive treatment, and presence of cardiovascular diseases. In another study brachial-ankle PWV was compared between patients with AD, vascular dementia, and cognitively normal age-matched controls. Arterial stiffness was higher in patients with vascular dementia than in those with AD or those without dementia. Another study reported an inverse correlation, adjusted for age, sex, mean arterial pressure, and antihypertensive treatment, between heart–brachial PWV and cognitive function, measured by the Hasegawa Dementia Scale Revised, in non-vascular dementia patients and persons with mild cognitive impairment. In our prospective study, we did not find an association between arterial stiffness and risk of dementia or cognitive decline. However, we cannot completely rule out an association between increased arterial stiffness and risk of vascular dementia because of the low number of incident vascular dementia cases.

Arterial stiffness is strongly associated with hypertension and atherosclerosis that have both been related to an increased risk of dementia. Therefore, an association of increased arterial stiffness with cognitive decline and dementia seemed plausible. Mechanisms for such an association include cerebrovascular disease (for instance, lacunar infarction or white matter lesions) and cerebral hypoperfusion.
Though previous studies suggested that arterial stiffness might provide additional value above other cardiovascular risk factors in relation to cognitive decline or dementia, our data do not support this hypothesis. To conclude, we did not identify arterial stiffness as an independent risk factor of cognitive decline or risk of dementia.
REFERENCES


3.3

Total cerebral blood flow in relation to cognitive function

ABSTRACT

Cerebral hypoperfusion has been associated with worse cognitive function. We investigated the association between cerebral blood flow and cognition and whether this association is independent of brain volume. In 892 participants, aged 60 to 91 years of the population-based Rotterdam Scan Study, we measured total cerebral blood flow (tCBF) and brain volume using magnetic resonance imaging. Lower tCBF was associated with worse information processing speed, executive function and global cognition. However, after correcting tCBF for brain volume, these associations disappeared. The association between tCBF and cognition may be mediated or confounded by brain atrophy. Future studies on tCBF should take into account brain atrophy.
INTRODUCTION

Elderly persons often suffer from deterioration of cognitive function. Vascular risk factors may contribute to cognitive impairment by affecting blood flow to the brain.¹ Moreover, it has been suggested that cerebral hypoperfusion precedes and possibly contributes to the onset of clinical dementia.²

To assess perfusion at the brain tissue level is difficult as most measurement techniques are invasive and complex. Phase-contrast magnetic resonance imaging (MRI) enables fast and accurate measurement of total cerebral blood flow (tCBF) and has shown to be applicable in population-based studies.³ Previous studies showed that lower tCBF assessed with phase-contrast MRI was related to poorer cognition, in particular information processing speed, and dementia.⁴⁻⁵ However, these studies did not assess whether this association was independent of brain atrophy. It can be hypothesized that smaller brain volume leads to decreased cerebral metabolic demand, and as such confounds the association between tCBF and cognitive function. Thus, the aim of our study was to investigate whether diminished tCBF is associated with specific domains of cognitive function independent of brain volume.

METHODS

Participants
This study is embedded within the Rotterdam Study, a large population-based cohort study in the Netherlands.⁶ The original study population consisted of 7983 participants aged 55 years and older from the Ommoord area, a suburb of Rotterdam. In the year 2000, the cohort was expanded with the addition of 3011 persons (≥55 years).⁶ From August 2005 to May 2006, we randomly selected 1073 members of this cohort expansion for participation in the Rotterdam Scan Study, a population-based brain-imaging study. After exclusion of individuals who were demented or had MRI contraindications, 975 persons were found to be eligible, of whom 907 participated and gave written informed consent. Because of physical abilities (e.g., back pain), imaging could not be performed or completed in 12 individuals. Therefore, a total of 895 complete MR examinations were performed. The institutional review board approved the study.
Magnetic Resonance Imaging Scan Protocol

Magnetic resonance imaging of the brain was performed on a 1.5T MRI scanner (General Electric Healthcare, Milwaukee, WI, USA), using an 8-channel head coil. For flow measurement, 2D phase-contrast imaging was performed as described previously. In brief, a sagittal 2D phase-contrast MRI angiographic scout image was performed. On this scout image, a transverse imaging plane perpendicular to both the precavernous portion of the internal carotid arteries and to the middle part of the basilar artery was chosen for a 2D gradient-echo phase-contrast sequence (repetition time=20ms, echo time=4 ms, field of view=19 cm², matrix=256x160, flip angle=8°, number of excitations=8, bandwidth=22.73 kHz, velocity encoding=120 cm/sec, slice thickness=5 mm). For an example, see Vernooij et al. Acquisition time was 51 secs and no cardiac gating was performed. We further performed three high-resolution axial MRI sequences, that is, a T1-weighted sequence, a proton density-weighted sequence, and a fluid attenuated inversion recovery sequence.

Measurement of Total Cerebral Blood Flow and Total Brain Perfusion

Flow was calculated from the phase-contrast images using interactive data language-based custom software (Cinetool version 4, General Electric Healthcare). Two independent, experienced technicians drew all the manual regions of interest (ROI) and performed subsequent flow measurements (inter rater correlations $N=533 >0.94$ for all vessels). In 3 persons, tCBF could not be measured because of incorrect positioning of the phase-contrast imaging plane, leaving a total of 892 persons in our analysis. We calculated total brain perfusion (in mL/min per 100 mL) by dividing tCBF (mL/min) by each individual’s brain volume (mL) and multiplying the obtained result by 100.

Assessment of Brain Volume

For the assessment of brain volume, the structural MRI scans (T1-weighted, PD-weighted, and fluid attenuated inversion recovery) were transferred to a Linux workstation. Preprocessing steps and the classification algorithm have been described elsewhere. In summary, preprocessing included coregistration, non-uniformity correction and variance scaling. We used the k-nearest-neighbor classifier to classify scans into brain tissue and cerebrospinal fluid using the multispectral MR intensities. All segmentation results were visually inspected and if needed manually corrected. To remove non-cerebral tissue, for example, eyes, skull, and cerebellum, we applied non-
rigid registration\textsuperscript{10} to register to each brain a template scan in which these tissues were manually masked. Brain volume was calculated by summing up all the voxels across the whole brain to yield volumes in milliliters.

**Cognitive Function**

Cognitive function was assessed with a neuropsychological test battery comprising the MMSE (mini-mental state examination), the Stroop test, the LDST (letter-digit substitution task; number of correct digits in 1 min), the WFT (word fluency test; animal categories), and a 15-WLT (15-word verbal learning test; based on Rey’s recall of words).\textsuperscript{11} For each participant Z-scores were calculated for each test separately (individual test score minus mean test score divided by the standard deviation), except for MMSE. To obtain more robust measures, we constructed compound scores for information-processing speed, executive function, memory and global cognitive function. The compound score for information processing speed was the average of the Z-scores for the Stroop reading and Stroop color-naming subtask and the LDST. Executive function included the Z-scores of the Stroop interference subtask, the LDST and the WFT (number of animals in 1 min). The compound score for memory was the average of the Z-scores for the immediate and delayed recall of the 15-WLT. For global cognitive function, we used the average of the Z-scores of the Stroop test (average of the reading, color-naming and interference subtask), the LDST, the WFT, and the immediate and delayed recall of the 15-WLT.\textsuperscript{11}

**Covariates**

We assessed the level of education and current smoking by interview. Systolic and diastolic blood pressures were measured twice on the right arm with a random-zero sphygmomanometer. The mean of the two readings was used in the analyses. Diabetes mellitus was defined as the use of blood glucose-lowering medication or fasting serum glucose level $\geq 7.0$ mmol/L. Carotid plaque score was assessed by Doppler ultrasound.\textsuperscript{12}

**Data Analysis**

We evaluated the association of both tCBF (mL/min) and total brain perfusion (mL/min per 100 mL brain tissue) per standard deviation (SD) increase with cognitive function using multiple linear regression models. All analyses were adjusted for age, sex and education. To examine whether associations were independent of vascular risk factors, we additionally adjusted for current smoking, systolic and diastolic blood pressure, diabetes mellitus and carotid plaque score.
RESULTS

Characteristics of the study population are shown in Table 1. Lower tCBF was associated with worse performance on tests of information-processing speed, executive function and global cognition, but not with the MMSE score and memory performance (Table 2).

Total brain volume was a strong determinant of tCBF (per SD increase in brain volume 36.00 mL/min increase in tCBF; 95% confidence interval, 30.00;42.10). The associations of tCBF with cognition disappeared on correcting for brain volume (Table 2). Adjustments for vascular risk factors did not change any of these associations (Table 2).

Table 1. Characteristics of the study population (N=892)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men, N (%)</td>
<td>441 (49.4)</td>
</tr>
<tr>
<td>Age, years (SD)</td>
<td>67.5 (5.5)</td>
</tr>
<tr>
<td>Primary education, N (%)</td>
<td>38 (4.4)</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg (SD)</td>
<td>143.8 (18.5)</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg (SD)</td>
<td>81.0 (10.2)</td>
</tr>
<tr>
<td>Diabetes mellitus, N (%)</td>
<td>85 (9.6)</td>
</tr>
<tr>
<td>Current smokers, N (%)</td>
<td>267 (29.9)</td>
</tr>
<tr>
<td>Plaques in carotid artery, range: 0-12*</td>
<td>3.0 (1.0-5.0)</td>
</tr>
<tr>
<td>Mini mental state examination, score (SD)</td>
<td>27.9 (1.8)</td>
</tr>
<tr>
<td>Brain volume, mL (SD)</td>
<td>976.8 (114.0)</td>
</tr>
<tr>
<td>Total cerebral blood flow, mL/min (SD)</td>
<td>497.4 (86.2)</td>
</tr>
<tr>
<td>Total brain perfusion, mL/min per 100mL brain tissue (SD)</td>
<td>51.2 (8.8)</td>
</tr>
</tbody>
</table>

Values are means (SD) or numbers (percentages).

* Median (interquartile range).
Table 2. Association of tCBF and total brain perfusion with cognitive function (Z-scores) using linear regression models (N=892)

<table>
<thead>
<tr>
<th>tCBF</th>
<th>MMSE</th>
<th>Z-score information executive memory glob</th>
<th>Z-score global cognition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td>0.08 (-0.04;0.19)</td>
<td>0.08 (0.03;0.14)</td>
<td>0.00 (-0.07;0.06)</td>
</tr>
<tr>
<td></td>
<td>0.07 (0.02;0.12)</td>
<td>0.06 (0.01;0.11)</td>
<td>0.05 (0.01;0.10)</td>
</tr>
<tr>
<td>Model 2</td>
<td>0.09 (-0.03;0.20)</td>
<td>0.07 (0.02;0.13)</td>
<td>0.00 (-0.07;0.06)</td>
</tr>
<tr>
<td></td>
<td>0.06 (0.01;0.11)</td>
<td>0.05 (0.01;0.10)</td>
<td></td>
</tr>
</tbody>
</table>

CI: confidence interval, MMSE: mini-mental state examination, tCBF: total cerebral blood flow.
Model 1: adjusted for age, sex and level of education.
Model 2: additionally adjusted for systolic blood pressure, diastolic blood pressure, current smoking, diabetes mellitus and plaque score.
DISCUSSION

We found that persons with low tCBF performed significantly worse on tasks assessing information-processing speed, executive function, and global cognitive function compared with persons with higher tCBF. However, total brain perfusion, indicating the flow in mL per 100 mL of brain tissue volume, was not associated with cognitive function. Adjustments for vascular risk factors did not change the results.

Before interpreting the results, some methodological issues need to be addressed. The strengths of our study are its population-based setting, the high response rate and the large sample size. A limitation is the cross-sectional design, which restricts our interpretation of the data with respect to cause and consequence. Furthermore, we only assessed average brain perfusion. Hence, we cannot exclude that brain perfusion in distinct brain regions may relate differently to cognitive performance. Finally, we could not measure blood flow into the cerebellum as we measured blood flow in the basilar artery at the level after the anterior and posterior inferior cerebellar arteries arise.

It can be hypothesized that cerebral hypoperfusion causes brain atrophy that subsequently leads to cognitive decline.1,13 Conversely, it may also be that because of a diminished demand, brain atrophy itself affects CBF. Thus, the association between tCBF and cognitive function may be mediated or confounded by brain atrophy.

In the past, CBF velocity measured by transcranial Doppler ultrasonography has been used as a proxy measure for CBF. Several studies using CBF velocity reported that subjects with greater CBF velocity were less likely to have dementia.2 Furthermore, a greater CBF velocity was found to be related with larger hippocampal and amygdalar volumes.2 More recently, associations of tCBF with speed, executive function,4 and dementia5 were found using phase-contrast MRI. Our data are in line with these studies, as we also found the strongest associations for cognitive domains of speed and executive function.4,5 However, none of those previous studies assessed whether the associations between tCBF and cognitive function were independent of brain volume. We went a step further by correcting for brain volume, and found no associations between total brain perfusion and cognitive function. Thus far, only a few small studies reported that regional patterns of hypoperfusion in the brain may relate to
cognitive decline or dementia independent of global differences. As mentioned, we could not evaluate this in our study. Further studies are needed to investigate this.

In conclusion, our findings show that the relation between tCBF and worse performance on several domains of cognitive function is dependent on brain volume. Our study emphasizes that future studies on tCBF should take into account brain atrophy.
REFERENCES


Assessment of cerebral small vessel disease predicts individual stroke risk

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Chapter 4

Incidental Findings on Brain MRI
4.1 Prevalence, determinants and clinical course of incidental findings on brain MRI

Submitted

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Chapter 5

General Discussion
Cerebral small vessel disease – i.e., pathologic alterations in the small penetrating arteries and arterioles of the brain – can be detected on magnetic resonance imaging (MRI) as lacunar infarcts, white matter lesions, and more recently, as cerebral microbleeds.\textsuperscript{1-2} Cerebral small vessel disease occupies an important position within the spectrum of presymptomatic brain pathology as it has been associated with both dementia and stroke.\textsuperscript{3-5}

The main objective of this thesis was to gain new insights into the pathophysiological processes underlying cerebral small vessel disease and the ways through which these are linked to dementia and stroke. This was accomplished by (i) examining cerebral microbleeds as new imaging marker of small vessel disease; (ii) identifying new vascular risk factors for cerebral small vessel disease; and (iii) exploring how these vascular risk factors and imaging markers of cerebral small vessel disease relate to clinical outcomes. All studies described in this thesis have been conducted as part of the Rotterdam Scan Study, a prospective population-based brain imaging study that aims to investigate causes and consequences of age-related brain changes using advanced MRI sequences.\textsuperscript{6-7}

In this chapter, I first summarize and review the main findings in the context of current knowledge. Next, I discuss the methodological issues of these studies that may have consequences for an accurate interpretation of our findings. Finally, suggestions for future research are given, and the potential clinical implications of our observations are discussed.

### InterpretaTion of Main Findings

**Cerebral Microbleeds**

Cerebral microbleeds are small foci of chronic blood products in normal (or near normal) brain tissue that can be detected \textit{in vivo} as small hypointense dots on T2*-weighted MR sequences.\textsuperscript{8-9} Over the past decade, they have emerged as an important new marker of cerebral small vessel disease.\textsuperscript{2,10} They may predict future hemorrhagic or ischemic stroke; they possibly contribute to cognitive impairment and dementia; and they are a potential key link between vascular and neurodegenerative patholo-
gies.\textsuperscript{11-16} As a result, there is an urgent need to establish their pathophysiology and clinical significance.

A previous study from the Rotterdam Scan Study reported on the high prevalence of cerebral microbleeds in 1062 community-dwelling people aged 60 years and older.\textsuperscript{17} During recent years, the Rotterdam Scan Study has been expanded with persons of 45 years and older. Information on microbleeds was now available for almost 4000 participants. In paragraph 2.1, we found that microbleeds are already present at middle age and that their prevalence gradually rises with age from 6.5\% in the age category of 45 to 50 years old to 35.7\% in participants of 80 years and older. The reported frequencies of microbleeds in the general population vary widely among studies which makes comparison difficult.\textsuperscript{18-21} These differences can be largely explained by heterogeneity in study populations, and more importantly, by differences in MRI scanning protocols.\textsuperscript{2,18-21}

From previous reports, it was also suggested that microbleeds are closely linked to underlying vasculopathies, in particular hypertensive arteriolosclerosis and cerebral amyloid angiopathy (CAA).\textsuperscript{2,17} In this expanded population-based cohort of middle aged and elderly persons, we confirmed our previous findings that cardiovascular risk factors, white matter lesions and lacunar infarcts are associated with microbleeds in a deep or infratentorial region, whereas APOE $\varepsilon 4$, a known risk factor for CAA, is related to microbleeds in a strictly lobar location (paragraph 2.1).\textsuperscript{17} A recent large meta-analysis also showed a robust association between APOE $\varepsilon 4$ and strictly lobar microbleeds.\textsuperscript{22} These results support the notion that the spatial distribution of microbleeds, in accordance with symptomatic intracerebral hemorrhage, likely reflects differences in underlying etiology.

More indirect support for the presumed association of lobar microbleeds with underlying CAA comes from our study on the spatial distribution of lobar microbleeds (paragraph 2.2). We found that lobar microbleeds, after correction for lobe volume and clustering effects, show a predilection for the posterior brain regions, particularly the temporal lobes. These posterior regions are known to be most affected in CAA.\textsuperscript{23-24}

From the above, it can be concluded that cerebral microbleeds likely reflect underlying vascular pathologies, i.e., CAA and hypertensive vasculopathy. However,
some researchers argue that the small microbleeds detected using more advanced imaging methods are either artifacts or may not share the same extent of underlying pathology as microbleeds that are more easily apparent on more conventional MR imaging. In 200 participants from our study who were scanned both with a conventional and high-resolution sequence, we found that participants who were rated positive for microbleed presence only on the high-resolution sequence were more alike in risk factor profile and risk of new microbleeds to persons who had microbleeds on both imaging sequences than to persons without microbleeds (paragraph 2.3). Although the microbleeds we find on our high-resolution sequence may still show only a small part of the underlying vascular pathology, our results indicate that it brings about clinically relevant information in community-dwelling elderly. Improved detection of cerebral microbleeds may thus lead to more accurate identification of persons with underlying vascular pathology. This is especially important in light of the fact that more advanced imaging techniques, such as susceptibility weighted imaging, are finding their way to clinical practice.

Assessment of underlying vascular pathology progression over time allows more precise analysis of the development of disease, and may be reflected by the development of new microbleeds. In paragraph 2.4, we showed that 10.2% of individuals developed new microbleeds on MRI over a 3-year interval, and that microbleeds generally do not disappear over time. Moreover, risk factors for incident microbleeds are similar to those for prevalent microbleeds and differed according to microbleed location, suggesting that assessment of microbleeds on T2*-weighted MRI over time may serve as a possible marker of progression of both CAA and hypertensive vasculopathy.

We – and others – reported previously that low serum total cholesterol levels are associated with an increased risk of symptomatic intracerebral hemorrhage. More recently, low cholesterol levels were also found to be related to the presence of cerebral microbleeds. However, the relative contributions of lipid fractions to these associations were unclear. In paragraph 2.5, we found that triglycerides were strongly and inversely associated with intracerebral hemorrhage, independent of HDL-cholesterol and LDL-cholesterol. Triglycerides were also associated with deep or infratentorial microbleeds, but not with strictly lobar microbleeds. These results provide additional support for a common underlying pathology for microbleeds and symptomatic intracerebral hemorrhage.
Concurrent with the growing body of literature on the high frequency of micro-bleeds in community-dwelling elderly and their potential link with specific underlying vascular pathological changes, some clinical studies suggested that microbleeds may play a role in impaired cognition. In almost 4000 middle aged and elderly non-demented persons, we studied how the presence and location of microbleeds related to various domains of cognitive function (paragraph 2.6). We found that presence of multiple microbleeds, especially in a strictly lobar location, was associated with worse performance on tests measuring cognitive function, even after adjustments for vascular risk factors, brain atrophy, lacunar infarcts and white matter lesions. These cross-sectional findings need yet to be confirmed in longitudinal studies, but do suggest an independent role for microbleed-associated vasculopathy in cognitive impairment. Two mechanisms are proposed by which microbleeds may influence cognitive function. Firstly, they may reflect focal damage of brain tissue and when located in strategic areas may interfere with cognitive processes. The alternative and more likely explanation is that microbleeds are a more general marker for the severity of small vessel disease and its underlying pathology, and as such may influence cognition. This last hypothesis could be especially interesting as it may help unravel the common pathophysiology of cerebrovascular and neurodegenerative disease. Location of microbleeds should thus be taken into account in future studies to examine the effects of underlying vasculopathy and amyloid deposition to cognitive impairment and dementia.

**Vascular Risk Factors and Clinical Outcomes**

Arterial stiffening (arteriosclerosis) is one of the earliest detectable manifestations of adverse structural and functional changes within the vessel wall. It reflects age-related generalized vascular disease, a process that is accelerated by hypertension. Carotid-femoral pulse wave velocity is the best validated method to non-invasively quantify arterial stiffness and has shown to be applicable in population-based studies. The interest in arterial stiffness arose when a high carotid-femoral pulse wave velocity was found to be associated with an increased risk of coronary heart disease and stroke in the general population independent of classic cardiovascular risk factors.

Potential mechanisms for this association between arterial stiffness and stroke involve pathways that include cerebral small vessel disease. We examined the relation between arterial stiffness, measured by pulse wave velocity, and cerebral small vessel
In paragraph 3.1, we found that persons with high arterial stiffness had a higher burden of small vessel disease, particularly among persons with uncontrolled hypertension. It is hypothesized that arterial stiffening exposes especially the microcirculation of the brain to abnormal flow pulsations, and as such contribute to the pathogenesis of cerebral small vessel disease. Shared underlying pathological mechanisms, however, may also be a possible explanation for the associations we found between arterial stiffness and markers of cerebral small vessel disease.

As vascular factors and stroke have been associated with cognitive function and dementia, an association of high arterial stiffness with cognitive decline and dementia was suggested. In paragraph 3.2, we examined this relation using carotid-femoral pulse wave velocity measurements, but did not identify arterial stiffness as an independent risk factor of cognitive decline or dementia. Several other studies did find an association between arterial stiffness and cognition over the past years. This discrepancy may be explained in several ways. First, selective attrition may have affected the results of our analyses regarding change in cognitive function; analyses regarding cognitive decline may have been affected by regression to the mean; and the low number of incident vascular dementia cases in our study does not completely rule out a possible association between increased arterial stiffness and risk of vascular dementia. On the contrary, more extensive adjustments compared to other studies may also explain why we did not find an association between arterial stiffness and cognition or dementia independent of other known risk factors for cognitive decline and dementia.

Another pathway through which underlying vascular mechanisms, such as hemodynamic changes and atherosclerosis, may contribute to cognitive impairment is hypoperfusion of the brain. Total cerebral blood flow, an indirect measure of brain perfusion, can be fast and accurately measured by phase-contrast MRI. In paragraph 3.3, we assessed the association between total cerebral blood flow and cognitive function. We found reduced cerebral blood flow to be associated with worse information processing speed, executive function and global cognition. However, after correcting total cerebral blood flow for brain volume, these associations disappeared. The association between total cerebral blood flow and cognition may thus be mediated or confounded by brain atrophy, indicating that future studies on total cerebral blood flow should take into account brain volume in their analyses.
Cerebral small vessel disease may be considered an intermediate phenotype between vascular risk factors on the one hand and cerebral end-organ damage and clinical outcomes on the other hand. Therefore, the assessment of cerebral small vessel disease may be relevant in predicting individual stroke risk. We investigated in paragraph 3.4 whether the presence of silent brain infarcts and white matter lesions on brain MRI could improve the prediction of stroke beyond a prediction based on the classic stroke risk factors from the Framingham Stroke Risk Function. We found that knowledge on presence or absence of small vessel disease improved the estimation of 10-year stroke risk, especially for women with an intermediate stroke risk. When we can use MRI to better distinguish people who are at high risk of stroke from those who are at low risk – especially in persons who are categorized as having an intermediate stroke risk based on the Framingham Stroke Risk Function – earlier and more extensive preventive treatment can be focused on those who will benefit from it most. This will improve primary stroke prevention and will save considerable costs. Cost-effectiveness analyses, however, will have to show whether these cost reductions counterbalance the costs of a two-stepped screening.

**Incidental Findings on Brain MRI**

From August 2005 onwards, more than 5000 individuals have undergone brain MRI as part of the Rotterdam Scan Study. An unintended, but inevitable consequence of the high resolution, state-of-the-art MRI techniques applied in our study in combination with this large number of scans acquired, is the detection of unexpected asymptomatic brain abnormalities unrelated to the purpose of our study. No established guidelines exist regarding their management as data on their prevalence and clinical course are limited. In paragraph 4.1, we examined the prevalence, determinants and clinical course of these incidental findings in the Rotterdam Scan Study. In total, 8.4% of participants had brain abnormalities on MRI, and cerebral aneurysms and meningiomas accounted for half of these findings. However, in most cases (85%) this remained without clinical consequences after persons were referred for further diagnostic examination. Older age, female sex and diabetes were related to the presence of meningiomas, whereas older age, female sex, smoking, use of blood pressure-lowering medication and HDL-cholesterol were all related to the presence of aneurysms. Modification of cardiovascular risk factors may therefore serve as target for the prevention of intracerebral aneurysms in the general population. However, this depends on whether the natural course and risk of incidentally discovered unruptured
aneurysms warrants any prevention at all. Further insights into the course of these incidental findings and whether persons will benefit from interventions are therefore needed.

**METHODOLOGICAL CONSIDERATIONS**

**Population-based Cohort Studies**

The Rotterdam Scan Study is embedded in the Rotterdam Study, a prospective population-based cohort study. Because of their typical high costs (due to the requirement of large sample sizes with long follow-up periods) and logistic complexities (due to the need for reassessment of exposure and outcome on a frequent basis), population-based cohort studies are not frequently conducted. Nevertheless, population-based cohort studies are informative for efficiently studying a wide range of exposure-disease associations, and are praised for their high degree of external validity.

Population-based cohort studies may be subject to biases including selection bias, information bias and confounding. Although we try to minimize these biases in our study by aiming at high response rates, minimal loss to follow-up, acquisition and analyses of data blinded to other parameters, and adjustment for known confounders, our results may still be subject to biases. Selective drop-out of individuals with cognitive impairment or physical constraints, for example, will result in an underestimation of the true effect. In addition, repeated cognitive testing is associated with learning effects that reduces sensitivity to longitudinal decline in cognitive functioning.

**Population-based Imaging**

The large-scale assessment of presymptomatic brain pathology in the general population requires standardized non-invasive imaging. To date, MRI is the most sensitive non-invasive imaging tool available to study brain pathology. From August 2005 onwards, a dedicated 1.5 Tesla MRI scanner is operational in the research center of the Rotterdam Study. All scans are collected according to a standardized imaging protocol, and no hardware or software alterations are allowed to ascertain comparability of scans between individuals and over time.
The continuous outcomes obtained from observations on brain MRI are generally more sensitive and powerful than the dichotomous outcomes obtained from disease outcomes. In addition, validity and reliability of MRI data is ensured by standardized acquisition as well as standardized evaluation of data. Nowadays, several measures are quantified with validated (fully) automated techniques (e.g., white/grey matter and white matter lesion volumes); this gives us a quick, reproducible and quantitative measure. However, automated evaluations are not yet available for some other measures, such as cerebral microbleeds and infarcts. For these evaluations, strict procedural guides are needed to avoid disagreement among readers. Although intra- and interreader reliabilities have shown to be consistently high in our study, visual readings are time consuming and very labor intensive. Currently, automated methods for microbleed detection are underway, and improving technologies may provide possibilities for automated detection of other imaging markers in the short term. These automated methods for detection of brain pathology will expand the opportunities for investigation as they may quick and reproducibly allow the comparison of anatomical distributions of brain pathology between individuals or groups.

This standardized acquisition and analysis of brain scans allows reliable cross-sectional assessment of presymptomatic brain pathology, but more importantly, also allows objective and reproducible assessment of brain pathology over time. In this thesis, most data were acquired cross-sectionally precluding the interpretation of cause and consequence. In the coming years, more prospective data will become available in our study that will contribute to the understanding of cause and effect relationships between potential risk factors and brain pathology. In addition, despite the large size of our study cohort, the number of people who developed incident dementia or stroke is currently small. More follow-up time is needed to reliably investigate the relation between imaging markers of brain pathology and clinical outcomes.

More widespread use of these standardized protocols for acquisition and analysis of data will facilitate pooling of data from different centers and over different studies; this will help studying rare exposures, rare outcomes and small effect sizes, e.g., genome-wide association studies of brain pathology burden in large international consortia.
The ultimate aim of epidemiological research is the prevention or effective control of human disease. With as many as 35 million people worldwide afflicted with dementia and 15 million people that suffer from stroke each year, there is still a long way to go.\textsuperscript{52-53} Although epidemiological studies on cerebral small vessel disease – by us and others – have added important knowledge to our understanding of both cerebrovascular and neurodegenerative disease, several aspects still remain unclear. These unanswered questions open new avenues for future research and a selection of them are described below. Finally, some recent findings that may change management in clinical trials as well as in clinical practice are discussed.

Cerebral Microbleeds

Cerebral microbleeds are primarily a radiological construct which are thought to represent hemosiderin deposits in the brain.\textsuperscript{2} In this thesis, indirect but robust support was found for a relation between cerebral microbleeds and underlying vascular pathology, in particular CAA and hypertensive vasculopathy. However, evidence from autopsy studies about the origin of these hemosiderin deposits and its relation with underlying pathology is still very limited.\textsuperscript{8-9,54} There is a preliminary but important indication that what is perceived as a ‘microbleed’ on MRI, i.e., a small hypointense focus on T2*-GRE imaging, may pathologically correspond to a more diverse spectrum of pathology, including not only microhemorrhages, but also for example, microinfarcts.\textsuperscript{55} Further autopsy studies and animal experiments are clearly warranted to study the pathogenesis of microbleeds.

In light of their underlying pathology, cerebral microbleeds may be crucial in further unraveling the relationship between CAA, hypertensive vasculopathy and Alzheimer's disease (AD). It is proposed that microbleeds may be a common downstream product of the amyloid cascade and vascular pathology and in this way play a crucial role in the pathophysiology of AD.\textsuperscript{16} Several findings from our studies underscore the possible high relevance of microbleeds in CAA and AD: APOE ε4, a risk factor for both CAA and AD, was related to strictly lobar microbleeds; the lobar distribution of microbleeds had, in accordance with findings in CAA and AD patients, a posterior predominance; and numerous microbleeds, especially in a strictly lobar location, were associated with worse cognitive function. In coming years, longitudinal data will have
to show whether microbleeds are also associated with cognitive decline and, ultimately, the risk of AD.

Another clinically relevant question needing a definite answer is whether microbleeds are a marker of an increased risk of (recurrent) hemorrhagic or ischemic stroke. Although some small clinical studies indeed suggested an association between microbleeds and risk of (recurrent) stroke, to date only one large prospective study has been performed in healthy elderly individuals. The authors found that the presence of microbleeds was associated with an excess risk of ischemic stroke and — even higher — risk of intracerebral hemorrhage. Replication of these results in prospective population-based studies is required since, if indeed true, this will raise questions about the safety of antithrombotic drug use in individuals with presence of microbleeds.

Several, mostly cross-sectional studies investigating the association between use of antithrombotic or thrombolytic drugs and cerebral microbleeds have been performed, but yielded inconclusive results. Recently, a large meta-analysis in transient ischemic attack patients and ischemic or hemorrhagic stroke patients suggested that warfarin may be harmful in patients with presence of microbleeds. In this pooled analysis, also a weak association was found between the presence of microbleeds and risk of intracerebral hemorrhage in antiplatelet users versus non-users. This warrants additional research determining the risks and benefits of antithrombotic or thrombolytic treatment in individuals with presence of microbleeds.

Cholesterol is suggested to play an important role in maintaining the integrity of small cerebral vessels. We found an inverse association of triglycerides with deep or infratentorial microbleeds and risk of intracerebral hemorrhage. In addition, the Stroke Prevention by Aggressive Reduction in Cholesterol Levels (SPARCL) study showed a reduction in risk for stroke and transient ischemic attack, but also a slightly increased risk of intracerebral hemorrhage among patients receiving high-dose statin treatment. This has raised concerns over the use of statins in persons with presence of microbleeds or a history of intracerebral hemorrhage. Future research is needed to assess whether the protective effects of statins for ischemic stroke outweigh the possible excess risk of intracerebral hemorrhage in these persons, especially in the long term.
**Vascular Risk Factors and Clinical Outcomes**

Arterial stiffness is associated with an excess risk of stroke and – possibly – dementia independent of the classic cardiovascular risk factors. A possible role for cerebral small vessel disease in these associations is suggested by us and others. These findings may encourage assessment of arterial stiffness routinely in clinical practice. However, the value of arterial stiffness for the reduction of these clinical outcomes under treatment needs yet to be demonstrated. To determine this, clinical trials need to investigate whether a reduction in arterial stiffness is associated with a concomitant reduction in clinical outcomes, independently of the normalization of classic cardiovascular risk factors. Assessment of cerebral small vessel disease on MRI may play a role in these trials as this will allow evaluating effects earlier than by clinical observations alone. A lack of standard methodology for measuring pulse wave velocity and good reference values has hampered the current use of arterial stiffness in clinical trials, but reference and normal values for pulse wave velocity from a large multicenter European cohort were recently published; this may encourage the future use of arterial stiffness in clinical trials.

Cerebral perfusion and its autoregulation aim to stabilize blood flow to the brain during variations in perfusion pressure. This vital mechanism is found to be impaired in patients with ischemic stroke as well as in AD. The relationship between cerebral perfusion and AD is likely to be bidirectional. Cerebrovascular disease, through chronic hypoperfusion and oxidative stress, modulates amyloid deposition and may as such initiate or aggravate AD pathology. On the other hand, AD in itself may lead to vascular disease, as excess amyloid depositions exert detrimental effects on cerebrovascular function. Disentangling these effects in longitudinal studies will give more insight in the complex interaction between cerebrovascular and neurodegenerative disease. Advanced measurements of brain perfusion using arterial spin labeling (ASL) or dynamic susceptibility contrast (DSC) with MRI may have added value as both methods provide information on regional cerebral perfusion that is not provided by the phase contrast method we used to measure total cerebral blood flow. Between the two methods, ASL may be more suitable for implementation in population-based studies since this method is non-invasive, whereas DSC requires intravenous contrast.

In order to effectively prevent a disease, one may target preventive therapies to a whole population or to those who have the highest risk of developing disease.
Although a risk factor may be significantly related to a disease, this does not necessarily mean that this risk factor has additive value for individual risk prediction. In this thesis, we found that addition of silent brain infarcts and white matter lesions to the Framingham Stroke Risk Function improved the prediction of stroke. The predictive value can possibly further be improved by adding cerebral microbleeds and other newly emerging imaging characteristics to this prediction model. Unfortunately, data on these imaging markers in combination with long-term clinical outcome measures are not yet available in our population under study. Future studies should continue the search for risk factors that may improve individual risk prediction, and investigate whether these risk prediction tools may be useful in clinical practice. However, cost-effectiveness analyses and evaluation of advances in treatment options will have to show whether brain MRI may be a worthwhile tool for identifying people at high risk of stroke in (specific subgroups of) the general population.

**Incidental Findings on Brain MRI**

In the coming years, the number of studies using brain MRI will only increase, resulting in an increasing number of incidental findings being detected. Currently, the way researchers handle these findings varies considerably. Yet, as incidental findings are highly prevalent in adult subjects, there is need to establish evidence-based protocols for the management of incidental findings. Such protocols should take into account the current ethical principles, the level of duty of care a researcher has to the research participant, and the likely risk of missing serious incidental findings or of incorrectly diagnosing inconsequential incidental findings. However, at the same time these protocols should be feasible and practical within research practice. Discussion of research practice for handling of incidental findings is needed among a broad cross section of experts (i.e., legal, ethical, medical and patient perspectives) in order to develop and review these protocols according to up-to-date knowledge and new insights. The data described in this thesis provide insights in the prevalence, determinants and clinical course of incidental brain findings to fuel this discussion.

**Advanced Imaging Modalities**

MR imaging methods are continuously being improved resulting in higher field strengths, thinner slices, and better tissue contrasts. It is likely that the measured prevalence and severity of presymptomatic brain pathology will continue to increase as more advanced imaging modalities are being developed and used. For example, choice of MRI
parameters as well as higher field strength and use of advanced postprocessing (e.g., susceptibility weighted imaging) substantially affect the detection rate of microbleeds. Moreover, markers of small vessel disease that are only detectable on advanced MRI techniques, e.g., the microstructural integrity of white matter, are suggested as more sensitive estimates of tissue damage and may as such contribute to our understanding of the early development of cerebral small vessel disease. In line with this, several population-based prospective autopsy studies suggested that cortical microinfarcts, another expression of cerebral small vessel disease, may be a major determinant of dementia. These microinfarcts, however, are not readily detectable on MRI.

Besides improvements in image acquisition, major improvements have also been made in image processing and analysis. One of these improvements is automatic segmentation of the brain into its main tissues and anatomical structures, e.g., white and grey matter segmentation and cortical thickness measurements. These image processing and analyzing techniques are now increasingly used in research settings, and are also being introduced into clinical practice. In our institute, for example, a Computer Aided Diagnosis (CAD) system is being implemented that will allow clinicians to compare data on brain pathology of a single patient with reference data from a population-based setting, hereby correcting for sex, age and other metadata.

Advances are also made by the development of new techniques to image specific underlying pathology. A recent major breakthrough in CAA and AD research is the development of amyloid imaging techniques that enable in vivo detection of amyloid deposition in the brain. Positron Emission Tomography (PET) imaging with amyloid ligand Pittsburgh compound B, for example, has shown to detect CAA as well as plaque amyloid in both patients with AD as well as in healthy elderly. PET imaging may not be easily implemented in large population-based cohort studies, but may help to establish where exactly microbleeds fit in the pathogenesis of CAA.

With more advanced imaging techniques and more markers of small vessel disease emerging, there is need for studies that combine these different markers and elucidate their relative contribution to clinical outcomes. There is also a need to investigate regional differences in the brain (e.g., regional atrophy and regional perfusion) as it is suggested that regional patterns may relate to clinical outcomes independent of global differences.
Clinical Trials and Clinical Practice

Several clinical trials investigating new treatment approaches for dementia and stroke are in progress.  However, large sample sizes are required to determine treatment efficacy when using stroke and dementia as endpoints in clinical trials. This is due to several issues that complicate treatment efficacy: the risk of (recurrent) stroke is relatively low; the rate of progression to dementia is slow; and assessment of cognitive decline is complicated by learning effects that reduces sensitivity. This has led to the suggestion that trials with clinical endpoints could use the progression of cerebral small vessel disease as an additional outcome variable to evaluate therapeutic effects earlier than by clinical observations alone. These objective and reproducible imaging measures may provide additional insights into the mechanisms of clinical interventions in therapeutic trials. To date, few longitudinal imaging data have been obtained in large stroke and dementia trials, but this is likely to grow rapidly in the coming years.

As cerebral microbleeds and its underlying pathology are suggested to play an important role in the development of AD, they may be of importance with regard to new therapies tested in clinical trials. For example, microbleeds may be used as a marker of disease progression in a trial. However, there are also indications that microbleed presence may be a risk factor to develop drug-related complications, for example amyloid-related imaging abnormalities in immunotherapy trials or bleeding in aspirin trials. Therefore, researchers have been hesitant to include persons with microbleeds in clinical trials on drugs for AD. It is been suggested that presence of multiple, especially lobar, microbleeds may be used as exclusion criteria for these trials, but exact numbers and guidelines are lacking. Yet, as microbleeds are highly prevalent in AD patients, excluding all persons with microbleeds from such trials should only be based on firm evidence. More data about the influence of prevalence, number and location of microbleeds on efficacy of new therapies is required to guide the design of future clinical trials.

In clinical practice, the relatively arbitrary separation of AD and vascular dementia may no longer be the best approach as increasing evidence suggests a continuum of AD pathology and vascular brain disease. It may be more informative to distinguish disease in clinical practice on the basis of MRI findings without the assumption that these subgroups indeed reflect clearly separable diagnostic entities. In line with this
perspective, treatment options may possibly be best based on MRI findings, and MRI markers may be used to evaluate treatment efficacy and disease progression.\textsuperscript{108} It should be aimed to combine several imaging markers and modalities together as the clinical utility of MRI markers rests largely on whether they offer additional information beyond which can be obtained from other imaging markers and clinical information. There are high hopes that this approach – possibly in combination with other biomarkers such as cerebrospinal fluid biomarkers and PET imaging – will improve the diagnosis and treatment of the individual patient with dementia in coming years.
REFERENCES


80. Wellcome Trust, Sinapse, and The Royal College of Radiologists. Management of incidental findings detected during research imaging. 2011.


Summary / Samenvatting
Chapter 1, the general introduction, provides the background and aim of this thesis. Dementia and stroke are common disorders in the elderly that have great impact on brain functioning and the way people live their lives. Since few therapeutic possibilities exist for these neurological diseases, effective prevention strategies are urgently needed. In order to develop such strategies for dementia and stroke, it is crucial to explore the early presymptomatic phases of these diseases. Magnetic resonance imaging (MRI) has proven to be a very suitable technique, as it offers detailed information about presymptomatic pathology in the brain without exposure to radiation.

Cerebral small vessel disease – i.e., pathologic alterations in the small penetrating arteries and arterioles of the brain – can be detected on MRI as lacunar infarcts, white matter lesions, and more recently, as cerebral microbleeds. Cerebral small vessel disease occupies an important position within the spectrum of presymptomatic brain pathology as it has been associated with both dementia and stroke. A better understanding of the pathophysiology of cerebral small vessel disease may therefore provide new insights in the connections between both diseases.

The main objective of this thesis was to gain new insights into the pathophysiology of small vessel disease in the aging brain. This was accomplished by (i) examining cerebral microbleeds as new imaging marker of small vessel disease; (ii) identifying new vascular risk factors for cerebral small vessel disease; and (iii) exploring how these vascular risk factors and imaging markers of cerebral small vessel disease relate to clinical outcomes. All studies described in this thesis have been conducted as part of the Rotterdam Scan Study, a large-scale prospective population-based imaging study that aims to investigate causes and consequences of age-related brain changes.

Chapter 2 focuses on cerebral microbleeds, small foci of chronic blood products in normal (or near normal) brain tissue, that can be detected \textit{in vivo} as small hypointense dots on T2*-weighted MR sequences. Over the past decade, cerebral microbleeds have emerged as an important new marker of cerebral small vessel disease. A previous study from the Rotterdam Scan Study reported on the high prevalence of cerebral microbleeds in 1062 community-dwelling persons aged 60 years and older. In paragraph
2.1, this prevalence estimate was updated in a larger and younger cohort consisting of almost 4000 persons aged 45 years and older. We found that microbleeds are already present at middle age and that prevalence gradually rises with age from 6.5% in the age category of 45 to 50 years old to 35.7% in participants of 80 years and older. Furthermore, we confirmed our previous findings that cardiovascular risk factors, white matter lesions and lacunar infarcts are associated with microbleeds in a deep or infratentorial region, whereas APOE ε4 – a known risk factor for cerebral amyloid angiopathy (CAA) – is related to microbleeds in a strictly lobar location. These results support the notion that microbleeds – in accordance to symptomatic intracerebral hemorrhages – are closely linked to underlying vasculopathies, in particular hypertensive arteriolosclerosis and CAA. More indirect support for the presumed association of lobar microbleeds with underlying CAA comes from our study on the spatial distribution of lobar microbleeds in the general population. (paragraph 2.2) We found that lobar microbleeds show a predilection for the posterior brain regions, particularly the temporal lobes, which are known to be most affected in CAA.

Microbleed detection is heavily dependent on MRI parameters, magnetic field strength and post-processing algorithms. However, it has been debated whether improved microbleed detection also results in clinically relevant data. In 200 study participants who were scanned with both a conventional and high-resolution MR sequence, we found that participants who were rated positive for microbleed presence only on the high-resolution sequence were more alike in risk factor profile and risk of new microbleeds to persons who had microbleeds on both imaging sequences than to persons without microbleeds. (paragraph 2.3) Improved detection of cerebral microbleeds may thus lead to more accurate identification of persons with underlying vascular pathology.

Assessment of underlying vascular pathology progression over time allows more precise analysis of the development of diseases, and may be reflected by development of new microbleeds. In paragraph 2.4, we showed that 10.2% of all individuals developed new microbleeds on MRI over a 3-year interval, and that microbleeds rarely disappeared over time. Moreover, risk factors for incident microbleeds were similar to those for prevalent microbleeds and differed according to microbleed location, suggesting that assessment of microbleeds on T2*-weighted MRI over time may serve as a possible marker of both CAA and hypertensive vasculopathy progression.
Low serum total cholesterol levels are associated with an increased risk of symptomatic intracerebral hemorrhage as well as with the presence of cerebral microbleeds. The relative contribution of lipid fractions to these associations, however, was unclear. We reported in paragraph 2.5 that low triglyceride levels are associated with presence of deep or infratentorial microbleeds as well as the risk of intracerebral hemorrhage. These results support the connection between asymptomatic microbleeds and symptomatic intracerebral hemorrhage.

Vascular pathology plays a prominent role in impaired cognitive function. Cerebral microbleeds may reflect underlying vascular disease, but their role in cognitive function was largely unknown. In paragraph 2.6, we found that presence of multiple microbleeds, especially in a strictly lobar location, was associated with worse performance on tests measuring cognitive function, even after adjustments for vascular risk factors, brain atrophy and other imaging markers of small vessel disease. These findings suggest an independent role for microbleed-associated vasculopathy in cognitive impairment.

Chapter 3 is dedicated to new vascular risk factors for cerebral small vessel disease and to how these vascular risk factors and imaging markers of cerebral small vessel disease relate to clinical outcomes. Arterial stiffness – a relatively novel marker of cardiovascular damage – has been associated with an excess risk of stroke in the general population, independent of the classic stroke risk factors. Potential mechanisms for this association between arterial stiffness and stroke involve pathways that include cerebral small vessel disease. We examined the relation between arterial stiffness, measured by carotid-femoral pulse wave velocity, and cerebral small vessel disease in paragraph 3.1. We found arterial stiffness to be related to cerebral small vessel disease, and that associations were most pronounced in persons with uncontrolled hypertension.

As vascular factors and stroke have been associated with cognitive function and dementia, an association between increased arterial stiffness and cognitive decline or dementia has been suggested. In paragraph 3.2, we examined this relation, but did not identify arterial stiffness as an independent risk factor of cognitive decline or dementia.

Another pathway through which underlying vascular mechanisms may contribute to cognitive impairment is hypoperfusion of the brain. Total cerebral blood flow, an indirect measure of brain perfusion, can be fast and accurately measured by phase-contrast MRI.
In paragraph 3.3, we assessed the association between total cerebral blood flow and cognitive function. We found reduced cerebral blood flow to be associated with worse information processing speed, executive function and global cognition. However, after correcting total cerebral blood flow for brain volume, these associations disappeared. The association between total cerebral blood flow and cognition may thus be mediated or confounded by brain atrophy.

Cerebral small vessel disease may be considered an intermediate phenotype between vascular risk factors on the one hand and cerebral end-organ damage and clinical outcomes on the other hand. Therefore, the assessment of cerebral small vessel disease may be relevant in predicting individual stroke risk. We investigated in paragraph 3.4 whether the assessment of cerebral small vessel disease on brain MRI could improve the prediction of stroke beyond a prediction model based on the classic stroke risk factors from the Framingham Stroke Risk Function. We found that knowledge on presence or absence of small vessel disease improved the estimation of 10-year stroke risk, especially for women with an intermediate stroke risk. However, cost-effectiveness analyses and evaluation of advances in treatment options have to show whether brain MRI may be a worthwhile tool to identify people at high risk of stroke in (specific subgroups of) the general population.

From August 2005 onwards, more than 5000 individuals have undergone brain MRI as part of the Rotterdam Scan Study. An unintended but inevitable consequence of the high resolution, state-of-the-art MRI techniques applied in our study in combination with this large number of scans acquired, is the detection of unexpected asymptomatic brain abnormalities unrelated to the purpose of our study. In chapter 4, the prevalence, determinants and clinical course of these incidental findings in the Rotterdam Scan Study were examined. In total, 8.4% of participants had brain abnormalities on MRI. Cerebral aneurysms and meningiomas accounted for half of these findings. In most cases (85%) this remained without clinical consequences after persons were referred for further diagnostic examination or treatment. Older age, female sex and diabetes were related to the presence of meningiomas, whereas older age, female sex, smoking, use of blood pressure-lowering medication and low high-density lipoprotein cholesterol were all related to the presence of aneurysms. Our data provide insights in the prevalence, determinants and clinical course of incidental brain findings on which to base future recommendations for referral and clinical management of incidental findings.
In chapter 5, the general discussion, the main findings are reviewed in the context of current knowledge. Also, methodological considerations with regard to the studies in this thesis are described; this includes aspects of population-based imaging, cross-sectional versus longitudinal data and automated rating versus visual readings of brain pathology. Finally, future research directions and clinical implications are discussed, such as the use of cerebral small vessel disease as surrogate marker in clinical trials and the shifting paradigm of the arbitrary separation of Alzheimer's disease pathology and vascular brain disease into a more continuous scale of pathologies.
SAMENVATTING

In hoofdstuk 1, de algemene introductie, wordt de achtergrond van het onderzoek in dit proefschrift beschreven en worden de doelstellingen nader toegelicht. Dementie en beroerte zijn aandoeningen die onder ouderen vaak voorkomen en die grote invloed hebben op de hersenfuncties en de kwaliteit van leven. Omdat de behandelingen voor beide hersenaandoeningen op dit moment nog beperkt zijn, is het van groot belang om succesvolle mogelijkheden te vinden om deze ziektes te voorkomen. Om dit te kunnen doen, is het essentieel om de vroege fase van dementie en beroerte te onderzoeken waarin men nog geen klinische symptomen heeft. Op dit moment is het afbeelden van de hersenen met behulp van Magnetic Resonance Imaging (MRI) de meest geschikte techniek voor het onderzoeken van presymptomatische afwijkingen in de hersenen, omdat MRI gedetailleerde informatie geeft over hersenstructuren zonder dat hiervoor schadelijke straling nodig is.

Kleine herseninfarcten, wittestofafwijken en sinds kort ook kleine hersenbloedingen (zogenaamde microbloedingen) die met behulp van MRI te zien zijn, duiden microvasculaire schade in de hersenen aan, die ook wel cerebrale microangiopathie wordt genoemd. Cerebrale microangiopathie neemt een belangrijke positie in binnen het spectrum van presymptomatische afwijkingen in de hersenen, omdat het geassocieerd is met zowel dementie als beroerte. Een beter begrip van cerebrale microangiopathie kan daarom mogelijk bijdragen aan nieuwe inzichten in de relatie tussen beide ziektes. In dit proefschrift onderzocht ik dit door (i) het onderzoeken van microbloedingen als nieuwe marker van cerebrale microangiopathie; (ii) het identificeren van nieuwe vasculaire risicofactoren (risicofactoren met betrekking tot de bloedvaten) voor cerebrale microangiopathie; en (iii) het bestuderen van de klinische betekenis van deze nieuwe risicofactoren en van MRI markers van cerebrale microangiopathie. Alle studies beschreven in dit proefschrift maken deel uit van de Rotterdam Scan Study, een groot schaalig en langlopend onderzoek naar hersenafwijkingen in de algemene bevolking, waarbij gebruik wordt gemaakt van MRI-technieken. Het doel van de Rotterdam Scan Study is om oorzaken en gevolgen van aan veroudering gerelateerde hersenafwijkingen te onderzoeken.

Hoofdstuk 2 is in zijn geheel gewijd aan cerebrale microbloedingen. Dit zijn zeer kleine bloedingen in de hersenen die tijdens het leven kunnen worden gezien als
kleine, donkere puntjes op een zogenaamde T2*-gewogen MRI sequentie. Pas in het laatste decennium is men deze microbloedingen gaan zien als een belangrijke marker van cerebrale microangiopathie. Uit een eerdere studie binnen de Rotterdam Scan Study onder 1062 personen van de algemene bevolking bleek dat microbloedingen relatief vaak voorkomen bij mensen van 60 jaar en ouder. In paragraaf 2.1 staan de resultaten van ons onderzoek onder een nog grotere en jongere groep deelnemers beschreven. Uit onderzoek binnen deze groep van bijna 4000 mensen van de algemene bevolking met een leeftijd van 45 jaar en ouder is gebleken dat microbloedingen vaak al aanwezig zijn op middelbare leeftijd. Daarnaast is ook duidelijk geworden dat microbloedingen vaker voorkomen naarmate men ouder wordt: van 6.5% in de leeftijdsgroep 45-50 jaar tot 35.7% in de leeftijdsgroep van 80 jaar en ouder. We bevestigden in dit onderzoek ook de bevindingen van onze eerdere studie dat risicofactoren voor hart- en vaatziekten, kleine herseninfarcten en wittestofafwijkingen gerelateerd zijn aan microbloedingen die zich diep in de hersenen of in de kleine hersenen (infratentorieel) bevinden. Deze bevinding suggereert dat diepe of infratentoriële microbloedingen mogelijk een gevolg zijn van vaatschade door hoge bloeddruk en verstijving van de wand van de kleine bloedvaten, ook wel hypertensieve vasculopathie genaamd. Daarnaast hebben wij ook een verband gevonden tussen het apolipoproteine E gen en lobeire microbloedingen (microbloedingen in de kwabben van de grote hersenen). Het apolipoproteine E gen is een risicofactor voor cerebrale amyloid angiopathie, een aandoening waarbij de kleine bloedvaten in de hersenen door stapeling van amyloid-eiwit broos worden en daardoor gemakkelijk bloeden. Onze bevindingen bevestigen dat cerebrale amyloid angiopathie mogelijk een belangrijk mechanisme is dat tot lobeire microbloedingen kan leiden.

Om meer bewijs te vinden voor de hypothese dat microbloedingen zijn gerelateerd aan specifieke onderliggende vaatschade in de hersenen, onderzochten wij de verdeling van microbloedingen over de verschillende hersenkwabben. (paragraaf 2.2) Daaruit bleek dat microbloedingen vaker voorkomen in de achterste regionen van de grote hersenen. Aangezien een vergelijkbare verdeling van afwijkingen over de hersenkwabben wordt gevonden in patiënten met cerebrale amyloid angiopathie, wijzen deze bevindingen ook in de richting van een mogelijke relatie tussen cerebrale amyloid angiopathie en lobeire microbloedingen.
Doordat MRI-technieken steeds geavanceerder zijn geworden, worden er ook steeds meer microbloedingen opgespoord. Het is echter de vraag of deze verbeterde opsporing ook klinisch belangrijk is. Dit hebben wij getest door 200 oudere deelnemers zowel te scannen met behulp van de standaard T2*-gewogen MRI sequentie als met de hoge-resolutie T2*-gewogen MRI sequentie, waarmee meer microbloedingen kunnen worden opgespoord. Wij vonden dat deelnemers waarbij microbloedingen alleen zichtbaar waren op de hoge-resolutie sequentie voor wat betreft risicoprofiel en kans op nieuwe microbloedingen het meest overeenkwamen met deelnemers waarbij op beide sequenties microbloedingen werden opgespoord. (paragraaf 2.3) Onze conclusie is dat op de hoge-resolutie T2*-gewogen MRI sequentie meer microbloedingen worden gevonden die waarschijnlijk ook klinisch belangrijk zijn.

Door het volgen van de ontwikkeling van schade aan de hersenvaten kunnen we meer te weten komen over het ontstaan van deze schade. Microbloedingen kunnen mogelijk een teken zijn van toename van microvasculaire schade in de hersenen. Echter, de frequentie waarmee nieuwe microbloedingen ontstaan, oftewel de incidentie, was in de algemene bevolking onbekend. In paragraaf 2.4 tonen wij dat bij 10.2% van oude personen uit de algemene bevolking over een periode van drie jaar nieuwe microbloedingen op de scan zichtbaar zijn en dat microbloedingen niet lijken te verdwijnen over tijd. Risicofactoren voor deze nieuwe microbloedingen waren gelijk aan de risicofactoren die we vonden voor al aanwezige microbloedingen (zie paragraaf 2.1) en verschillen naar gelang de locatie waar de bloedingen zich in de hersenen bevonden. Deze bevindingen suggereren dat microbloedingen mogelijk als marker kunnen dienen van toename van onderliggende schade aan de hersenvaten.

Lage cholesterolwaarden in het bloed zijn eerder in verband gebracht met de aanwezigheid van microbloedingen in de hersenen en met een hoger risico op een symptomatische hersenbloeding. Het was echter niet bekend welke soorten vetten (lipiden) een belangrijke rol spelen in deze relatie. Wij vonden dat lage waarden van triglyceriden in het bloed gerelateerd zijn aan de aanwezigheid van diepe en infratentoriële microbloedingen en dat deze lage triglyceridenwaarden ook een hoger risico geven op een hersenbloeding. (paragraaf 2.5) Deze bevinding vormt een aanvullend bewijs dat microbloedingen en symptomatische hersenbloedingen mogelijk dezelfde oorzaken hebben.
Vaatschade is een belangrijke oorzaak van een slechtere hersenfunctie (cognitie) in ouderen. Zoals eerder beschreven zijn cerebrale microbloedingen mogelijk een afspiegeling van schade aan de kleine hersenvaten. Lang was echter onbekend welke invloed deze microbloedingen op het cognitieve functioneren hebben. Wij onderzochten in bijna 4000 personen van middelbare en oudere leeftijd of cerebrale microbloedingen gerelateerd zijn aan cognitief functioneren. (paragraaf 2.6) We vonden dat personen met microbloedingen, en vooral lobeire microbloedingen, slechter presteren in tests die het cognitief functioneren meten, zelfs wanneer we corrigeerden voor de aanwezigheid van risicofactoren voor hart- en vaatziekten, hersenvolume, kleine herseninfarcten en wittestofafwijkingen. Deze bevindingen suggereren dat microbloedingen (als afspiegeling van onderliggende vaatschade) bijdragen aan slechtere cognitieve functies in ouderen, onafhankelijk van andere schade in de hersenen.

Hoofdstuk 3 richt zich op nieuwe vasculaire risicofactoren en de klinische betekenis van cerebrale microangiopathie. Een hoge vaatwandstijfheid – een relatief nieuwe meting om vroege schade in de slagaders van het lichaam aan te duiden – bleek al eerder een verhoogd risico te geven op een beroerte, onafhankelijk van de meest bekende risicofactoren voor hart- en vaatziekten. In deze relatie speelt schade aan de kleine hersenvaten mogelijk een rol. Daarom onderzochten wij of vaatwandstijfheid gerelateerd is aan de aanwezigheid van cerebrale microangiopathie. (paragraaf 3.1) Wij constateerden dat dit verband er is, en de bevindingen waren het meest uitgesproken bij personen met een hoge bloeddruk.

Aangezien zowel vasculaire factoren als een beroerte een hoger risico geven op dementie, was het aannemelijk dat er mogelijk ook een verband zou zijn tussen vaatwandstijfheid en cognitief functioneren of dementie. Wij hebben dit binnen onze studiepopulatie onderzocht, maar geen verbanden gevonden. (paragraaf 3.2)

Een andere manier waarop onderliggende vasculaire mechanismen mogelijk kunnen bijdragen aan slechtere cognitief functioneren, is een slechte doorbloeding van de hersenen. In onze studiepopulatie vonden we dat een lage hersendoorbloeding inderdaad gerelateerd was aan slechtere prestaties op diverse cognitieve gebieden (snelheid van informatieverwerking, uitvoerende functies en globale cognitie). (paragraaf 3.3) Wanneer we echter rekening hielden met het totale hersenvolume in de
berekening van de hersendoorbloeding, dan verdwenen deze associaties. Het verband tussen hersendoorbloeding en cognitie wordt dus mogelijk vertroebeld of veroorzaakt door atrofie (verlies) van hersenweefsel.

De ernst van cerebrale microangiopathie laat mogelijk zien hoe de hersenen reageren op risicofactoren voor hart- en vaatziekten. Daarom kan het evalueren van cerebrale microangiopathie mogelijk bijdragen aan het voorspellen van wie een beroerte zal krijgen en wie niet. In paragraaf 3.4 onderzochten wij of de aanwezigheid van stille herseninfarcten (zonder symptomen) en wittestofafwijkingen bijdraagt aan het herkennen van personen met een verhoogd risico op een beroerte. Hiervoor werden deze hersenafwijkingen toegevoegd aan de Framingham Stroke Risico Functie, een risicofunctie waarin de meeste bekende risicofactoren voor hart- en vaatziekten zijn verwerkt. We vonden dat kennis over de aanwezigheid van stille herseninfarcten en wittestofafwijkingen bijdraagt aan een betere voorspelling van wie wel en wie geen beroerte zal krijgen binnen tien jaar. De grootste verbetering in voorspelling werd behaald bij vrouwen die op basis van de Framingham Stroke Risico Functie een gemiddeld risico hadden op een beroerte. Aangezien MRI echter een kostbaar onderzoek is, moet verder onderzocht worden of deze extra kosten voor MRI opwegen tegen het voordeel van snellere opsporing en vroegere behandeling van personen met een verhoogd risico op een beroerte in (een specifiek deel van) de algemene bevolking.

Sinds de start van de Rotterdam Scan Study in augustus 2005 hebben meer dan 5000 personen een MRI ondergaan binnen onze studie. Als MRI-onderzoek van de hersenen bij onderzoeksdeelnemers wordt uitgevoerd, kan dit leiden tot de ontdekking van tot dan toe niet bij de deelnemer bekende hersenafwijkingen die geen verband hebben met het doel van het onderzoek, maar die mogelijk wel klinische betekenis hebben. In hoofdstuk 4 vonden wij dat 8.4% van alle deelnemers aan ons onderzoek een zogenaamde toevalsbevinding had, waarbij aneurysmata oftewel uitstulpingen in de wand van de bloedvaten (2.3%) en goedaardige hersentumoren (1.9%) voor de helft van het aantal bevindingen zorgden. Bijna drie procent van onze onderzoeksdeelnemers werd doorverwezen naar een medisch specialist voor verder onderzoek en eventuele behandeling van deze bevinding. Uit onze studie bleek echter ook dat in de meerderheid van deze doorverwijzingen (85%) geen verdere medische behandeling noodzakelijk was. Wij constateerden ook dat het hebben van een goedaardige tumor gerelateerd is aan hogere leeftijd, geslacht (vrouwen hebben meer kans op een
goedaardige tumor) en het hebben van diabetes (suikerziekte). Voor aneurysmata vonden wij een relatie met hogere leeftijd, het vrouwelijk geslacht, roken, het gebruik van bloeddrukverlagende medicatie en een lagere waarde van het HDL-cholesterol (‘goede’ cholesterol). Onze onderzoeksgereviews geven meer inzicht in de frequentie van voorkomen, de risicofactoren en het beloop van toevalsbevingingen. Dit inzicht kan gebruikt worden voor het ontwikkelen van richtlijnen voor verwijzing en handelwijze rondom toevalsbevingingen.

In hoofdstuk 5, de algemene discussie, worden de belangrijkste bevindingen besproken in het licht van de huidige kennis. Daarnaast worden methodologische afwegingen besproken die betrekking hebben op de studies die beschreven zijn in dit proefschrift. Dit omvat aspecten van bevolkingsonderzoeken met beeldvorming; het doen van dwarsdoorsnede-onderzoek evenals het vervolgen van deze bevindingen door de tijd; en de beperkingen van het visueel beoordelen van hersenschade ten opzichte van geautomatiseerde technieken hiervoor. Ook worden mogelijke richtingen van toekomstig onderzoek besproken en tot slot bespreek ik de mogelijke klinische consequenties van het onderzoek. Voorbeelden daarvan zijn het gebruik van cerebrale microangiopathie als eindpunt in klinisch onderzoek, en de verschuiving van het willekeurige onderscheid tussen afwijkingen die bij de ziekte van Alzheimer optreden aan de ene kant en vasculaire hersenschade aan de andere kant, naar een meer continue schaal van deze afwijkingen.
Dankwoord
DANKWOORD

‘If I have seen further than others, it is by standing upon the shoulders of giants.’
Isaac Newton (1642-1727)

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Hoewel de ‘neuro-asolunch’ een begrip is geworden, waren de lunches, borrels en etentjes toch het leukst met alle (voormalige) promovendi en post-docs van de afdeling, en in het bijzonder: Gabriëlle, Virginie, Bouwe, Toke, Daan, Eline, Rikje, Quirijn, Germaine, Mariana, Theun, Abbas, Maryam, Maarten, Henriët, Lies, Mark, Charlottte, Janine, Monique, Monika, Lintje en Wishal.

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Lieve Ernst, jij haalt het beste uit mijn brein. Dank je wel voor alles... en zoveel meer!
List of Publications
LIST OF PUBLICATIONS

Publications and Manuscripts Based on the Studies Described in this Thesis


Other Publications


PhD Portfolio
PHD PORTFOLIO

PhD Training

Research Skills
2008 Biomedical English Writing and Communication
2004-2007 Master of Science in Clinical Epidemiology, Netherlands Institute for Health Sciences, Rotterdam, the Netherlands

In-depth Courses
2010 Advances in Epidemiologic Study Design, Nihes, Rotterdam, the Netherlands
2008 R-course, Erasmus MC, Rotterdam, the Netherlands
2008 Principles of Epidemiologic Data-analysis, Nihes, Rotterdam, the Netherlands
2007 Advances in Clinical Neuroepidemiology, Nihes, Rotterdam, the Netherlands

Invited Lectures and Seminars
2010 27th Princeton Conference on Cerebrovascular Disease, Boston, USA
Oral presentation: Microbleeds: Pathophysiology, imaging, and prognosis
2008 1st Cerebral Microbleed Consortium Meeting, Chicago, USA

(International) Conferences
2011 Alzheimer's Association International Conference, Paris, France
Oral presentation: Cerebral microbleeds are associated with worse cognitive function. The Rotterdam Scan study
2011 Alzheimer's Imaging Consortium, Paris, France
Oral presentation: Cerebral microbleeds are associated with worse cognitive function. The Rotterdam Scan study
2011 International Stroke Conference, Los Angeles, USA
Oral presentation: Incidence of cerebral microbleeds in the Rotterdam Scan study
2010 Radiological Society of North America (RNSA), 96th scientific meeting, Chicago, USA
Poster presentation: Incidence of cerebral microbleeds in the general population: The Rotterdam Scan study

2010 7th Forum of European Neuroscience, Federation of European Neuroscience Societies (FENS), Amsterdam, the Netherlands

2010 European Stroke Conference, Barcelona, Spain
Oral presentation: Cerebral small vessel disease in the prediction of stroke
Poster presentation: Lobar distribution of cerebral microbleeds: The Rotterdam Scan study

2009 International Conference on Alzheimer’s Disease and Related Disorders (ICAD), Vienna, Austria
Poster presentation: Lobar distribution of cerebral microbleeds: The Rotterdam Scan study

2009 Alzheimer’s Imaging Consortium, Vienna, Austria
Poster presentation: Lobar distribution of cerebral microbleeds: The Rotterdam Scan study

2008 American Academy of Neurology, 60th Annual Meeting, Chicago, USA
Poster presentation: Cerebral blood flow, white matter lesion volume and cognitive function

2007 WEON: Vereniging voor Epidemiologie, Maastricht, the Netherlands
Poster presentation: Cerebral blood flow, white matter lesion volume and cognitive function

Other

2008-current Referee activities for various international scientific journals (e.g., Stroke, Neurology)
Teaching Activities

*Lecturing*

2009-2010 Clinical Epidemiology, 4th year medical students, Erasmus MC, Rotterdam, the Netherlands

*Supervising Master of Science Students/Medical Students*

2010-2011 Supervisor Kèren Zaccai, Clinical Epidemiology, Nihes, Rotterdam. Thesis topic: Arterial stiffness and cerebral small vessel disease

2008 Supervisor Dymph Mesker, Medicine, Leiden University. Thesis topic: Lobar distribution of cerebral microbleeds
About the Author
ABOUT THE AUTHOR

Mariëlle Poels was born on May 30, 1984 in Venray, the Netherlands. In 2002, she graduated from grammar school at Raayland College, Venray, and started her medical studies in Rotterdam. In her second year of medical school, Mariëlle was invited to participate in the Master of Science program in Clinical Epidemiology at the Netherlands Institute for Health Sciences. During this program she received her initial training in Epidemiology and worked for one year under the supervision of prof.dr. Monique M.B. Breteler and dr. Marieke van Oijen at the Department of Epidemiology (head: Prof.dr. Albert Hofman) of Erasmus MC, Rotterdam. As part of this research training she also attended a summer school in Epidemiology at Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA.

Mariëlle received both her Master’s degree in Medicine and in Clinical Epidemiology in 2007. That same year, the Gerrit Jan Mulder Stichting awarded her the yearly prize for best medical student research project of Erasmus MC. At the end of 2007, she interrupted her medical internships and started her PhD study under the supervision of prof.dr. Monique M.B. Breteler, prof.dr. Aad van der Lugt and dr. Meike W. Vernooij at the Department of Epidemiology in close collaboration with the Department of Radiology (head: Prof.dr. Gabriel P. Krestin). During the second part of her medical internships (2008-2009), Mariëlle conducted research on surgical outcomes of the Baerveldt glaucoma implant at the Rotterdam Eye Hospital (supervisors: Prof.dr. Hans G. Lemij, dr. Antoinette G.J.E. Niessen, drs. Peter W.T. de Waard). She obtained her medical degree in 2009.

In April 2010, Mariëlle was invited to give a lecture at the Princeton Conference on Cerebrovascular Disease, Boston, MA, USA. Her abstract entitled ‘Incidence of cerebral microbleeds in the Rotterdam Scan Study’ was rated among the 20 best abstracts at the International Stroke Conference 2011, Los Angeles, CA, USA. Based on this abstract, she was invited to publish an article in Stroke.

From May 2012 onwards, Mariëlle will do her residency training in Ophthalmology (head: Prof.dr. Jan C. van Meurs) at the Rotterdam Eye Hospital.
The subject matter of this thesis is symbolically represented in its cover image. The blue illustration of the brain in the background symbolizes the imaging techniques that have been used to conduct most of the research that was done for this thesis. The tiny punch holes in the section of the brain that is placed prominently in the foreground represent cerebral microbleeds. Glancing through these punch holes, one will discover a bright pink color typical of good health, as most people with cerebral microbleeds initially remain in good health and do not suffer from their disorder. Finally, a dotted line connects the title of this thesis to the brain stem; the most essential part of the brain.