Prevalence and Risk Factors of Cerebral Microbleeds
An Update of the Rotterdam Scan Study

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Background and Purpose—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. (Stroke. 2010;41[suppl 1]:S103-S106.)

Key Words: gradient echo MRI ■ intracerebral hemorrhage ■ microbleeds

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.10 We have since extended our cohort with participants of 45 years of age or older, and we currently have 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. 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 multisquence 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 (ie, 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.

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.

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).
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, vs men‡</td>
<td>0.95 (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, vs 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, vs 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, vs 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, vs 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>

*With or without lobar microbleeds; †adjusted for sex; ‡adjusted for age.

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.

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

Table 4. Cardiovascular Determinants and the Presence of Cerebral Microbleeds

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<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP† 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>Diastolic BP† 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>Pulse pressure† 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>Hypertension</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Mild, vs 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>Severe, vs none</td>
<td>1.38 (1.06–1.81)</td>
<td>1.29 (0.94–1.76)</td>
<td>1.66 (1.07–2.59)</td>
</tr>
<tr>
<td>Smoking, ever vs 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>Diabetes, yes vs 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.

*With or without lobar microbleeds; †additionally adjusted for the use of blood pressure-lowering medication; ‡additionally adjusted for the use of lipid-lowering drugs.

BP indicates blood pressure; HDL, high-density lipoprotein.
Table 5. Cerebral Vascular Disease and the Presence of Cerebral Microbleeds

<table>
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</tr>
</thead>
<tbody>
<tr>
<td>Cortical infarcts, vs 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>Lacunar infarcts, vs 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>White matter lesion volume† 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. *With or without lobar microbleeds; †natural log–transformed.

APOE ε4 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). However, we consistently find the association between APOE ε4 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 AGES² and Framingham study, which did not find clear associations of cardiovascular factors with CMBs, but is in line with data from most other studies. 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. 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. Furthermore, hypertension is known by its long, clinically asymptomatic period, in which arteriolsclerosis of small, deep-penetrating arteries can already be proven in autopsy tissue. Prevention strategies for both hypertensive and amyloid angiopathy should thus start early in life and may be aided by noninvasive 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.

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Disclosures

None.

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