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Cerebral microbleeds are associated with progression of ischemic vascular lesions

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Abstract

Background—Despite their different appearance on imaging, hemorrhagic and ischemic vascular lesions frequently co-occur in the brain and are hypothesized to progress concurrently. Although silent hemorrhagic and ischemic vascular brain lesions are highly prevalent in the general population, the concomitant progression of these lesions have only been studied limitedly in this population. We therefore aimed to investigate whether pre-existing and incident cerebral microbleeds relate to progression of ischemic lesions in the general population.

Methods—In the prospective population-based Rotterdam Scan Study, 803 people aged 60 years and over underwent serial magnetic resonance imaging, with an average interval of 3.4 years. Presence of microbleeds and lacunes were visually rated by trained research-physicians and white

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matter lesions were automatically segmented at both time points. Logistic regression was used to investigate the association of microbleeds with incident lacunes, and linear regression was used to investigate the relation between microbleeds and progression of white matter lesion volume. All analyses were adjusted for age, sex, and time-interval between baseline and follow-up scan. Analyses were repeated after additional adjustments for cardiovascular risk, namely: blood pressures, total and high-density lipoprotein cholesterol, smoking, diabetes mellitus, lipid lowering, antihypertensive and antiplatelet medication, and *apolipoprotein E ε4*. Analyses involving white matter lesions were also adjusted for intracranial volume.

Results—We found that pre-existing microbleeds in any location of the brain related to a higher incidence of lacunes (age, sex, and scan interval adjusted odds ratio 4.67, 95% CI 1.84;11.85). Pre-existing microbleeds were not related to progression of white matter lesion volume (mean difference in white matter lesion volume increase −0.03, 95% CI −0.15;0.09). Additional adjustments for cardiovascular risk did not change the results meaningfully. Incident microbleeds in any location of the brain associated with a higher incidence of lacunes (odds ratio 9.18, 95% CI 3.61;23.35), whereas only incident microbleeds located in cortico-subcortical regions related to progression of white matter lesion volume (mean difference in white matter lesion volume increase 0.41, 95% CI 0.21;0.62). Again, adjustments for cardiovascular risk did not change the results significantly.

Conclusions—Our findings suggest that in the general population cerebral microbleeds serve as a predictor of ischemic brain lesions, and may represent an imaging marker of active vasculopathy. These results support the hypothesis of a common underlying pathway in the development of ischemic and hemorrhagic brain lesions.

Keywords

Cerebral microbleeds; white matter lesions; lacunes; magnetic resonance imaging

Introduction

Vascular brain pathology is highly prevalent in aging populations and may clinically manifest itself as stroke or dementia. Before onset of clinical disease, vascular pathology accumulates over the course of several years, during which it can be visualized on magnetic resonance imaging (MRI) as lacunes of vascular origin (henceforth lacunes) [1], white matter lesions (WML), and cerebral microbleeds (CMBs) [2].

Based on pathologic features, lacunes and WML are presumed to indicate ischemic vascular pathology in the brain, whereas microbleeds are thought to represent a bleeding-prone state. Despite their different appearance on imaging, lacunes, WML and microbleeds very often co-occur in the brain [3–6]. Moreover, the many shared risk factors for lacunes, WML and microbleeds suggest that these lesions are at least partly affected by common pathological mechanisms [4–9]. Therefore, in the progression of vascular brain pathology one would expect that one common pathological pathway ultimately diverges into two distinct pathways leading to the different phenotypes, i.e., ischemic or hemorrhagic events (Figure 1). However, evidence in support of this ‘diverging pathway’ hypothesis is scarce [3, 10]. Hemorrhagic and ischemic brain lesions and their progression are often studied as separate

entities and typically in clinical populations, even though both types of lesions are also highly prevalent in the general population [11].

We have previously shown that, in a population-based setting, pre-existing microbleeds increase the risk of developing new microbleeds [12]. In addition, we showed that pre-existing ischemic lesions, i.e., lacunes and WML, were related to both the presence and incidence of cerebral microbleeds [12–14]. Thus far no study in the general population has explored the opposite association. Evidence for microbleeds predicting and simultaneously progressing with silent ischemic vascular lesions would strengthen the argument of a shared etiological pathway. We therefore investigated, in the population-based Rotterdam Scan Study, whether pre-existing microbleeds and incident microbleeds were related to incident lacunes and progression of WML volume.

Methods

Participants

The study was conducted in the population-based Rotterdam Scan Study [15], a neuroimaging study embedded in the larger prospective Rotterdam Study [16]. Between 2005 and 2006, 1375 non-demented study participants were randomly invited to undergo baseline brain MRI scanning. The institutional review board approved the study. A total of 146 participants were ineligible to undergo MRI (typically due to MRI contra-indications). Of the 1229 eligible participants at baseline, 1062 people (86.4%) underwent complete MRI scanning after informed consent was retrieved. Subsequently, participants were reinvited to undergo a repeat brain MRI between 2008 and 2010, i.e., 3–4 years after baseline. Of the 1062 participants with baseline MRI examinations, 982 people were eligible to participate in follow-up scanning and 848 (86.4%) gave written informed consent. Physical disabilities prohibited completion of scanning in 14 people. Of the 834 complete scans, 3 had to be excluded because of artifacts, leaving a total of 831 scans for analyses.

Assessment of MRI markers of cerebrovascular disease

Both baseline and follow-up scans were performed on a 1.5-Tesla MRI scanner (GE Healthcare Milwaukee, WI) [15]. Presence of MRI markers of cerebrovascular disease was rated by one of 5 trained research-physicians. Raters were blinded to clinical data. The presence, number and location of cerebral microbleeds was rated on three-dimensional T2* gradient-recalled echo (3D T2* GRE) weighted MR imaging at both time points [13]. Baseline or follow-up scans that showed one or more CMBs were rated blinded for time point in a side-by-side comparison [12]. Participants were categorized into groups of people who had microbleeds in lobar brain regions only, and people who had microbleeds in deep or infratentorial regions (regardless of the presence of lobar microbleeds). The presence of lacunes and cortical infarcts was rated on fluid-attenuated inversion recovery (FLAIR), proton density weighted and T1-weighted sequences [13]. Participants with one or more infarcts at any of the two time points were included in a side-by-side comparison to assess the final number of infarcts on each scan. Lacunes were defined as focal lesions of 3mm and <15mm in size with the same signal intensity as cerebrospinal fluid on all sequences and a hyperintense rim on the FLAIR (when located supratentorially). Infarcts showing

involvement of grey matter were classified as cortical infarcts [13]. Quantitative measures of WML volume (in mL) and intracranial volume (in mL) were obtained at both time points using validated automated post-processing steps that include conventional k-nearest-neighbor brain tissue classifier extended with WML segmentation [17, 18].

Assessment of cardiovascular risk factors

All participants were examined by trained personnel [16, 19]. We used standardized interviews as well as laboratory and physical examinations around the time of baseline MRI scan to assess cardiovascular risk. Blood pressure measurements were averaged over two readings, measured on the right arm with a random-zero sphygmomanometer. Serum total and high-density lipoprotein cholesterol were determined using an automated enzymatic procedure [20]. Smoking habits were defined as “ever” or “never” smoking. Participants were considered diabetic if fasting glucose levels were ≥ 7.0 mmol/L, or the use of any glucose lowering medication. The use of lipid-lowering medication and blood-pressure lowering medication was assessed by interviews during home visits. Information on antiplatelet medication use (i.e., aspirin or carbasalate calcium preparations) was obtained from automated pharmacy records, and categorized as ever versus never use before MRI. *Apolipoprotein E (APOE)* genotyping was done on coded genomic DNA samples [21], with allele frequencies being in Hardy-Weinberg equilibrium.

Data analysis

We investigated microbleed status dichotomously and by location (strictly lobar versus no CMBs, and deep or infratentorial versus no CMBs), as described previously [13]. Additionally we investigated categories of microbleed count, namely single versus no CMBs and multiple versus no CMBs. Logistic regression models adjusted for age, sex, and scan interval (in years) were used to investigate the association between pre-existing and incident microbleeds with incident lacunes. WML progression (in mL/years) was assessed by calculating the differences in lesion load volume (in mL) on baseline and follow-up scan, and subsequently dividing this number by the time interval between the two scans (in years). We applied linear regression models to investigate the association between pre-existing and incident microbleeds and progression of WML volume. All analyses with WML were adjusted for age, sex, scan interval and intracranial volume. Analyses were repeated after adjustments for cardiovascular risk, adjustments for *APOE* $\epsilon 4$, and finally after stratification by *APOE* $\epsilon 4$ carriership. People with cortical infarcts at baseline or follow-up scan were excluded from all analyses (N=28) because tissue loss and the gliosis surrounding cortical infarction may influence image registration to an extent that white matter lesion segmentation measures becomes unreliable. For analyses with WML an additional N=27 scans had to be excluded due to segmentation errors in baseline or follow-up scan. Analyses were performed using statistical software package SPSS 21.0 (SPSS Inc, Chicago, IL), using an α -value of 0.05.

Results

Characteristics of the study population are shown in Table 1. Mean age was 68.3 years, and 410 (51.1%) were women. Microbleeds were present in 195 (24.3%) participants at baseline,

and 78 (9.7%) developed one or more new microbleeds during follow-up. Incident microbleeds were located in strictly lobar regions in 49 (6.1%) participants and in deep or infratentorial brain regions in 29 (3.6%) participants. At baseline, 59 (7.3%) participants had lacunes, and 20 (2.5%) participants developed one or more new lacunes. The median baseline volume for WML was 3.7 mL and a median increase of 0.18 mL/year was seen during a mean scan interval of 3.4 years.

Table 2 shows the association between cerebral microbleeds and incident lacunes. Pre-existing microbleeds, at any location in the brain, were associated with incident lacunes. Although deep or infratentorial microbleeds were more strongly associated with incident lacunes after adjustments for cardiovascular risk factors (odds ratio [OR] for incident lacunes 6.10, 95% CI 1.61; 23.14), strictly lobar microbleeds were strongest associated with incident lacunes after adjusting for *APOE* ϵ 4 carriership (OR 4.79, 95% CI 1.46; 15.67). Also, incident microbleeds at any location in the brain associated strongly with incident lacunes (OR 9.18, 95% CI 3.61; 23.35). Adjusting for baseline WML volume did not alter these results (data not shown). Both single and multiple pre-existing or incident microbleeds increased the risk of incident lacunes, and though the numbers were small associations were strongest for multiple incident microbleeds (Supplementary Table 1).

The association between cerebral microbleeds and WML volume progression is shown in Table 3. Pre-existing microbleeds did not associate with WML volume progression. However, progression of strictly lobar microbleeds strongly associated with the progression of WML volume (mean difference in WML volume increase 0.41, 95% CI 0.21; 0.62). This was true for people with a single incident microbleed but even more so for those with multiple incident strictly lobar microbleeds (Supplementary Table 2). Adjusting for baseline lacunes did not alter these results (data not shown). Although statistical significance was not reached it appeared that there was more WML volume progression in people with incident strictly lobar microbleeds who carried the *APOE* ϵ 4 allele compared to non-carriers (P-interaction 0.14) (Supplementary Table 3).

Discussion

In our longitudinal population-based study, we found that both pre-existing and incident microbleeds were related to incident lacunes and progression of WML volume.

Strengths of our study are its population-based setting and the large number of participants with a follow-up scan. Also, we performed an identical MRI protocol on the same MRI-scanner at both time points without software or hardware alterations to optimize comparability between scans over time. A possible limitation of the study is that selective dropout may have influenced our results, as participants who underwent follow-up MRI scanning were younger and healthier than those who refused or were ineligible to undergo a second MRI scan. However, if present, selective dropout would have underestimated the true strength of the association between microbleeds and ischemic vascular lesions in our study because people that would have dropped out are more likely to have a worse cardiovascular risk profile. As for the stratified analysis by *APOE* ϵ 4, the sample size was rather small and these results should be interpreted with caution.

We found that incident microbleeds, which pathologically correspond to hemorrhagic lesions [22–24], related to an increased risk of progression of ischemic vascular lesions. This was particularly true for people with multiple incident microbleeds. Unfortunately, our study setting did not allow us to identify the chronological order of lesion occurrence. However, the co-occurrence of incident cerebral microbleeds, incident lacunes, and progression of WML does suggest that accumulation of these vascular pathologies follows a common pathway, which may depend on shared pathophysiological mechanisms. Person-specific modifiable risk factors (e.g., oral anticoagulant drug use) or non-modifiable risk factors (e.g., genetic variations) may then tilt people towards a final pathway of either major symptomatic hemorrhagic events or major symptomatic ischemic events. We previously showed that in preclinical, asymptomatic people pre-existing ischemic vascular lesions were related to the presence and incidence of microbleeds [13, 14]. Taken together with our current findings, this strengthens the suggestion of a common pathogenic pathway with a divergence for ischemic and hemorrhagic lesions. Of note is that the progression of hemorrhagic and ischemic vascular brain lesions did not solely depend on shared cardiovascular risk factors, as adjusting for these factors did not change our findings. Although the underlying mechanism explaining this diverging pathway remains unclear, we speculate that advanced cerebral amyloid angiopathy (CAA) pathology or hypertensive arteriopathy may give rise to both incident CMBs and incident ischemic lesions. Additionally, in small vessel disease impaired vessel tone and impaired autoregulation may lead to hypoperfusion after vessels rupture [1, 25, 26]. Finally, proinflammatory pathways may be triggered in the vasculopathy advocating the synchronic progression of both hemorrhagic and ischemic lesions [27]. Interestingly, there are also observations from pathology suggesting that microbleeds themselves have an ischemic origin [28]. Microbleeds may not exclusively reflect extravasation of erythrocytes but may partly signify the inability of the aging brain to store ferritin iron released from ischemic damaged brain cells [28].

Consistent evidence from observational and pathology studies implies that CAA is the prevailing pathology underlying microbleeds confined to cortico-subcortical (i.e., lobar) regions of the brain, whereas microbleeds located in deeper or infratentorial regions of the brain are more suggestive of hypertensive arteriopathy [13, 22, 29]. These observations have led to the assumption that depending on the location of microbleeds in the brain, and thus on their underlying pathology, microbleeds may differentially relate to ischemic small vessel lesions, i.e., lacunes or WML. In the current study we found that both pre-existing lobar and deep or infratentorial microbleeds increased the risk of incident lacunes. Also, the development of lobar microbleeds concurred with a higher progression of white matter lesion volume. In previous work, we showed that higher white matter lesion load increased the risk of both deep or infratentorial as well as lobar microbleeds [12], and that pre-existing microbleeds increased the risk of new microbleeds [12]. Taken together, these findings suggest that the pathology underlying microbleeds – be it hypertensive arteriopathy, amyloid angiopathy, or a combination of both – may thus contribute to both the progression of ischemic lesions as well as hemorrhagic lesions. In CAA, vascular β -amyloid accumulates and might eventually lead to destruction of vessel lumen, which leads to the development of lobar microbleeds [30]. However, vascular amyloid deposition may also cause stenosis,

occlusion, loss of the contractile components of the vessel wall, and impaired reactivity to physiologic stimulation [3]. Abnormal relaxation and constriction of small vessel may in turn contribute to repetitive hypoperfusion and possibly ischemic events [31]. Indeed, in patients with CAA related hemorrhages, progression of ischemic brain lesions on MRI have increasingly been recognized [9, 27, 32, 33]. Given our previous [12–14] and new findings, we might consider that in the general population possible CAA related microhemorrhages and ischemic lesions develop more steadily throughout the ‘common pathway’. We did not find an association between deep or infratentorial CMBs and WML volume progression. As participants with deep or infratentorial CMBs have higher WML volumes compared to those without microbleeds in these locations [12–14], a possible ‘ceiling effect’ may be created in this group. This group nonetheless developed more lacunes, another likely ischemic manifestation.

In conclusion, we found support for a common underlying pathway in the development of ischemic and hemorrhagic vascular brain lesions. Furthermore, our findings suggest that cerebral microbleeds may represent an imaging marker of active vasculopathy, which serves as a predictor of ischemic brain lesions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Accumulation of ischemic and hemorrhagic vascular pathology

The figure above shows a hypothetical scheme of accumulating vascular brain pathology. In a first stage, subclinical ischemic and hemorrhagic vascular pathology may occur concurrently due to common risk factors or pathophysiological mechanisms (images from left to right: microbleed on T2* weighted sequence; white matter lesions on fluid-attenuated inversion recovery (FLAIR); lacune of presumed vascular origin on FLAIR). In a later stage, a common pathway may diverge into distinct final pathways in which ischemic or hemorrhagic specific risk factors cause one overall clinical phenotype of vascular brain disease.

Table 1

Characteristics of the study population.

	N= 803
Age, years	68.3 (6.2)
Women	410 (51.1)
Pre-existing microbleeds	195 (24.3)
Strictly lobar	129 (16.1)
Deep or infratentorial	66 (8.2)
Incident microbleeds	78 (9.7)
Strictly lobar	49 (6.1)
Deep or infratentorial	29 (3.6)
Baseline white matter lesion volume, mL	3.7 (2.2; 7.1)
White matter lesions volume progression ^a	0.18 (0.03; 0.53)
Pre-existing lacunes	59 (7.3)
Incident lacunes	20 (2.5)
Scan interval, years	3.4 (0.2)
Intracranial volume, mL	1119.1 (115.6)
Systolic blood pressure, mmHg	143.7 (18.0)
Diastolic blood pressure, mmHg	80.7 (10.3)
Total cholesterol, mmol/L	5.7 (1.0)
High-density lipoprotein cholesterol, mmol/L	1.4 (0.4)
Smoking	564 (70.2)
Diabetes mellitus	64 (8.0)
Lipid lowering medication use	170 (21.2)
Antihypertensive medication use	282 (35.1)
Antiplatelet medication use	207 (25.8)
<i>Apolipoprotein E</i> e4 carriership	198 (24.7)

Data are presented as mean (standard deviation) for continuous variables, number (%) for categorical variables.

^aWhite matter lesions are presented as median (interquartile range). The following variables had missing data: systolic and diastolic blood pressure N=3, total and high-density lipoprotein cholesterol N=6, smoking N=8, diabetes mellitus N=11, lipid lowering and antihypertensive medication N=8, *apolipoprotein E* genotype N=4.

Table 2

Microbleeds and incident lacunes.

	n/N	Incident lacunes		
		Model 1	Model 2	Model 3
No pre-existing microbleeds	8/608	Reference	Reference	Reference
Pre-existing microbleeds (all)	12/195	4.67 (1.84; 11.85)	4.25 (1.56; 11.60)	4.67 (1.59; 13.74)
Pre-existing strictly lobar	6/129	3.56 (1.18; 10.74)	3.27 (0.97; 11.00)	4.79 (1.46; 15.67)
Pre-existing deep or infratentorial	6/66	6.60 (2.17; 20.09)	6.10 (1.61; 23.14)	4.16 (0.96; 17.93)
No incident microbleeds	10/725	Reference	Reference	Reference
Incident microbleeds (all)	10/78	9.18 (3.61; 23.35)	6.28 (2.26; 17.47)	8.41 (2.82; 25.10)
Incident strictly lobar	4/49	5.60 (1.63; 19.30)	4.19 (1.03; 17.07)	7.75 (2.07; 28.98)
Incident deep or infratentorial	6/29	17.09 (5.60; 52.20)	10.43 (2.80; 38.77)	9.25 (2.17; 39.38)

Model 1: adjusted for age, sex, and scan interval.

Model 2: as model 1, additionally adjusted for blood pressures, total and HDL cholesterol, smoking, diabetes mellitus, lipid lowering medication, antihypertensive medication, and antiplatelet medication.

Model 3: as model 1, additionally adjusted for *apolipoprotein Eε4* genotype.

Values represent odds ratios for incident lacunes in participants with pre-existing and incident microbleeds compared to no microbleeds.

Abbreviations: n= number of participants with incident lacunes; N= total number of participants.

Table 3

Microbleeds and progression of WML volume.

	Difference in annual progression of WML volume		
	Model 1	Model 2	Model 3
No pre-existing microbleeds	Reference	Reference	Reference
Pre-existing microbleeds (all)	-0.03 (-0.15; 0.09)	-0.05 (-0.17; 0.08)	-0.01 (-0.14; 0.11)
Pre-existing strictly lobar	-0.04 (-0.18; 0.10)	-0.06 (-0.20; 0.08)	-0.07 (-0.21; 0.08)
Pre-existing deep or infratentorial	0.01 (-0.19; 0.18)	-0.01 (-0.21; 0.18)	0.09 (-0.11; 0.28)
No incident microbleeds	Reference	Reference	Reference
Incident microbleeds (all)	0.23 (0.05; 0.40)	0.19 (0.01; 0.37)	0.22 (0.04; 0.41)
Incident strictly lobar	0.41 (0.21; 0.62)	0.39 (0.18; 0.61)	0.33 (0.10; 0.55)
Incident deep or infratentorial	-0.10 (-0.37; 0.17)	-0.17 (-0.45; 0.11)	0.02 (-0.28; 0.32)

Model 1: adjusted for age, sex, scan interval, and intracranial volume.

Model 2: as model 1, additionally adjusted for blood pressures, total and HDL cholesterol, smoking, diabetes mellitus, lipid lowering medication, antihypertensive medication, and antiplatelet medication.

Model 3: as model 1, additionally adjusted for *apolipoprotein Eε4* genotype.

Values represent differences in annual white matter lesion volume progression in participants with pre-existing and incident microbleeds compared to no microbleeds.

Abbreviations: WML= white matter lesion.