Microbleeds Versus Macrobleeds
Evidence for Distinct Entities

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Background and Purpose—Small, asymptomatic microbleeds commonly accompany larger symptomatic macrobleeds. It is unclear whether microbleeds and macrobleeds represent arbitrary categories within a single continuum versus truly distinct events with separate pathophysologies.

Methods—We performed 2 complementary retrospective analyses. In a radiographic analysis, we measured and plotted the volumes of all hemorrhagic lesions detected by gradient-echo MRI among 46 consecutive patients with symptomatic primary lobar intracerebral hemorrhage diagnosed as probable or possible cerebral amyloid angiopathy. In a second neuropathologic analysis, we performed blinded qualitative and quantitative examinations of amyloid-positive vessel segments in 6 autopsied subjects whose MRI scans demonstrated particularly high microbleed counts (>50 microbleeds on MRI, n=3) or low microbleed counts (<3 microbleeds, n=3).

Results—Plotted on a logarithmic scale, the volumes of 163 hemorrhagic lesions identified on scans from the 46 subjects fell in a distinctly bimodal distribution with mean volumes for the 2 modes of 0.009 cm³ and 27.5 cm³. The optimal cut point for separating the 2 peaks (determined by receiver operating characteristics) corresponded to a lesion diameter of 0.57 cm. On neuropathologic analysis, the high microbleed-count autopsied subjects showed significantly thicker amyloid-positive vessel walls than the low microbleed-count subjects (proportional wall thickness 0.53±0.01 versus 0.37±0.01; P<0.0001; n=333 vessel segments analyzed).

Conclusions—These findings suggest that cerebral amyloid angiopathy-associated microbleeds and macrobleeds comprise distinct entities. Increased vessel wall thickness may predispose to formation of microbleeds relative to macrobleeds. (Stroke. 2009;40:00-00.)

Key Words: cerebral amyloid angiopathy ■ intracerebral hemorrhage ■ microbleeds

The widespread availability of MRI techniques with high sensitivity for hemosiderin has stimulated increasing interest in cerebral microbleeds.1–3 Microbleeds appear as small, rounded hypointense lesions on gradient-echo T2*-weighted MRI sequences and as clusters of hemosiderin-containing macrophages, often perivascular, on histopathologic examination.4 Microbleeds can be seen in healthy aging5–7 and at higher frequencies in ischemic stroke and particularly hemorrhagic stroke, in which the prevalence is approximately 50% to 70%.1–3 Cerebral microbleeds have been implicated as a marker of, and possibly a contributor to,8–10 small vessel brain disease.

The cerebrovascular pathologies that give rise to microbleeds such as hypertensive vasculopathy and cerebral amyloid angiopathy (CAA) are also responsible for larger symptomatic intracerebral hemorrhages (ICHs). This raises the question of whether microbleeds and their larger macrobleed counterparts are simply 2 arbitrarily defined categories from what is actually a continuum of hemorrhage sizes resulting from vessel rupture. Alternatively, it is possible that there are key pathogenic steps that fundamentally differentiate a macrobleed from a microbleed. There is neuropathological evidence, for example, that the pathogenesis of large symptomatic ICH entails not only the initial rupture of a blood vessel, but also secondary mechanical shearing of surrounding blood vessels to reach full size.11

The current study sought to explore the relationship between microbleeds and macrobleeds in patients with CAA. We specifically addressed the following questions: (1) among patients presenting with ICH, do hemorrhage volumes occur as a single unimodal distribution or is there a natural division between the volumes of microbleeds and macrobleeds suggestive of distinct pathogeneses for these 2 types of hemorrhage? (2) Are there pathological characteristics of diseased
blood vessels that predispose to microbleeds rather than macrobleeds (or vice versa)?

Materials and Methods
More detailed information on methods for measurement and quantitative analysis of hemorrhage volumes and arteriolar wall involvement can be found in the Supplemental Materials and Methods available online.

Measurement and Analysis of Hemorrhage Volumes
Analysis of hemorrhage volumes was performed on consecutive patients admitted to Massachusetts General Hospital between January 1998 and December 2002 with symptomatic primary lobar intracerebral hemorrhage diagnosed as probable or possible CAA by the pathologically validated Boston criteria. Inclusion criteria were age >55 years and MRI with gradient-echo (GRE) sequences (performed as part of the admission evaluation as previously reported), using a 1.5-T GE magnet with repetition time = 750 ms, echo time = 25 ms, flip angle = 20°, number of excitations = 2, matrix size 256 x 256, slice thickness 5 mm with 1-mm interslice gap) within 90 days of presentation. Subjects were excluded for unavailability of digital images or poor GRE image quality that precluded measurement of lesion volumes. Of 57 potentially eligible subjects with primary lobar ICH and gradient-echo MRI, 11 were excluded for poor image quality, yielding 46 subjects for analysis (Table 1).

Clinical characteristics of subjects and use of antiplatelet agents (aspirin in all cases) or anticoagulant agents (warfarin) were determined at the time of presentation as described.

Hemorrhagic lesions were identified as hypointensities that were largest on the GRE images, excluding signal suggestive of vessel flow voids, mineralization of the basal ganglia, cranial sinuses, or extension of a larger hemorrhage. Segmentation of hemorrhages, determination of hemorrhage volumes, statistical analysis of the distribution of hemorrhage volumes, and determination of the optimal cut point between the 2 observed populations of hemorrhages are described in the Supplemental Materials and Methods.

Neuropathology of Cerebral Arterioles
Cerebral arteriolar pathology was analyzed in autopsied subjects for its association with the presence of high or low numbers of microbleeds. Neuropathological analysis was performed on consecutive patients admitted to Massachusetts General Hospital between January 1998 and December 2003 with symptomatic primary lobar ICH. GRE MRI performed before death demonstrating particularly high (defined as >30) or low (defined as <3) microbleed counts, and subsequent full brain autopsy confirming definite CAA-related ICH. Six such subjects were identified, 3 with high microbleed counts and 3 with low microbleed counts (Table 2). (Three of the 6 were among the 46 subjects analyzed for hemorrhage volume; the remaining 3 were not included in the hemorrhage volume analysis because of absence of adequate GRE MRI images as described previously).

Table 1. Subjects Analyzed for Hemorrhagic Lesion Volume

<table>
<thead>
<tr>
<th>Age, mean ± SD*</th>
<th>74.8 ± 7.7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female, n (%)</td>
<td>23 (50)</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>26 (57)</td>
</tr>
<tr>
<td>Antiplatelet use, n (%)†</td>
<td>22 (48)</td>
</tr>
<tr>
<td>Anticoagulant use, n (%)†</td>
<td>4 (9)</td>
</tr>
<tr>
<td>No. of hemorrhagic lesions per scan, median (25th, 75th percentiles)</td>
<td>11 (3, 26)</td>
</tr>
</tbody>
</table>

*Age refers to time of MRI scan. †Use of antiplatelet or anticoagulant agents refers to time of initial presentation with symptomatic ICH.

Table 2. Autopsied High- and Low-Microbleed Count Subjects

<table>
<thead>
<tr>
<th>Subject/ Age/Sex</th>
<th>Antiplatelet Use†</th>
<th>Hypertension</th>
<th>Microbleeds</th>
<th>Macrobleeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>High microbleed count</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. 76 F</td>
<td>–</td>
<td>+</td>
<td>62</td>
<td>1</td>
</tr>
<tr>
<td>2. 75 M</td>
<td>+</td>
<td>–</td>
<td>58</td>
<td>1</td>
</tr>
<tr>
<td>3. 87 M</td>
<td>+</td>
<td>–</td>
<td>57</td>
<td>2</td>
</tr>
<tr>
<td>Low microbleed count</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. 67 F</td>
<td>–</td>
<td>+</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>5. 66 F</td>
<td>+</td>
<td>+</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>6. 76 F</td>
<td>–</td>
<td>+</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

*Age refers to age at death. †Antiplatelet use (aspirin in all cases) refers to time of initial presentation with symptomatic ICH. None of the autopsied subjects used anticoagulants. F indicates female; M, male.

Paraffin-embedded sections of occipital (Brodmann areas 17/18) and frontal cortex (areas 8/9) were stained for ß-amyloid (DAKO). Qualitative analysis of vessel characteristics was performed by review of all sections by a neuropathologist (M.P.F.) without knowledge of subject or neuroimaging information. In addition to this qualitative analysis, a single rater (P.D.), also blinded to subject information, performed systematic quantitative analysis of CAA-positive vascular wall thickness and circumferential involvement. Methods for measurement of vascular wall thickness and circumferential involvement and statistical comparisons between the high and low microbleed-count subjects by linear mixed effects models are described in the Supplemental Materials and Methods.

Results
Volume of Hemorrhagic Lesions
To assess whether CAA-related microbleeds and macrobleeds represent distinct size categories, we analyzed the distribution of hemorrhagic lesion volumes among 46 consecutive elderly subjects with probable or possible CAA and suitable GRE MRI images. Characteristics of the analyzed subjects are shown in Table 1 and the volumes of the 163 hemorrhages detected on these scans are plotted on a logarithmic scale as the histogram in Figure 1.

Rather than forming a unimodal distribution (the null hypothesis of a single normal distribution could be rejected with P<0.0001), the hemorrhagic lesion volumes distributed into 2 peaks. Mean volume of the higher-volume peak was 27.5 cm³ (corresponding to a diameter of 3.75 cm, assuming spherical shape). Mean volume of the lower volume peak was 0.009 cm³ (corresponding to a spherical diameter of 0.26 cm).

The optimal threshold that correctly classified (with 99% probability) a hemorrhagic lesion from either subpopulation was 0.098 cm³, corresponding to a spherical diameter of 0.57 cm (vertical dashed line in Figure 1). Exclusion of the 20 hemorrhagic lesions identified in 4 subjects taking warfarin at the time of presentation (Table 1) did not alter the bimodal appearance of the histogram (not shown).

Pathology of Amyloid-Positive Vessels in High Versus Low Microbleed-Count CAA
We next examined the structural basis by which CAA-affected vessels are predisposed to give rise to microbleeds as
opposed to macrobleeds. We approached this question by identifying autopsied CAA subjects (Table 2) with large numbers of microbleeds (50 on GRE MRI; Figure 2A) or few microbleeds (3; Figure 2B). Visual inspection of sections of frontal and occipital cortex performed without knowledge of patient characteristics found noticeably increased wall thickness of amyloid-positive vessels among the high microbleed-count subjects relative to the low microbleed subjects (Figure 3). No other qualitative pathological differences such as the frequency or type of amyloid-positive vessels or other secondary vascular changes such as dyschroic amyloid deposits, concentric splitting of the vessel wall, or loss of smooth muscle cells were observed.

Increased wall thickness of amyloid-positive vessel segments in high microbleed versus low microbleed subjects was confirmed by blinded quantitative measurement. Proportional wall thickness (mean ± SD) was 0.53 ± 0.01 for the high microbleed subjects versus 0.37 ± 0.01 for the low microbleed subjects (P < 0.0001). The association between high microbleed count and increased proportional wall thickness remained independent (P < 0.005) in an additional mixed effects model controlling for subject age and hypertension as fixed effects. A separate analysis of amyloid-negative white matter vessels found no difference in proportional wall thickness (0.40 ± 0.04 for high microbleed subjects, 0.36 ± 0.04 for low microbleed; P = 0.45). Quantitative analysis of the circumferential extent of amyloid found circumferential involvement to be near complete in most amyloid-positive vessels (90% of 287 vessel analyzed segments scoring 12 on the 12-point scale) without a significant difference between high and low microbleed subjects (P = 0.19).

Discussion

We report 2 lines of evidence suggesting that CAA-related microbleeds and macrobleeds, although arising from a common vascular disease, represent distinct pathophysiological events. Findings supporting this inference are that (1) hemorrhage volumes appear to fall into a bimodal distribution best modeled as a mixture of 2 separate populations; and (2) CAA subjects with many microbleeds demonstrate significantly thicker amyloid-positive vessels than those with few microbleeds. These essentially independent observations support a model by which symptomatic macrobleeding and asymptomatic microbleeding are distinct entities with characteristic pathophysiology.

It is notable that the cut point between the 2 hemorrhage volume populations determined by receiver operating characteristic analysis of the estimated 2-component mixture model (Figure 1) corresponded to a spherical diameter of 0.57 cm, a value very close to the upper size limits of 0.5 to 1 cm traditionally chosen to define microbleeds. The reasonably
Hemorrhage volume measurements on GRE MRI overestimate the true size of the microbleeds because of the susceptibility ("blooming") artifact. A recent comparison of hemorrhage volumes on GRE MRI and CT suggested a correction factor of 0.8.\textsuperscript{15}

The overall relationship between microbleeds and macrobleeds remains an active area of investigation. Some previous studies have suggested that the number of microbleeds predicts risk of future symptomatic ICH.\textsuperscript{13,16} In the case of symptomatic hemorrhage after thrombolytic treatment for ischemic stroke, however, the presence of microbleeds appears not to be predictive.\textsuperscript{17,18}

The observation of increased vessel wall thickness among high relative to low microbleed-count subjects suggests that thicker vessels may render vessels more likely to produce microbleeding when they rupture. Thickening of the vessel wall and narrowing of the vascular lumen has long been noted as characteristic of CAA,\textsuperscript{14,19} but the factors determining degree of wall thickness are largely unknown. Severe wall thickening occurs in Iowa-type hereditary CAA,\textsuperscript{20} a familial form of CAA also characterized by multiple microbleeds without symptomatic hemorrhage among 10 affected members of the originally identified pedigree. Whether similar considerations of microbleeding and vessel wall thickness apply to vasculopathies other than CAA such as hypertensive hemorrhage\textsuperscript{11} remains to be determined. In cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy, another vascular pathology associated with severe thickening of the arterial wall and loss of normal wall elements, microbleeds are common but symptomatic macrobleeds are rare,\textsuperscript{9,21–23} suggesting possible similarities to the CAA high microbleed group.

There are important limitations to the current analysis. Hemorrhages and hemorrhage volumes were measured by MRI (using relatively thick slices) rather than neuropathologically, yielding potential radiographic artifacts and mismeasurements, but also allowing systematic detection of hemorrhage throughout the full cerebral cortices. Also, because most of our CAA subjects are identified after presentation to a tertiary referral center with symptomatic ICH, our study cohort was likely biased to have more and larger macrobleeds than would be observed in a community-based analysis. Recent data from the population-based Brain Attack Surveillance in Corpus Christi (BASIC) study, for example, found a 25th percentile for ICH volumes of 3 cm\textsuperscript{3}, indicating a substantial number of smaller ICHs.\textsuperscript{24} It is nonetheless unlikely that a bias toward more and larger macrobleeds would account for the overall bimodal distribution of hemorrhage volumes (Figure 1), which is caused by relatively underpopulated bins at even smaller volumes (approximately 0.14 to 2.7 cm\textsuperscript{3}) than those detected in the BASIC study. We also note that the volumes of the presenting ICH were similar between subjects with and without accompanying microbleeds (P=0.62 by Wilcoxon rank sum test) and that there was no association between number of microbleeds and the volume of the presenting macrobleed (Kendall’s tau = −0.065, P=0.57), suggesting that the observed bimodal distribution was not the result of combining 2 separate subtypes of ICH patients. Finally, our neuropathologic analysis involved only a small number of subjects, although with a large enough number of vessel segments to allow robust statistical comparisons using a mixed effects model to account for correlations within subjects and tissue sections. Because this analysis was performed at a single time point for each subject, we cannot exclude the possibility that proportional wall thickness changes with increasing disease duration.

Summary

Volume measurements from subjects diagnosed with CAA suggest that microbleeds and macrobleeds represent 2 separate categories of hemorrhagic events. Subjects with the highest microbleed counts had significantly thicker amyloid-positive vessel wall segments than those with low microbleed counts, indicating that a vessel’s tendency to give rise to microbleeds may be driven by distinct pathological features. These findings are particularly relevant to our understanding of the relationship between microbleeds and macrobleeds, a question of growing importance with increasing recognition of microbleeds in the healthy aging population.\textsuperscript{5–7}

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Disclosures

None.
References


Supplemental Materials and Methods

Measurement and Analysis of Hemorrhage Volumes

Hemorrhages were manually segmented using MRICro (Version 1.40; University of South Carolina) imaging tools. For larger hemorrhages appearing on multiple slices, total volume was determined from the sum of segmented volumes on each slice by MRICro software accounting for the interslice gap. Microbleeds were typically visible on just a single slice. To estimate their volume, we assumed the shape of the small hemorrhages was spherical and that the area visible on a single slice corresponded to its central cross-sectional area. The number of pixels visible inside the hemorrhage was counted (Digital Imaging software, Version 1.4.1; McConnell Brain Imaging Center, Montreal Neurological Institute) and multiplied by the pixel cross-sectional area to obtain the total lesion cross-sectional surface area. The in-plane radius was then calculated from this area using the formula for the area of a circle \( \text{radius} = \sqrt{\frac{\text{area}}{\pi}} \) and the volume of the assumed sphere calculated as \( \frac{4}{3} \pi \text{(radius)}^3 \).

Volumes of all observed hemorrhagic lesions were plotted on a natural logarithmic scale to evaluate their distribution. Based on the bimodal appearance of the nonparametric histogram (Figure 1), we fit a 2-component mixture model using maximum likelihood and considered normal and double exponential distributions for each component. Receiver operating characteristic curve methods were used to identify the optimal cut point between the 2 distributions that would maximize the “distance” from zero for the probability of correctly classifying hemorrhages as belonging to one component distribution versus the other. The estimated parameters of the component distributions for the mixture model were used to compute probabilities of correct classification. To assess for possible within-subject correlation of hemorrhage volumes, we extended the mixture component distributions for the mixture model were used to compute probabilities of correct classification. To assess for possible within-subject correlation of hemorrhage volumes, we extended the mixture model to include a random subject effect for mean hemorrhage size.

Volumes of all observed hemorrhagic lesions were plotted on a natural logarithmic scale to evaluate their distribution. Based on the bimodal appearance of the nonparametric histogram (Figure 1), we fit a 2-component mixture model using maximum likelihood and considered normal and double exponential distributions for each component. Receiver operating characteristic curve methods were used to identify the optimal cut point between the 2 distributions that would maximize the “distance” from zero for the probability of correctly classifying hemorrhages as belonging to one component distribution versus the other. The estimated parameters of the component distributions for the mixture model were used to compute probabilities of correct classification. To assess for possible within-subject correlation of hemorrhage volumes, we extended the mixture model to include a random subject effect for mean hemorrhage size.

The variance of this random effect (0.07) was small relative to the fixed means; however, implying that correlation was not a large factor in the volume distribution. The R statistical package (www.r-project.org/) and SAS programming language were used for these procedures.

Neuropathology of Cerebral Arterioles

Contiguous low-powered (4×) fields were photographed from each cortical area (occipital and frontal) starting at the superficial edge of a gyrus and extending to a width of 4 to 6 fields and a depth of 2 fields generating a total of 8 to 12 fields per slide for vessel analysis. All vessels showing any amyloid staining within these fields were individually photographed at high power (60×), yielding 20 to 60 vessels per slide (50 to 90 vessels per case). For comparison, vessels from white matter (frontal and occipital subcortical white matter as well as the anterior limb of the internal capsule at the level of the nucleus accumbens) were photographed, because these white matter regions are consistently free of involvement by amyloid angiopathy but are affected by other small vessel pathologies such as arteriosclerosis. For each photographed vessel, 2 lines were superimposed on a central spot in the lumen: one oriented along the widest dimension of the vessel lumen and the second perpendicular to the first. These 2 perpendicular lines were used to measure the vessel’s outer (external) diameter and inner (luminal) diameters. The perpendicular outer and inner diameter measurements for each vessel were then averaged, and these averages used to calculate the proportional wall thickness defined as \((\text{average outer diameter} - \text{average inner diameter})/\text{average outer diameter}\). Values for proportional wall thickness could thus range from near zero (if nearly the entire width of the vessel was comprised of lumen) to one (if the entire width of the vessel was comprised of wall). To measure the circumpolar extent of amyloid, each vessel wall was divided into 12 equal segments and the extent of Abeta involvement scored as the presence or absence of Abeta deposition in each segment (0=absent, 1=present) and summed over the 12 segments to yield a score of zero to 12.

Vessel segments were excluded from quantitative analysis if sectioned obliquely (defined as an outer diameter along the vessel’s widest dimension more than twice the outer diameter in the perpendicular dimension) or if stain quality was inadequate for measurement. Variable stain quality occurred in 2 cases that required immunohistochemistry be performed on previously stained sections. As a result, proportional wall thickness measurements could not be performed in one case (with high microbleed count) and circumferential measurements could not be performed in 2 cases (one high microbleed and one low microbleed count). A total of 333 vessel segments were analyzed for proportional wall thickness (149 from low microbleed cases, 184 from high microbleed cases) and a total of 287 for circumpolar involvement (149 low microbleeds, 138 high microbleeds). Outer diameters were similar between the 2 groups, indicating that similar distributions of vessels were sampled: median (25th, 75th percentile) outer vessel diameter 46 \(\mu\text{m}\) (37.5, 55.3) for the low microbleed group and 46 \(\mu\text{m}\) (33.5, 63.0) for the high microbleed group (\(P=0.7\) by Wilcoxon test).

Comparison of proportional wall thickness and circumferential amyloid between the high and low microbleed-count subjects was performed by linear mixed effects models. Group category (high versus low microbleed count) and anatomic section (frontal versus occipital) served as fixed effects and individual subjects and anatomic section nested within subject served as random effects. Additional models were performed that also included age and hypertension as fixed effects. For comparison of amyloid-negative white matter vessels, a multivariable linear model was fit for proportional wall thickness as a function of group category, location, and subject. The reported probability values are for the effect of high versus low microbleed group category.
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