# Microbleeds on MRI are associated with microinfarcts on autopsy in cerebral amyloid angiopathy

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## ABSTRACT

**Objectives:** To identify in vivo MRI markers that might correlate with cerebral microinfarcts (CMIs) on autopsy in patients with cerebral amyloid angiopathy (CAA).

**Methods:** We included patients with neuropathologic evidence of CAA on autopsy and available antemortem brain MRI. Clinical characteristics and in vivo MRI markers of CAA-related small vessel disease were recorded, including white matter hyperintensities, cerebral microbleeds, cortical superficial siderosis, and centrum semiovale perivascular spaces. In addition, the presence of intracerebral hemorrhage on MRI was assessed. Evaluation of the presence and number of CMIs was performed in 9 standard histology sections.

**Results:** Of 49 analyzed patients with CAA, CMIs were present in 36.7%. The presence of ≥1 CMIs on autopsy was associated with higher numbers of microbleeds on antemortem MRI (median 8 [interquartile range 2.5–33.0] vs 1 [interquartile range 0–3], $p = 0.003$) and with the presence of intracerebral hemorrhage (44.4% vs 16.1%, $p = 0.03$). No associations between CMIs and other in vivo MRI markers of CAA were found. In a multivariable model adjusted for severe CAA pathology, higher numbers of microbleeds were independent predictors of the presence of CMIs on pathology.

**Conclusions:** CMIs are a common finding at autopsy in patients with CAA. The strong association between MRI-observed microbleeds and CMIs at autopsy may suggest a shared underlying pathophysiologic mechanism between these lesions. *Neurology*® 2016;87:1488–1492

## GLOSSARY

**Aβ** = β-amyloid; **CAA** = cerebral amyloid angiopathy; **CMI** = cerebral microinfarct; **CS-PVS** = centrum semiovale perivascular space; **cSS** = cortical superficial siderosis; **PVS** = perivascular space; **SVD** = small vessel disease; **WMH** = white matter hyperintensity.

Cerebral microinfarcts (CMIs) are defined as microscopic lesions of ischemic tissue damage on standard histopathologic sections (figure, A). The prevalence of microinfarcts at autopsy is reported to range from 16% to 48% in different populations and has been associated with cognitive impairment.[1] Several pathologic studies found CMIs to be more common in patients with cerebral amyloid angiopathy (CAA) compared to those without CAA.[2] Although to date it is not feasible to fully quantify CMI burden, it has been suggested that ≥1 CMIs seen in routinely sampled sections are indicative of hundreds to thousands CMIs throughout the entire brain.[3] It is hypothesized that these high numbers and the widespread distribution of CMIs can affect cortical networks as a substantial contributor to neurologic dysfunction and dementia.[1,4]

In the context of CAA, MRI markers of cerebral small vessel disease (SVD) such as lobar cerebral microbleeds, cortical superficial siderosis (cSS), centrum semiovale perivascular spaces (CS-PVSs), and white matter hyperintensities (WMHs) appear to predict clinical outcomes in the disease (figure, B–E).[5] The relationship of CMIs to these established MRI markers has

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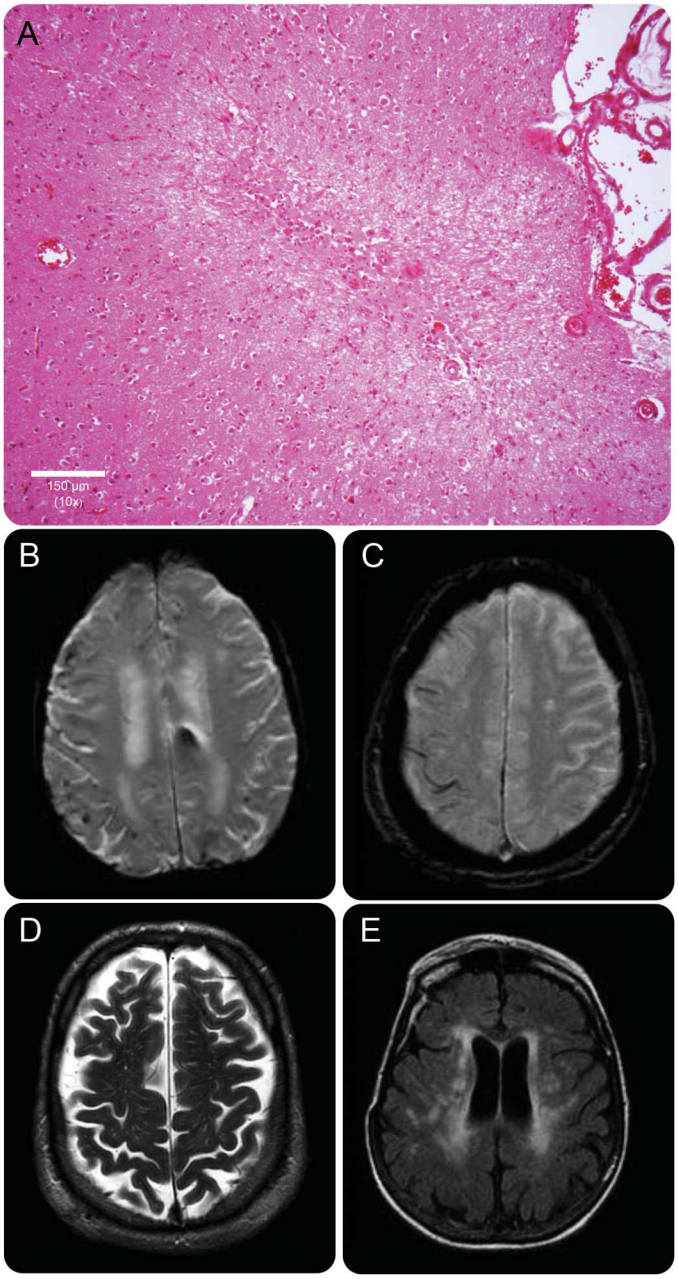
Figure Microinfarct and MRI markers of small vessel disease




(A) Example of a cerebral microinfarct identified on a cortical section (hematoxylin and eosin stain), (B) lobar microbleeds, and (C) superficial siderosis on T2*-weighted MRI. (D) Dilated centrum semiovale perivascular spaces on T2-weighted MRI. (E) Extensive white matter hyperintensities on fluid-attenuated inversion recovery MRI.

not yet been defined. The purpose of this study is to explore in vivo clinical MRI markers and their correlation with CMI found on autopsy in patients with CAA.

## METHODS

**Standard protocol approvals, registrations, and patient consents.** This study was performed with approval of the institutional review board of the Massachusetts General Hospital (protocol No. 2015P001177).

**Patients.** Patients were retrospectively identified from a neuropathologic CAA cohort (1997–2012) by a systematic keyword search of pathology reports and prospective clinical databases based on both pathologic evidence of mild, moderate, or severe CAA (from routinely collected brain autopsy) and available antemortem brain MRI sequences as previously described.[6]

**Pathologic data.** Morphologic assessment was performed with hematoxylin-eosin, Congo red, and β-amyloid (Aβ) immunohistochemistry stains, according to standardized neuropathologic assessment. Severity of vascular Aβ deposition was rated and graded according to the method described by Vonsattel et al[7] by experienced neuropathologists. We categorized cases as severe CAA if the characteristic vessel-in-vessel appearance caused by amyloid deposition was observed in the examined Aβ-stained sections. Nine standard hematoxylin-eosin–stained sections (6 cortical regions: midfrontal, middle-superior temporal, inferior parietal, cingulate, hippocampus, and entorhinal; and 3 deep brain regions: anterior basal ganglia, anterior thalamus, and midbrain) were reviewed for the presence and number of CMIs as previously described.[3] Lesions were identified by finding cavitation, puckering, or discrete regions of pallor with cell loss and gliosis (screened with ×10, confirmed with ×20 objectives) and manually counted by 2 readers (S.J.v.V, trained in CMI reading, and C.M.W, a board-certified neuropathologist) blinded to in vivo MRI and clinical data (interrater agreement: Cohen $\kappa = 0.75$). A consensus meeting was performed that included a third reader (A.L., trained in CMI reading) in cases of disagreement ($n = 5$).

**Neuroimaging data and analysis.** Imaging for all patients consisted of MRI scans performed with 1.5T or 3T scanners and included an axial T2*-weighted sequence, a sagittal T1, and an axial fluid-attenuated inversion recovery sequence. The MRI closest to death was examined if multiple MRIs were available. All MRIs were reviewed by researchers blinded to clinical and pathologic data by trained observers, according to STRIVE (Standards for Reporting Vascular Changes on Neuroimaging).[8] In short, microbleed presence and number were evaluated according to current consensus criteria, and on the basis of previously described increased risk of cognitive impairment, patients were dichotomized into ≤4 vs >4 microbleeds.[4] The presence and number of ICHs (>10 mm in diameter on T2*-weighted MRI scans) were also noted. The severity of cSS, defined as curvilinear patterns of low signal on T2*-weighted sequences in the superficial layers of the cerebral cortex, was classified as focal (restricted to ≤3 sulci) or disseminated (≥4 sulci) in the absence of contiguous connection with a lobar ICH. We assessed CS-PVSs on axial T2-weighted MRIs using a previously described,[9] validated, 5-point visual rating scale (0 = no PVSs, 1 ≤ 10 PVSs, 2 = 11–20 PVSs, 3 = 21–40 PVSs, and 4 ≥ 40 PVSs). Volume of WMH was quantified with the use of a semiautomated, multistep protocol on axial fluid-attenuated inversion recovery sequences.

**Statistical analysis.** Categorical variables were compared by use of $\chi^2$ tests. Normally distributed continuous variables were compared with the unpaired $t$ test, and skewed distributed data were analyzed with the Mann-Whitney $U$ test. Frequency distribution of CMI in cortical vs deep brain regions was compared by use of the Fisher exact test. Forced entry logistic regression analysis was used to determine whether microbleeds were independently associated with the presence of CMIs. Entered variables are presented with their odds ratio, standard error, 95% confidence interval, and $p$ value after adjustment for confounding variables. A value of $p < 0.05$ was considered statistically significant. Statistical analyses were performed with SPSS 22.0.

**RESULTS** We identified 49 patients with CAA who met the inclusion criteria. Table 1 shows the baseline characteristics of this cohort. Patients initially presented with cognitive impairment (53.0%), ICH (22.4%), or ischemic stroke (10.2%). One or more CMIs on pathology were present in 36.7% of our study population (multiple CMIs in 12.2%, range 1–4). CMIs were distributed equally between cortical and deep brain regions (9 CMIs in the sections targeting deep area vs 24 CMIs in the sections targeting cortical areas, $p = 0.699$). Patients with or without CMIs on pathology did not differ by age, sex, or time from evaluated MRI to death (table 1). Severe CAA pathology was more common in patients with vs without CMIs (61.1% vs 29.0%, $p = 0.04$), but Alzheimer pathology (Braak stage III or greater) was not different (72.2% vs 74.2%, $p = 0.72$). Previous ICH on MRI (44.4% vs 16.1%, $p = 0.03$) and higher numbers of microbleeds (median 8 [interquartile range 2.5–33.0] vs 1 [interquartile range 0–3], $p = 0.001$) were found more frequently in patients who were CMI positive. No other neuroimaging markers of CAA-related SVD were found to be related to CMI on pathology (table 1). In a multivariable model including age and presence of severe CAA, >4 microbleeds on MRI remained significantly associated with CMI on pathology (table 2).

**DISCUSSION** Our data suggest an association between MRI-visible microbleeds and CMIs found on pathology in patients with CAA independently of neuropathologic disease severity. This supports

**Table 1** Baseline characteristics and comparison of microinfarct presence vs absence in postmortem pathology

| | Microinfarcts present (18) | Microinfarcts absent (31) | *p* Value |
|---|---|---|---|
| **Baseline characteristics** | | | |
| **Clinical characteristics** | | | |
| Age at MRI, mean ± SD, y | 75.5 ± 10.72 | 74.6 ± 7.9 | 0.732 |
| Time from MRI to death, mean ± SD, d | 896 ± 996 | 988 ± 863 | 0.419 |
| Female sex, n (%) | 8 (44.4) | 10 (32.3) | 0.394 |
| Hypertension, n (%) | 12 (66.7) | 24 (77.4) | 0.411 |
| CAD, n (%) | 5 (27.8) | 10 (32.2) | 0.743 |
| Atrial fibrillation, n (%) | 3 (16.7) | 3 (9.7) | 0.472 |
| Diabetes mellitus, n (%) | 2 (11.1) | 1 (3.2) | 0.267 |
| Elevated cholesterol, n (%) | 1 (5.6) | 7 (22.6) | 0.120 |
| Antiplatelet, n (%) | 3 (17.6) | 12 (41.4) | 0.097 |
| Anticoagulant, n (%) | 2 (11.8) | 4 (13.8) | 0.844 |
| **Pathologic findings** | | | |
| Alzheimer disease; Braak stage III or greater, n (%) | 13 (72.2) | 23 (74.2) | 0.719 |
| Severe CAA, n (%) | 11 (61.1) | 9 (29.0) | 0.038 |
| **In vivo neuroimaging characteristics** | | | |
| **MRI field strength, n (%)** | | | |
| 1.5T | 16 (94.1) | 30 (93.8) | 0.938 |
| 3.0T | 1 (5.9) | 2 (6.3) | 0.938 |
| ICH on MRI, n (%) | 8 (44.4) | 5 (16.1) | 0.030 |
| Microbleeds lobar, median n (IQR) | 7 (1.75–32.5) | 1 (0–3.0) | 0.003 |
| >4 Microbleeds, n (%) | 13 (72.2) | 6 (19.4) | <0.001 |
| WMH volume, median (IQR) | 32.5 (10.8–43.9) | 14.9 (9.1–28.5) | 0.250 |
| Disseminated cSS, n (%) | 4 (22.2) | 4 (12.9) | 0.320 |
| CS-PVS, median, N (IQR) | 3 (2.0–4.0) | 2 (1.0–3.0) | 0.147 |
| CS-PVS score >2, n (%) | 13 (72.2) | 14 (45.2) | 0.082 |

Abbreviations: CAA = cerebral amyloid angiopathy; CAD = coronary artery disease; CS-PVS = centrum semiovale perivascular space; cSS = cortical superficial siderosis; ICH = intracerebral hemorrhage; WMH = white matter hyperintensity. When appropriate, n refers to the number of patients. Denominators may vary slightly as a result of unknown or missing data. Data are reported as raw quantiles, raw frequencies with weighted percentages, and weighted means and SEs.

**Table 2** Multivariable analysis of predictors of microinfarct presence

| Multivariable analysis of microinfarct presence | OR | 95% CI | *p* Value |
|---|---|---|---|
| Age at death | 1.008 | 0.921–1.102 | 0.863 |
| Severe CAA (yes vs no) | 1.769 | 0.266–11.770 | 0.555 |
| Microbleeds >4 (yes vs no) | 8.622 | 1.781–41.737 | 0.007 |
| Previous ICH (yes vs no) | 0.562 | 0.069–4.542 | 0.589 |

Abbreviations: CAA = cerebral amyloid angiopathy; CI = confidence interval; ICH = intracerebral hemorrhage; OR = odds ratio.

the concept of mixed SVD: simultaneously occurring ischemic and hemorrhagic disease in a single SVD-related disorder. The association of CMIs with microbleeds could be related to damage to vessel continuity caused by CAA pathology. While the characteristic accumulation of Aβ within the arterial media and adventitia likely leads to vessel fragility and hemorrhage, amyloid deposits are also thought to lead to ischemia by thickening of the vessel wall and vessel occlusion in CAA.[3] Our results suggest that CMI burden in patients with CAA may in part be related to similar pathologic mechanisms underlying microbleeds, representing another consequence of vascular Aβ accumulation. Indeed, a recent study has shown that the histopathologic substrate underlying MRI-defined microbleeds is heterogeneous and includes vasculopathies and hemorrhagic microinfarctions.[10]

We did not observe an association with other MRI markers of CAA-related SVD. The reason may be that these other markers have distinct pathophysiologic mechanisms. Blood leaking from CAA-affected superficial cortical and leptomeningeal vessels is thought to give rise to the signal abnormalities characteristic of cSS on in vivo MRI, while CS-PVS enlargement could be related to drainage impairment of interstitial fluid and WMH to chronic ischemic changes in deeper regions of the brain.[2,11] Similar to microinfarcts, WMHs are considered to be of ischemic nature; however, we did not find an association between these 2 lesions. This is consistent with a previous pathologic study in which increased WMH burden was not associated with the presence of CMI on pathology, although there appeared to be a trend toward greater CAA severity.[12] The data may suggest that these lesions have different underlying mechanisms.

We found CMIs in more than one-third of the CAA cases included in this study. This high prevalence of CMI pathology is in line with previous studies and may indicate an overall burden of several hundreds to thousands in the entire brain.[1,3] Because the majority of CMIs remain undetected by conventional MRI, a surrogate marker to indirectly quantify or monitor growing CMI burden could be of great value.[1,2,8]

Limitations of this study lie in its retrospective nature and possible selection bias in this hospital-based cohort. The relatively small number of patients, the interval between neuroimaging and pathologic evaluation, and the cross-sectional design limit generalizability regarding the prognostic significance of microbleeds for the presence of CMIs. The strength of this work is the combination of detailed pathologic examinations and in vivo imaging data in a defined patient population.

In conclusion, CMIs are common in patients with CAA, and there is a strong association between CMI at autopsy and microbleeds on MRI obtained during life. This suggests a shared underlying pathophysiologic mechanism. Further studies are needed to evaluate whether microbleeds observed on MRI represent a robust surrogate for CMI burden and to explore the association between microbleeds and cognitive impairment.

## AUTHOR CONTRIBUTIONS

Dr. Lauer: data collection, analysis and interpretation of data, and drafting of the manuscript. Dr. van Veluw: data collection and revising the manuscript. Dr. William: data collection and revising the manuscript. Dr. Charidimou: critical revision of the manuscript for important intellectual content. Dr. Roongpiboonsopit: data collection and revising the manuscript. A. Vashkevich: data collection and revising the manuscript. A. Ayres: data collection and revising the manuscript. Dr. Martinez-Ramirez: data collection and revision of the manuscript. Drs. Gurol, Biessels, Frosch, and Greenberg: critical revision of the manuscript for important intellectual content. Dr. Viswanathan: study concept and design, analysis and interpretation of data, revising manuscript, and study supervision.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## DISCLOSURE

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