Incidence of cerebral microbleeds in preclinical Alzheimer disease

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ABSTRACT

Objective: We sought to determine the incidence and associations of lobar microbleeds (LMBs) in a longitudinal cohort with ^{11}C–Pittsburgh compound B (PiB) PET imaging.

Methods: One hundred seventy-four participants from the observational Australian Imaging, Biomarkers and Lifestyle Study of Ageing (97 with normal cognition [NC], 37 with mild cognitive impairment [MCI], and 40 with Alzheimer disease [AD] dementia) were assessed at 3 time points over 3 years with 3-tesla susceptibility-weighted MRI and ^{11}C-PiB PET. MRIs were inspected for microbleeds, siderosis, infarction, and white matter hyperintensity severity, blind to clinical and PiB findings. Neocortical PiB standardized uptake value ratio, normalized to cerebellar cortex, was dichotomized as positive or negative (PiB+/−, standardized uptake value ratio >1.5). Annualized LMB incidence was calculated, and logistic regression was used to determine the association of incident LMBs with PiB, *APOE* ε4+ status, and cerebrovascular disease.

Results: LMBs were present in 18.6% of NC, 24.3% of MCI, and 40% of AD participants ($p < 0.05$ vs NC). LMB incidence was 0.2 ± 0.6 per year in NC participants, 0.2 ± 0.5 in MCI, and 0.7 ± 1.4 in AD ($p < 0.03$ vs NC) and was 6-fold higher in PiB+ than PiB-NC. Incident LMBs were associated with age, *APOE* ε4+, PiB+, and baseline LMBs. Incidence of multiple LMBs was also associated with lacunar infarction and white matter hyperintensity severity.

Conclusions: Older age, baseline LMBs, higher β-amyloid burden, and concomitant cerebrovascular disease may all confer higher risk of incident LMBs. This should be considered when designing protocols for amyloid-modifying clinical trials. ***Neurology*® 2014;82:1266–1273**

GLOSSARY

Aβ = β-amyloid; **AD** = Alzheimer disease; **AIBL** = Australian Imaging, Biomarkers and Lifestyle Study of Ageing; **CAA** = cerebral amyloid angiopathy; **CI** = confidence interval; **FLAIR** = fluid-attenuated inversion recovery; **GRE** = gradient-recall echo; **LMB** = lobar microbleed; **MCI** = mild cognitive impairment; **MP-RAGE** = magnetization-prepared rapid-acquisition gradient echo; **NC** = normal cognition; **OR** = odds ratio; **PiB** = Pittsburgh compound B; **SS** = superficial siderosis; **SUVR** = standardized uptake value ratio; **SWI** = susceptibility-weighted imaging; **TE** = echo time; **TR** = repetition time; **VRF** = vascular risk factor; **WMH** = white matter hyperintensity.

Cerebral microbleeds[1] and superficial siderosis (SS)[2] are frequently identified using T2* gradient-recall echo (GRE) or susceptibility-weighted MRI sequences in the setting of microvascular disease due to either hypertensive arteriosclerosis or cerebral amyloid angiopathy (CAA).[3] Microbleeds in a lobar, cortico-subcortical distribution and SS[4] are frequently due to CAA[1] and are associated with *APOE* ε4+ status, severe white matter hyperintensity (WMH),[5] and in vivo evidence of β-amyloid (Aβ) with PET.[6–8]

Microbleeds have also been noted in some treatment trials for Alzheimer disease (AD), hypothesized to be from altered vascular permeability from mobilization of parenchymal or vascular Aβ.[9,10] Because of safety concerns, it is recommended that individuals with multiple

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microbleeds be excluded from immunotherapy studies[9]; however, there is limited information regarding the natural history of these lesions to guide clinical trial design.

Carbon 11 Pittsburgh compound B (^{11}C-PiB) PET imaging has demonstrated co-occurrence of Aβ with microbleeds (particularly lobar microbleeds [LMBs]) in asymptomatic older controls[7] and intracranial hemorrhage,[6] and increased PiB retention at sites of subsequent incident microbleeds,[11] implicating the presence of Aβ with risk of future microbleeds. Although there are several prospective studies of microbleeds,[11–15] the incidence of microbleeds in preclinical AD (i.e., normal cognition [NC] with presence of an Aβ biomarker[16]) is unknown.

We therefore investigated the incidence of LMBs and risk factors for incident LMBs over 3 years in a cohort with ^{11}C-PiB PET imaging.

METHODS

Setting and participants. Participants studied were from the Melbourne Neuroimaging Cohort of the Australian Imaging, Biomarkers and Lifestyle Study of Ageing (AIBL), comprising 174 individuals who underwent susceptibility-weighted MRI, PiB PET, and clinical assessment, and had blood drawn for biomarkers and *APOE* genotype sequencing.[17]

At baseline there were 97 control participants with NC, 37 participants with mild cognitive impairment (MCI), and 40 participants with dementia due to AD. Of these, 123 had at least 2 susceptibility-weighted imaging (SWI) scans over 36 months of follow-up (123 had 2 and 87 had 3 scans).

Inclusion criteria and methodology for this cohort has been previously described. Of note, individuals with a history of large stroke or alcohol abuse were excluded.[18]

Standard protocol approvals, registrations, and patient consents. Approval for the study was obtained from the Austin Health Human Research Ethics Committee. Written informed consent was obtained from all participants.

Clinical assessment. Each individual underwent a neuropsychological test battery (including Folstein Mini-Mental State Examination[19] and Clinical Dementia Rating[20]) and panel discussion, blinded to neuroimaging findings. Individuals met National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer's Disease and Related Disorders Association[21] and Petersen[22] criteria for AD and MCI, respectively, and controls performed within age-matched norms.[18]

As previously described,[7] the following vascular risk factors (VRFs) were identified from self-report, physical examination, and laboratory findings: hypertension, diabetes mellitus, hypercholesterolemia, current smoker, cardiovascular disease, and atrial fibrillation.

MRI acquisition and interpretation. All MRIs were performed on the same 3-tesla Siemens TRIO MRI system (Siemens AG, Erlangen, Germany). High-resolution T1-weighted magnetization-prepared rapid-acquisition gradient echo (MP-RAGE), fluid-attenuated inversion recovery (FLAIR), and SWI were performed as follows: T1 MP-RAGE: matrix 160 × 240 × 256, voxel size 1.2 × 1 mm (sagittal × coronal), slice thickness 1 mm, repetition time (TR)/echo time (TE) 2,300/2.98 milliseconds, flip angle 9°; FLAIR: matrix 176 × 240 × 256, voxel size 0.90 × 0.97 mm, slice thickness 0.97 mm, TR/TE 6,000/421 milliseconds, flip angle 120°, TI 2,100 milliseconds; and SWI: matrix 176 × 256 × 80, voxel size 0.94 × 0.94 mm, slice thickness 1.75 mm, no gap, TR/TE 27/20 milliseconds, flip angle 15°.

MRIs were inspected for microbleeds, siderosis, infarction, and WMH severity, blinded to clinical and PiB findings. Microbleeds were defined as round or ovoid (nonlinear) hypointense lesions ≤10 mm[1] (figure 1). Lesions were tracked through multiple slices to exclude pial blood vessels. First, 2 neuroradiologists independently read all SWIs separately (in random order). A consensus was reached for presence and number of lesions on each scan. The agreement between readers 1 and 2 was 83% (κ = 0.65); reader 1 × consensus 91% (κ = 0.8); and reader 2 × consensus 91% (κ = 0.83). An additional blinded assessment was performed to confirm these ratings, with agreement of 82% with reader 1 (κ = 0.56), 78% with reader 2 (κ = 0.52), and 83% with consensus (κ = 0.61).

Second, images were read sequentially (i.e., baseline, 18 months, 36 months) to document the appearance or disappearance of lesions over the 3 time points, with T1 and T2 FLAIR images available for comparison. Lesions were categorized by region into lobar or nonlobar (deep or infratentorial). Lesions in the cerebellum were counted as "lobar" because this is an area in which CAA can occur.[23] Presence of SS (figure 2), curvilinear hypointensity in the subarachnoid space, was counted in analyses as equivalent to an LMB (herein "LMB" includes SS unless specified otherwise).[4] According to established rating scales,[24,25] lesions were stratified into "possible" and "definite." Only definite microbleeds were included in analyses. For incidence analyses, incident LMBs included both newly identified LMBs (including SS) and those that were reclassified to definite from possible on a prior scan. To confirm the validity of this method, we recalculated the incidence rates excluding incident LMBs that were identified as "possible" at baseline with no significant change to the results.

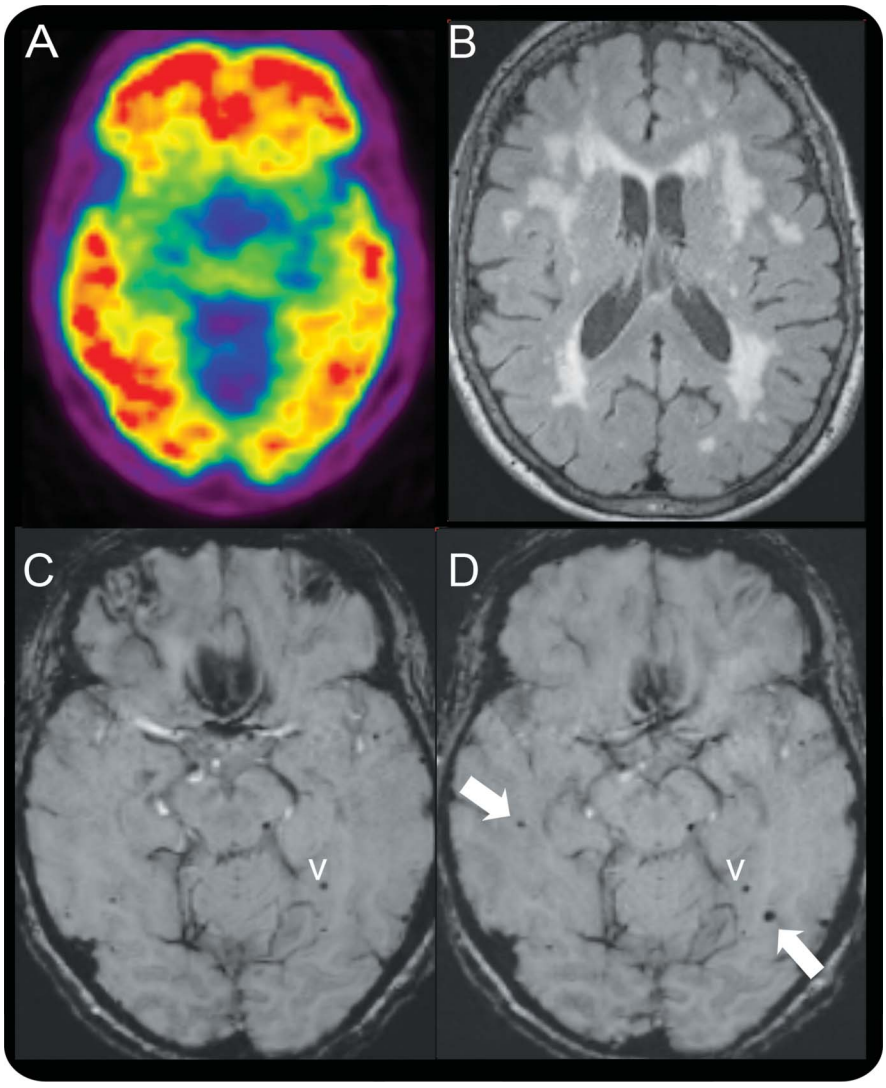
Lacunar infarcts were defined as foci 3 to 20 mm in size, hypointense on T1, and hypointense with perifocal high signal on T2 FLAIR.[26,27] Cortical infarction was defined as cortical lesions with increased FLAIR, isointense or hypointense T1 signal.[28] Hemorrhage was defined as SWI hypointensity >10 mm in diameter.[29] Deep and periventricular WMH severity was rated visually using a validated rating scale.[30]

PET acquisition and interpretation. Each participant received approximately 370 MBq of ^{11}C-PiB IV over 1 minute. A 30-minute acquisition, 40 minutes post-injection, was performed using a Philips Allegro PET camera (Philips Healthcare, Andover, MA). A transmission scan was performed for attenuation correction. Cortical-to-cerebellar gray matter ratios (standardized uptake value ratio [SUVR]) were generated for regions of interest. Neocortical Aβ burden was expressed as the average SUVR of frontal, superior parietal, lateral temporal, lateral occipital, and anterior and posterior cingulate regions. A threshold neocortical SUVR of 1.5 was used to indicate significant PiB retention (PiB+/−).[17]

Statistical methods. Groups were compared using χ^2, analysis of variance, or Kruskal-Wallis tests, with Bonferroni correction for multiple comparisons.

To compare incidence between participants with different follow-up periods, the annual incidence for LMBs (including SS) was calculated using the total number of incident lesions and maximum time interval between baseline and final scans. Incidence rates were not calculated for non-LMBs because of the small number of these lesions. Spearman correlations were performed among LMB incidence, PiB SUVR, and number of

Figure 1 Multiple incident microbleeds in a PiB+ cognitively normal control



Images from an 86-year-old participant with a baseline Mini-Mental State Examination score of 30/30 and Clinical Dementia Rating of 0. (A) ^{11}C-PiB PET: extensive cortical PiB retention (standardized uptake value ratio = 2.15). (B) Fluid-attenuated inversion recovery MRI: severe periventricular and deep white matter hyperintensities. (C, D) Baseline and 18-month susceptibility-weighted MRI demonstrating a vessel (v) and multiple incident microbleeds (arrows). PiB = Pittsburgh compound B.

baseline LMBs. Logistic regression was used to determine association of clinical and imaging variables with incident LMBs (including SS). To account for potential bias, all regression analyses were adjusted for age, sex, and scan interval.

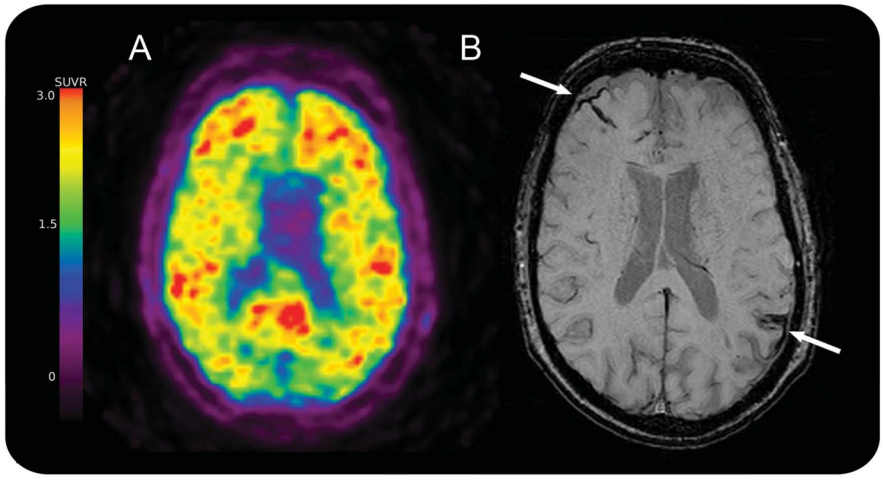
To determine whether the association between Aβ burden and incident LMBs was influenced by *APOE* ε4 status, logistic regression was performed including PiB status, *APOE* ε4, and an interaction term, *APOE* ε4 × PiB.

RESULTS The median duration between baseline PiB and MRI was 0 days (interquartile range 20.3). There were no significant differences in age, sex, number of VRFs, or antiplatelet medications among NC, MCI, and AD groups (table 1). In addition, there was no significant difference in prevalence of VRFs among NC, MCI, and AD groups or when stratified by PiB+/− (data not shown).

Five individuals reported a history of stroke or TIA (1 stroke, 4 TIA), of whom 3 had radiologic evidence of infarction, and clinically silent infarction was identified in 14 individuals. Nine participants had lacunar infarction, 8 had cortical infarction, and 3 had hemorrhage. Two participants had both lacunar infarction and hemorrhage and one had both lacunar and cortical infarction. There was no difference in prevalence of infarction among NC, MCI, and AD groups. Periventricular WMH severity and prevalence of SS were both greater in AD than in NC participants ($p < 0.05$, table 1).

Baseline analysis: Microbleed prevalence. The prevalence of microbleeds (in any location) was 21.6% in NC, 24.3% in MCI, and 42.5% in AD participants

Figure 2 Superficial siderosis in a PiB+ participant with Alzheimer disease



Images from an 83-year-old participant with Alzheimer dementia, baseline MMSE score = 17/30, CDR = 1, and CDR-SOB = 5. At 3 years: MMSE = 10/30, CDR = 2, and CDR-SOB = 12. (A) ^{11}C-PiB PET: extensive cortical PiB retention (SUVR = 2.96). (B) Susceptibility-weighted MRI: superficial siderosis in right frontal and left parietotemporal regions (arrows). CDR-SOB = Clinical Dementia Rating–Sum of Boxes; MMSE = Mini-Mental State Examination; PiB = Pittsburgh compound B; SUVR = standardized uptake value ratio.

($p < 0.05$ AD vs NC, $\chi^2 = 4.3$). The range of microbleeds per participant was 0–19 in NC, 0–4 in MCI, and 0–16 in AD. LMBs (including SS) were present in 18.6% of NC, 24.3% of MCI, and 40% of AD participants ($p < 0.05$ vs NC, $\chi^2 = 7.0$). Deep or infratentorial microbleeds were infrequent (NC 10.3%, MCI 5.4%, AD 2.5%, $p > 0.05$, $\chi^2 = 2.8$).

LMB prevalence in PiB+ NC participants was 41.4% (12/29) compared with 8.8% (6/68) in PiB-NC participants ($p < 0.001$, $\chi^2 = 14.3$). LMB prevalence was higher in individuals with lacunar infarction than in those without (55.6%, 5/9 vs 23.0%, 38/165, $p = 0.04$, $\chi^2 = 4.9$). No difference was seen with or without cortical infarcts or hemorrhage.

Longitudinal analysis. One hundred twenty-three participants (70.7%) had sequential imaging. Participants who withdrew before follow-up were significantly older, had poorer cognition, more severe WMH, and higher prevalence of PiB+ and microbleeds (table e-1 on the *Neurology*® Web site at Neurology.org). Discontinuation from imaging was attributable to the following factors: participant declining participation (10.9%), progressive cognitive impairment (9.2%), new MRI contraindication (8.0%), other illness (6.9%), scan pending or postponed (4.6%), clinical trial (4.0%), deceased (4.0%), lost contact (1.7%), and missing MRI sequence (0.6%).

Microbleed incidence. Thirty participants had incident LMBs (range 0–16 per participant) and 6 had incident non-LMBs. Two cases of incident SS occurred (both AD participants), and all (3/3) participants with baseline SS with longitudinal imaging demonstrated incident LMBs. The incidence of LMBs was higher in participants with AD than in those with NC or MCI (table 2).

LMB incidence was higher in PiB+ than PiB− and in those with multiple baseline LMBs, and was higher in *APOE* ε4+ than ε4− status (0.5 ± 1.0 vs 0.1 ± 0.3 per year, $p < 0.01$).

Participants with NC. In participants with NC, there was a correlation between LMB incidence (LMBs/year) and both PiB SUVR ($\rho = 0.27$, $p = 0.01$) and baseline LMBs ($\rho = 0.35$, $p < 0.001$). Incident LMBs were associated with age, *APOE* ε4 carrier status, PiB+ status, and presence of baseline LMBs, but not sex, VRFs, antiplatelet use, WMH ratings, lacunar infarction, or cortical infarction. The odds ratio (OR) for incident LMBs was higher with multiple baseline LMBs compared with one or more. An association with presence of lacunar infarction was of trend-level significance (table e-2). In participants with NC, the incidence of multiple LMBs was associated with age, *APOE* ε4 status, PiB+ status, and presence of LMBs and lacunar infarction at baseline (table e-2).

All participants. When all participants were included in the model (NC, MCI, and AD), incident LMBs were associated with age (OR 1.0, 95% confidence interval [CI] 1.0–1.1), *APOE* ε4 (OR 3.8, 95% CI 1.6–9.1), PiB+ (OR 2.5, 95% CI 1.1–5.9), and baseline LMBs (OR 5.6, 95% CI 2.0–15.3), as well as periventricular (OR 1.5, 95% CI 1.0–2.3) but not deep WMH (OR 1.1, 95% CI 0.6–2.0). Incidence of multiple LMBs was also associated with age, *APOE* ε4 status, PiB+ status, baseline LMBs, lacunar infarction, and periventricular (OR 1.9, 95% CI 1.1–3.5) and deep WMH (OR 3.4, 95% CI 1.4–8.1).

Table 1 Clinical and neuroimaging characteristics of participants

	NC (n = 97)	MCI (n = 37)	AD (n = 40)
Clinical characteristics			
Age, y	74.2 ± 7.3	75.8 ± 6.9	74.6 ± 8.4
Males	46 (47.4)	21 (56.8)	16 (40.0)
APOE ε_4+	33 (34.0)	16 (44.4)	28 (70.0)[a]
Vascular risk factors	1.3 ± 1.2	1.5 ± 1.1	1.3 ± 1.1
Antiplatelet medication	36 (37.1)	13 (35.1)	10 (25.0)
MMSE score	28.9 ± 1.3	27.4 ± 1.9[a]	21.4 ± 5.2[a]
CDR-SOB	0.1 ± 0.2	1.1 ± 0.8[a]	5.0 ± 2.8[a]
Neuroimaging characteristics			
PiB+	29 (29.9)	21 (56.8)[a]	40 (100)[a,b]
PiB SUVR	1.4 ± 0.4	1.8 ± 0.7[a]	2.4 ± 0.4[a,b]
Any stroke	11 (11.3)	1 (6.3)	6 (15.0)
Lacunar infarction	5 (5.2)	1 (2.7)	3 (7.5)
Cortical infarction	6 (6.2)	0 (0.0)	2 (5.0)
Hemorrhage	1 (1.0)	0 (0.0)	2 (5.0)
Periventricular WMH severity	1.1 ± 1.0	1.2 ± 1.1	1.5 ± 1.0[a]
Deep WMH severity	1.0 ± 0.7	1.1 ± 0.8	1.2 ± 0.8
Microbleed prevalence	21 (21.6)	9 (24.3)	17 (42.5)[a]
Lobar[c]	18 (18.6)	9 (24.3)	16 (40.0)[a]
Nonlobar	10 (10.3)	2 (5.4)	1 (2.5)
Superficial siderosis	1 (1.0)	0 (0.0)	7 (17.5)[a,b]

Abbreviations: AD = Alzheimer disease; CDR-SOB = Clinical Dementia Rating–Sum of Boxes; MCI = mild cognitive impairment; MMSE = Mini-Mental State Examination; NC = normal cognition; PiB = Pittsburgh compound B; SUVR = standardized uptake value ratio; WMH = white matter hyperintensity.
Data are presented as mean ± SD or prevalence (%).
[a] Significantly different from NC, $p < 0.05$.
[b] Significantly different from MCI, $p < 0.05$.
[c] Lobar microbleed prevalence does not include superficial siderosis.

Table 2 LMB incidence overall and stratified by PiB status and baseline LMBs

	LMB incidence (lesions/y)			
	NC (n = 83)	MCI (n = 23)	AD (n = 17)	All participants (n = 123)
Overall	0.2 ± 0.6	0.2 ± 0.5	0.7 ± 1.4[a]	0.3 ± 0.8
PiB status				
PiB−	0.1 ± 0.2	0.1 ± 0.2	—	0.1 ± 0.2
PiB+	0.6 ± 1.1[b]	0.3 ± 0.6	0.7 ± 1.4	0.5 ± 1.1[b]
Baseline LMBs				
0	0.1 ± 0.2[c]	0.1 ± 0.3	0.03 ± 0.1[c]	0.1 ± 0.2[c]
1	0.2 ± 0.3[c]	0.5 ± 0.8	1.0 ± 1.7	0.4 ± 0.8[c]
≥2	2.4 ± 1.3	0.0 ± 0.0	2.7 ± 2.0	2.5 ± 1.5

Abbreviations: AD = Alzheimer disease; LMB = lobar microbleed; MCI = mild cognitive impairment; NC = normal cognition; PiB = Pittsburgh compound B.
Data are mean ± SD. LMBs include superficial siderosis. Analysis of variance was used for statistical analyses.
[a] Significantly different from NC, $p < 0.05$.
[b] Significantly different from PiB−, $p < 0.01$.
[c] Significantly different from ≥2 LMBs, $p < 0.001$.

***APOE* status, global Aβ burden, and LMBs.** Sixty-three percent of individuals with (any) incident LMB and 85.7% of individuals with multiple incident LMBs were *APOE* ε4+. Incident LMBs were associated with *APOE* ε4+ in univariate analysis; however, with *APOE* ε4+ and PiB+ in a regression model together, PiB+ remained significant (OR 5.6, 95% CI 1.1–31.5) whereas *APOE* ε4+ did not (OR 3.2, 95% CI 0.7–15.9). The interaction term *APOE* ε4 × PiB was not associated with incident LMBs. *APOE* ε2 was not associated with baseline or incident LMBs.

DISCUSSION This study assessed the annual incidence of LMBs in a cohort of older adults with concurrent PiB imaging over a 3-year follow-up period. We found that individuals with high Aβ burden or prior microbleeds were more likely to develop new lesions over time and that the incidence was related to the number of LMBs at baseline, Aβ burden, age, and other markers of cerebral small-vessel disease. When analyzed together, high global Aβ burden was independently associated with incident LMBs, whereas *APOE* ε4 carriage was not.

Our cohort included individuals (PiB+ with NC) meeting criteria for preclinical AD, a category for which several amyloid-modifying trials are proposed.[16] However, because conventional amyloid PET imaging cannot distinguish between parenchymal and vascular Aβ, it is theoretically possible that a proportion of this group may have predominantly vascular pathology (i.e., "preclinical CAA"). Longitudinal follow-up of clinical outcomes will be enlightening in this cohort.

To date, there are limited but growing prospective data on the natural history of microbleeds to guide clinicians and safety monitoring in clinical trials in AD and CAA. Separating definite from possible microbleeds[24,25] and reading images sequentially[31] have been shown to improve rater confidence and inter-rater agreement. Current guidelines for assessment of microbleeds in AD clinical trials focus predominantly on T2* GRE rather than SWI, because this sequence has traditionally been more widely available.[9] SWI permits greater contrast by image postprocessing, allowing greater definition of vascular structures and higher sensitivity for detection of microbleeds.[32]

Using SWI, participants with 2 or more baseline LMBs (i.e., satisfying the Boston criteria for probable CAA[33]) had a significantly higher rate of incident lesions than those with one, and the LMB incidence rate correlated with global Aβ burden.

Individuals with higher Aβ burden, particularly those with probable CAA, may be at greater risk of amyloid-related imaging abnormalities in the setting of immunotherapy. In designing clinical trials in preclinical AD, a more stringent exclusion threshold of ≥2 lesions may be reasonable if SWI, rather than T2* GRE, is used for participant screening. This should be tested by designing future randomized controlled trials with safety endpoints aimed to prospectively assess incident amyloid-related imaging abnormality events.

The mean incidence of LMBs was also higher in AD participants (0.7/y) than in NC (0.2/y) or MCI participants (0.2/y). Incidence rates in AD have not been reported to date; however, the incidence of microbleeds (any site) in acute stroke has been reported to be 0.8/y, increasing to 5.4/y in those with ≥5 microbleeds at baseline.[13]

In cross-sectional studies, LMBs have been associated with increasing age,[7,34] presence of *APOE* ε2[35] and *APOE* ε4,[5,34,36] and high PiB retention on PET imaging.[7] Over longitudinal follow-up, incident LMBs have also been associated with the presence of baseline microbleeds and severity of white matter disease.[15,37] We saw a significant association between incident LMBs and baseline Aβ burden, corroborating our previous cross-sectional findings.[7] Age and *APOE* ε4 carrier status were also associated with incident LMBs, and WMH and lacunar infarction were associated with development of multiple LMBs.

The findings are consistent with preclinical studies demonstrating that both age-related vascular changes and *APOE* ε4 carriage may increase the risk of CAA through disruption of perivascular drainage of soluble Aβ from the brain interstitium.[38,39] Presence of severe WMH may reflect impaired perivascular drainage in the setting of severe CAA, with stasis of interstitial fluid, and chronic white matter edema.[40,e1] In addition, severe subcortical small-vessel disease (encompassing lacunar infarction and WMH) may act in concert with CAA to increase risk of LMBs.[e2] However, without histopathologic correlation, it is not clear whether our findings are attributable to concomitant arteriosclerotic disease, more advanced CAA, or both.

In this study, incident LMBs were more strongly associated with global brain Aβ burden than *APOE* ε4+ status, and there was no significant interaction between them on incidence of new lesions. Consistent with our baseline results and the findings from the larger Alzheimer's Disease Neuroimaging Initiative, this suggests that the influence of *APOE* ε4 on LMBs may be effected through its influence on total brain Aβ. *APOE* ε2 carriage has also been associated with intracerebral hemorrhage in the setting of CAA, and this may be through mechanisms independent of Aβ.[8] However, because our sample contained few *APOE* ε2 carriers, no conclusion could be drawn about this.

The baseline prevalence of LMBs among participants with NC in our study (21.6%) is similar to that

reported by the Rotterdam Study[15] but higher than several earlier reports using T2* GRE.[e3–e5] Along with differences in MRI parameters (e.g., field strength, coil technology, higher-resolution SWI), our sample demographics may also be implicated. The NC group in this study had a higher prevalence of *APOE* ε4 carriage (34%) compared with the general population (because of intentional selection criteria applied for the AIBL). In addition, they were predominantly of Caucasian extraction, were relatively well-educated and affluent, and were screened to exclude macrovascular stroke. These differences may explain the relative paucity of deep or infratentorial microbleeds compared with community, stroke, or vascular dementia groups[5,13,e6] and may limit generalizability of the findings.

Survivor bias may also be present. As in the Rotterdam Study, participants who withdrew from imaging were older and had greater prevalence of LMBs than those continuing.[15] This may have led to an underestimation of the true incidence of LMBs in the AD and MCI groups where most of the withdrawals occurred.

Incidence of LMBs (including SS) in cognitively normal individuals is associated with the presence of Aβ on PiB-PET and presence of baseline LMBs. Evidence of severe microvascular disease (WMH and lacunar infarcts) may also confer additional risk of incident LMBs. Future work with larger cohorts is necessary to corroborate these findings. Appreciation of the natural history of microbleeds and siderosis is fundamental to future trials of AD and CAA therapies that target Aβ, and these findings should be considered in the design of study protocols.

AUTHOR CONTRIBUTIONS

Dr. Yates analyzed data, conducted statistical analysis, drafted the manuscript, and was responsible for the final version. Prof. Desmond and Dr. Phal contributed to analysis or interpretation of data, writing of the manuscript. Dr. Steward contributed to acquisition of data, analysis or interpretation of data. Associate Prof. Szoeke contributed to the study supervision, writing of the manuscript, and obtained funding. Dr. Salvado contributed to study concept/design, analysis or interpretation of data, study supervision, writing of the manuscript, and obtained funding. Dr. Ellis contributed to study concept/design, acquisition of data, study supervision, writing of the manuscript, and obtained funding. Prof. Martins contributed to recruitment of patients, study supervision, and obtained funding. Prof. Masters contributed to study concept/design, study supervision, writing of the manuscript, and obtained funding. Prof. Ames contributed to study concept/design, recruitment of patients, writing of the manuscript, and obtained funding. Associate Prof. Villemagne contributed to study concept/design, study supervision, acquisition of data, analysis or interpretation of data, and writing of the manuscript. Prof. Rowe contributed to study concept/design, recruitment of patients, study supervision, analysis or interpretation of data, writing of the manuscript, and obtained funding.

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