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ABSTRACT

BACKGROUND AND PURPOSE: Cerebral microbleeds are associated with aging, hypertension, and Alzheimer disease. Microbleeds in a lobar distribution are believed to reflect underlying amyloid angiopathy, whereas microbleeds in the deep gray matter and infratentorial brain are commonly seen with hypertension. However, it is unknown how microbleeds in either distribution are related to Alzheimer pathogenesis. The purpose of this analysis was to test whether lobar and deep gray/infratentorial microbleeds demonstrate differential associations with CSF amyloid-β and phosphorylated tau 181 protein levels and longitudinal cognitive decline.

MATERIALS AND METHODS: A total of 626 subjects (151 cognitively normal, 389 with mild cognitive impairment, and 86 with Alzheimer disease) from the Alzheimer's Disease Neuroimaging Initiative who had undergone 3T MR imaging and lumbar puncture were included in the analysis. The number and location of microbleeds were assessed visually. Associations between lobar or deep gray/infratentorial microbleeds with CSF amyloid-β levels, abnormal CSF phosphorylated tau 181 protein levels, and longitudinal cognitive decline were assessed by using ordinary least-squares, logistic, and mixed-effects regression models while adjusting for covariates.

RESULTS: Having $\geq$3 lobar microbleeds was associated with lower levels of CSF amyloid-β ($P = .001$). After adjusting for CSF amyloid-β level, lobar microbleeds were independently associated with a higher likelihood of having an abnormal CSF phosphorylated tau 181 protein level ($P = .004$). Lobar microbleeds were associated with accelerated longitudinal cognitive decline ($P = .007$). Deep gray/infratentorial microbleeds revealed no significant associations.

CONCLUSIONS: The distribution of microbleeds revealed different associations with amyloid-β and phosphorylated tau 181 protein levels and cognition. Lobar and deep gray/infratentorial microbleeds should be considered separately with regard to Alzheimer disease pathogenesis.

ABBREVIATIONS: Aβ = amyloid-β; AD = Alzheimer disease; ADAS = Alzheimer Disease Assessment Scale; ADNI = Alzheimer's Disease Neuroimaging Initiative; APOE = apolipoprotein E; p-tau = phosphorylated tau 181 protein

Cerebral microbleeds, typically associated with aging, hypertension, and Alzheimer disease (AD), are common findings on gradient recalled-echo and susceptibility-weighted MR imaging sequences. In a population-based study in which a gradient recalled-echo sequence at 1.5T was used, approximately 36% of the people >80 years old were found to have microbleeds, compared with only 7% of the people who were 45–50 years old.[1] In a systematic review of studies that used echo-planar and gradient recalled-echo sequences at 0.5–3T, hypertensive individuals were reported to be 4 times more likely than those in the general population to have microbleeds,[2] particularly in association with other signs of small-vessel disease, such as white matter hyperintensities and lacunar infarcts.[3,4] In a review of multiple studies that used gradient recalled-echo and SWI sequences at 1.5T and 3T, people with mild cognitive impairment and Alzheimer disease were reported to have microbleeds at a prevalence of 20%–43% and 18%–32%, respectively, compared with 0%–19% in cogni-

tively normal individuals.[5] In the setting of AD, microbleeds are associated with global brain amyloidosis, seen with increased uptake on positron-emission tomography scans by using [18F]florbetapir[6] and decreased levels of CSF amyloid-β (Aβ).[5]

The anatomic distribution of microbleeds is believed to reflect their underlying pathology. Microbleeds located in the deep gray matter and infratentorial brain are typically seen in hypertensive individuals[2,7] and correspond to foci of hemosiderin leakage from small abnormal blood vessels.[8] Conversely, microbleeds in the setting of aging and AD are typically lobar, at the corticosubcortical junction,[2,7] and correspond to amyloid-β deposition along vessel walls,[9] also known as amyloid angiopathy. However, it remains unclear whether microbleeds, either from hypertension or amyloid angiopathy, relate to tau pathology or cognitive changes that lead to Alzheimer pathogenesis[10] independent of global brain amyloidosis.

The purpose of our analysis was to determine whether the distribution of microbleeds, either lobar or deep gray/infratentorial, has differential associations with downstream events in Alzheimer pathogenesis. Specifically, by using data from the multicenter Alzheimer's Disease Neuroimaging Initiative (ADNI) (adni.loni.usc.edu),[11] we tested the hypotheses that lobar microbleeds 1) are associated with brain amyloidosis, reflected by lower CSF amyloid-β levels, 2) predict the presence of tau pathology after overall brain amyloidosis is adjusted for, and 3) predict greater longitudinal cognitive decline.

MATERIALS AND METHODS

Subjects

The subjects of these analyses were 626 ADNI participants who had undergone 3T MR imaging and lumbar puncture for CSF analysis (151 cognitively normal, 389 with mild cognitive impairment, and 86 with AD). The ADNI is a longitudinal multicenter observational cohort study designed to identify imaging and biochemical biomarkers for the diagnosis and monitoring of AD.[12] The study was approved by the institutional review board of each of the participating institutions. Informed written consent was obtained from each participant at each site. Each of the subjects who enrolled in the ADNI-2 or ADNI–Grand Opportunity underwent a T2* gradient recalled-echo sequence, which was used to enumerate the microbleeds in the brain. Subjects were between the ages of 55 and 90 years and showed no clinical or structural evidence of a significant neurologic or psychiatric disease and no systemic medical illness or laboratory abnormalities that would interfere with follow-up. To reduce confounding by comorbid vascular disease, a subject was enrolled in the ADNI only if he or she had a low modified Hachinski score of ≤4.[13] Cognitive function was assessed by using the Alzheimer Disease Assessment Scale (ADAS),[14] which is the most widely used measure for clinical trials.

MR Image Acquisition

Each subject underwent a standardized 3T MR imaging protocol, which included T2* gradient recalled-echo and T1-weighted 3D MPRAGE sequences (http://adni.loni.usc.edu/methods/documents/mri-protocols/). This standardized protocol was distributed digitally to all ADNI-certified scanners and included the following

parameters for the gradient recalled-echo sequence: TE, 20 ms; TR, 650 ms; flip angle, 20°; section thickness, 4 mm; section gap, 0 mm. Before any subject was scanned using this protocol, an ADNI phantom was used to assess linear and nonlinear spatial distortion, signal-to-noise ratio, and image contrast, which were reviewed by a single quality-control center to ensure harmonization among the sites.[15]

Microbleeds were quantified visually by a board-certified neuroradiologist with subspecialty certification (G.C.C.). Microbleeds were defined as hypointense lesions within the brain parenchyma that measured <10 mm on the gradient recalled-echo sequence. Only microbleeds that were considered definite were included in the analysis.

Microbleeds were classified visually by location as 1) deep gray matter/infratentorial, if they involved the basal ganglia, thalami, brain stem, or cerebellum, or 2) lobar, if they involved other regions of the brain parenchyma.

CSF Biomarkers

Each subject included in this analysis underwent lumbar puncture to obtain CSF samples for quantifying levels of CSF amyloid-β and phosphorylated tau 181 protein (p-tau).[16] Briefly, each lumbar puncture was performed with a 20- or 24-gauge spinal needle at the baseline visit after an overnight fast. The CSF samples were transferred into polypropylene transfer tubes, frozen on dry ice within 1 hour after collection, and shipped on dry ice overnight to a single designated laboratory. After thawing for 1 hour at room temperature and gentle mixing, 0.5-mL aliquots were prepared from these samples. The aliquots were then stored in bar-code–labeled polypropylene vials at −80°C and measured by using the xMAP Luminex platform (Luminex, Austin, Texas) with INNO-BIA AlzBio3 (Innogenetics, Ghent, Belgium) immunoassay kit-based reagents. Monoclonal antibodies specific for Aβ and p-tau, which have been found to be useful in predicting AD, were used as reagents.[17] Total CSF tau level was not included in this analysis, because it was not available for some of the participants. In addition, p-tau has a higher specificity and negative predictive value for ruling out the presence of AD with 90% probability.[18]

Apolipoprotein E Genotyping

Each participant underwent apolipoprotein E (APOE) genotyping at the baseline visit. Approximately 6 mL of blood was obtained from each participant in an ethylenediamine tetraacetic acid tube, gently mixed by inversion, and shipped at ambient temperature to a single designated laboratory within 24 hours of collection for genotyping analysis.

Statistical Analysis

All statistical analyses were programmed in STATA version 13 (StataCorp, College Station, Texas). Comparisons of baseline variables among the groups were performed by using the Wilcoxon rank sum, Fisher exact, Kruskal Wallis, or χ^2 test, depending on the number of groups and type and distribution of the variables.

To test the hypothesis that lobar microbleeds are associated with overall brain amyloidosis, we used ordinary least-squares regression with age, sex, years of education, history of hyperten-

Table 1: Baseline group characteristics

			Microbleeds Present			
Characteristic	Microbleeds Absent	P Value[a]	Lobar Microbleeds Only	Mixed Lobar and Deep Gray/ Infratentorial Microbleeds	Deep Gray/Infratentorial Microbleeds Only	P Value[b]
No. of subjects	407		163	29	27	
Mean age (SD), y	72 (7.6)	<.001[c]	74 (7.0)	77 (6.4)	77 (9.0)	.06
Sex, male/female	218:189	.15	100:63	17:12	14:13	.64
Mean education (SD), y	16 (2.6)	.24	16 (2.9)	16 (2.9)	16 (2.3)	.78
% with HTN	47	.28	46	72	67	.009[d]
% with APOE4	43	.15	53	52	26	.03[d]
% with APOE2 allele	7	>.99	6	7	19	.06
% with NL	26	.14	20	10	33	.10
% with MCI	61	.80	61	79	56	.33
% with AD	12	.18	18	10	11	.41

Note:—HTN indicates hypertension; MCI, mild cognitive impairment; NL, normal cognitive function; SD, standard deviation.
[a] P values compare baseline variables between those without microbleeds and those with microbleeds (combining the 3 groups with different types of microbleeds).
[b] P values compare baseline variables among the 3 groups with microbleeds.
[c] Significance according to the Wilcoxon rank sum test.
[d] Significance according to the χ^2 test.

sion, APOE2 and APOE4 status, and diagnostic group (normal cognitive function, mild cognitive impairment, or AD) as covariates. CSF Aβ values were log transformed for normality and included as the outcome variable. Lobar microbleeds were dichotomized as 0 or 1 (indicating presence or absence, respectively) and used as the predictor. The number of lobar microbleeds was then categorized as an ordinal variable of 0, 1, 2, 3, or >3 to determine whether increasing numbers of lobar microbleeds were associated with greater brain amyloidosis, reflected by decreased CSF Aβ levels. The regressions were then repeated by using deep gray/infratentorial microbleeds, categorized as dichotomous or ordinal variables, to differentiate between microbleeds that may have been associated with hypertension and those associated with amyloid angiopathy.

To determine whether lobar microbleeds are associated with tau pathology, we used logistic regression, adjusting for age, sex, years of education, history of hypertension, APOE2 and APOE4 status, and diagnostic group. CSF Aβ level was also included as a covariate, dichotomized as normal or abnormal on the basis of the previously published cutoff of 192 pg/mL,[19] to determine the effect of microbleeds independent of global brain amyloidosis. We then dichotomized abnormal CSF p-tau levels by using a cutoff of 23 pg/mL,[19] which was used as the outcome variable. Lobar versus deep gray/infratentorial microbleeds were again included as the predictor, either as a dichotomous or ordinal variable. The sensitivity, specificity, positive predictive value, and negative predictive value of lobar microbleeds in predicting an abnormal CSF p-tau level were also calculated post hoc.

To determine whether lobar microbleeds are associated with a longitudinal change in cognition, we used the following linear mixed-effects model: $ADAS_{ij} = (B_0 + \beta_0) + \beta_1 MCH_i + (\beta_2 + \beta_3 MCH_i) t_{ij} + covariates + \varepsilon_{ij}$. $ADAS_{ij}$ represents the ADAS score of subject i at time point j, MCH_i represents the presence or absence of lobar microbleeds in each subject, and t_{ij} represents the time interval between ADAS tests. B_0 and β_0 are the coefficients for the random and fixed variations in baseline ADAS scores, respectively. The coefficient β_1 represents the fixed effect of having lobar microbleeds at baseline. Finally, $\beta_2 + \beta_3$ are the coefficients for time-dependent changes in ADAS scores, irrespective or respec-

tive, of the presence of microbleeds. The error term ε_{ij} represents random noise.

RESULTS

Subject characteristics are presented in Table 1. Of the 626 subjects, 407 (65%) had no microbleeds, whereas 219 (35%) had at least one microbleed. Of the 219 subjects with microbleeds, 192 (88%) had at least one lobar microbleed, and 27 (12%) had only deep gray/infratentorial microbleeds. As expected, the subjects with microbleeds were older ($P < .001$).[1] Those with lobar microbleeds, either alone or in combination with deep gray/infratentorial microbleeds, were more likely to be APOE4 carriers ($P = .03$), which has also been described previously.[1] Finally, those with mixed lobar and infratentorial or only infratentorial microbleeds were more likely to have a history of hypertension ($P = .009$).

Having ≥3 Lobar Microbleeds Was Associated with Abnormal Levels of CSF Aβ, Whereas Having Deep Gray/Infratentorial Microbleeds Was Not

After adjusting for covariates, having at least one lobar microbleed was associated with greater brain amyloidosis, reflected by a lower CSF Aβ level, though this association did not reach statistical significance (coefficient = −0.04; $P = .08$). However, accounting for increasing numbers of microbleeds, having 3 (coefficient = −0.30; $P = .001$) or >3 (coefficient = −0.18; $P = .001$) lobar microbleeds was also associated with lower levels of CSF Aβ (Table 2). Deep gray/infratentorial microbleeds were not associated with CSF Aβ levels ($P = .64$), and increasing numbers of deep gray/infratentorial microbleeds also were not associated with CSF Aβ levels ($P = .29–.97$).

Lobar Microbleeds Are Associated with a Higher Likelihood of Having an Abnormal CSF p-Tau Level, Independent of the CSF Aβ Level; Deep Gray/Infratentorial Microbleeds Are Not Associated with Abnormal CSF p-Tau Levels

Using logistic regression after adjusting for the CSF Aβ level, having at least one lobar microbleed was associated with more than

Table 2: Regression model demonstrating association between lobar microbleeds and CSF amyloid-β level (log transformed)

CSF Aβ (Log Transformed)	Coefficient (95% CI)	P Value
No. of lobar microbleeds		
1	−0.005 (−0.057 to 0.047)	.85
2	−0.030 (−0.12 to 0.056)	.49
3	−0.30 (−0.48 to −0.12)	.001
>3	−0.18 (−0.29 to −0.068)	.002
Age	−0.007 (−0.0098 to −0.0041)	<.001
Male sex	−0.013 (−0.055 to −0.030)	.57
Years of education	0.003 (−0.0046 to 0.011)	.40
APOE4	−0.23 (−0.28 to −0.19)	<.001
APOE2	0.059 (−0.022 to 0.14)	.15
MCI	−0.078 (−0.13 to −0.028)	.002
AD	−0.26 (−0.33 to −0.19)	<.001
History of hypertension	−0.011 (−0.052 to 0.031)	.61

Note:—MCI indicates mild cognitive impairment.

Table 3: Regression model demonstrating association between lobar microbleeds and likelihood of an abnormal CSF p-tau level

Abnormal CSF p-Tau Level	Odds Ratio (95% CI)	P Value
No. of lobar microbleeds		
1	2.82 (1.52–5.24)	.001
2	1.24 (0.51–3.01)	.64
3	0.37 (0.081–1.73)	.21
>3	3.22 (0.40–25.9)	.27
Abnormal CSF Aβ level	3.40 (2.16–5.34)	<.001
Age	1.01 (0.99–1.04)	.32
Male sex	0.93 (0.61–1.42)	.74
Years of education	0.98 (0.90–1.06)	.56
APOE4	2.33 (1.42–3.84)	.001
APOE2	1.02 (0.52–2.02)	.95
MCI	1.05 (0.67–1.67)	.83
AD	3.5 (1.15–10.7)	.03
History of hypertension	0.98 (0.65–1.48)	.92

Note:—MCI indicates mild cognitive impairment.

Table 4: Mixed-effects regression model demonstrating an association between lobar microbleeds and longitudinal change in ADAS

No. of Lobar Microbleeds	Annual Change in ADAS (95% CI)	P Value
0	0.78 (0.52 to 1.03)	<.001
1	1.35 (0.92 to 1.78)	.025
2	0.36 (−0.61 to 1.32)	.41
3	0.50 (−2.07 to 3.07)	.83
>3	2.34 (1.51 to 3.18)	<.001

DISCUSSION

The following are the major findings of our analysis: 1) having ≥3 lobar microbleeds is associated with global brain amyloidosis, whereas having deep gray/infratentorial microbleeds is not; 2) lobar microbleeds, unlike deep gray/infratentorial microbleeds, are associated with elevated CSF p-tau levels but with no dose-response relationship; and 3) lobar microbleeds, unlike deep gray/infratentorial microbleeds, are associated with accelerated longitudinal cognitive decline. Overall, the results of our analysis suggest a differential association of lobar versus deep gray/infratentorial microbleeds with Alzheimer pathogenesis, which reveals the importance of lobar microbleeds in prognostication, independent of CSF Aβ levels.

The first major finding, that lobar microbleeds are associated with greater overall brain amyloidosis, is concordant with the results of previous studies that showed lower CSF Aβ levels[3,20,21] and higher uptake on PET amyloid scans[5,6] in people with microbleeds. Previous studies have also suggested that lobar microbleeds are more suggestive of underlying amyloid angiopathy,[2,7,9] which is seen concomitantly in 78%–98% of postmortem examinations of the brains of people with Alzheimer disease.[22] The finding that only higher numbers of lobar microbleeds were found to be associated with abnormal CSF Aβ levels suggests that severe amyloid angiopathy is related more to overall brain amyloidosis and may contribute to the disease process. On the other hand, deep gray/infratentorial microbleeds, which are associated more typically with hypertension, were not associated with CSF Aβ levels. Previous work also demonstrated that deep gray, not lobar, microbleeds are associated with small-vessel disease.[23] Therefore, hypertension may produce deep gray/infratentorial microbleeds and small-vessel changes but is likely involved in Alzheimer pathogenesis from a nonamyloid pathway, such as decreased cognitive reserve.

The second major finding is that having at least one lobar microbleed, unlike having deep gray/infratentorial microbleeds, was associated with greater odds of having an abnormal CSF p-tau level, after adjusting for CSF Aβ levels. Furthermore, in post hoc analyses, we found that the presence of at least one lobar microbleed had a high positive predictive value for an abnormal CSF p-tau level. However, the absence of lobar microbleeds had a low negative predictive value for excluding the presence of an abnormal CSF p-tau level. A recent study in a memory clinic population found a similar association between microbleeds and CSF total tau, but not CSF p-tau, levels in individuals without dementia.[21] The fact that they did not find an association between microbleeds and p-tau levels may reflect technical differences in the assay or the fact that p-tau is more specific for neurodegeneration in

double the odds of having an abnormal CSF p-tau level (P = .004). The sensitivity and specificity of detecting an abnormal CSF p-tau level with the presence of at least one lobar microbleed were 35% and 82%, respectively. The positive and negative predictive values of at least one lobar microbleed for an abnormal CSF p-tau level were 86% and 29%, respectively.

Having deep gray/infratentorial microbleeds was not associated with abnormal CSF p-tau levels (P = .97). Unlike with CSF Aβ levels, no dose-response relationship was seen; having one lobar microbleed was most associated with an abnormal CSF p-tau level, with an odds ratio of 2.8 (P = .001) (Table 3).

Lobar Microbleeds Are Associated with Accelerated Longitudinal Cognitive Decline

Using a linear mixed-effects model and adjusting for covariates, including CSF Aβ level and diagnostic group, having at least one lobar microbleed was significantly associated with an accelerated longitudinal change in ADAS score (P = .007) of 1.4 points per year compared with 0.8 points per year for those without lobar microbleeds. Furthermore, having >3 lobar microbleeds was significantly associated with an increase of 2.3 points per year (P < .001) (Table 4). Deep gray/infratentorial microbleeds were not associated with change in ADAS scores (P = .31).

cohorts with more comorbid vascular disease. Nonetheless, both of our studies revealed an association between microbleeds and elevated levels of forms of CSF tau. One hypothesis is that microbleeds might reflect damaged microvasculature, resulting in decreased blood flow to neurons, ischemia, neuronal degeneration, and increased tau pathology. In postmortem studies, p-tau seems to have increased aggregation around arteries and arterioles with Aβ in the vessel walls.[24] Alternatively, microbleeds might induce enough inflammation, without duration, to cause an elevated CSF p-tau level. In an animal model, microhemorrhages were seen to trigger inflammation and activated microglia, macrophages, and lymphocytes[9,25] rather than cell death or ischemia. This inflammation may be sufficient to produce neuronal release of tau into the extracellular space. This inflammation may also contribute to further Alzheimer pathogenesis. Finally, the presence of lobar microbleeds, reflecting amyloid angiopathy, may suggest that a patient is farther along in the Alzheimer disease cascade.[10] In this case, lobar microbleeds, in addition to an abnormal CSF Aβ level, may signal more severe underlying disease.

The third major finding is that having at least one lobar microbleed is associated with accelerated longitudinal cognitive decline. Results discussed in the literature evaluating the association between microbleeds and cognitive decline have been variable, depending on the cohort studied (community-based populations,[26-29] people with a history of stroke or suspected stroke,[30-33] or subjects in a memory clinic who had mild cognitive impairment/AD[3,34-38]) and whether cognition was being evaluated on a cross-sectional[26,30-32,34,35] or longitudinal[27,33,36-39] basis. Most of these studies demonstrated an association between microbleeds and either global or executive impairment[3,26,28,30-32] cross-sectionally, as well as increased progression of memory impairment,[38] greater longitudinal changes in Mini-Mental State Examination scores,[36] higher likelihood of mild cognitive impairment converting to AD,[27] and increased risk of developing incident dementia.[39] One study also found cognitive improvement after stroke if no microbleeds were present,[33] which suggests a detrimental role of microbleeds on cognitive recovery. However, none of these studies adjusted for concomitant Alzheimer pathology, which may have driven the longitudinal changes in cognition. In our study, the association between lobar microbleeds and accelerated cognitive decline persisted after adjusting for CSF Aβ levels, which suggests that microbleeds alone, perhaps reflecting underlying amyloid angiopathy, lead to cognitive impairment. This finding mirrors that of a postmortem study that found that moderate-to-severe amyloid angiopathy is associated with perceptual speed and episodic memory, even after adjusting for concomitant AD pathology.[40] We also found that having ≥3 lobar microbleeds was more associated with decreasing cognition, similar to the results of previous studies that found greater cognitive decline with ≥2[39] or ≥5[28] lobar microbleeds. The finding that deep/infratentorial microbleeds were not associated with cognitive decline is concordant with results of a previous study[32] but differs from those of another study that found mixed, not strictly lobar, microbleeds were associated with cognitive decline.[39] Again, the differential effect on cognitive decline secondary to microbleed location may hint at differing etiologies of microbleeds.

Our study has several limitations. First, the ADNI is not a community sample. The cohort consisted of more white people, people who were more highly educated, and people who had fewer comorbidities than a community population at this age.[11] Furthermore, subjects were excluded from the ADNI if they had significant comorbid vascular disease, reflected by a modified Hachinski score of >4.[13] Therefore, although we did not find a significant effect on hypertension-related deep gray/infratentorial microbleeds on tau pathology and cognition, a significant association may be found in people with more significant vascular disease. As a result, generalization of these findings should be approached with caution, and further validation in prospective population-based cohorts, particularly those with concomitant vascular disease, is required.

CONCLUSIONS
The distribution of microbleeds provides clinically significant information, not only in suggesting differences in underlying etiology (ie, amyloid angiopathy versus hypertension) but also in demonstrating different associations with downstream events in Alzheimer pathogenesis (ie, tau and cognitive decline). Those who perform neuroimaging evaluations of older individuals who are being assessed for cognitive decline should consider microbleed distribution in their reports.

mild cognitive impairment, and people with early AD. The follow-up duration of each group is specified in the protocols for ADNI-1, ADNI-2, and ADNI–Grand Opportunity. Subjects originally recruited for ADNI-1 and ADNI–Grand Opportunity had the option to be followed in ADNI-2. For up-to-date information, please see www.adni-info.org.

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