Microbleed Topography, Leukoaraiosis, and Cognition in Probable Alzheimer Disease From the Sunnybrook Dementia Study

Jacqueline A. Pettersen, MD, MSc, FRCPC; Gayathri Sathiyamoorthy, BSc; Fu-Qiang Gao, MD; Gregory Szilagyi; Neelesh K. Nadkarni, MD; Peter St George-Hyslop, MD, DSc; Ekaterina Rogaeva, PhD; Sandra E. Black, MD, FRCPC

Cerebral microbleeds are hemosiderin deposits around small vessels and are well visualized with T2*-weighted gradient-recalled echo (GRE) imaging. In the presence of hypertension, microbleeds occur more commonly in centrencephalic regions (the basal ganglia, thalamus, and cerebellum) in arterioles at high pulse pressures. In contrast, multiple, strictly lobar hemorrhages, or microbleeds, are highly specific for cerebral amyloid angiopathy (CAA) in elderly patients. Amyloid-β, which accumulates in small vessels in areas of decreased pulse pressure and interstitial fluid pumping (ie, lobar, rather than centrencephalic). Both hypertensive and amyloid pathologies are associated with smooth-muscle loss, decreased vasoreactivity, and rupture resulting in leukoaraiosis and microbleeds. Indeed, leukoaraiosis occurs in 80% of CAA cases and correlates with the number of microbleeds. Although leukoaraiosis has been implicated in cognitive impairment and decline in CAA, it is unclear whether microbleeds affect cognition. Among patients with cerebrovascular disease (ie, prior stroke or transient ischemic attack) or vascular dementia, microbleeds independently predicted cognitive dysfunction—specifically, executive impairment if frontal and basal ganglia regions were involved—memory, attention/executive, and visuospatial functioning when microbleeds involved temporoparietal regions. Importantly, CAA and AD frequently co-occur and have both been associated with cognitive decline and dementia. Autopsy series estimate that 82% to 98% of AD cases have associated CAA pathology of varying severity. It is possible that microbleeds are only associated with more severe CAA pathology. To date, 4 studies have assessed microbleeds in AD, with prevalences of 12.5% to 32%. Interestingly, although lobar predominance has been reported, cerebral topography has not been systematically assessed. Similar to individuals with CAA, AD patients with mi-
Microbleeds are more likely to have associated leukoaraiosis, but its topography in relation to microbleeds has not been evaluated either. It remains unclear whether microbleeds are independently associated with cognitive decline, as only Mini-Mental State Examination scores have been assessed and were found not to be associated. Finally, the absence of healthy controls potentially limits the interpretation of results. Given these literature deficiencies, the objectives of our investigation were to determine frequency and topography of microbleeds in patients with AD and healthy controls and to assess the association of microbleeds with leukoaraiosis and cognition in AD.

METHODS

STUDY POPULATION

Participants consisted of 105 patients recruited from the Cognitive Neurology Clinic at Sunnybrook Health Sciences Centre for the prospective Sunnybrook Dementia Study who had GRE imaging (incorporated into standard imaging protocol in 2002). All patients underwent standardized dementia assessments, including medical history and examination, blood tests, single-photon emission computed tomography, magnetic resonance imaging, and neuropsychological testing measuring global cognition and 6 domains of neuropsychological functioning. Consecutive patients who had diagnoses of probable AD (n=80) according to National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association criteria were recruited from 2002 to 2006. Controls (n=25) were community-dwelling, healthy elderly volunteers with normal baseline neuropsychological test results and no vascular risk factors, prior stroke, or head injury. Demographic and comorbid disease data were collected on patients with AD, including age, sex, education, vascular risk factors (hypertension, dyslipidemia, diabetes, cardiovascular disease, peripheral vasculopathy, smoking, and prior stroke or transient ischemic attack), and head injury. The APOE gene was genotyped in 70 (88%) patients with AD according to a previously described method. Our hospital research ethics board approved the project and all participants provided informed consent.

NEUROPSYCHOLOGICAL TESTS

Patients underwent formal cognitive testing at baseline and annually thereafter. To determine an association between microbleeds and cognition, the results of the set of tests performed closest (≤ 90 days) to GRE imaging that assessed microbleeds were used. We tested the following: (1) global cognition (Dementia Rating Scale and the Mini-Mental State Examination), (2) language (Boston Naming Test and semantic fluency), (3) executive functioning (phonemic fluency and the Wisconsin Card Sorting Task), (4) learning/memory (California Verbal Learning Test and the Logical Memory Delayed Recall subtest of the Wechsler Memory Scale–Revised), (5) visuospatial ability (the Benton Judgment of Line Orientation Test), (6) working memory (Trails B Test), and (7) praxis (Western Aphasia Battery Apraxia Subtest).

MAGNETIC RESONANCE IMAGING PROTOCOL

Magnetic resonance imaging was performed on a 1.5-T Signa system (GE Healthcare, Chalfont St Giles, England). Testing consisted of axial T2*-weighted GRE sequences (18 slices; field of view, 20 mm; matrix, 256 × 256; slice thickness, 6 mm; interslice gap, 2 mm; echo time/repetition time, 33 ms/775 ms; flip angle, 20°), axial spin–echo acquisition, and T2-weighted and proton density–weighted (20 slices; field of view, 20 × 20 cm; matrix, 256 × 192; number of excitations, 0.5; echo time/repetition time, 80 ms/3000 ms) and T1-weighted 3-dimensional volumetric spoiled GRE sequences (124 slices; matrix, 256 × 192; slice thickness, 1.2 mm; number of excitations, 1; echo time/repetition time, 35 ms/5 ms; flip angle, 35°).

MICROBLEEDS

Microbleeds were defined as small, rounded areas of marked homogeneous signal loss 10 mm in diameter or smaller on GRE imaging. Signal loss in sulci, symmetric globus pallidus hypointensities, and cortical artery flow voids were not considered microbleeds. With regards to topography, microbleeds were allocated to 6 regions: frontal, parietal, occipital, basal ganglia/thalamus, and infratentorial (brainstem and cerebellum). At least 2 of 3 independent raters (G.S., F-Q.G., N.K.N.) blinded to clinical information had to agree for a microbleed to be counted (κ=0.61). If repeat GRE imaging was performed on the same patient (ie, the patient had multiple years of follow-up with 1 GRE scan per year), the scan representing the maximum number of microbleeds was used. Multiple (≥ 4) scans were reviewed in 45 participants. The corresponding T2-weighted and proton density–weighted scans were used for Age-Related White Matter Changes Rating Scale (ARWMC) scoring and neuropsychological tests from that same time (ie, within 90 days of GRE imaging) were used. A 3-dimensional composite brain image was constructed to illustrate lobar microbleed topography. The center of each microbleed was traced in a template image used in MRicro (http://www.sph.sc.edu/comd/rorden/mricro.html) guided by anatomical landmarks using Analyze.

ARWMC SCORE

White matter change (ie, leukoaraiosis) was assessed on T2-weighted and proton density–weighted images using ARWMC, which assigns a rating on a 4-point scale (0, no lesions; 1, focal lesions; 2, increasing confluence; 3, diffuse involvement) in each of 5 regions in both hemispheres separately (frontal, parietooccipital, temporal, infratentorial, and basal ganglia). Two experienced raters blinded to clinical information assessed scans in random order. Interrater (intrarater correlation coefficient=0.97) and intrarater reliability (intrarater correlation coefficient=0.94) revealed high levels of agreement.

<table>
<thead>
<tr>
<th>Location</th>
<th>Controls (n=3)</th>
<th>Patients With AD (n=23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal ganglia/thalamus</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Infratentorial/thalamus</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Lobar</td>
<td>3</td>
<td>90</td>
</tr>
<tr>
<td>Frontal</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>Temporal</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>Parietal</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Occipital</td>
<td>3</td>
<td>51</td>
</tr>
<tr>
<td>Total</td>
<td>4</td>
<td>98</td>
</tr>
</tbody>
</table>
STATISTICAL ANALYSIS

Version 13 of SPSS (SPSS Inc, Chicago, Illinois) was used. Differences between discrete and continuous variables were assessed using Fisher exact and $t$ tests and Spearman rank correlations, respectively. Comparisons on neuropsychological tests between AD patients with and without microbleeds were controlled for age and education using analysis of variance. Given the exploratory nature, statistical significance was defined as $P < .05$, despite multiple comparisons.

RESULTS

Compared with healthy controls, patients with AD had lower Mini-Mental State Examination scores (mean [SD], 21.4 [6.5] vs 28.7 [1.2], $P < .001$) but did not differ in age (mean, [SD], 71.6 [11] years, overall), sex, or years of education. A total of 4 microbleeds were seen in 3 (12%) of the controls and 98 were seen in 23 (29%) patients with AD. Although prevalence of microbleeds appeared

Figure 1. Lobar topography of microbleeds in Alzheimer disease. Lobar microbleeds ($n = 90$) are displayed in sagittal (A) and axial (B) 3-dimensional images. Sagittal (C) and axial (D) views without the cortical surface are also shown. Each red dot represents the center rather than the full extent of individual microbleeds. A indicates anterior (frontal); CS, central sulcus; L, left; P, posterior (occipital); R, right; and SF, sylvian fissure.
to be greater in AD cases, this difference was not statistically significant \((P = .11)\). Microbleeds were frequently multiple, particularly in patients with AD: more than 1 microbleed was seen in 48% of AD patients with microbleeds compared with 33% of controls with microbleeds, and 3 or more microbleeds were observed in 35% of AD patients with microbleeds (1 patient had 36 microbleeds) but none of the controls. The topography of microbleeds revealed lobar predominance in 92% of AD patients, with occipital lobes accounting for 57% of these microbleeds (Table 1 and Figure 1). While 1 AD patient had 27 of 36 microbleeds in the occipital lobes, even excluding this data, occipital lobe prevalence remained. Among controls, the occipital lobe was also the most common location for microbleeds.

Leukoaraiosis severity, as assessed by ARWMC scores, did not differ between controls (mean [SD], 5.8 [4.6]) and patients with AD (5.6 [5.5]) \((P = .8)\). Its presence was not associated with worse performance on any of the neuropsychological test scores in either group.

Among AD patients, those with microbleeds were more likely to be older than 75 years (78.3% vs 52.6%, \(P < .05\)). There were no other significant group differences in demographics or comorbidities. Regarding APOE, allelic frequency conformed to the Hardy-Weinberg equilibrium. There were no differences in APOE\(^*4\) frequency between groups; approximately 70% had at least 1 APOE\(^*4\) allele, with homozygosity occurring in 20% in both groups \((P = .6)\). There were too few patients with APOE\(^*2\) alleles to perform statistical analyses \((n = 3)\).

Presence of microbleeds was associated with more severe leukoaraiosis: AD patients with microbleeds had a higher ARWMC score (mean [SD], 7.8 [4.6]) than those without \(4.6 \pm 4.5\), \(P < .04\); the number of microbleeds were significantly correlated with ARWMC scores \((r = 0.39, P = .01)\) (Figure 2), even with outliers removed \((r = 0.33, P < .05)\). Interestingly, AD patients with microbleeds had significantly higher ARWMC scores within the frontal \((P < .05)\) and parietooccipital \((P < .05)\) regions but not in other regions. The number of microbleeds were also significantly correlated with parietooccipital ARWMC scores \((r = 0.28, P < .01)\), even with the most extreme outliers removed \((r = 0.24, P < .05)\), but not with frontal (or other brain region) ARWMC scores. Within the control group, the number of microbleeds were also significantly correlated only with occipital ARWMC scores \((r = 0.4, P < .05)\). The presence of microbleeds, like the presence of leukoaraiosis, was not associated with worse performance on neuropsychological testing (Table 2).

**COMMENT**

To our knowledge, occipital predominance of microbleeds has not been well described in prior AD imaging studies. Microbleeds in AD were lobar in 92% of patients and occurred in centrencephalic regions much less frequently. This relative lobar distribution has been reported in AD\(^{13-18}\), however, occipital lobe predominance has not been so well described. With the exception of the study by Nakata and colleagues,\(^{19}\) who noted 8 occipital microbleeds compared with 6 parietal, 4 temporal, and 3 frontal microbleeds in a small sample, other studies have not assessed lobar topography. Our findings correspond with the lobar predilection found in patients with probable CAA\(^{5,24}\) and interestingly our 3-dimensional cerebral representation is remarkably similar to that of Rosand and colleagues\(^{25}\) regarding occipital predominance of microbleeds. This further suggests that microbleeds in AD are associated with amyloid angiopathy, but it remains unclear why occipital lobes are most affected. A recent investigation revealed that severity of CAA pathology was particularly increased in occipital lobes in brains with more severe AD pathology.\(^{25}\) Amyloid-\(\beta\) accumulation may be associated with decreased pulse pressure and interstitial fluid pumping with presumably lower clearance of vascular amyloid.\(^{1}\) These processes may be most markedly reduced in occipital lobes.

Microbleeds were frequent and often multiple: 29% of patients with AD were shown to have cerebral microbleeds, 48% of whom had more than 1, and 35% of those with microbleeds had 3 or more in total. These findings are consistent with other AD studies demonstrating prevalences of 16% to 32% and multiplicity (>1 microbleeds) in 32% to 45% of cases.\(^{15,16,18}\) While only 12% of controls had microbleeds, this rate is somewhat higher than in other studies of healthy patients (3.1%-8.5%).\(^{20}\) An important factor appears to be age. While the rate of microbleeds was 4.7% in the offspring cohort of the Framingham Study,\(^{27}\) overall it was 12.6% in patients 75 years of age or older. Given that the mean age of our controls was 71.3 (SD, 1.0) years, the prevalence of 12% is consistent with the literature. Among AD patients, those with microbleeds were also likely to be older, replicating results from a previous study.\(^{18}\) Hypertension or other vascular risk factors did not differ between AD groups,
The presence of APOE*4 alleles was not associated with microbleeds in our study nor in another recent study but despite a purported association with CAA. The APOE*2 alleles, which have been associated with CAA-related hemorrhage, occurred too infrequently in our sample (3 of 70 patients) to demonstrate any association with microbleeds.

Presence and frequency of microbleeds predicted more severe leukoaraiosis, consistent with previous AD and CAA studies. Interestingly, the topographic location of microbleeds (ie, predominantly occipital) was correlated with that of leukoaraiosis (ie, parietooccipital) in our study. Similar to our findings, periventricular leukoaraiosis has been shown to be most prominent in the posterior brain regions of patients with CAA, corresponding to the area particularly affected by microbleeds in this population. While the etiology of leukoaraiosis in this context is not entirely clear, the similarity distribution to microbleeds suggests that amyloid deposition, rather than hypertensive vasculopathy, may be importantly implicated. In further support of this association, Nakata-Kudo and colleagues found evidence of leukoaraiosis in 57% of AD patients with microbleeds who were free of cerebrovascular disease vs 0% of those who had cerebrovascular disease.

Neither microbleeds nor leukoaraiosis accounted for additional cognitive impairment as assessed by neuropsychological testing. However, our tests, and possibly sample size, may have lacked sensitivity to measure such a difference. Nevertheless, our findings are consistent with 2 AD studies evaluating effects of microbleeds on only Mini-Mental State Examination scores. In contrast, impaired cognition was demonstrated in cerebrovascular patients with frontal and basal ganglia microbleeds and vascular dementia patients with temporo-parietal predominance of microbleeds.

Limitations of our study include the relative thickness of T2*-weighted GRE slices and interslice gaps. While our measurement of a 6-mm slice and a 2-mm interslice gap is consistent with at least 1 other investigation, other studies have used slightly thinner measurements. Our reported numbers of microbleeds may therefore be conservative in comparison. However, when multiple GRE scans were performed, the one with the most microbleeds was chosen, so this potential underestimation was likely minimized and may possibly even represent an upward bias. Finally, our study is limited by the absence of neuropathology to confirm amyloid angiopathy.

Microbleeds are frequent in AD and given their location, predominantly occipital location (ie, similar to CAA) and lack of association with hypertension, they likely represent amyloid vasculopathy. Amyloid deposition in small vessels may lead not only to microbleeds but also to altered white matter perfusion and ischemia, contributing to leukoaraiosis in AD. Our findings illustrate the complexity of AD vasculopathy and the need for additional studies in both dementia and stroke populations.

**Accepted for Publication:** October 3, 2007.

**Correspondence:** Jacqueline A. Pettersen, MD, MSc, FRCP, Division of Neurology, Department of Medicine, Sunnybrook Health Sciences Centre, A421-2075 Bayview Ave, Toronto, ON M4N 3M5, Canada (jacqui.pettersen@utoronto.ca).

**Author Contributions:** Dr Pettersen had full access to all of the data in the study and takes responsibility for its integrity and the accuracy of the analysis. Study concept and design: Pettersen, Nadkarni, and Black. Acquisition of data: Pettersen, Sathiyanamorthy, Gao, Nad...
karni, St. George-Hyslop, Rogaeva, and Black. Analysis and interpretation of data: Pettersen, Szilagyi, and Black. Drafting of the manuscript: Pettersen. Critical revision of the manuscript for important intellectual content: Sathiyamoorthy, Gao, Szilagyi, Nadkarni, St. George-Hyslop, Rogaeva, and Black. Statistical analysis: Pettersen. Obtained funding: Rogaeva and Black. Administrative, technical, and material support: Pettersen, Sathiyamoorthy, Szilagyi, Nadkarni, St. George-Hyslop, and Black. Study supervision: Gao and Black.

Financial Disclosure: None reported.

Funding/Support: This study was supported by the Canadian Institute of Health Research and Alberta Heritage Foundation for Medical Research fellowships (Dr Pettersen); Sunnybrook Research Institute summer scholarships (Ms Sathiyamoorthy and Mr Szilagyi); the Heart and Stroke Foundation Centre for Stroke Recovery (Drs Gao and Nadkarni); and the Canadian Institute of Health Research, the Heart and Stroke Foundation Canada, and the Alzheimer’s Association (Dr Black).

Additional Contributions: Mario Masellis, MD, MSc, FRCP, provided genetics data analysis input.

REFERENCES