Analysis of Cerebral Lobar Microbleeds and a Decreased Cerebral Blood Flow in a Memory Clinic Setting

Hikaru Doi, Saeko Inamizu, Ban-Yu Saito, Hiroyuki Murai, Takehisa Araki and Jun-Ichi Kira

Abstract

Objective  Cerebral microbleeds (MBs) have been previously associated with cognitive dysfunction, including Alzheimer’s disease. In the present study, we aimed to clarify the relationship between cerebral lobar MBs and the regional cerebral blood flow (CBF).

Methods  We investigated the data obtained from 122 patients in our memory clinic who were examined by both MRI and 99mTc-ethyl cysteinate dimer (ECD)-single photon emission computed tomography (SPECT). Patient brain scans were superimposed and brain regions containing both decreased CBF and MBs were visually identified. For each patient eight brain regions were evaluated, comprising the right and left frontal, temporal, parietal, and occipital lobes.

Results  Cerebral MBs were detected in 36 of the 122 (29.5%) patients. Of these 36 patients, 23 had detectable lobar MBs, which were primarily distributed in the occipital lobe in 19 of the 46 (41.3%) regions with lobar MBs. The frequency of MBs accompanied by a decreased CBF in the parietal and occipital lobes was significantly higher than that observed in the frontal lobe (73.3% vs. 27.3%, p<0.05, and 73.7% vs. 27.3%, p<0.05, respectively). Additionally, a decreased CBF was observed significantly more frequently in the brain regions with 5 or more MBs compared to the regions with one microbleed (83.3 vs. 25.0%, p<0.0005).

Among the 17 patients with observable MBs accompanied by a decreased CBF, none were initially diagnosed with either subjective complaints or mild cognitive impairment.

Conclusion  We determined that the cerebral lobar MBs located in the parietal and occipital lobes, and the lobar regions with a large number of MBs, were significantly more likely to be accompanied by a decreased CBF.

Key words: cerebral microbleeds, cerebral blood flow, ECD-SPECT, Alzheimer’s disease, dementia with Lewy bodies


Introduction

Cerebral microbleeds (MBs) are known as the clinical manifestations of small-vessel disease, similar to lacunar infarctions and white matter lesions (1), and they are detected by small rounded, homogeneous regions of hypointensity on T2*-weighted gradient-recalled echo (T2*-GRE) magnetic resonance imaging (MRI), 3D T2*-weighted MR angiography (SWAN), or susceptibility-weighted imaging (SWI) (2). MBs are primarily caused by hypertensive arteriopathy and cerebral amyloid angiopathy (CAA) (3). While hypertensive arteriopathy-related MBs are mainly located in the deep regions of the brain, CAA-related MBs are primarily observed in the lobar brain regions (1, 3).

CAA is a common progressive disease of the elderly that can cause cerebral hemorrhage, including MBs, in the lobar brain regions (4). Interestingly, recent studies have revealed that CAA is a crucial pathology of Alzheimer’s disease (AD) (5-7) and that 23% of patients diagnosed with AD...
have detectable MBs (8). Lobar MBs are more common than deep MBs and are predominantly located in the temporal and occipital lobes (9, 10). It remains controversial as to whether the existence, location or number of MBs affect the cognitive function of healthy subjects or patients with AD, including those patients with mild cognitive impairment (MCI) (11-16). Furthermore, the relationship between the location and number of MBs and the regional cerebral blood flow (CBF) has not yet been elucidated.

In this study, we focused on the location and number of cerebral lobar MBs detected by MRI, and a decreased CBF as analyzed by single photon emission computed tomography (SPECT), to clarify how MBs influence the regional CBF.

Materials and Methods

Subjects

A total of 192 Japanese patients who were examined by a neurologist in a memory clinic at the Department of Neurology, Hiroshima Red-Cross Hospital and Atomic-Bomb Survivors Hospital between November 2011 and August 2013, were enrolled in the present study. Of these 192 patients, we investigated those patients who were previously examined by both MRI and SPECT using \(^{99m}\text{Tc}\)-labeled ethyl cysteinate dimer (\(^{99m}\text{Tc}\)-ECD). All examinations, including MRI and SPECT, were carried out as part of routine patient care. The medical records from these patients were retrospectively evaluated for patient medical history, diagnosis, the mini-mental state examination score, initial diagnosis, location and number of MBs, and the presence of a decreased CBF. The original diagnosis was made by the neurologist who initially examined each patient. This diagnosis was based on the diagnostic guidelines published by the National Institute on Aging-Alzheimer’s Association workgroups for AD and MCI (17, 18), and the third report published by the DLB Consortium for Dementia with Lewy bodies (DLB) (19). This study was approved by the Ethics Committee at the Hiroshima Red-Cross Hospital and Atomic-Bomb Hospital on January 20, 2014.

Brain imaging

MRI was performed using a General Electric Signa HDxt 1.5T (Fairfield, U.S.A.). T2* and SWAN sequences with a time of repeat (TR)/time of echo (TE)/flip angle/slice thickness of 600/17/20/5 and 76.30/48.90/20/3 mm, were performed using the conventional MRI protocol, including T1-weighted, T2-weighted and fluid attenuated inversion recovery imaging. The severity of the volume of interest (VOI) atrophy was evaluated using a voxel-based specific regional analysis system for AD (VSRAD) advance, based on the Statistical Parametric Mapping 8 (SPM8) and the Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra (DARTEL) (20).

SPECT examinations were performed using a Siemens Symbia T6/T16 (München, Germany). The patients were examined in the supine resting position with their eyes closed in a dimly-lit quiet room, and a dose of approximately 600 MBq of \(^{99m}\text{Tc}\)-ECD was injected intravenously. Images were acquired 5 minutes post \(^{99m}\text{Tc}\)-ECD administration with patients remaining in the supine position in the scanner with their arms at their sides. The head was placed in a natural position to alleviate discomfort and minimize motion during the image acquisition. The imaging data were acquired at 30 minutes post tracer injection, with a maximum scan duration of 30 minutes. The entire brain, including the cerebellum was included in the field of view of the image. The results were analyzed using an easy Z-score Imaging System (eZIS) and an eZIS Specific VOI Analysis (eZIS-SVA) was performed to evaluate the severity, extent, and ratio of the VOI for AD (21).

The MBs and decreased CBF were evaluated in eight brain regions in each patient, comprising the right and left frontal, temporal, parietal, and occipital lobes. The location and number of MBs in each region were identified in each patient. The MRI images and the eZIS mapping were superimposed, and a decreased CBF was visually defined as a Z-score of 2 or more within a circle of 1-centimeter radius of the center of the MB within the brain parenchyma (Fig. 1). An MB adjacent to another MB within 1-centimeter was defined as one region for the evaluation of decreased CBF. The detection of MBs and the determination of a decreased CBF was performed by two experienced neurologists. If different results were found by the two neurologists, then the corresponding areas were excluded from further analyses, and were defined as normal observations.

Statistical analysis

The SAS JMP9 software program was used to perform the statistical analyses. Statistical assessment to determine whether any differences between the lobar regions was significant, was performed using either the chi-square test (Pearson) or Fisher’s exact probability test, when the criteria for the chi-square test were not fulfilled to compare categorical variables. The Kruskal-Wallis H test was used for non-normally distributed variables. Values of p<0.05 were considered to be statistically significant.

Results

Background data of the patients

We investigated the MBs and the presence of a decreased CBF using the data gathered from 122 Japanese patients, comprising 47 men and 75 women, who underwent both MRI and \(^{99m}\text{Tc}\)-ECD-SPECT (Table 1). The mean age ± SD of the patients was 78.8±7.8 years. MBs were detected in 36 of the 122 (29.5%) patients. The 36 patients with MBs had a significantly longer duration of disease course and were significantly older in age at the disease onset, compared to the 86 patients without MBs (2.6±2.1 vs. 1.6±1.6 years, p<
Figure 1. Example of typical cases. The left figure represents T2* or SWAN by MRI, and the right figure represents the region of the relatively decreased CBF on the eZIS by SPECT. A and B: MBs with decreased CBF. C and D: MBs without decreased CBF. CBF: cerebral blood flow, MBs: microbleeds, SWAN: T2*-weighted MR angiography, SPECT: single photon emission computed tomography

Table 1. Demographic Features of the Total Patients and Those with Microbleeds

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Microbleeds (+)</th>
<th>Microbleeds (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=122</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>78.8±7.8</td>
<td>81.3±5.4*</td>
<td>77.7±8.4*</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>47/75</td>
<td>14/22</td>
<td>33/53</td>
</tr>
<tr>
<td>Duration of the disease</td>
<td>1.9±1.8</td>
<td>2.6±2.1*</td>
<td>1.6±1.6*</td>
</tr>
<tr>
<td>years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Education year</td>
<td>11.2±2.5</td>
<td>10.8±2.6</td>
<td>11.4±2.5</td>
</tr>
<tr>
<td>MMSE</td>
<td>21.8±5.2</td>
<td>21.3±5.0</td>
<td>22.0±5.3</td>
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<tr>
<td>VSRAD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Z-score</td>
<td>2.0±5.2</td>
<td>2.4±2.8</td>
<td>1.8±0.9</td>
</tr>
<tr>
<td>eZIS-SVA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>severity</td>
<td>1.2±0.5</td>
<td>1.2±0.6</td>
<td>1.2±0.4</td>
</tr>
<tr>
<td>extent</td>
<td>12.9±12.1</td>
<td>13.0±11.4</td>
<td>12.9±12.4</td>
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<tr>
<td>ratio</td>
<td>2.1±1.7</td>
<td>1.9±1.4</td>
<td>2.2±1.8</td>
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<tr>
<td>Clinical Diagnosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subjectively complaint</td>
<td>18</td>
<td>2</td>
<td>16</td>
</tr>
<tr>
<td>MCI</td>
<td>14</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Alzheimer's disease</td>
<td>72</td>
<td>24</td>
<td>48</td>
</tr>
<tr>
<td>(with VaD)</td>
<td>(12)</td>
<td>(7)</td>
<td>(5)</td>
</tr>
<tr>
<td>VaD</td>
<td>8</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>DLB</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Others</td>
<td>5</td>
<td>0</td>
<td>5</td>
</tr>
</tbody>
</table>

Others: corticobasal degeneration (n = 1); semantic dementia (n = 1); frontotemporal dementia (n = 1); idiopathic normal pressure hydrocephalus (n = 2).
DLB: dementia with Lewy bodies, eZIS-SVA: easy Z-score imaging system specific VOI analysis, MCI: mild cognitive impairment, MMSE: mini-mental status examination, VaD: vascular dementia, VSRAD: voxel-based specific regional analysis system for Alzheimer's disease. *p < 0.05
0.005, and 81.3±5.4 vs. 77.7±8.4 years, p<0.05, respectively). Additionally, cerebral lobar MBs were detected in 23 of the 36 (63.9%) patients, including 11 patients with lobar-type MBs and 12 patients with mixed-type MBs, whereas, deep-brain MBs were detected in 13 of the 36 (36.1%) patients. Of the 23 patients with cerebral lobar MBs, five patients had one MB, three patients had 2-4 MBs, six patients had 5-9 MBs, and nine patients had 10 or more MBs in the whole lobar region. The initial diagnosis of the 23 patients with lobar MBs is as follows: 18 patients were diagnosed with AD including three patients comorbid with vascular dementia, three patients were diagnosed with DLB, one patient was diagnosed with vascular dementia, and one patient was diagnosed with a subjective complaint.

**Locations of MBs and decreased CBF**

Location analysis of MBs determined that of the 184 regions in 23 patients with lobar MBs (46 regions in each lobe), MBs were detected in 11 of the 46 (23.9%) regions in the frontal lobes, 14 of the 46 (30.4%) regions in the temporal lobes, 15 of the 46 (32.6%) regions in the parietal lobes, and 19 of the 46 (41.3%) regions in the occipital lobes. Although MBs in the occipital lobes were more commonly observed compared with the other lobar regions, the statistical analyses did not identify any significant differences. MBs accompanied by decreased CBF were detected in 3 of 11 (27.3%) regions in the frontal lobes, 8 of 14 (57.1%) regions in the temporal lobes, 11 of 15 (73.3%) regions in the parietal lobes, and 14 of 19 (73.7%) regions in the occipital lobes (Fig. 2). Statistical analyses of these data determined that MBs accompanied by decreased CBF were significantly more frequently detected in the parietal and occipital lobes, as compared with the frontal lobe (73.3% vs. 27.3%, p<0.05, and 73.7% vs. 27.3%, p<0.05, respectively).

**Numbers of MBs and decreased CBF**

Of the 184 regions evaluated in the 23 patients with lobar MBs, one MB was detected in 20 of 184 (10.9%) regions, 2-4 MBs were detected in 15 of 184 (8.2%) regions, and 5 or more MBs were detected in 24 of 184 (13.0%) regions. MBs accompanied by a decreased CBF were detected in 5 of 20 (25.0%) regions with one MB, 11 of 15 (73.3%) regions with 2-4 MBs, and 20 of 24 (83.3%) regions with 5 or more MBs (Fig. 3). Statistical analyses showed that regions with 5 or more MBs were significantly more likely to be accompanied by a decreased CBF than regions with only one MB (83.3% vs. 25.0%, p<0.0005). When the evaluation was restricted to only the occipital and parietal lobes, MBs accompanied with a decreased CBF were detected in 4 of 9 (44.4%) regions, in regions with one MB in total, 7 out of 9 (77.8%) regions, in regions with 2-4 MBs, and 14 out of 16 (87.5%) regions, in regions with 5 or more MBs, respectively (p=0.0608). Furthermore, the 17 patients displaying a decreased CBF also had significantly increased severity and extent values as compared to the 6 patients without a decreased CBF (1.2±0.3 vs. 1.0±0.2, p<0.05 and 14.2±9.3 vs. 6.7±5.5%, p<0.05, respectively) (Table 2). None of the 17 patients with MBs accompanied by a decreased CBF were initially diagnosed with either subjective complaints or MCI. However, multiple lobar MBs accompanied with a decreased CBF predominantly in the occipital lobe, were observed in all three patients initially diagnosed with DLB.

**Discussion**

In the current study we determined that cerebral lobar
MBs were distributed predominantly in the occipital lobe, as previously reported (9, 10), and that the MBs in the parietal and occipital lobes were significantly more often accompanied by a decreased CBF as compared with MBs in the frontal lobe. Furthermore, the brain regions with higher numbers of MBs were more often accompanied by a decreased CBF in the same region, than regions with only one MB, in our memory clinic setting.

### Lobar MBs and decreased CBF

The majority of patients in this study were diagnosed with AD. A previously published imaging study of patients with probable CAA, showed that MBs as well as intracerebral hemorrhage occurred more often in the temporal and occipital lobes (10). Additionally, in a study on AD, brain MBs showed cortico-subcortical predominance in 92% of the AD patients with the occipital lobes accounting for 57% of these brain MBs (9). In contrast, the Rotterdam Scan Study of 198 healthy subjects with MBs found that the MB’s were predominantly found in the temporal lobe (22). The discrepancy in the distribution of MBs between healthy subjects and AD patients cannot be explained by vulnerability of the blood-brain barrier in the occipital lobe region, as suggested by the pathogenesis of posterior reversible encephalopathy syndrome (23).

In the present study, lobar MBs were predominantly distributed in the occipital lobe, as was previously reported in the probable CAA and AD studies (9, 10) and consistent with the majority of patients in this study having a primarily diagnosis of AD. However, this is the first study to investigate the relationship between MBs and regional CBF both in healthy subjects and patients diagnosed with AD. CAA is a common small vessel disease that is associated with deposition of amyloid-β protein in the walls of small-to-medium-sized arteries, arterioles and capillaries (4). MBs are identi-
fied by hemosiderin leakage from small vessels that is accompanied by macrophages in the brain parenchyma, a phenomenon typically seen in patients with CAA (24). Furthermore, CAA was almost invariably found at autopsy in more than 90% of cases of AD (5-7). The results from these studies support a strong association between AD and CAA. Furthermore, patients with AD have a 2- to 3-fold greater Carbon11-labeled Pittsburgh Compound B (PiB) retention on positron emission tomography (PET) scans in brain areas known to contain large amounts of fibrillar amyloid-β plaques, such as the frontal and temporal cortex (25, 26), and the distribution of amyloid-β protein is well correlated with post-mortem examination. In addition, a previous study focusing on MBs and the deposition of amyloid detected by PiB-PET, found that the amyloid burden was only related to lobar MBs (27). Although the pathological relationship between lobar MBs and deposition of amyloid-β is supported by these studies, the posterior-dominant distribution of MBs is not consistent with the frontal and temporal lobe-dominant distribution of amyloid-β protein.

In the present study, none of the patients with MBs accompanied by a decreased CBF were previously diagnosed with MCI or had any subjective complaints. Furthermore, the patients with MBs accompanied by decreased CBF displayed higher severity and extent values of the VOI analyzed by eZIS-SVA than those without decreased CBF, although the number of patients examined was insufficient for statistical significance to be demonstrated. In early AD patients, decreases in the regional CBF in the posterior cingulate gyri, precunei, and parietal cortices have been reported by SPECT (28, 29). Accordingly, the distribution of MBs is related to the functional aspect seen by a SPECT examination of AD rather than by the distribution of amyloid-β protein, although the relationship between cognitive impairment and MBs in location and number remains controversial, thus resulting from directly opposing results found by studies in both healthy subjects and patients with AD, including MCI (11-16). Collectively, the results of the current study highlight the need for further studies to clarify the importance of occipital lobe-dominant multiple MBs relevant to a decreased CBF, rather than the reflection of SPECT typically seen in AD, as this study focused on subjects in a memory clinic setting.

**MBs in patients with DLB**

All three patients who were initially diagnosed with DLB showed multiple MBs accompanied by a decreased CBF predominantly in the occipital lobe. In a previous study that compared MBs between patients with AD and DLB, the prevalence of MBs in DLB was 16.9% and the number of MBs was higher than that in AD (30). Furthermore, the presence of MBs increased the risk of progression of non-AD disease despite the finding that medial temporal lobe atrophy was associated with a progression of AD in patients with MCI (31). These studies raise the possibility that a strong relationship may therefore exist between MBs and DLB. However, given the small numbers of patients with DLB in the present study, the presence of MBs in patients with dementia, including DLB, should be interpreted with caution.

**Limitations**

There were several technical points that still need to be overcome regarding the present study. First, this study retrospectively evaluated data from the patients examined with both MRI and SPECT, therefore some selection bias of the patients may exist. Secondly, the number of patients evaluated was small, especially for comparisons between patients with MBs, with or without decreased CBF. Thirdly, eZIS mapping uses reconstructive brain remodeling, thereby preventing precise matching with the MBs identified in the MRI. Finally, although the regions demonstrating a decreased CBF were visually detected using an eZIS by two experienced neurologists, the results were qualitative and therefore some bias may exist. To resolve these shortcomings, further studies are needed which incorporate quantitative analyses.

**Author’s disclosure of potential Conflicts of Interest (COI).**
Jun-Ichi Kira: Honoraria, Biogen Idec Japan, Novartis Pharma Japan and Mitsubishi Tanabe Pharma; Research funding, Pfizer Japan and the Japan Blood Products Organization.

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easy Z-score imaging system for multicenter brain perfusion

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