

# Patients With Alzheimer Disease With Multiple Microbleeds Relation With Cerebrospinal Fluid Biomarkers and Cognition

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**Background and Purpose**—Microbleeds (MBs) are commonly observed in Alzheimer disease. A minority of patients has multiple MBs. We aimed to investigate associations of multiple MBs in Alzheimer disease with clinical and MRI characteristics and cerebrospinal fluid biomarkers.

**Methods**—Patients with Alzheimer disease with multiple ( $\geq 8$ ) MBs on T2\*-weighted MRI were matched for age, sex, and field strength with patients with Alzheimer disease without MBs on a 1:2 basis. We included 21 patients with multiple MBs ( $73 \pm 7$  years, 33% female) and 42 patients without MBs ( $72 \pm 7$  years, 38% female). Mini-Mental State Examination was used to assess dementia severity. Cognitive functions were assessed using neuropsychological tests. Medial temporal lobe atrophy (0 to 4), global cortical atrophy (0 to 3), and white matter hyperintensities (0 to 30) were assessed using visual rating scales. In a subset, apolipoprotein E genotype and cerebrospinal fluid amyloid  $\beta$  1-42, total  $\tau$  and  $\tau$  phosphorylated at threonine 181 were determined.

**Results**—Patients with multiple MBs performed worse on Mini-Mental State Examination (multiple MB:  $17 \pm 7$ ; no MB:  $22 \pm 4$ ,  $P < 0.05$ ) despite similar disease duration. Atrophy was not related to presence of MBs, but patients with multiple MBs had more white matter hyperintensities (multiple MB:  $8.8 \pm 4.8$ ; no MB:  $3.2 \pm 3.6$ ,  $P < 0.05$ ). Adjusted for age, sex, white matter hyperintensities, and medial temporal lobe atrophy, the multiple MB group additionally performed worse on Visual Association Test object naming and animal fluency. Patients with multiple MBs had lower cerebrospinal fluid amyloid  $\beta$  1-42 levels ( $307 \pm 61$ ) than patients without MBs ( $505 \pm 201$ ,  $P < 0.05$ ). Adjusted for the same covariates, total  $\tau$ , and  $\tau$  phosphorylated at threonine 181 were higher in the multiple MB group.

**Conclusion**—Microbleeds are associated with the clinical manifestation and biochemical hallmarks of Alzheimer disease, suggesting possible involvement of MBs in the pathogenesis of Alzheimer disease. (*Stroke*. 2009;40:3455-3460.)

**Key Words:** CSF biomarkers ■ dementia ■ magnetic resonance imaging ■ microbleeds

Alzheimer disease (AD) is the main cause of dementia in the elderly. The pathological hallmarks are neuritic plaques (amyloid  $\beta$ ) and neurofibrillary tangles ( $\tau$ ).<sup>1</sup> Furthermore, in 70% to 98% of patients with AD, intravascular amyloid  $\beta$  deposition is found at autopsy.<sup>2</sup> Atrophy, especially of the medial temporal lobes, constitutes the radiological hallmark of AD on MRI. In addition, white matter hyperintensities (WMH) have been shown to be more prevalent in patients with AD than in control subjects. Microbleeds (MBs) can be observed on T2\*-weighted gradient-echo MRI. Histologically, they represent hemosiderin-laden macrophages resulting from leakage from cerebral small vessels.<sup>3-5</sup>

Recent findings indicate that MBs in the general elderly population are relatively common and are even more frequently observed in patients with AD.<sup>6-9</sup> In our memory clinic population, prevalence of one or more MBs in patients with AD was 18%.<sup>10</sup> Among those patients, the majority had

only one or a few MBs. However, a subgroup of patients with AD shows many MBs. Lobar MBs in corticosubcortical locations with a posterior predilection are thought to be an expression of underlying bleeding-prone cerebral amyloid angiopathy (CAA), especially in patients with AD.<sup>6,9,11,12</sup> Because CAA has been related previously to low amyloid levels in cerebrospinal fluid (CSF), presumably resulting from increased intravascular amyloid deposition,<sup>13</sup> we hypothesized that amyloid levels in CSF would be reduced in patients with AD with multiple MBs as a result of intravascular amyloid pathology.

The occurrence of MBs seems to increase with age and is believed to coincide with hypertension,<sup>14</sup> ischemic and hemorrhagic stroke, lacunes, and the extent of white matter disease.<sup>15</sup> MBs have been associated with cognitive decline in patients with stroke and subcortical vascular dementia and in the general elderly population.<sup>16-19</sup> In AD, however, the clinical significance of MBs remains elusive.<sup>6,10,15,20</sup>

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Former studies finding no correlation of MBs with clinical manifestation included many patients with one or just a few MBs. We took a proof-of-principle approach by comparing patients with AD with many MBs with patients with AD without any MBs maximizing possible differences between patient groups. We aimed to investigate the associations of multiple MBs in patients with AD with clinical, neuropsychological, and MRI characteristics and levels of CSF biomarkers.

## Materials and Methods

### Patients

From our database of patients who underwent dementia screening at the Alzheimer Center of the VU University Medical Center Amsterdam memory clinic, we retrospectively reviewed presence and number of MBs in patients with a diagnosis of probable AD visiting between May 2001 and July 2008 (n=427). Of these, 350 patients (82%) had no MBs and 67 patients (18%) had one or more MBs present on baseline T2\*-weighted gradient-echo imaging. For the current study, we selected patients with  $\geq 8$  MBs. This cutoff was specified in advance before any statistical analysis was performed and resulted in 21 patients, representing the 5% of patients with AD with the most severe MB burden (multiple MB group; age  $73 \pm 7$  years, 33% female). The patients were matched on a 1:2 basis for age, sex, and MRI field strength with patients with AD without any MBs. These "control" patients with AD without MBs were selected to have complete data of interest (no MB group: n=42; age  $72 \pm 7$  years, 38% female). All patients underwent standardized dementia screening, including medical history, physical, neurological, and neuropsychological examination. Patients were considered as having arterial hypertension, diabetes mellitus, or hypercholesterolemia if they had a known history of the disease or were receiving drug treatment. Furthermore, screening involved MRI and routine laboratory examinations. Diagnoses of probable AD were made in a multidisciplinary consensus meeting according to the clinical criteria of the National Institute of Neurological Disorders and Stroke–Alzheimer's Disease and Related Disorders Association.<sup>1</sup> The level of education was classified using the system of Verhage, ranging from 1 (low) to 7 (high).<sup>21</sup> The study was approved by the ethical review board of the VU University Medical Center and all subjects gave written informed consent.

### Neuropsychological Assessment

The neuropsychological test battery was designed to screen the major cognitive functions. Two experienced neuropsychologists were responsible for testing. All testing was done on the same day as MRI scanning as part of our screening routine, except for 3 patients, who were tested within 2 months of scanning. Dementia severity was assessed using the Mini-Mental State Examination (MMSE).<sup>22</sup> For memory, the Visual Association Test (VAT) was used.<sup>23</sup> Patients are shown cue cards with an object and association cards with the previously seen object plus an interacting object and are asked to name them. Subsequently, the cue cards are shown without delay and patients are asked to recall the now missing interacting objects (score 0 to 12). VAT object naming was used as a measure for language (0 to 12). Language functions were additionally assessed using category fluency, in which patients have to produce as many animals as possible in a timespan of 60 seconds. In addition, the Trail Making Test was used.<sup>24</sup> The simple Part A provides a measure of psychomotor speed. It requires the connection by pencil of numbers (1 to 20) positioned randomly on a sheet of paper. The more complex Part B requires patients to alternate between numbers and letters (eg, 1–A–2–B–3–C–. . .) and was used to evaluate executive functioning. For both conditions, the time required for completion is recorded. Additionally, digit span (forward and backward) was used to assess working memory. Ongoing efforts to optimize the neuropsychological screening protocol resulted in a slightly varying content of the neuropsychological battery over the years. As a result, availability of the data varied per test from 43 patients on the Trail Making Test B

test, digit span forward and backward (n=56), Trail Making Test A (n=57), VAT naming (n=58), animal fluency (n=59), MMSE (n=60), and VAT (n=61).

### MRI Protocol

The majority of scans (14 in the multiple MB group and 28 in the no MB group) were obtained on a 1.0-T scanner (Magnetom Impact; Siemens, Erlangen, Germany). Twenty-one scans (7 in the multiple MB group and 14 in the no MB group) were obtained on a 1.5-T platform (Siemens Sonata Syngo [n=17] or Siemens Avanto [n=4]). Scan protocol included (1) axial T2\*-weighted gradient-echo images for MB detection (19 to 23 slices, field of view 250 mm, matrix  $256 \times 192$  to 256, slice thickness: 5 mm, interslice gap: 1 to 1.5 mm, repetition time: 415 to 800 ms, echo time: 22 to 25 ms, flip angle  $15^\circ$  to  $20^\circ$ ); (2) coronal T1-weighted 3-dimensional magnetization-prepared rapid acquisition gradient-echo volumes (single slab, 160 to 176 sections; matrix size  $256 \times 192$  to 256; field of view  $250 \times 250$  mm; voxel size  $1 \times 1 \times 1.5$  mm; repetition time 2700 ms, echo time 5.2 to 7 ms, TI 300 to 950 ms; flip angle  $8^\circ$  to  $15^\circ$ ); (3); axial 2-dimensional fluid-attenuated inversion recovery (field of view 250 mm, matrix  $256 \times 184$  to 256, slice thickness 5.0 mm, interslice gap 1.5 mm, echo time 89 to 108 ms, repetition time 9000 ms, TI 2200 to 2500 ms, and flip angle  $150^\circ/180^\circ$ ); and (4) axial T2-weighted turbo spin echo (19 to 21 slices, field of view 250 mm, matrix  $512 \times 512$ , slice thickness 4 to 5 mm, interslice gap 1 mm, echo time 114 to 199 ms, repetition time 4590 to 5775 ms, flip angle  $180^\circ$ ).

### MRI Assessment

MRI rating was performed blinded to the patients' clinical data. MBs were defined as rounded hypointense homogeneous foci up to 10 mm in size in the brain parenchyma on T2\*-weighted images. Lesions in sulci possibly representing flow voids from pial vessels and symmetrical lesions in the basal ganglia, supposedly representing iron or calcium deposits, were excluded. Hyposignals inside a lesion compatible with an infarct were not counted as MBs, but regarded to be probable hemorrhagic transformations. Cavernous angiomas were not taken into account. MBs were counted in 4 lobar regions: frontal, parietal, temporal, and occipital and in 2 nonlobar regions: basal ganglia (including thalamus) and infratentorial. In addition, on the fluid-attenuated inversion recovery sequence WMH were assessed using the age-related white matter change scale.<sup>25</sup> The age-related white matter change scale has scores from 0 to 3 (none, punctuate, early confluent, and confluent) given in 5 regions, each left and right, adding up to a total range from 0 to 30. In addition, the presence of large-vessel and lacunar infarcts was assessed. Large-vessel infarcts were rated as present or absent based on hyperintensity of the lesion on both fluid-attenuated inversion recovery and T2-weighted sequences. Lacunar infarcts were defined as deep lesions from 3 to 15 mm with low signal on fluid-attenuated inversion recovery and T1 sequences and high signal on T2-weighted images. Lacunar infarcts were scored as present or absent. Furthermore, 2 widely used visual rating scales for the assessment of atrophy were used. Medial temporal lobe atrophy (MTA) was rated using a 5-point rating scale (0 to 4) using oblique reconstructions of the magnetization-prepared rapid acquisition gradient-echo volume sequences perpendicular to the long axis of the hippocampus.<sup>26</sup> In the analysis, the average MTA score for the left and right side was used. Global cortical atrophy was assessed on the fluid-attenuated inversion recovery sequence. The global cortical atrophy scale ranges from 0 to 3.<sup>27</sup> On both scales maximal atrophy is represented by the highest score.

### Apolipoprotein E Genotyping

DNA was isolated from 10 mL of ethylenediaminetetra-acetic acid/blood and was available from 55 of 63 patients (87%). apolipoprotein E (APOE) genotype was determined with the light cycler APOE mutation detection method (Roche Diagnostics GmbH, Mannheim, Germany). Patients were categorized according to APOE  $\epsilon 4$  status (noncarriers, heterozygous and homozygous).

### CSF Analysis

In a subset of patients 51 of 63 patients (81%), CSF was obtained by lumbar puncture between the L3/L4 or L4/L5 intervertebral space, using a 25-gauge needle, and collected in 10-mL polypropylene tubes. Within 2 hours, CSF samples were centrifuged at 1800 g for 10 minutes at 4°C. A small amount of CSF was used for routine analysis, including total cells (leukocytes and erythrocytes), total protein, and glucose. CSF was aliquoted in polypropylene tubes of 0.5 or 1 mL and stored at -80°C until further analysis. CSF amyloid- $\beta$ 1-42 (A $\beta$ 42), total  $\tau$  ( $\tau$ ) and  $\tau$  phosphorylated at threonine 181 (p $\tau$ -181) were measured with Innostest sandwich enzyme-linked immunosorbent assay as described previously.<sup>28</sup> Because the manufacturer does not supply controls, the performance of the assays was monitored with pools of surplus CSF specimens. In the study period, multiple specimens with various concentrations, which were included in 7 to 18 runs, have been used for this purpose. The interassay coefficients of variation were (mean $\pm$ SD) 11.3% $\pm$ 4.9% for A $\beta$ 42; 9.3% $\pm$ 1.5% for  $\tau$  and 9.4% $\pm$ 2.5 for p $\tau$ -181. The team involved in the CSF analysis was not aware of the clinical diagnosis.

### Statistical Analysis

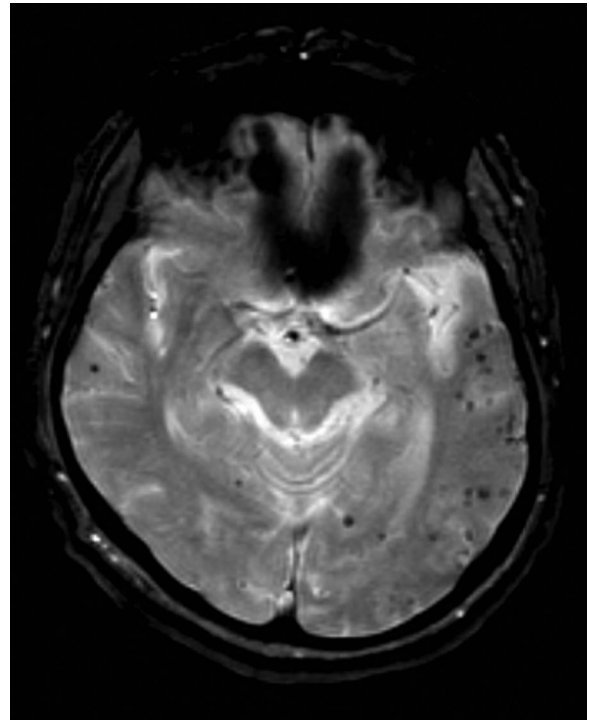
For statistical analysis, SPSS 14.0 for Windows (Chicago, Ill) was used. Categorical data were analyzed by  $\chi^2$  tests. Comparison between groups for continuous variables was executed by Student *t* tests. Analysis of variance as used to adjust for age, sex, WMH, and MTA. We used logistic regression to investigate the association between MBs and APOE  $\epsilon$ 4 status adjusted for age, sex, WMH, and MTA. The correlation between number of MBs and MMSE was investigated using Spearman correlation coefficient. Significance was set at  $P < 0.05$ .

### Results

In the group of 21 patients with multiple MBs, the total number of MBs was 491 with a median of 15 MBs (range, 8 to 104). Lobar locations accounted for 94% of the total number of MBs. Percentage of total MB count per lobe was: temporal 32%, parietal 24%, occipital 19%, and frontal 19%. Only 6% of all detected MBs were in nonlobar locations with 4% in the basal ganglia and thalamus and 2% in infratentorial regions. Eleven patients in the multiple MB group (52%) had strictly lobar MBs (Figure 1). Six patients (29%) had a few nonlobar MBs next to mainly lobar MBs, 3 patients (14%) had comparable numbers of nonlobar and lobar MBs, whereas only one patient (5%) presented more nonlobar than lobar MBs.

MRI characteristics, medical history, and medication use are shown in Table 1. Patients with multiple MBs showed more severe WMH than those without MBs ( $P < 0.001$ ). The presence of lacunar or large-vessel infarcts did not differ between groups. No difference between groups regarding atrophy (global cortical or medial temporal lobe) was found. There was no difference in disease duration, level of education, or medical history between groups. Patients with multiple MBs more often used antiplatelet medication, whereas there were no differences in other medications.

Neuropsychological and laboratory results are shown in Table 2. Patients with multiple MBs had lower MMSE than patients with AD without MBs (17 $\pm$ 7 versus 22 $\pm$ 4,  $P < 0.005$ ). Furthermore, within the group of patients with multiple MBs only, we found a correlation between the total number of MBs and MMSE (Spearman  $r = -0.47$ ;  $P < 0.05$ ; Figure 2) with more MBs being associated with lower MMSE scores. After removal of one outlier with a MMSE of 2 and 38 MBs, the correlation remained of moderate strength, although



**Figure 1.** Example of axial T2\*-weighted gradient echo image of a patient with multiple MBs. Multiple MBs in the occipital and temporal lobes, mainly on corticosubcortical junctions, with the left side more affected. The MBs were strictly lobar in location, suspect for probable CAA.

significance was lost (Spearman  $r = -0.38$ ,  $P > 0.05$ ). Unadjusted, there was no group difference for any of the neuropsychological tests. After adjustment for age, sex, MTA, and WMH, patients with multiple MBs additionally performed worse on animal fluency, VAT object naming, and digit span (forward and backward) than the group without MBs ( $P < 0.05$ ). There were no associations between age or sex and any of the neuropsychological measures. Medial temporal lobe atrophy was associated with lower VAT memory scores (Spearman  $r = -0.26$ ,  $P < 0.05$ ), but there were no associations with WMH.

Patients with multiple MBs had lower CSF levels of A $\beta$ 42 than patients without MBs in univariate analysis ( $P < 0.01$ ). Adjusted for age, sex, MTA, and WMH, patients with multiple MBs additionally had higher levels of CSF  $\tau$  and p $\tau$ -181. The aforementioned covariates had no univariate association with CSF A $\beta$ 42 levels. CSF levels of  $\tau$  and p $\tau$ -181, however, were both associated with WMH (Spearman  $r = -0.36$ ,  $P < 0.01$  and Spearman  $r = -0.34$ ,  $P < 0.05$ , respectively) and MTA (Spearman  $r = -0.30$ ,  $P < 0.05$  and Spearman  $r = -0.28$ ,  $P < 0.05$ , respectively), albeit in the counterintuitive direction as higher CSF p $\tau$  levels were observed in patients with relatively little MTA and WMH.

Patients with multiple MBs were more often homozygous for APOE  $\epsilon$ 4 (31% versus 17%), although this difference was not significant ( $P = 0.55$ ). When we used logistic regression to adjust for age, sex, MTA, and WMH, we found that homozygous APOE  $\epsilon$ 4 carriers had an increased risk to have multiple MBs (OR [95% CI], 16 [0.9 to 276]), almost reaching significance. After removing 4 patients with multiple deep



**Table 1. MRI Characteristics, Medical History, and Medication**

Variable	AD $\geq$ 8 MBs (n=21)	AD No MBs (n=42)
<b>MRI characteristics</b>		
MBs	24 $\pm$ 24	0
ARWMC score	8.8 $\pm$ 4.8	3.2 $\pm$ 3.6*
Lacunar infarcts	5 (24%)	6 (14%)
Large-vessel infarcts	2 (3%)	1 (2%)
Global cortical atrophy score	1.1 $\pm$ 0.8	1.1 $\pm$ 0.7
MTA score	2.0 $\pm$ 0.9	1.6 $\pm$ 0.9
<b>Medical history</b>		
Level of education (1–7)†	4.5 $\pm$ 1.7	5.1 $\pm$ 1.2
Duration of symptoms, years	3 $\pm$ 3	3 $\pm$ 2
Hypertension	6 (29%)	16 (38%)
Stroke or transient ischemic attack	3 (14%)	2 (5%)
Peripheral or cardiac vasculopathy	2 (10%)	6 (14%)
Diabetes mellitus	2 (10%)	3 (7%)
Hypercholesterolemia	0 (0%)	3 (7%)
<b>Medication</b>		
Nonsteroidal anti-inflammatory drugs	0 (0%)	1 (2%)
Anticoagulation	0 (0%)	0 (0%)
Antiplatelet medication	6 (29%)	7 (17%)*

Data are represented as no. of patients with the variable present (%) or mean $\pm$ SD.

\* $P<0.01$ .

†Level of education according to Verhage.

ARWMC indicates age-related white matter changes.

MBs from analysis, we found an even higher OR of 19 (CI, 1.0 to 377;  $P=0.05$ ). On visual inspection, we found that all homozygous APOE  $\epsilon$ 4 carriers in the multiple MB group had no or only very little WMH (age-related white matter change score  $\leq$ 4). There was no association between heterozygous APOE  $\epsilon$ 4 carriership and multiple MBs (OR [95% CI], 1.6 [0.1 to 19]).

## Discussion

In this proof-of-principle case–control study, we showed the relationship between multiple MBs and more pronounced impairment in a number of cognitive domains. Moreover, we found that multiple MBs were associated with lower CSF levels of A $\beta$ 42 and higher levels of  $\tau$  and p $\tau$ -181. Although MBs were associated with more severe WMH, this did not explain the more pronounced cognitive decline or abnormal CSF biomarker levels. Furthermore, our findings could not be explained by longer disease duration, level of education, or more severe atrophy.

We found an association of multiple MBs with lower CSF A $\beta$ 42, suggesting a direct link between MBs and amyloid- $\beta$ , one of the key proteins involved in AD. A postmortem study showed a relationship between the severity of CAA and lower A $\beta$ 42 levels, which could not be explained by amyloid plaque or tangle burden.<sup>13</sup> The authors hypothesized that low levels of A $\beta$ 42 may reflect increased deposition of amyloid  $\beta$  not only in plaques, but also in cerebral vessels. Accumulation of Alzheimer-related amyloid pathology in the vessels may lead

**Table 2. Neuropsychology and Laboratory Results**

Variable	AD $\geq$ 8 MBs (n=21)	AD No MBs (n=42)	Unadjusted	Adjusted*
<b>Neuropsychological data</b>				
MMSE score	17 $\pm$ 7	22 $\pm$ 4	§	§
VAT	4 $\pm$ 4	6 $\pm$ 4		†
VAT object naming	10 $\pm$ 3	12 $\pm$ 1		§
Animal fluency	11 $\pm$ 6	13 $\pm$ 5		‡
Trail Making Test A	127 $\pm$ 78	97 $\pm$ 88		
Trail Making Test B	401 $\pm$ 335	331 $\pm$ 251		
Digit span forward	10 $\pm$ 2	11 $\pm$ 2		‡
Digit span backward	6 $\pm$ 3	7 $\pm$ 2		‡
<b>Laboratory results</b>				
CSF A $\beta$ 42, pg/mL	307 $\pm$ 61	505 $\pm$ 201	§	§
CSF $\tau$ , pg/mL	940 $\pm$ 708	597 $\pm$ 298		§
CSF p $\tau$ -181	110 $\pm$ 64	85 $\pm$ 41		‡
APOE $\epsilon$ 4: noncarriers	23%	32%		
Heterozygous	46%	51%		
Homozygous	31%	17%		

Data are represented as mean $\pm$ SD unless indicated otherwise. Availability for incomplete data for multiple MB patients: blood pressure measurements 17/21 mm Hg, MMSE, and VAT naming 18/21, VAT 19/21, animal fluency 17/21, Trail Making Test A 15/21, Trail Making Test B 11/21, APOE 13/21, CSF 9/21.

\*Adjusted for WMH, MTA, age, and sex.

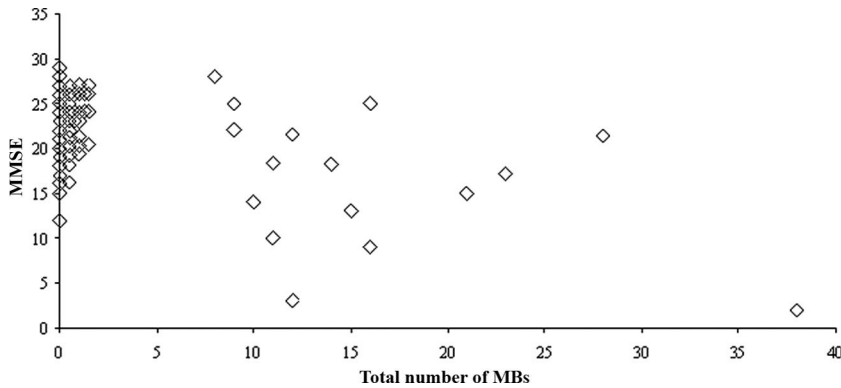
† $P<0.10$ .

‡ $P<0.05$ .

§ $P<0.01$ .

to reduced vessel wall integrity, which in turn may result in MBs. Alternatively, the more abnormal CSF levels may have been caused indirectly through vascular risk factors and associated WMH.<sup>29</sup> This seems unlikely, however, because results remained unchanged after adjustment for WMH. Moreover, when we inspected the univariate associations between CSF levels of p $\tau$  and WMH, we found that patients with higher (ie, more abnormal) p $\tau$  -181 levels had less WMH. A possible explanation for this seemingly counterintuitive finding could be that less WMH implies more pure AD associated with more abnormal Alzheimer biomarkers. The combination of high CSF  $\tau$  levels and low-grade periventricular WMH has been described before in patients with mild cognitive impairment converting to AD.<sup>30</sup> We therefore feel that the association between MBs and  $\tau$  after adjustment for WMH provides additional support for the notion that MBs may have a role in the pathogenesis of AD.

Earlier studies reporting on prevalence of MBs in AD (with prevalences ranging from 17% to 32%)<sup>6,7,10,12,15,20</sup> were not able to show any relationship between MB occurrence and cognitive performance. In these studies, most patients had only one or a few MBs. We reasoned that having one or only a few MBs is not sufficient for any measurable clinical effect. Therefore, we took a different approach by selecting the 5% of patients with AD with the most severe MB burden and comparing these with patients with AD without any MBs. In



**Figure 2.** Scatterplot demonstrating the association with total number of MBs per patient and MMSE scores in patients with AD. Correlation between the total number of MBs and MMSE in the multiple MB group (Spearman  $r = -0.47$ ;  $P < 0.05$ ). For 2 patients with  $>40$  MBs, MMSE data were missing.

this way, we aimed to maximize the supposed deleterious effects associated with MBs and to increase the power to demonstrate clinical correlates of MBs. In this way, we were able to show that patients with AD with multiple MBs present with more severe cognitive impairment. This finding could not be explained by a more advanced disease stage as illustrated by disease duration or degree of atrophy. Our findings are in line with a recent study that showed a relationship between number of MBs and cognitive impairment in subcortical vascular dementia.<sup>18</sup> Like our study, the number of observed MBs was high (median number, 13). In contrast with our study, patients were of Asian origin and were diagnosed with subcortical vascular dementia, although the authors mention that underlying Alzheimer pathology could not be excluded.

The vast majority of MBs in our population was located in lobar regions. The locations of MBs in our study are in line with other studies that suggested CAA as underlying vasculopathy of lobar MBs in AD.<sup>6,7,9,11,12</sup> Only a small minority of MBs was found in the basal ganglia, thalamic, or infratentorial regions. MBs in the deep gray matter structures have been associated with vascular risk factors and hypertensive microangiopathy at autopsy.<sup>3,4,9,31,32</sup> Furthermore, lobar MBs have been associated with APOE  $\epsilon 4$  status in community-based elderly population studies, whereas pure nonlobar MBs lack an association with APOE  $\epsilon 4$ .<sup>8,9</sup> In our current study, the relative prevalence of homozygous APOE  $\epsilon 4$  was nearly twice as high in the multiple MB group as in the no MB group, although the observed difference did not reach significance, probably due to lack of power. Moreover, when patients with multiple deep MBs were excluded, the risk became even higher. These findings support the notion that strictly lobar MBs are indeed CAA-related and as a result have a stronger association with homozygous APOE carrier-ship. Remarkably, all homozygous  $\epsilon 4$  carriers with multiple MBs had low WMH scores, seemingly suggesting separate pathophysiological mechanisms for MBs presenting with and without WMH.

Limitations of the study include the relatively small sample size, cross-sectional design, and the retrospective nature of the study. The retrospective design resulted in varying extent of incomplete data. Furthermore, our ongoing efforts to optimize the neuropsychological screening protocol have resulted in a slightly varying order and content of the neuropsychological evaluation during the years. We did not

account statistically for the missing data, in this way choosing to stay close to the original data. We feel that given the relatively large amount of missing data, imputation of missing data would potentially have added too much noise.

Furthermore, different scanners may have induced variability in the results, because detection of MBs presumably depends largely on imaging parameters.<sup>5</sup> The most prominent differences in MB detection, however, are reported on comparing images with postprocessing (ie, susceptibility-weighted imaging) with regular T2\*-weighted pulse sequences without postprocessing, varying echo times to a greater extent than was the case in our study, and doubling of field strengths, that is, 1.5 T versus 3 T. First, on all scanners, T2\*-weighted gradient-recalled echo was performed as opposed to susceptibility-weighted imaging that results in considerably higher prevalence of MBs. Second, regarding scanning parameters, slice thickness was constant on all machines, interslice gaps were comparable (1 to 1.5 mm), flip angle, and, most importantly, echo time were of comparable order (22 to 25 ms) in our protocols. Finally, by matching for field strength, we minimized the supposed effect of higher field strengths. Moreover, field strengths did not include 3 T, but only 1 and 1.5 T.

According to our proof-of-principle approach, we compared patients with many MBs with patients without any MBs to maximize supposed effects associated with MBs. Because, to our knowledge, a firm definition of “many MBs” does not exist, we had to use an arbitrary cutoff. We included the 5% of patients with AD with the most severe MB burden with the concomitant cutoff of  $\geq 8$  MBs. The main outcomes remained unchanged, however, when we used  $\geq 5$  MBs or  $\geq 10$  MBs as the cutoff, illustrating the robustness of our findings. These findings must be confirmed in a larger cohort, ideally pathology confirmed, including patients with AD with one or a few MBs to answer the question whether our findings are specific for the small subgroup of patients with AD with many MBs or that there is a dose–response relationship with patients with one or a few MBs being in between. The critical question raised by these findings is whether MBs are part of a spectrum of abnormalities in AD or reflect a distinct pathological subgroup.

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## Disclosure

None.

## References

- McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology*. 1984;34:939-944.
- Attems J. Sporadic cerebral amyloid angiopathy: pathology, clinical implications, and possible pathomechanisms. *Acta Neuropathol*. 2005;110:345-359.
- Fazekas F, Kleinert R, Roob G, Kleinert G, Kapeller P, Schmidt R, Hartung HP. Histopathologic analysis of foci of signal loss on gradient-echo T2\*-weighted MR images in patients with spontaneous intracerebral hemorrhage: evidence of microangiopathy-related microbleeds. *AJNR Am J Neuroradiol*. 1999;20:637-642.
- Tatsumi S, Shinohara M, Yamamoto T. Direct comparison of histology of microbleeds with postmortem MR images: a case report. *Cerebrovasc Dis*. 2008;26:142-146.
- Greenberg SM, Vernooij MW, Cordonnier C, Viswanathan A, Al-Shahi SR, Warach S, Launer LJ, van Buchem MA, Breteler MM. Cerebral microbleeds: a guide to detection and interpretation. *Lancet Neurol*. 2009;8:165-174.
- Pettersen JA, Sathiyamoorthy G, Gao FQ, Szilagyi G, Nadkarni NK, St George-Hyslop P, Rogaeva E, Black SE. Microbleed topography, leuko-araiosis, and cognition in probable Alzheimer disease from the Sunnysbrook dementia study. *Arch Neurol*. 2008;65:790-795.
- Atri A, Locascio JJ, Lin JM, Yap L, Dickerson BC, Grodstein F, Irizarry MC, Growdon JH, Greenberg SM. Prevalence and effects of lobar microhemorrhages in early-stage dementia. *Neurodegener Dis*. 2005;2:305-312.
- Sveinbjornsdottir S, Sigurdsson S, Aspelund T, Kjartansson O, Eiriksdottir G, Valtysdottir B, Lopez OL, van Buchem MA, Jonsson PV, Gudnason V, Launer LJ. Cerebral microbleeds in the population based AGES-Reykjavik study: prevalence and location. *J Neurol Neurosurg Psychiatry*. 2008;79:1002-1006.
- Vernooij MW, van der LA, Ikram MA, Wielopolski PA, Niessen WJ, Hofman A, Krestin GP, Breteler MM. Prevalence and risk factors of cerebral microbleeds: the Rotterdam Scan Study. *Neurology*. 2008;70:1208-1214.
- Cordonnier C, van der Flier WM, Sluimer JD, Leys D, Barkhof F, Scheltens P. Prevalence and severity of microbleeds in a memory clinic setting. *Neurology*. 2006;66:1356-1360.
- Lee SH, Kim SM, Kim N, Yoon BW, Roh JK. Cortico-subcortical distribution of microbleeds is different between hypertension and cerebral amyloid angiopathy. *J Neurol Sci*. 2007;258:111-114.
- Nakata-Kudo Y, Mizuno T, Yamada K, Shiga K, Yoshikawa K, Mori S, Nishimura T, Nakajima K, Nakagawa M. Microbleeds in Alzheimer disease are more related to cerebral amyloid angiopathy than cerebrovascular disease. *Dement Geriatr Cogn Disord*. 2006;22:8-14.
- Strozyk D, Blennow K, White LR, Launer LJ. CSF Abeta 42 levels correlate with amyloid-neuropathology in a population-based autopsy study. *Neurology*. 2003;60:652-656.
- Cordonnier C, Al-Shahi SR, Wardlaw J. Spontaneous brain microbleeds: systematic review, subgroup analyses and standards for study design and reporting. *Brain*. 2007;130:1988-2003.
- Hanyu H, Tanaka Y, Shimizu S, Takasaki M, Abe K. Cerebral microbleeds in Alzheimer's disease. *J Neurol*. 2003;250:1496-1497.
- Greenberg SM, Eng JA, Ning M, Smith EE, Rosand J. Hemorrhage burden predicts recurrent intracerebral hemorrhage after lobar hemorrhage. *Stroke*. 2004;35:1415-1420.
- Werring DJ, Frazer DW, Coward LJ, Losseff NA, Watt H, Cipolotti L, Brown MM, Jager HR. Cognitive dysfunction in patients with cerebral microbleeds on T2\*-weighted gradient-echo MRI. *Brain*. 2004;127:2265-2275.
- Won SS, Hwa LB, Kim EJ, Chin J, Sun CY, Yoon U, Na DL. Clinical significance of microbleeds in subcortical vascular dementia. *Stroke*. 2007;38:1949-1951.
- Yakushiji Y, Nishiyama M, Yakushiji S, Hirotsu T, Uchino A, Nakajima J, Eriguchi M, Nanri Y, Hara M, Horikawa E, Kuroda Y. Brain microbleeds and global cognitive function in adults without neurological disorder. *Stroke*. 2008;39:3323-3328.
- Nakata Y, Shiga K, Yoshikawa K, Mizuno T, Mori S, Yamada K, Nakajima K. Subclinical brain hemorrhages in Alzheimer's disease: evaluation by magnetic resonance T2\*-weighted images. *Ann N Y Acad Sci*. 2002;977:169-172.
- Verhage F. *Intelligence and Age: Study With Dutch People Aged 12-77* [in Dutch]. Assen: Van Gorcum; 1964.
- Folstein MF, Folstein SE, McHugh PR. 'Mini-Mental State.' A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res*. 1975;12:189-198.
- Lindeboom J, Schmand B, Tulner L, Walstra G, Jonker C. Visual association test to detect early dementia of the Alzheimer type. *J Neurol Neurosurg Psychiatry*. 2002;73:126-133.
- Reitan RM. Validity of the Trail Making Test as an indicator of organic brain damage. *Percept Mot Skills*. 1958;8:271-276.
- Wahlund LO, Barkhof F, Fazekas F, Bronge L, Augustin M, Sjogren M, Wallin A, Ader H, Leys D, Pantoni L, Pasquier F, Erkinjuntti T, Scheltens P. A new rating scale for age-related white matter changes applicable to MRI and CT. *Stroke*. 2001;32:1318-1322.
- Scheltens P, Launer LJ, Barkhof F, Weinstein HC, van Gool WA. Visual assessment of medial temporal lobe atrophy on magnetic resonance imaging: interobserver reliability. *J Neurol*. 1995;242:557-560.
- Pasquier F, Leys D, Weerts JG, Mounier-Vehier F, Barkhof F, Scheltens P. Inter- and intraobserver reproducibility of cerebral atrophy assessment on MRI scans with hemispheric infarcts. *Eur Neurol*. 1996;36:268-272.
- Bouwman FH, Schoonenboom NS, Verwey NA, van Elk EJ, Kok A, Blankenstein MA, Scheltens P, van der Flier WM. CSF biomarker levels in early and late onset Alzheimer's disease. *Neurobiol Aging*. 2008 Apr 8 [Epub ahead of print].
- Stenset V, Johnsen L, Kocot D, Negaard A, Skinningsrud A, Gulbrandsen P, Wallin A, Fladby T. Associations between white matter lesions, cerebrovascular risk factors, and low CSF Abeta42. *Neurology*. 2006;67:830-833.
- Maruyama M, Matsui T, Tanji H, Nemoto M, Tomita N, Ootsuki M, Arai H, Sasaki H. Cerebrospinal fluid tau protein and periventricular white matter lesions in patients with mild cognitive impairment: implications for 2 major pathways. *Arch Neurol*. 2004;61:716-720.
- van Es AC, van der Grond J, de Craen AJ, Admiraal-Behloul F, Blauw GJ, van Buchem MA. Risk factors for cerebral microbleeds in the elderly. *Cerebrovasc Dis*. 2008;26:397-403.
- Tanaka A, Ueno Y, Nakayama Y, Takano K, Takebayashi S. Small chronic hemorrhages and ischemic lesions in association with spontaneous intracerebral hematomas. *Stroke*. 1999;30:1637-1642.