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Specific risk factors for microbleeds and white matter hyperintensities in Alzheimer's disease

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ABSTRACT

We investigated whether microbleeds and white matter hyperintensities (WMH) in Alzheimer's disease (AD) associate more with conventional vascular risk factors or with risk factors that reflect amyloid burden. A total of 371 patients with probable AD were included. WMH (Fazekas 2 or 3) were present in 107 (29%) patients and microbleeds were seen in 98 (26%). Patients with both microbleeds and WMH were older and presented more frequently with lacunes and multiple microbleeds than patients with microbleeds in isolation (all $p < 0.05$). Using multivariate regression models, we found that WMH presence showed independent associations with age, hypertension, current smoking, and lacune presence. Microbleeds were independently associated with male gender, higher blood pressure, lower cerebrospinal fluid Aβ42, and apolipoprotein E ε4 homozygosity. Separate analyses for microbleeds according to their location showed that these associations were driven by microbleeds in lobar locations. Our results suggest that, unlike WMH, microbleeds in AD are particularly associated with additional amyloid burden, and as such, may relate to cerebral amyloid angiopathy.

1. Introduction

Alzheimer's disease (AD) is essentially regarded as a neurodegenerative disorder, but increasing evidence suggests that vascular disease may play a role as well (Attems et al., 2011; de la Torre, 2002, 2004; Decarli, 2004; Kalaria, 2002; van der Flier et al., 2007). Cerebral amyloid angiopathy (CAA) or small vessel disease related to conventional vascular risk factors may explain vascular disease in AD (Cordonnier and van der Flier, 2011).

Microbleeds and white matter hyperintensities (WMH), both regarded as manifestations of vascular disease on magnetic resonance imaging (MRI), are more frequently observed in patients with AD compared with a general elderly population (Cordonnier et al., 2006; Scheltens et al., 1992). Microbleeds are presumed to reflect focal hemosiderin deposits resulting from small vessel blood leakage (Greenberg et al., 2009) and they have been associated with CAA as well as small vessel disease (Poels et al., 2010; Vernooij et al., 2008). WMH are assumed to be rather heterogeneous in nature and severity (Gouw et al., 2011) and they may also be seen in CAA (Charidimou et al., 2012; Viswanathan and Greenberg, 2011). In elderly populations, however, WMH are generally considered a consequence of small vessel disease and are particularly associated with hypertension (Basile et al., 2006).

On MRI, microbleeds may occur with or without additional WMH. Specifically microbleeds in nonlobar locations have been associated with WMH and these microbleeds are presumed to reflect small vessel disease (Goos et al., 2010; Poels et al., 2010; Vernooij et al., 2008). Previously, we observed in AD patients that apolipoprotein E (ApoE) ε4 carriers with multiple microbleeds presented with remarkably little WMH (Goos et al., 2009). ApoE e4 has been linked to microbleeds in lobar locations and CAA is suggested to be the underlying substrate of lobar microbleeds (Poels et al., 2010; Vernooij et al., 2008).

At autopsy, CAA is found in 80% to 95% of the AD cases (Jellinger, 2002). Most of these patients only have a mild form; approximately 25% have severe CAA (Ellis et al., 1996). The presence of CAA has important implications for anti-amyloid immunization trials. Both the presence of CAA at study entry or a transient increase in CAA by immunization may predispose to adverse treatment responses,

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called amyloid-related imaging abnormalities (ARIA) (Boche et al., 2008; Sperling et al., 2011; Weller et al., 2009). At present, it is therefore recommended that microbleeds are carefully detected at baseline and during these trials, as microbleeds are assumed to be a sign of CAA. However, many aspects of the etiology and clinical relevance of ARIA are still unknown and there are only limited data about the risk profiles of AD patients presenting spontaneously with microbleeds.

Our aim was to investigate whether microbleeds and WMH on MRI, in isolation or combined, associate with certain risk factor profiles in patients with AD. We were particularly interested in risk factors that reflect amyloid burden (low cerebrospinal fluid [CSF] Aβ42 and ApoE ε4 carriership) in relation to microbleeds. First, we compared AD patients who presented with microbleeds in isolation with AD patients who presented with microbleeds and WMH in combination. Moreover, we investigated independent risk factors for microbleeds and WMH. We also explored microbleed location, as different locations may reflect different risk factor profiles.

2. Methods

2.1. Patients

Patients were included from the memory clinic based Amsterdam Dementia Cohort. Patients visited either the Alzheimer Center or the Internal/Geriatric Medicine Department of the VU University Medical Center Amsterdam (VUmc) between August 2008 and August 2011. All patients underwent an extensive dementia screening, including medical history, neurological and physical examination, cognitive assessment, and brain MRI. The diagnosis of probable AD was made according to the NINCDS-ADRDA criteria (proposed by National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association), by consensus of a multidisciplinary team (McKhann et al., 1984). In total, 371 patients with probable AD for whom 3-T MRI fluid attenuated inversion recovery (FLAIR) and T2* sequence were available were included. Informed consent was obtained from all patients to use their clinical data for research purposes.

2.2. Vascular risk factors

History of hypertension, hypercholesterolemia, and diabetes were defined based on patient-reported medical history and medication use. In addition, blood pressure (systolic and diastolic) was measured manually using a sphygmomanometer, with an average of 2 measurements in supine position. For patients without supine measurements, blood pressure was measured once in sitting position. Smoking status was defined as never, former, or current.

2.3. MRI protocol

MRI of the brain was acquired on a 3-T whole-body magnetic resonance system (Signa, HDxt, General Electric Medical Systems, Milwaukee, WI, USA), using an 8-channel head coil. The MRI protocol included an axial two-dimensional T2* gradient echo with an echo-planar read out (EPI; matrix 256 × 480, field of view [FOV] 25 × 19 cm^2, slice thickness 3.0 mm, repetition time [TR] 5300 milliseconds, echo time [TE] 25 milliseconds, 2 excitations); a sagittal three-dimensional FLAIR (matrix 224 × 224, FOV 25 × 25 cm^2, slice thickness 1.2 mm, TR 8000 milliseconds, TE 140 milliseconds); an axial two-dimensional proton density/T2-weighted fast spin echo (PD-T2; matrix 384 × 384, FOV 25 × 19 cm^2, slice thickness 3.0 mm, TR 9100 milliseconds, TE 23/114 milliseconds), and a three-dimensional fast spoiled gradient-recalled echo–based sequence (FSPGR; matrix 256 × 256, FOV 25 × 25 cm^2, slice thickness 1 mm, TR 708 milliseconds) with oblique reconstructions.

2.4. MRI assessment

MRI rating was performed by an experienced neuroradiologist, blinded to the clinical data. Microbleeds were counted on T2* sequences and were defined as small round foci of hypointense signal, up to 10 mm in brain parenchyma. Lesions in sulci possibly representing flow voids were excluded, as well as symmetric lesions in the basal ganglia, suggestive of iron or calcium deposits. Hypointensities inside infarcts were regarded as probable hemorrhagic transformations, and not counted as microbleeds. Microbleeds were counted in lobar (frontal, parietal, temporal, and occipital) and nonlobar (basal ganglia including thalamus and infratentorial) regions. Microbleed presence was dichotomized into absent (MB−, 0 microbleeds) or present (MB+, ≥1 microbleed). In addition, in microbleed-positive patients, patients with 1 microbleed were compared with patients with multiple microbleeds (>1). Microbleeds were also analyzed according to their location: strictly lobar, strictly nonlobar, or mixed. On FLAIR sequences, WMH were rated using the Fazekas scale (Fazekas et al., 1987) and classified as punctuate (grade 1); beginning confluent (grade 2), or confluent (grade 3). The WMH score was dichotomized into minimal or absent WMH (WMH−, Fazekas <2; referred to as WMH absent) or WMH present (WMH+, Fazekas ≥2). Lacunar infarcts were defined as deep lesions (3–15 mm), with (CSF-like) low signal on T1-weighted sequences and high signal on T2-weighted sequences; they were scored as absent or present. Medial temporal lobe atrophy (MTA) was rated on the oblique reconstructions of the FSPGR sequence, using a 5-point rating scale (0–4) (Scheltens et al., 1995b). Global cortical atrophy (GCA) was assessed on the FLAIR sequence, with a 4-point rating scale (0–3) (Pasquier et al., 1996). In the analyses, MTA scores were dichotomized into absent (mean left and right <1.5) or present (mean left and right ≥1.5). Similarly, GCA scores were dichotomized into absent (GCA <2) or present (GCA ≥2).

2.5. CSF analyses

CSF was obtained by lumbar puncture between the L3/L4 or L4/L5 intravertebral space and was available for 239 (64%) patients. A 25-gauge needle was used and CSF was collected in 10-mL polypropylene tubes (Sarstedt, Nümbrecht, Germany). 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 analyses, including total cells (leukocytes and erythrocytes), total protein, and glucose. CSF was aliquoted in polypropylene tubes of 0.5 and 1 mL and stored at −20 °C until further analysis. CSF amyloid beta 1-42 (Aβ42) was determined with Innotest sandwich ELISA as described previously (Bouwman et al., 2007). The performance of the assays was monitored with internal quality controls consisting of pools of surplus CSF specimens. During the study period, multiple specimens with various concentrations were used. The interassay coefficient of variation for CSF Aβ42 was (mean ± SD) 11.3% ± 4.9%.

2.6. ApoE ε4 genotyping

DNA was isolated from 10 mL of ethylenediaminetetra-acetic acid/blood and was available for 283 (76%) patients. ApoE genotype was determined with the light cycler ApoE mutation detection method (Roche Diagnostics GmbH, Mannheim, Germany).