Purpose: To investigate whether the blood-brain barrier (BBB) leaks blood-circulating substances in patients with early forms of Alzheimer disease (AD), and if so, to examine the extent and pattern of leakage.

Materials and Methods: This study was approved by the local medical ethical committees of the Maastricht University Medical Center and Leiden University Medical Center, and written informed consent was obtained from all subjects. For this pilot study, 16 patients with early AD and 17 healthy age-matched control subjects underwent dynamic contrast material–enhanced magnetic resonance (MR) imaging sequence with dual time resolution for 25 minutes. The Patlak graphical approach was used to quantify the BBB leakage rate and local blood plasma volume. Subsequent histogram analysis was used to determine the volume fraction of the leaking brain tissue. Differences were assessed with linear regression analysis, adjusted for confounding variables.

Results: The BBB leakage rate was significantly higher in patients compared with that in control subjects in the total gray matter (P < .05) and cortex (P = .03). Patients had a significantly higher volume fraction of the leaking brain tissue in the gray matter (P = .004), normal-appearing white matter (P < .04), deep gray matter (P = .01), and cortex (P = .004). When all subjects were considered, scores on the Mini-Mental State Examination decreased significantly with increasing leakage in the deep gray matter (P = .007) and cortex (P < .05).

Conclusion: The results of this study showed global BBB leakage in patients with early AD that is associated with cognitive decline. A compromised BBB may be part of a cascade of pathologic events that eventually lead to cognitive decline and dementia.

Online supplemental material is available for this article.
Evidence is increasing that impairment of the cerebral microvasculature is a contributing factor in the pathophysiology of Alzheimer disease (AD) (1,2). However, the exact pathway remains unclear. Results of histologic evaluation and albumin sampling studies show that an increased permeability of the blood-brain barrier (BBB) is likely a key mechanism (3). The BBB is a collection of cells and subcellular structures in the cerebrovascular wall that separates the blood from the brain parenchyma. It regulates the delivery of important nutrients to the brain through active and passive transport mechanisms and prevents neurotoxins from entering the brain (4). It also has a clearance function, meaning that it removes surplus substances from the brain. A well-functioning BBB is essential to keeping the brain tissue in a healthy condition. Results of previous studies (5,6) suggest that deterioration of the BBB can cause an ill-conditioned environment for neuronal cells and other pathologic changes such as small-vessel abnormality, protein deposits, inflammation, and neuronal cell death. These changes eventually may lead to cognitive decline and dementia.

BBB degradation in the advanced stages of AD has been shown mainly by using histologic results and the albumin ratio measurement (7). The use of dynamic contrast material–enhanced magnetic resonance (MR) imaging for the detection of subtle BBB leakage in vivo is a well-established method in neurologic oncology due to relatively strong leakage of gadolinium contrast agent in high-grade tumors (8). With longer imaging times, it is also becoming a promising method for use in patients with neurodegenerative and cerebrovascular diseases in whom the leakage is expected to be much lower (9–12). To investigate whether BBB leakage contributes to the early pathophysiology of AD, we hypothesized that patients with early forms of AD already show increased BBB permeability in comparison with age-matched control subjects. For this pilot study, we used a dedicated dynamic contrast-enhanced MR imaging acquisition protocol with dual-time resolution that separates the filling of the blood vessels from the leakage (13). We also investigated differences in local blood plasma volume fraction, and the relationship between BBB permeability and global cognition.

Materials and Methods

Subjects

Patients with mild cognitive impairment (MCI) due to AD or patients at an early phase of AD were prospectively recruited at the memory clinic of the Maastricht University Medical Center and Leiden University Medical Center. Because MCI due to AD and AD comprise a continuum of cognitive decline, both groups were combined for our primary analysis. All patients were referred by general practitioners because of memory concerns. A multidisciplinary team at one the two centers made the diagnoses in consensus according to the Dubois criteria for MCI and the criteria of the National Institute on Aging and the Alzheimer’s Association for AD (14,15). Patients were included when they received a diagnosis of either dementia typical of AD or MCI due to AD, with a clinical dementia rating less than or equal to 1 to ensure that the patients had AD in its early stage (16). Healthy control subjects were recruited through advertisements in local newspapers. Exclusion criteria were contraindications for imaging (eg, brain surgery, cardiac pacemaker, metal implants, claustrophobia, large body tattoos); contraindications for the use of a contrast agent, such as renal failure defined as an estimated glomerular filtration rate of less than 30 mL/min or known allergy to gadolinium-based contrast agents; major vascular disorders such as stroke, heart disease, or other causes of vascular dementia; psychiatric disorders such as major depression, schizophrenia, bipolar disorder, psychotic disorder not otherwise specified or treatment for a psychotic disorder within the previous 12 months; epilepsy; Parkinson disease; multiple sclerosis; electroshock therapy; kidney dialysis; Meniere disease; infection, trauma, or major structural abnormalities of the brain; cognitive impairment due to alcohol and/or drug abuse or abuse of other substances; and absence of a reliable person who knows the patient and his or her concerns thoroughly. An overview of the results of neuropsychologic tests of the patients can be found in the Appendix E1 (online). This study was approved by the local medical ethical committees of both institutions. Written informed consent was obtained from all subjects. All subjects underwent the Mini-Mental State Examination before MR imaging (17). The patients had scores of...
Table 1

Subject Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients</th>
<th>Control Subjects</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no. of participants</td>
<td>16</td>
<td>17</td>
<td>NA</td>
</tr>
<tr>
<td>Patients with AD</td>
<td>7</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Patients with MCI</td>
<td>9</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>73.6 ± 7.9 (59–85)</td>
<td>75.8 ± 6.2 (65–85)</td>
<td>.4</td>
</tr>
<tr>
<td>Sex</td>
<td>.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>9 (56)</td>
<td>11 (65)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>7 (44)</td>
<td>6 (35)</td>
<td></td>
</tr>
<tr>
<td>Age of men (y)</td>
<td>71.0 ± 6.7 (59–81)</td>
<td>74.6 ± 5.6 (65–85)</td>
<td>.2</td>
</tr>
<tr>
<td>Age of women (y)</td>
<td>78.9 ± 8.6 (64–85)</td>
<td>78.2 ± 6.9 (66–86)</td>
<td>.8</td>
</tr>
<tr>
<td>Mini-Mental State Examination score</td>
<td>26.3 ± 1.9</td>
<td>29.5 ± 0.6</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>WM hyperintensity volume (cm³)</td>
<td>15.8 ± 15.8</td>
<td>7.8 ± 11.9</td>
<td>.11</td>
</tr>
<tr>
<td>Fazekas score</td>
<td>1.8 ± 0.9</td>
<td>1.4 ± 0.9</td>
<td>.16</td>
</tr>
<tr>
<td>Medial temporal lobe atrophy (average of left and right)</td>
<td>1.6 ± 1</td>
<td>0.6 ± 0.7</td>
<td>.003</td>
</tr>
<tr>
<td>Diabetes*</td>
<td>6 (38)</td>
<td>1 (6)</td>
<td>.03</td>
</tr>
<tr>
<td>Hypertension*</td>
<td>9 (56)</td>
<td>9 (53)</td>
<td>.8</td>
</tr>
<tr>
<td>Other vascular diseases†</td>
<td>5 (31)</td>
<td>3 (18)</td>
<td>.4</td>
</tr>
</tbody>
</table>

Note.—Unless otherwise indicated, data are means ± standard deviation, with the range in parentheses. NA = not applicable, WM = white matter.

* Data are number of participants, with percentage in parentheses.
† Includes cardiac arrhythmia, coronary disease, and atherosclerosis.

Blood-brain barrier leakage was assessed using a dynamic contrast-enhanced MR imaging protocol with dual-time resolution. The fast sequence provided a higher spatial resolution and allowed for a higher temporal resolution during initial arrival and recirculation of the contrast agent, while the slow sequence provided a higher spatial but lower temporal resolution during contrast agent distribution and washout (Fig 1). To include the most WM hyperintensity, the centers of the field of view in the fast and slow sequences were colocalized just under the genu of the corpus callosum. Additional information on the imaging protocol also can be found in the Appendix E1 (online).

**Imaging Protocol**

To detect BBB leakage, a dynamic contrast-enhanced MR imaging protocol with dual-time resolution was implemented with a 3-T MR imaging system (Achieva; Philips, Best, the Netherlands) at both sites. This protocol consisted of two nested pulse sequences, a slow and a fast sequence (13). The fast sequence was a saturation recovery gradient-recalled sequence (repetition time msec/echo time msec, 5.2/2.5; flip angle, 30°; field of view, 25.6 × 20 × 5 cm³; matrix, 256 × 200 × 10; dynamic imaging interval, 3.2 sec) with a saturation prepulse given with a delay time of 200 msec. It was used for 1.5 minutes, during which the bolus of 0.1 mmol/kg of gadobutrol was injected with a power injector and a flow rate of 3 mL/sec, followed by a 20-mL saline flush. The slow sequence was a saturation recovery gradient-recalled sequence (5.6/2.5; field of view, 25.6 × 25.6 × 10 cm³; matrix, 256 × 256 × 50; dynamic imaging interval 30.5 sec) with the same prepulse. The slow sequence started immediately after the fast sequence, for a total time of 25 minutes. In the dual-time resolution imaging, the fast sequence allowed for a higher temporal resolution during initial arrival and recirculation of the contrast agent, while the slow sequence provided a higher spatial but lower temporal resolution during contrast agent distribution and washout (Fig 1). To include the most WM

**MR Imaging Analysis**

All analyses were performed by H.J.v.d.H. (with 4 years of experience), with support from J.F.A.J. and W.H.B. (with 13 and 18 years of experience, respectively). Individual vascular input functions were extracted from the superior sagittal sinus by using an automated method (18). The sagittal sinus was chosen because it was the largest cerebral blood vessel in the field of view and has been used in other studies (13,19,20). A two-compartment pharmacokinetic model was applied per voxel by using the Patlak graphical approach (21), which was found to be the most appropriate model in a low-leakage regimen (22,23). The Patlak graphical approach provided the BBB leakage rate and the local blood plasma volume. The Patlak graphical approach is based on linear fitting of scatterplots. The slope of this fit is the BBB leakage rate (assuming a tissue density of 1 g/mL), and the intercept is the local blood plasma volume. However, the scatterplots were rather noisy due to the very low permeability of brain tissue and thus yielded not only positive but also negative slope values. To further increase the sensitivity of this leakage detection, a histogram approach was used. The histograms were normalized, and noise was estimated by assuming that negative slope values can only be attributed to noise, and that a similar distribution of noise is also present in positive slope values. The data were then corrected by subtracting the estimated noise from the measured histogram. The remaining cumulative sum of the bins was defined as the BBB leakage volume fraction (Fig 2).

**Statistical Analysis**

All statistical analyses were performed by using software (SPSS Statistics for Windows, Version 20.0; SPSS, Chicago, Ill). Group characteristics were less than or equal to 2 on the modified Hachinski scale, indicating that the dementia did not have a vascular origin. We included 18 patients and 19 healthy control subjects (13 patients and 15 healthy control subjects were from the Maastricht University Medical Center). Two patients were excluded because of incomplete MR imaging examinations and two control subjects were excluded because of severe motion-induced artifacts and low renal function results. Therefore, the data of 16 patients (seven with AD and nine with MCI) and 17 healthy control subjects were included for final analysis. Participant characteristics are provided in Table 1.

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Subjects were tested with an independent-sample two-sided t test. The uncorrected differences in leakage volume between the groups were first tested by using an independent-sample two-sided t test. Next, linear regression was used to correct for age, sex, relative WM hyperintensity volume (except for testing BBB leakage in the WM hyperintensity), diabetes status, and other noncerebral vascular disease. We also used linear regression to investigate the

Figure 1: Graph shows example curves of contrast agent concentration in venous blood used as vascular input function and entire normal-appearing WM and the total gray matter (GM) (deep and cortex) of a single subject (75-year-old man). Time axis is on logarithmic scale to emphasize rapid changes during contrast agent arrival and initial recirculation (moment of injection ± 1 minute and 45 seconds). Below time axis is a bar depicting when fast and slow temporal resolution parts of the sequence were performed.

Figure 2: Bar graphs show overview of noise estimation method. Noise estimation method was based on bins with negative slope values, which are considered noise. A. By assuming the same distribution on positive side, noise (dark gray bins in A) can be discerned from the detectable leakage (white bins in A). B. By subtracting the noise from the original histogram, the fraction of leaking tissue is obtained. $K_i =$ BBB leakage rate.
Investigation of the effect of diabetes and other noncerebral vascular diseases in the total model provided comparable results, with patients exhibiting a significantly higher leakage volume in the WM ($b = 0.125, P < .05$) and GM ($b = 0.2229, P = .005$) and also in the normal-appearing WM ($b = 0.128, P = .06$) and in WM hyperintensities ($P = .4$).

When adjustments were made for all covariates, the patients exhibited a significantly higher leakage volume in the WM ($\beta = 0.136$) and GM ($\beta = 0.214$) and also in the normal-appearing WM ($\beta = 0.139$), deep GM ($\beta = 0.182$), cortex ($\beta = 0.207$), but not in WM hyperintensities ($\beta = 0.075$, Table 2). Investigation of the effect of diabetes and other noncerebral vascular diseases in the total model provided comparable results, with patients exhibiting a significantly higher leakage volume in the WM ($\beta = 0.125, P < .05$) and GM ($\beta = 0.2229, P = .005$) and also in the normal-appearing WM ($\beta = 0.128, P = .06$).
rate correction) and cortex ($\beta = -0.033$, $P < .050$), but was not significant in the GM ($\beta = -0.033$, $P = .06$) normal-appearing WM ($\beta = -0.022$, $P = .09$), WM ($P = .1$), and WM hyperintensities ($P = .3$). In the subset of patients with MCI, leakage volume was also significantly higher than that for control subjects (Table 2) in all tissue classes.

When all subjects were considered, Mini-Mental State Examination scores decreased significantly with increasing leakage volume in the deep GM ($\beta = -0.039$, $P = .007$), and the difference remained significant after false discovery rate correction and cortex ($\beta = -0.033$, $P < .050$), but was not significant in the GM ($\beta = -0.033$, $P = .06$) normal-appearing WM ($\beta = -0.022$, $P = .09$), WM ($P = .1$), and WM hyperintensities ($P = .3$). In the subset of patients with MCI, leakage volume was also significantly higher than that for control subjects (Table 2) in all tissue classes.

Figure 5: Histograms show the fraction of leaking voxels for patients and control subjects in (a) WM and (b) GM. Bins containing noise have been removed, so only bins representing actual leakage are displayed. Note that patients have larger proportion of leaking voxels compared with control subjects. Individual bins were tested ($P < .05$) to illustrate that the nature of the difference between the groups is particularly in the low–leakage rate ($K_i$) range (these tests were not used to draw any further conclusions). The cumulative sum of the bins is defined as the fraction of leaking tissue, which represents a summary measure that is more sensitive than the median BBB leakage rate.
subjects in the deep GM (P = .02) and cortex (P < .05), with no significant differences in the GM (P = .05), WM (P < .1), and normal-appearing WM (P = .08). Patients with AD had significantly higher leakage volume than did the control subjects in the GM (P = .04) and cortex (P = .04), but the differences were not significant in the deep GM (P = .07) or in other tissue types (P > .1). In the comparison of patients with MCI to patients with AD, no significant differences were found in any of the tissue classes investigated (P > .6).

## Discussion

The results of this study showed increased BBB leakage in patients with early AD. The leakage was globally distributed throughout the cerebrum and was associated with declined global cognitive performance. By using dynamic contrast-enhanced MR imaging with dual-time resolution, we found an increased BBB leakage rate in the GM of patients with early AD. By also showing very subtle BBB impairment in the WM, leakage volume proved to be even more sensitive to the differences in BBB leakage than was the leakage rate. Not only did this show that the differences between patients with early AD and healthy control subjects were in the extent of the BBB leakage rather than the rate (ie, strength), but it also showed that the leakage was widespread rather than localized to a single tissue class such as WM hyperintensities, normal-appearing WM, or cortex. In addition, the BBB impairment did not fully originate from vascular abnormality, because adding diabetes and other noncerebral vascular diseases to the analysis model did not change the results. This suggested that the BBB impairment stemmed from the AD abnormality instead of from vascular comorbidities. To our knowledge, authors of only a few previous studies reported on BBB impairment with dementia by using contrast-enhanced MR imaging. Starr et al (25) investigated the BBB in patients with early AD. They found dynamic signal intensity enhancement differences that suggested altered blood-brain-cerebrospinal fluid compartment kinetics compared with those of healthy control subjects, but not direct evidence of increased BBB permeability. Wang et al (26) investigated patients with MCI by using dynamic contrast-enhanced MR imaging and also observed altered temporal enhancement patterns in the hippocampus (slower decay) indicative of increased BBB permeability. Recently, Montagne et al (11) used gadobenate dimeglumine as a contrast agent and found elevated BBB impairment in the hippocampus that increased with age in patients with MCI. Compared with the results of these studies, our findings were more widespread BBB impairment, analogous to previous findings in the different stages of vascular dementia (9,10,27). However, Taheri et al (9,10) found that the leakage appeared to be mostly in WM hyperintensities in patients with vascular dementia, which we did not find in our study. An explanation for this is that the current approach may be more sensitive to the very subtle BBB impairment in patients without obvious cerebrovascular abnormality, which would also make it applicable to other diseases that may express diffusely distributed leakage.

The leakage observed in this study can be explained as a breakdown of the BBB tight junctions. It has been shown in rodents that tight junction damage allows gadolinium leakage through the BBB (28). The regions with high BBB leakage were diffusely distributed throughout the brain, showing that BBB tight junctions were globally impaired. This could have allowed the passage of small and lipophilic molecules that could not cross a healthy BBB. The loss of tight junctions also changes cell polarity, which influences the expression of transporter complexes and thus indirectly affects active transport across the BBB (29). Therefore, both passive and active transport mechanisms may be impaired in patients with early AD, possibly disturbing homeostasis (1,11).

Patients with early AD exhibited a global reduction in local blood plasma volume fraction compared with control

### Table 2

<table>
<thead>
<tr>
<th>Region of Interest</th>
<th>Leakage Rate (× 10⁻⁴ min⁻¹)</th>
<th>Fractional Leakage Volume</th>
<th>Fractional Blood Plasma Volume (× 10⁻³)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients</td>
<td>Control Subjects</td>
<td>P Value</td>
</tr>
<tr>
<td>WM</td>
<td>0.66 ± 0.44</td>
<td>0.70 ± 0.64</td>
<td>.8</td>
</tr>
<tr>
<td>GM</td>
<td>0.89 ± 1.12</td>
<td>0.17 ± 0.81</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>Normal-appearing WM</td>
<td>0.65 ± 0.43</td>
<td>0.70 ± 0.64</td>
<td>.8</td>
</tr>
<tr>
<td>Deep GM</td>
<td>1.25 ± 1.15</td>
<td>0.84 ± 1.41</td>
<td>.4</td>
</tr>
<tr>
<td>Cortex</td>
<td>0.84 ± 1.14</td>
<td>0.08 ± 0.08</td>
<td>.03</td>
</tr>
<tr>
<td>WM hyperintensities</td>
<td>1.06 ± 1.11</td>
<td>0.61 ± 0.77</td>
<td>.19</td>
</tr>
</tbody>
</table>

Note.—All data (other than P values) are mean ± standard deviation.

* Adjusted for age, sex, and relative WM hyperintensity volume.
† Significant after correction for multiple comparisons using the false discovery rate approach.
‡ Significant after additional correction for diabetes and other noncerebrovascular disease.

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subjects. This finding suggests widespread cerebrovascular differences between the groups, which also is reflected in the diffusely distributed BBB leakage. The lower local blood plasma volume may be a sign of global hypoperfusion of the brain, which has already been shown with other techniques for measuring cerebral blood flow (30). Hypoperfusion or ischemia might be an underlying factor of diffuse cerebrovascular endothelial failure leading to leaky blood vessels, but many other pathologic mechanisms also have been suggested to contribute to small-vessel disease (31).

We found that cognitive decline was associated with stronger BBB leakage, and both the patients with MCI and those with early AD showed increased BBB leakage. These observations suggest that BBB impairment may be a contributing factor in the early pathophysiology of AD. A possible mechanism is that loss of tight junctions impairs the filter function of the BBB, leading to a toxic accumulation of substances in the brain. This, combined with the altered active transport systems, might add up to a substantial effect on neuronal function that eventually leads to dementia (5). Inserting this information into the hypothetical model by Jack et al (32) suggests that BBB impairment would manifest earlier than do other structural brain changes, although additional information is needed to compare BBB impairment to amyloid β and tau abnormalities. The current study does not give information on the interaction between amyloid β and the BBB, because amyloid β is actively transported across the BBB, whereas gadolinium leaks passively through the tight junctions (4). Previous work with positron emission tomographic data has shown that clearance of amyloid β is also impaired in patients with AD (33). An impaired clearance of amyloid β would mean that the BBB is impaired in different ways, contributing to the pathologic cascade leading to AD. Therefore, BBB leakage may help to provide a biomarker for early diagnosis, or at least a marker indicating vulnerability for the development of dementia. Successful prediction of dementia eventually might lead to optimized treatment, delay, or even prevention of the disease.

This study had some potential limitations. First, it was a pilot study with a limited group size. However, even in this relatively small group, highly significant differences were detected, which indicates that the effect is substantial and that the methods are sensitive. Second, the diagnosis of the patients was not confirmed at neuropathologic examination. However, the diagnosis was made by using the latest research criteria (14,15), which are stricter and more reliable than standard clinical criteria. In conclusion, in this pilot study, MR imaging was used to show global, diffusely distributed BBB leakage in patients with early AD, which suggests that a compromised BBB is part of the early pathology of AD and might be part of a cascade of pathologic events that eventually lead to cognitive decline.

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