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Blood–Brain Barrier Breakdown in Relationship to Alzheimer and Vascular Disease

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Abstract

Objective: Blood–brain barrier (BBB) breakdown has been suggested to be an early biomarker in human cognitive impairment. However, the relationship between BBB breakdown and brain pathology, most commonly Alzheimer disease (AD) and vascular disease, is still poorly understood. The present study measured human BBB function in mild cognitive impairment (MCI) patients on 2 molecular scales, specifically BBB’s permeability to water and albumin molecules.

Methods: Fifty-five elderly participants were enrolled, including 33 MCI patients and 22 controls. BBB permeability to water was measured with a new magnetic resonance imaging technique, water extraction with phase contrast arterial spin tagging. BBB permeability to albumin was determined using cerebrospinal fluid (CSF)/serum albumin ratio. Cognitive performance was assessed by domain-specific composite scores. AD pathology (including CSF A β and ptau) and vascular risk factors were examined.

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Author Contributions

Z.L., A.M., and H.L. contributed to the conception and design of the study. All authors contributed to the acquisition and analysis of data. Z.L., A.M., and H.L. contributed to drafting the text and preparing the figures. All authors edited and approved the manuscript.

Potential Conflicts of Interest

Nothing to report.

Results: Compared to cognitively normal subjects, BBB in MCI patients manifested an increased permeability to small molecules such as water but was no more permeable to large molecules such as albumin. BBB permeability to water was found to be related to AD markers of CSF A β and ptau. On the other hand, BBB permeability to albumin was found to be related to vascular risk factors, especially hypercholesterolemia, but was not related to AD pathology. BBB permeability to small molecules, but not to large molecules, was found to be predictive of cognitive function.

Interpretation: These findings provide early evidence that BBB breakdown is related to both AD and vascular risks, but their effects can be differentiated by spatial scales. BBB permeability to small molecules has a greater impact on cognitive performance.

Cognitive impairment and dementia affect more than 50 million people worldwide.¹ Recent literature suggests that damage to the blood–brain barrier (BBB) may play a key role in this process and present a novel target for therapeutic interventions.^{2–13} The BBB, formed by vascular endothelial and supporting cells, functions to regulate blood–brain exchange of substances and protect the central nervous system from neurotoxins and pathogens.¹⁴ Postmortem studies demonstrated an accumulation of blood-derived proteins in brain parenchyma, degeneration of BBB-specific cells, and injury of vascular endothelium in brains of dementia patients.^{15,16} In vivo studies also showed that cerebrospinal fluid (CSF) of cognitively impaired patients has a higher concentration of blood-derived albumin when compared with healthy controls, suggesting a BBB disruption.^{17–20} BBB breakdown and neuroinflammation may also be a critical component in the contribution of gut microbiota to dementia.²¹

However, the relationship between BBB breakdown and brain pathology in cognitive impairment is still poorly understood. Cognitive impairment can be attributed to different pathological mechanisms, with different treatment strategies. Alzheimer pathology and vascular risks as well as their mixed presentation account for more than 80% of patients with cognitive impairment.^{22–24} Some studies have shown that the BBB disruption is related to amyloid- β peptide (A β) accumulation,^{21,25,26} thereby linking BBB to Alzheimer disease (AD) pathology. Others showed that BBB breakdown in cognitively impaired subjects was independent of amyloid and tau.⁵ Recent literature has also suggested that BBB breakdown is related to vascular risks, as evidenced by a significant correlation between BBB permeability and white matter hyperintensities (WMH).^{3,8,27–29} Patients with enlarged perivascular space or microbleeds were shown to reveal higher BBB permeability, suggesting a link to vascular risks.^{30,31} It is therefore important to reconcile these discrepancies in terms of the relationship of BBB breakdown to AD and vascular risks.

We hypothesize that BBB breakdown in cognitive impairment is not a single-facet phenomenon and that AD and vascular risks cause BBB breakdown at different molecular levels, which can be distinguished by using tracers of varying sizes. Therefore, in the present study, we aim to assess BBB permeability in older individuals with and without cognitive impairment on 2 spatial scales. One is to use a novel noncontrast magnetic resonance imaging (MRI) technique, water extraction with phase contrast arterial spin tagging (WEPCAST) MRI, to measure the BBB permeability to water molecules.^{32,33} Because the molecular weight of water is only 18Da with a diameter of 275pm, measurement

of water exchange is expected to provide an assessment of BBB permeability to very small molecules. The other is the permeability to albumin, which has a molecular weight of 66,000Da and a size of 3,800pm, by measuring the concentration of albumin in CSF obtained from lumbar puncture (LP) and normalizing it against serum albumin. The BBB permeability to these 2 molecules in patients with mild cognitive impairment (MCI) was compared to that in age-matched, cognitively normal controls. The association between BBB breakdown and cognitive performance was examined. Furthermore, the relationships between BBB disruption and AD pathology as well as vascular risks were investigated.

Materials and Methods

Participants and Consensus Diagnosis

Fifty-five elderly participants were enrolled from the Johns Hopkins Alzheimer's Disease Research Center and local outpatient clinics (age = 68.4 ± 7.3 years, 26 males, 29 females), including 33 MCI patients and 22 cognitively normal controls. All participants signed institutional review board–approved consent forms before participating in the study. The diagnostic procedure followed the recommendations of the National Institute on Aging/Alzheimer's Association workgroups.³⁴

Cognitive Assessments

The neuropsychological tests were divided into 4 cognitive domains, and scores were generated for each cognitive domain, by creating a *z* score for each test score and averaging the *z* scores within each domain. The domains included: (1) verbal episodic memory (Logical Memory delayed-recall,³⁵ Hopkins Verbal Learning Test recall over trials 1–5³⁶), (2) executive function (Digit Span backward,³⁵ Trial Making Test part B,³⁷ Digit Symbol Test,³⁸ Stroop Color and Word score³⁹), (3) processing speed (Trial Making Test part A,³⁷ Stroop Color and Word score³⁹), and (4) language (Multilingual Naming Test,⁴⁰ letter [F & L] and category [animal, vegetables] fluency tasks⁴¹). A composite overall cognitive score was computed by averaging the 4 domain scores. The Montreal Cognitive Assessment (MoCA) was also performed to evaluate global cognition.

MRI Experiments

All MRI experiments were performed on a 3T magnetic resonance system (Philips Healthcare, Best, the Netherlands). BBB permeability to water was quantified by WEPCAST MRI. Details of the WEPCAST MRI technique can be found in Lin et al.^{32,33} Briefly, an MRI pulse sequence module similar to pseudocontinuous arterial spin labeling was used to label water in the arterial blood in the cervical spine region. At the capillary–tissue interface, water exchange across the BBB takes place and a fraction of the labeled water molecules is extracted into the tissue, whereas the remaining unextracted water is drained to the venous system. WEPCAST MRI selectively measures the amount of labeled water in the superior sagittal sinus (SSS), which can give an estimation of the global extraction fraction (*E*) of water. Together with global cerebral blood flow (CBF; *f*), BBB permeability can be assessed as permeability–surface area product (PS)⁴²:

$$PS = -\ln(1 - E) \cdot f \quad (1)$$

WEPCAST MRI was performed in midsagittal plane using a labeling duration of 2,000 milliseconds, a postlabeling delay (PLD) of 4,000 milliseconds, and an encoding velocity (V_{enc}) of 15cm/s. Other imaging parameters were as follows: single-shot gradient echo planar imaging readout, field of view (FOV) = $200 \times 200\text{mm}^2$, single slice, matrix = 64×64 , voxel size = $3.13 \times 3.13\text{mm}^2$, slice thickness = 10mm, SENSE factor = 2, flip angle = 90° , repetition time (TR) = 7,547 milliseconds, echo time (TE) = 13 milliseconds, number of control/label pairs = 10, scan duration = 5 minutes 9 seconds. An additional M_0 image with the same TE and V_{enc} and a long TR (10 seconds) was acquired for normalization.

Global CBF was measured by phase-contrast (PC) MRI at 4 main feeding arteries (left/right internal carotid arteries and left/right vertebral arteries)⁴³ and a T_1 -weighted magnetization-prepared rapid acquisition of gradient echo (MPRAGE) scan. The imaging planes of the PC MRI were placed perpendicular to the targeted arteries with the following parameters: TR = 19.8 milliseconds, TE = 9.68 milliseconds, flip angle = 15° , FOV = $200 \times 200 \times 5\text{mm}^3$, voxel size = $0.5 \times 0.5 \times 5\text{mm}^3$, single slice, $V_{\text{enc}} = 40\text{cm/s}$, and scan duration = 15.9 seconds.

A fluid-attenuated inversion recovery MRI scan was acquired to assess WMH with the following parameters: TR = 4,800 milliseconds, TE = 275 milliseconds, inversion time = 1,650 milliseconds, flip angle = 90° , FOV = $240 \times 240 \times 165\text{mm}^3$, voxel size = $1.1 \times 1.1 \times 1.1\text{mm}^3$, number of slices = 150, axial orientation, and scan duration = 4 minutes 14 seconds.

MRI Data Processing

All MRI data were processed using in-house MATLAB (MathWorks, Natick, MA) scripts. Details of WEPCAST processing can be found in Lin et al.^{32,33} Briefly, PC velocity-encoded images were collected for control and label conditions, and their subtraction yielded arterially labeled signals (M) at acquisition time t :

$$\Delta M = 2\alpha(1 - E)M_0 e^{-\frac{\delta_v}{T_{1b}}} c(t, \delta_v) \otimes G(t) \quad (2)$$

where E is the extraction fraction of water, α is labeling efficiency (assumed to be 86%⁴⁴), δ is bolus arrival time (BAT) of SSS, and T_{1b} is venous blood T_1 (calculated based on individual hematocrit level⁴⁵). M_0 is the equilibrium magnetization and was measured from the M_0 scan mentioned above. $c(t) = \begin{cases} 1, & \text{if } \delta_v < t < \delta_v + \tau \\ 0, & \text{otherwise} \end{cases}$ is the arterial input function, where

τ is labeling duration. $G(t) = \frac{1}{\sqrt{2\pi\sigma^2}} e^{-t^2/2\sigma^2}$ denotes the Gaussian kernel due to dispersion

of the labeled bolus (where σ is the standard deviation of the distribution, assumed to be 0.5, which is 2 times that in the arterial side⁴⁶) and \otimes denotes convolution operation. Thus, the only unknowns in Equation 2 are δ_v and E . We took advantage of having measured WEPCAST signal along the entire length of the SSS. Then the peak signal should appear at

the center of the labeling bolus, and the BAT of it is $PLD + \frac{\tau}{2}$. Once δ_v is known, E can be estimated using Equation 2. Then PS was estimated from E and CBF using Equation 1.

The MPRAGE images were segmented using an automatic processing tool, MRICloud (www.MRICloud.org, Johns Hopkins University, Baltimore, MD), for total brain volume quantification, which is needed for CBF quantification. Additionally, we quantified temporal lobe volume (relative to intracranial volume) as an index of brain atrophy.

Qualitative WMH were assessed by a board-certified neuroradiologist to obtain a Fazekas score indicating their severity. Quantitative WMH volume was obtained by a Bayesian-based automatic detection method as described in DeCarli et al.⁴⁷

CSF Sample Analysis

CSF was collected via LP. Thirty-four (age = 69.3 ± 7.3 years, 15 males, 19 females) of the 55 participants who received WEPCAST MRI underwent LP (62%).

CSF and blood albumin concentration were measured with the turbidimetric assay on the Tina-quant Albumin Gen.2 analyzer (Roche Diagnostics International, Rotkreuz, Switzerland). CSF/serum albumin ratio was calculated as the index of BBB permeability to albumin.

Additionally, AD biomarkers, including CSF A β 42, A β 40, total tau, and ptau-181 concentration (picograms/milliliter), were estimated in 32 participants (age = 69.1 ± 7.5 years, 15 males, 17 females) using the Neurology 3-plex Simoa immunoassay (Quanterix, Lexington, MA) on the SR-X platform. All samples were analyzed over 2 plates with the same lot number, and an average value for the 2 runs was recorded. The ratio between CSF A β 42 and A β 40 was used as an index of amyloid pathology. A CSF standard was run across both plates to ensure minimal plate-to-plate variability. Note that this group (n = 32) fully overlapped with the participants with CSF/serum albumin ratio (n = 34). Additionally, 12 participants (age = 69.9 ± 7.8 years, 5 males, 7 females, 4 controls, 8 MCI) who were recruited using the identical criteria but did not participate in the BBB study were included when making comparisons of the AD biomarkers between groups.

CSF neurofilament light chain (NFL) level was measured with the Simoa NF-Light Kit using the HD-X platform (Quanterix) in the same group of participants. Intra-assay and interassay coefficients of variation were <10% and <15% for quality control samples, respectively.

Vascular Risk Factors

Clinical evaluation of vascular risk factors was collected from all participants. There has not been a standard in the literature as to how the vascular risks should be quantified and combined; the present study used binary encoding of these variables based on procedures we employed previously.^{48,49} Specifically, 5 measures were considered: (1) hypertension (1 if recent, 0 if remote/absent), (2) hypercholesterolemia (1 if recent, 0 if remote/absent), (3) diabetes (1 if recent, 0 if remote/absent), (4) smoking (1 if smoked >100 cigarettes in his/her life, 0 if not), and (5) body mass index (BMI; calculated as weight in kilograms divided by

height in meters squared; 1 if BMI > 30, 0 if not). A composite vascular risk score (VRS) was calculated as the sum of the 5 measures^{48–51} and ranged from 0 to 5.

APOE Status.—Genomic DNA was extracted from buffy coat using the QIAamp DNA Mini QIAcube Kit (QIAGEN, Germantown, MD). Genotyping was performed using premade TaqMan single nucleotide polymorphism genotyping assays C-905013-10 (rs405509), C-3084793-20 (rs429358), and C-904973-10 (rs7412) using an Applied Biosystems (Foster City, CA) 7900HT Real Time PCR System. The *APOE4* carrier status was coded as follows: individuals with 2 E4 alleles were coded as 2, individuals with 1 E4 allele were coded as 1, and individuals with no E4 alleles were coded as 0.

Statistical Analysis

Group differences in demographic information (including age, sex, education, and *APOE4* status) were examined by 2-sample *t* test. Group differences in cognitive performance, brain volumes, and NFL level between MCI patients and controls were assessed by linear regression. When relevant, age, sex, and education were included as covariates.

To examine whether there was a difference in water BBB permeability between MCI and control groups, multilinear regression analysis was performed where PS was the dependent variable and group index was the independent variable, with age and sex as covariates. Similar analyses were performed to test the group differences in CSF/serum albumin ratio.

We assessed the association between BBB permeability and cognitive performance using linear regression in which the cognitive test scores were the dependent variables (separately for cognitive composite score, 4 domain scores, and MoCA score) and BBB permeability (PS or CSF/serum albumin ratio) was the independent variable, with age, sex, and education as covariates.

The relationship between BBB permeability and AD pathology (including A β 42/A β 40 ratio, total tau, and ptau level) was tested by linear regression analysis where PS or CSF/serum albumin ratio was the independent variable and AD pathology was the dependent variable. Age and sex were included as covariates. Similar analyses were performed to examine the relationship between BBB permeability and CSF NFL level.

To test the association between BBB permeability and vascular risks, linear regression analysis was performed using PS or CSF/serum albumin ratio as the dependent variable and vascular risk factors as the independent variable. Age and sex were included as covariates.

The relationship between BBB permeability and *APOE4* status was also assessed with linear regression, correcting for age and sex. The BBB group comparison analyses mentioned above were also repeated after adding *APOE4* status as a covariate.

In all analyses, a 2-tailed *p* value of 0.05 or less was considered statistically significant. For analyses of domain cognitive scores (ie, memory, executive function, language, and processing speed), Šidák correction was performed, that is, corrected *p* value = $1 - (1 - p \text{ value})^n$, where $n = 4$ to account for 4 domains. In addition, 98.75% confidence interval (CI) was calculated as corrected CI. Similarly, Šidák corrections were performed for analyses

involving domain vascular risks (ie, hypertension, hypercholesterolemia, diabetes, smoking, and BMI).

Results

Demographics, Cognitive Performance, and Brain Volume

Demographic information of the participants is summarized in Table 1. There were no differences between MCI and control groups in terms of age, sex, and education. However, the MCI group had a significantly higher fraction of participants with *APOE4* in comparison with the control group (95% CI = 0.0076–0.63, $p = 0.045$). As expected, significant differences were found in cognitive composite score (95% CI = –0.88 to –0.48, $p < 0.001$), episodic memory (95% CI = –1.19 to –0.53, $p < 0.001$), executive function (95% CI = –1.13 to –0.56, $p < 0.001$), language (95% CI = –0.68 to –0.13, $p = 0.019$), processing speed (95% CI = –1.02 to 0.21, $p = 0.014$), and MoCA score (95% CI = –3.33 to –0.99, $p < 0.001$) between the 2 groups, after adjusting for age, sex, and education, with the MCI group having poorer scores on all tests. MCI patients also had a significantly smaller normalized temporal lobe volume compared with controls (95% CI = –0.47 to –0.042, $p = 0.020$) after adjusting for age and sex.

BBB Permeability in MCI and Controls

Figure 1A shows WEPCAST MRI data from representative MCI and control participants. Quantitative analysis of the WEPCAST data yields an estimation of BBB permeability to water, that is, PS value. A higher PS indicates a leakier BBB. A box plot comparing water PS values between the MCI and control groups is shown in Figure 2A. The MCI patients revealed a higher PS value (ie, breakdown of BBB) compared to controls (MCI, 142.6 ± 25.6 ml/100g/min; control, 123.0 ± 26.0 ml/100g/min, 95% CI of the difference = 4.8–33.8 ml/100g/min, $p = 0.010$), after adjusting for age and sex.

Figure 1B illustrates the CSF collection and assay procedures. CSF/serum albumin ratio was found to be 5.36 ± 1.70 (mg/dl)/(g/dl) and 5.99 ± 1.64 (mg/dl)/(g/dl) in MCI and controls, respectively. Figure 2B shows a box plot of the data. There was no difference between the 2 groups (95% CI = –1.94 to 0.54 [mg/dl]/[g/dl], $p = 0.26$).

Relationship between BBB Permeability to Water and Albumin and Cognitive Performance

Table 2 summarizes the relationship between BBB permeability and cognition. After adjusting for age, sex, and education, regression analysis revealed a significant inverse association between water PS and episodic memory ($\beta = -0.0108$, 95% CI = –0.018 to –0.0039, $p = 0.011$; see Fig 2C) and composite score ($\beta = -0.0051$, 95% CI = –0.0099 to –0.00022, $p = 0.041$; see Fig 2D). Individuals with a higher PS, that is, BBB leakage, had poorer cognitive performance. For the case of episodic memory, each 93 ml/100g/min increase in PS will correspond to a decrease in memory performance by 1 standard deviation. Water PS also has a trend of negative association with language score ($\beta = -0.0065$, 95% CI = –0.012 to –0.0014, $p = 0.053$). However, it was not associated with other cognitive tests and domains, including MoCA ($\beta = -0.017$, 95% CI = –0.040 to 0.0066, $p = 0.16$), executive function ($\beta = -0.0025$, 95% CI = –0.0091 to 0.0042, $p = 0.92$), and

processing speed ($\beta = -0.00055$, 95% CI = -0.0085 to 0.0074 , $p = 1.00$), although in all results the coefficient values were negative (ie, higher PS corresponding to poorer cognitive scores).

No significant associations between CSF/serum albumin ratio and domain or global cognitive scores were found, suggesting that BBB permeability to large molecules is less sensitive in predicting cognitive function.

Relationship between BBB Permeability and Alzheimer Pathology

Figure 3A shows a scatter plot between water PS and CSF A β 42/A β 40 ratio. Linear regression revealed that higher water PS, that is, worse BBB breakdown, was associated with lower CSF A β 42/A β 40 ratio ($\beta = -0.00027$, 95% CI = -0.00045 to -0.000097 , $p = 0.0037$). Note that lower CSF A β 42 corresponds to higher brain A β 42.⁵² Thus, these data suggest that higher BBB permeability to water corresponds to more amyloid burden in the brain. Similarly, higher water PS was associated with a higher CSF ptau level ($\beta = 0.45$, 95% CI = 0.11 – 0.79 , $p = 0.012$; see Fig 3B). There was not an association between water PS and total tau level in the CSF ($\beta = 0.45$, 95% CI = -0.38 to 1.28 , $p = 0.27$).

There was not an association between CSF/serum albumin ratio and any of the AD pathological markers, including A β 42/A β 40 ratio ($\beta = 0.00060$, 95% CI = -0.0029 to 0.0041 , $p = 0.73$), total tau ($\beta = 1.68$, 95% CI = -12.67 to 16.02 , $p = 0.81$), and ptau ($\beta = -2.15$, 95% CI = -8.59 to 4.30 , $p = 0.50$).

Comparisons of AD markers between MCI and control participants showed a significant difference in ptau ($\beta = 22.94$, 95% CI = 1.49 – 44.38 , $p = 0.037$), but no differences in A β 42/A β 40 ratio or total tau (Fig 4A–C). When including the additional participants that provided CSF samples, A β 42/A β 40 ratio or total tau also showed a trend of difference, whereas ptau remained different (see Fig 4D–F).

Comparison of CSF NFL between participant groups showed no difference between MCI and controls in the subjects who received WEPCAST BBB assessment (see Table 1). However, when including the additional participants who provided CSF but did not receive WEPCAST, MCI participants revealed significantly higher NFL than controls (MCI, $2,988.82 \pm 1,223.90$ pg/ml; control, $2,356.93 \pm 825.35$ pg/ml, $\beta = 610.50$, 95% CI = 6.86 – $1,214.14$, $p = 0.048$). We did not find a significant association between CSF NFL levels and PS ($\beta = -3.26$, 95% CI = -12.74 to 6.23 , $p = 0.49$) or CSF/serum albumin ratio ($\beta = -86.04$, 95% CI = -74.93 to 247.03 , $p = 0.74$).

Relationship between BBB Permeability and Vascular Risks

Linear regression showed no significant associations between water PS and the composite VRS or any of the domain vascular risk factors.

Next, we examined the relationship between BBB permeability to albumin and vascular risk factors. It was found that CSF/serum albumin ratio was significantly associated with the composite VRS ($\beta = 0.79$, 95% CI = 0.19 – 1.39 , $p = 0.012$; see Fig 3C) and the domain of

hypercholesterolemia ($\beta = 1.74$, 95% CI = 0.67–2.80, $p = 0.011$; see Fig 3D). CSF/serum albumin ratio was not associated with the other domain vascular risks (Table 3).

We also assessed the relationship between BBB permeability and WMH. No significant association was found between Fazekas score and water PS ($\beta = -0.0010$, 95% CI = -0.0079 to 0.0059 , $p = 0.78$) or CSF/serum albumin ratio ($\beta = 0.054$, 95% CI = -0.087 to 0.20 , $p = 0.44$). Similarly, no significant association was found between WMH volume and water PS ($\beta = 0.077$, 95% CI = -0.028 to 0.18 , $p = 0.15$) or CSF/serum albumin ratio ($\beta = 0.43$, 95% CI = -2.22 to 3.09 , $p = 0.74$). However, we found a positive association between Fazekas score and VRS ($\beta = 0.18$, 95% CI = 0.039 – 0.32 , $p = 0.014$; see Fig 4G), and between WMH volume and VRS ($\beta = 2.74$, 95% CI = 0.51 – 4.96 , $p = 0.017$; see Fig 4H).

Relationship between BBB Permeability and APOE4 Status

Linear regression showed that, after correcting for age and sex, *APOE4* status was not associated with either PS ($\beta = 4.13$, 95% CI = -9.36 to 17.62 , $p = 0.54$) or CSF/serum albumin ratio ($\beta = -0.27$, 95% CI = -1.23 to 0.70 , $p = 0.58$). When adding *APOE4* status as a covariate, the abovementioned relationship between BBB permeability and participant diagnostic group remained.

Discussion

In this study, we investigated BBB breakdown on 2 spatial scales in older individuals with MCI who have varying degrees of AD pathology and vascular risks. Our findings provide early evidence that, relative to cognitively normal subjects, BBB in MCI patients manifested an increased permeability to small molecules such as water but was no more permeable to large molecules such as albumin. BBB permeability to water was found to be related to AD markers of CSF A β and ptau. On the other hand, BBB permeability to albumin was found to be related to vascular risk factors, in particular hypercholesterolemia, but was not related to AD pathology. Moreover, BBB permeability to small molecules, but not to large molecules, was found to be predictive of cognitive function.

Disrupted BBB in cognitive impairment and dementia has been shown by recent literature.^{2,5,6,8,9,11} However, the mechanism and relationship of BBB breakdown to AD and vascular risks are still poorly understood and somewhat controversial. In this study, we showed that BBB breakdown is a multifaceted manifestation and that both AD and vascular risks can cause BBB disruption, but on different molecular scales. AD pathology is accompanied by increased BBB permeability to small molecules such as water. Vascular risk factors, on the other hand, are associated with elevated BBB permeability to larger molecules such as albumin. We note that BBB permeability to small and large molecules could be physiologically decoupled. For example, as shown in an illustration in Figure 5B, minor but pervasive BBB breakdown in brain capillaries would result in increased permeability to water but not albumin, as large molecules like albumin are unable to leak through the minor breakdown. On the other hand, as illustrated in Figure 5C, sparse but severe BBB damage in a small fraction (one-fifth in the example illustrated, but it could be fewer) of capillaries would result in leakage of large molecules such as albumin into

parenchyma and CSF, but will have negligible impact on BBB permeability to water, because healthy BBB is already highly permeable to water and the leakage points are only present in a small subset of capillaries. Therefore, it appears that vascular risks cause isolated but severe BBB damage, whereas AD is accompanied by minor but widespread BBB breakdown. It should be pointed out that the sample size of participants with CSF/serum albumin ratio is relatively small due to the need to perform LP. Thus, the level of statistical significance was lower than some of the previous studies.^{3,20,53–55} However, other studies with larger sample sizes have confirmed that vascular dementia patients had significantly higher CSF/serum albumin ratios compared to non-vascular-type dementia patients.^{3,20,53–55} Those studies also observed a lack of association between albumin permeability and AD biomarkers.^{53,55}

Other potential reasons could explain the findings in the current study. The increased BBB permeability to water may also represent the dysregulation of the aquaporin-4 (AQP4) channels on the astrocyte endfeet.⁵⁶ Both increased AQP4 expression and reduction of AQP4 polarization have been reported in aging and AD, which may have different effects on the water permeability.^{12,57,58} Future studies are needed to further understand the specific role of AQP4 in BBB dysfunction in dementia. Similarly, albumin in the plasma can enter the central nervous system (CNS) not only by crossing the BBB to the interstitial fluid and CSF, but also by directly crossing the blood–CSF barrier (BCSFB).⁵⁹ Thus, the CSF/serum albumin ratio may also provide information on the BCSFB, which is a topic under intensive investigation in the aging and dementia field.^{60,61}

Although the present work is a cross-sectional study, it is useful to discuss the potential causal relationship between BBB function and other pathological processes in cognitive impairment. Given the observed association between BBB disruption and A β 42 and that amyloid accumulation is an early process in the pathogenesis of AD, it is plausible that amyloid deposition may cause damage to the BBB, such as diminished endothelial transport, loss of tight junction integrity, and pericyte and astrocyte degeneration, which results in BBB leakage.^{4,26,62,63} Additionally, amyloid may be deposited in cerebral vessels, causing cerebral amyloid angiopathy,⁶⁴ which may cause further BBB damage. As an alternative hypothesis, it is also possible that BBB dysfunction is an upstream process for A β accumulation in CNS,^{21,65} which would suggest that A β accumulation partly reflects an immune response of the brain.²¹ Future studies are needed that examine BBB permeability in AD/MCI/high-risk patients with a variety of severities to elucidate the temporal relationship between BBB damage and AD pathological markers.

Our study has several limitations. First, the water permeability MRI technique is a global method with no regional information. Future studies are needed to develop a more region-specific technique for water BBB permeability measurement. Additionally, it should be pointed out that the technique used in this study, WEPCAST MRI, was only recently developed^{32,33}; thus, further experience from more investigators/laboratories is needed to confirm these findings. Validation with invasive methods in animal models is also desirable. Second, this study has not collected longitudinal data; thus, we do not know whether the BBB disruption in older individuals can predict cognitive decline, which remains to be explored in future studies. Third, only a subset of participants received CSF-based

measurements, which may have led to a lower sensitivity in the statistical analyses. Future studies in larger populations are needed to further verify the sensitivity of WEPCAST in assisting the diagnosis of AD.

In conclusion, this study assessed BBB breakdown in MCI patients on 2 molecular scales. We provided early evidence that MCI patients had higher BBB permeability compared with controls. Increased permeability to small molecules such as water was associated with lower A β 42 and high ptau in CSF and poorer cognitive function. On the other hand, BBB permeability change to larger molecules such as albumin was associated with vascular risk factors. These findings suggested that BBB breakdown is related to both AD and vascular risks, but their effects can be differentiated by spatial scales.

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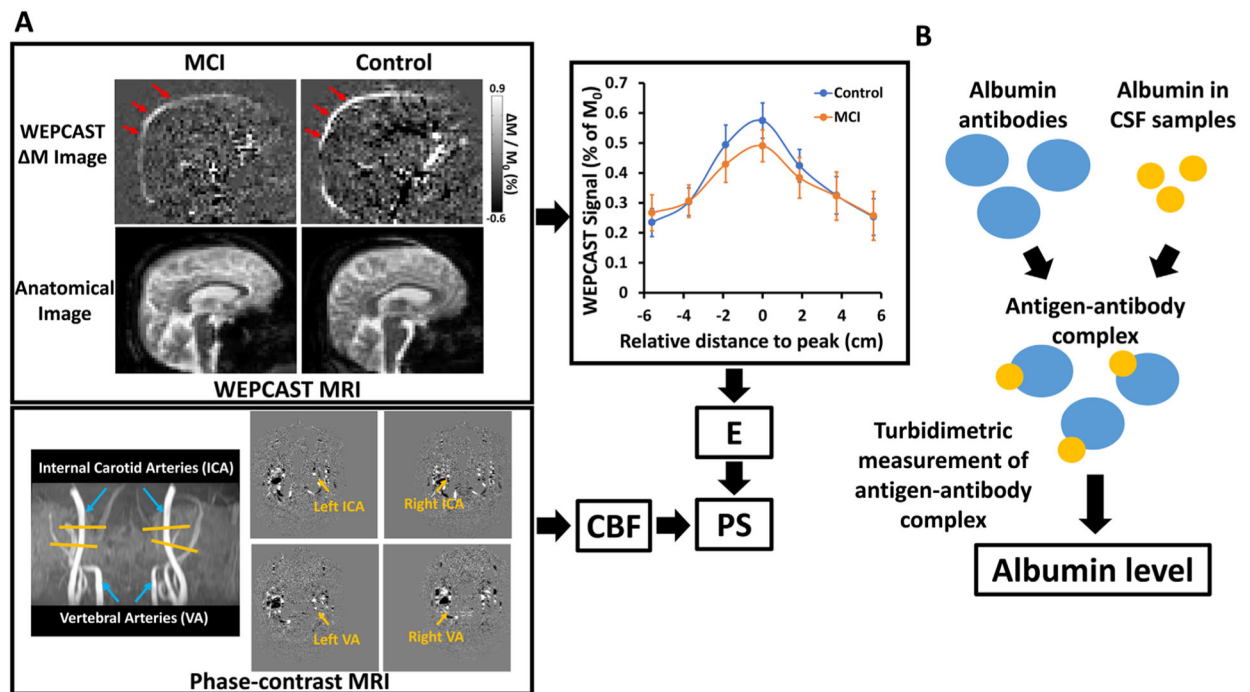
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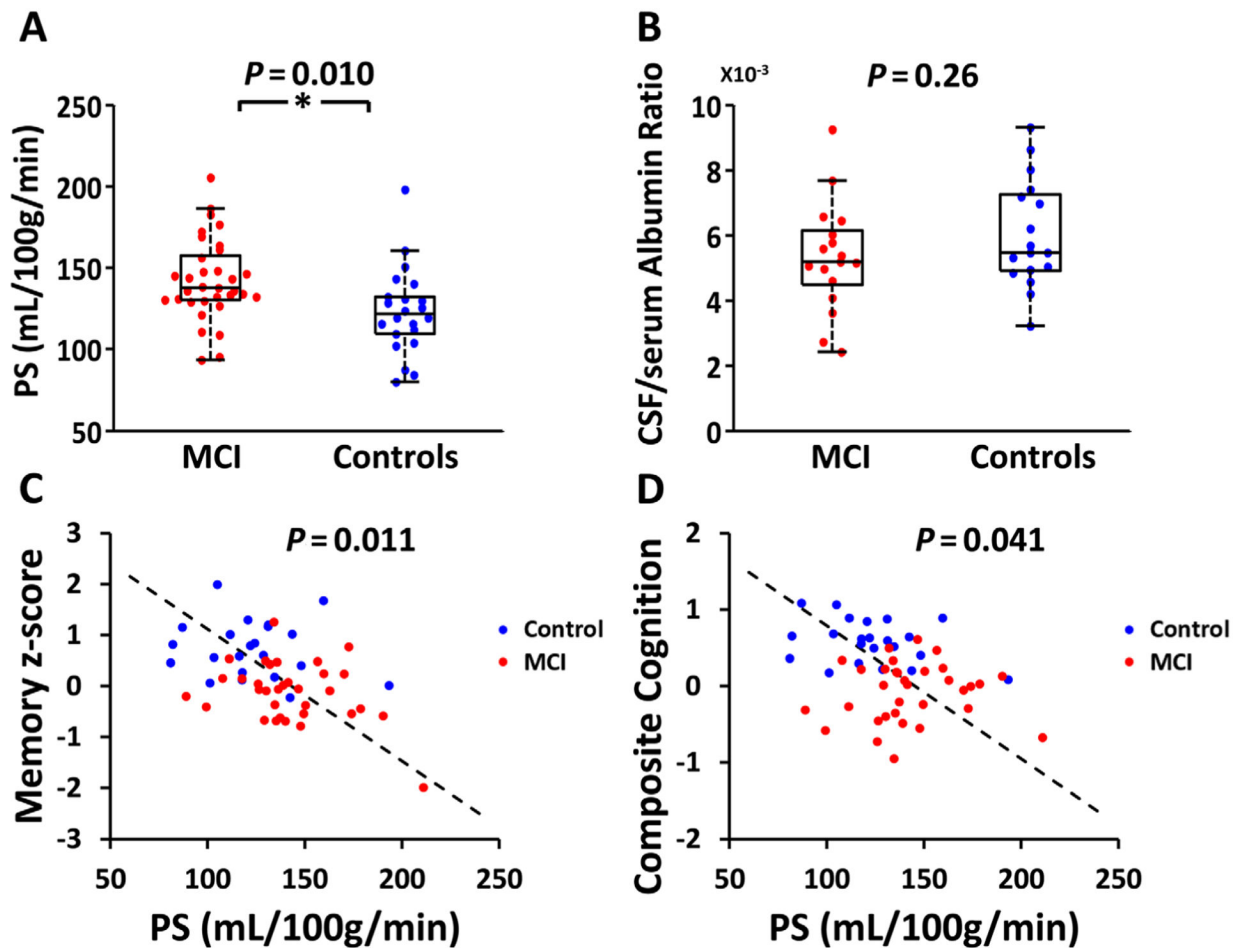
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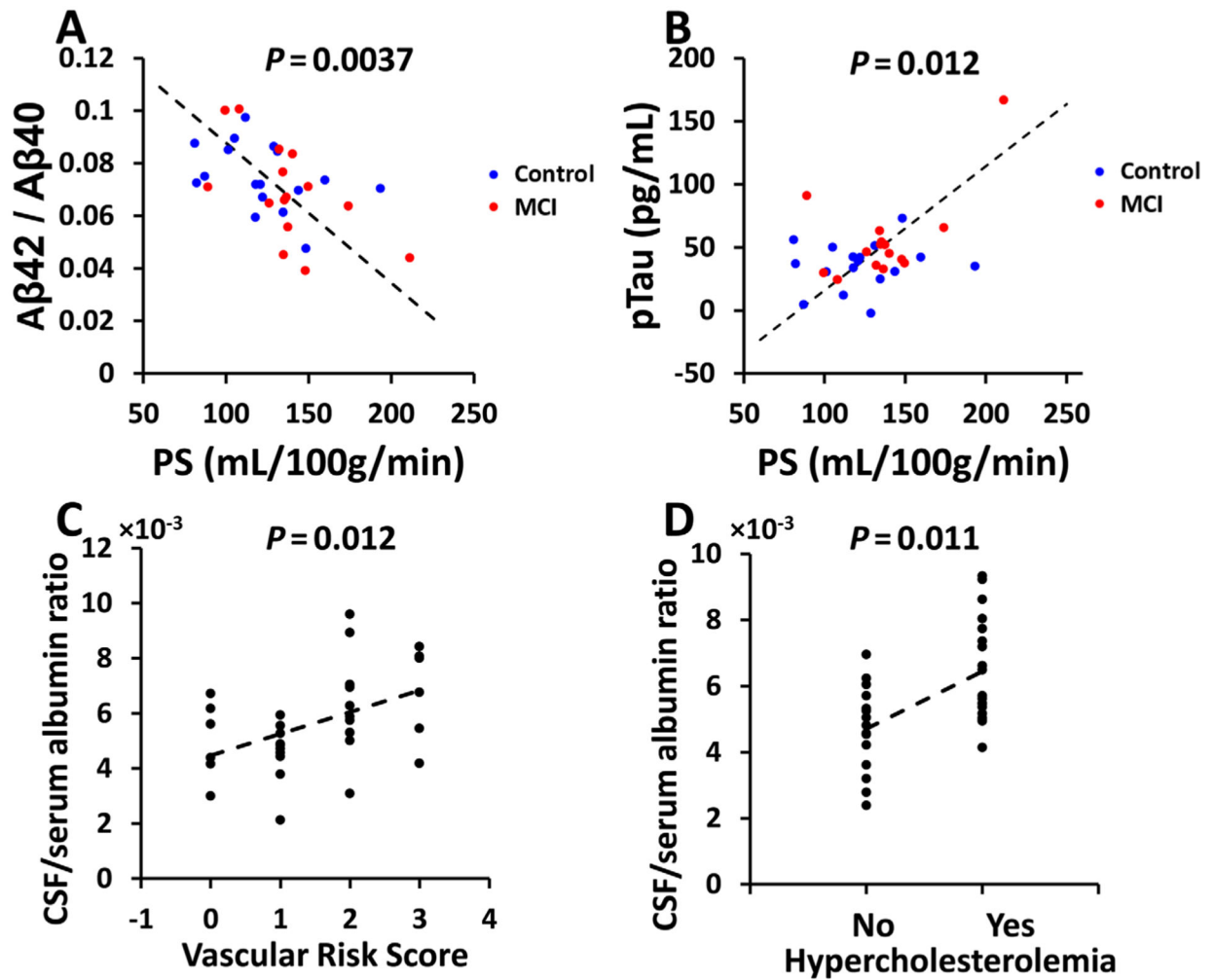
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**FIGURE 1:**

Measurement of blood–brain barrier (BBB) permeability to water and albumin. (A) Water extraction with phase contrast arterial spin tagging (WEPCAST) magnetic resonance imaging (MRI) for the assessment of BBB permeability to water. M image in a representative mild cognitive impairment (MCI) and control subject and corresponding anatomic images are shown. WEPCAST difference signal curves along superior sagittal sinus (SSS) averaged for MCI and control groups are also shown. Error bar denotes standard error across participants. Peak SSS signal was used to calculate the water extraction fraction (E). Together with cerebral blood flow (CBF) measured by phase-contrast MRI, BBB permeability to water (ie, permeability–surface area product [PS] value) can be calculated. (B) Measurement of albumin level with turbidimetric assay. CSF = cerebrospinal fluid.

**FIGURE 2:**

Relationship of blood-brain barrier permeability with clinical diagnosis cognition. (A) Box plot of water permeability-surface area product (PS) between mild cognitive impairment (MCI) and control groups. ($*P < 0.05$). (B) Box plot of cerebrospinal fluid (CSF)/serum albumin ratio between MCI and control groups. (C) Scatter plot between water PS and memory z score. (D) Scatter plot between water PS and composite cognition.

**FIGURE 3:**

Relationship of blood–brain barrier permeability to Alzheimer disease pathology and vascular risks. (A) Scatter plot between permeability–surface area product (PS) and cerebrospinal fluid (CSF) $A\beta_{42}/A\beta_{40}$ level. (B) Scatter plot between PS and CSF ptau level. (C) Scatter plot between CSF/serum albumin ratio and vascular risk score. (D) Scatter plot between CSF/serum albumin ratio and hypercholesterolemia. MCI = mild cognitive impairment.

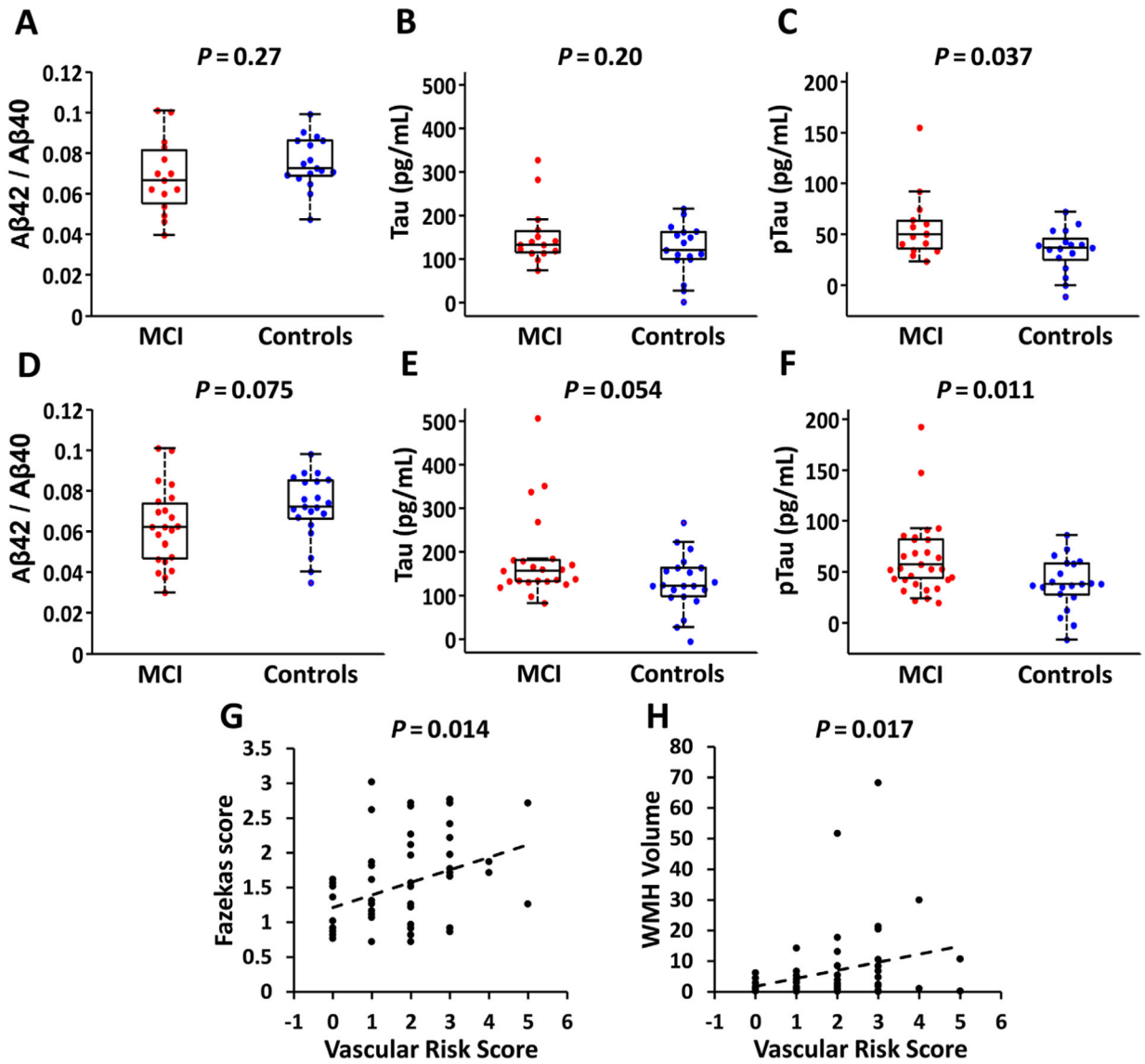


FIGURE 4: Summary of Alzheimer disease (AD) markers, vascular risks, and white matter hyperintensities (WMH) in the participants. (A–C) Box plots of AD pathological markers (Aβ42/Aβ40, tau, and ptau) between mild cognitive impairment (MCI) and control groups who received water extraction with phase contrast arterial spin tagging (WEPCAST) magnetic resonance imaging (MRI; n = 32, 15 MCI and 17 controls). (D–F) Box plots of AD pathological markers between MCI and control groups after including additional participants who provided cerebrospinal fluid samples but did not receive WEPCAST MRI (n = 44, 23 MCI and 21 controls). (G) Scatter plot between vascular risk factors and Fazekas score in the WEPCAST cohort. (H) Scatter plot between vascular risk factors and WMH volume in the WEPCAST cohort.

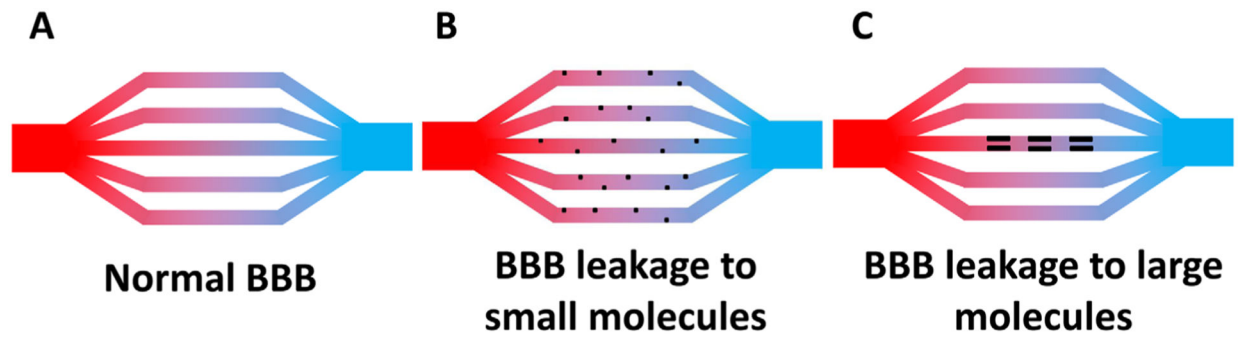


FIGURE 5:

An illustration that blood–brain barrier (BBB) leakage can be specific to small and large molecules. (A) Normal BBB. (B) BBB that is leaky to small molecules, for example water, but not to large molecules. Black dots indicate leakage points. Numerous small leakages are present throughout the capillaries. (C) BBB that is leaky to large molecules, for example albumin. A limited number of leakage points are present, causing slow leaking of albumin, but their impact on water permeability is small because the leakage points are only present in a subset of capillaries (in this example, one-fifth).

TABLE 1.
Demographic, Clinical, and Biomarker Information of All Participants (Mean ± Standard Deviation)

Characteristic	All Participants		Participants with CSF Samples		P
	MCI	Controls	MCI	Controls	
n	33	22	17	17	
Age, yr	68.2 ± 8.1	68.5 ± 6.2	68.1 ± 8.9	70.6 ± 5.4	0.32
Females, n (%)	16 (48.5%)	13 (59.1%)	8 (47.1%)	11 (64.7%)	0.31
Education, yr	15.6 ± 3.6	16.6 ± 2.6	14.9 ± 3.8	16.5 ± 2.8	0.17
Episodic memory	-0.15 ± 0.65	0.76 ± 0.52	-0.34 ± 0.74	0.79 ± 0.53	<0.001
Executive function	-0.24 ± 0.55	0.64 ± 0.41	-0.35 ± 0.55	0.62 ± 0.44	<0.001
Language	-0.0096 ± 0.59	0.48 ± 0.54	-0.042 ± 0.54	0.50 ± 0.55	0.025
Processing speed	-0.055 ± 0.78	0.58 ± 0.58	-0.31 ± 0.80	0.50 ± 0.47	0.0048
Composite cognition	-0.11 ± 0.44	0.62 ± 0.29	-0.26 ± 0.45	0.60 ± 0.31	<0.001
MoCA	25.39 ± 2.47	27.59 ± 1.72	25.35 ± 2.74	27.59 ± 1.94	0.0013
Vascular risk score	1.91 ± 1.26	1.73 ± 1.24	1.71 ± 0.99	1.35 ± 1.00	0.31
Hypercholesterolemia, n (%)	20 (60.6%)	13 (59.1%)	11 (64.7%)	8 (47.1%)	0.31
Hematocrit, %	41.8 ± 3.6	42.2 ± 3.0	41.4 ± 3.5	42.0 ± 2.8	0.56
Temporal lobe, %	9.25 ± 0.40	9.47 ± 0.35	9.19 ± 0.44	9.42 ± 0.35	0.067
CBF, ml/100g/min	49.68 ± 9.47	47.39 ± 8.47	50.33 ± 10.78	47.95 ± 9.39	0.66
<i>APOE4</i> status	no E4, n = 21 1 E4, n = 9 2 E4, n = 3	no E4, n = 19 1 E4, n = 3 2 E4, n = 0	no E4, n = 9 1 E4, n = 5 2 E4, n = 3	no E4, n = 14 1 E4, n = 3 2 E4, n = 0	0.035
CSF NFL, pg/ml			2,578.5 ± 802.5	2,281.0 ± 850.2	0.11
Had AD pathology samples, n			15	17	
Aβ42/Aβ40			0.070 ± 0.022	0.073 ± 0.013	0.27
Total tau, pg/ml			141.32 ± 86.08	133.35 ± 56.38	0.20
ptau, pg/ml			49.87 ± 47.99	40.66 ± 15.05	0.037

AD = Alzheimer disease; CBF = cerebral blood flow; CSF = cerebrospinal fluid; MCI = mild cognitive impairment; MoCA = Montreal Cognitive Assessment; NFL = neurofilament light chain.

TABLE 2.
Relationship between Blood–Brain Barrier Permeability to Water (PS) and Cognition

Cognition	Coefficient for PS	95% CI	Corrected CI	Uncorrected <i>p</i>	Corrected <i>p</i>
Episodic memory	-0.011	-0.018 to -0.0039	-0.020 to -0.0019	0.0028	0.011
Executive function	-0.0025	-0.0091 to 0.0042	-0.011 to 0.0061	0.46	0.92
Language	-0.0065	-0.012 to -0.0014	-0.013 to 0.000082	0.014	0.053
Processing speed	-0.00055	-0.0085 to 0.0074	-0.011 to 0.0098	0.89	1.00
Composite score	-0.0051	-0.0099 to -0.00022	-0.0099 to -0.00022	0.041	0.041
MoCA score	-0.017	-0.040 to 0.0066	-0.040 to 0.0066	0.16	0.16

The coefficient of the relationship is written in the unit of z score per ml/100g/min.

CI = confidence interval; MoCA = Montreal Cognitive Assessment; PS = permeability–surface area product.

Relationship between Blood–Brain Barrier Permeability to Albumin (CSF/Serum Albumin Ratio) and Vascular Risks

TABLE 3.

Vascular Risks	Coefficient for Vascular Risks	95% CI	Corrected CI	Uncorrected <i>p</i>	Corrected <i>p</i>
Hypertension	0.83	−0.43 to 2.09	−0.86 to 2.53	0.19	0.65
Hypercholesterolemia	1.74	0.67 to 2.80	0.30 to 3.17	0.0023	0.011
Diabetes	N/A ^a	N/A	N/A	N/A	N/A
Smoking	0.39	−0.96 to 1.74	−1.43 to 2.21	0.56	0.96
Body mass index	−0.20	−2.07 to 1.67	−2.72 to 2.31	0.83	1.00
Vascular risk score	0.79	0.19 to 1.39	0.19 to 1.39	0.012	0.012

^aNone of the diabetic participants received LP due to their use of anticoagulants such as warfarin and dabigatran or antiplatelets such as Plavix, which represented a contraindication to LP. Thus, the association of CSF/serum albumin ratio with diabetes was not studied.

CI = confidence interval; CSF = cerebrospinal fluid; LP = lumbar puncture; N/A = not applicable.