Blood-brain barrier integrity impacts the use of plasma amyloid-β as a proxy of brain amyloid-β pathology

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Abstract

Introduction: Amyloid-β (Aβ) and tau can be quantified in blood. However, biological factors can influence the levels of brain-derived proteins in the blood. The blood-brain barrier (BBB) regulates protein transport between cerebrospinal fluid (CSF) and blood. BBB altered permeability might affect the relationship between brain and blood biomarkers.

Methods: We assessed 224 participants in research (TRIAD, n = 96) and clinical (BIODEGMAR, n = 128) cohorts with plasma and CSF/positron emission tomography Aβ, p-tau, and albumin measures.

Results: Plasma Aβ42/40 better identified CSF Aβ42/40 and Aβ-PET positivity in individuals with high BBB permeability. An interaction between plasma Aβ42/40 and BBB permeability on CSF Aβ42/40 was observed. Voxel-wise models estimated that the association of positron emission tomography (PET), with plasma Aβ was most affected by BBB permeability in AD-related brain regions. BBB permeability did not significantly impact the relationship between brain and plasma p-tau levels.

Discussion: These findings suggest that BBB integrity may influence the performance of plasma Aβ, but not p-tau, biomarkers in research and clinical settings.

Highlights: BBB permeability affects the association between brain and plasma Aβ levels. BBB integrity does not affect the association between brain and plasma p-tau levels. Plasma Aβ was most affected by BBB permeability in AD-related brain regions. BBB permeability increases with age but not according to cognitive status.

Keywords: Alzheimer's disease; amyloid-β; blood biomarkers; blood-brain barrier; confounding factors.

Introduction

Alzheimer's disease (AD) is the most prevalent neurodegenerative disease worldwide, and the presence of hallmark proteinopathies - amyloid-β (Aβ) and tau tangles - is required for *postmortem* diagnosis (1). Recent advances in neuroimaging and fluid biomarkers made it possible to diagnose and monitor these proteins in living individuals (2). High accuracy in detecting brain levels of AD pathophysiology *in vivo* is achieved by measuring Aβ and tau proteins in the cerebrospinal fluid (CSF) and using positron emission tomography (PET). Although reliable, their use in clinical practice and trials is a challenge due to their invasiveness, availability, and cost (3).

While robust results have been reported using plasma Aβ and phosphorylated tau (p-tau) for detecting brain AD pathophysiology (4–6), significant variability in biomarkers performance is observed among clinical cohorts (7,8). Biological factors may account for this variability observed between individuals and, therefore, limit the performance of AD plasma biomarkers (9). In this regard, because the brain is not in direct contact with the periphery, the blood-brain barrier (BBB) is one biological factor that is very likely influencing the relationship between brain and blood biomarkers levels.

The BBB is a highly-specialized structure composed of endothelial cells, astrocytes, and pericytes that maintain the brain's chemical composition by regulating the exchange of nutrients, inflammatory mediators, and other proteins between the brain and blood (10). Several *in vitro* and animal studies revealed a major role of the BBB in determining concentrations of proteins in biofluids by regulating its specialized transport (11–13). In this sense, the transport across the BBB represents up to 75% of the Aβ isoforms exchange between

the brain and circulatory system (14–16), while the clearance of tau is believed to occur mostly through the interstitial fluid (ISF) bulk flow into the CSF. Altogether, these studies suggest that the loss of BBB integrity can potentially create an unbalance in the transport of proteins (especially Aβ) between the brain and blood, affecting the relationship of biomarkers between these compartments.

Here, we evaluated the impact of BBB permeability, as measured by the validated CSF/serum albumin ratio (17), on the associations between plasma and brain (CSF/PET) AD core biomarkers across the aging and AD spectrum. We hypothesize that individuals with increased BBB permeability will present stronger associations between brain and plasma AD proteins.

Results

Participants

We studied 95 individuals, including 21 young CU adults (age = 23.6 ± 2.8), 48 elderly CU (age = 69.4 ± 8.7) and 26 CI individuals (age = 70.3 ± 7.2). The elderly cognitively unimpaired (CU) and cognitively impaired (CI) individuals were divided into low and high BBB permeability based on their CSF/serum albumin ratio. No significant differences regarding age, APOE ε4 carrier status, fluid and imaging Aβ and tau levels, and cognitive status were observed between individuals with low and high BBB permeability (**Table 1**). The demographic and clinical characteristics of the elderly participants enrolled in this study are summarized in **Table 1**. Demographics of CU young individuals are depicted in **Supplemental Table 1**.

BBB permeability associated with age but not cognitive status

We found a significant increase in CSF/serum albumin ratio in elderlies (CU and CI) individuals compared to CU young individuals ($P < 0.0001$, **Fig. 1A**), but no difference between CU elderlies and CI participants ($P = 0.997$, **Fig. 1A**). Furthermore, we observed a positive correlation between CSF/serum albumin ratio and age ($r = 0.45$, $P < 0.0001$, **Fig. 1B**), but no correlation was found with MMSE score ($P = 0.86$, **Fig. 1C**).

BBB permeability affected the association between brain and plasma Aβ levels

We first tested the direct association of CSF/serum albumin ratio with AD core biomarkers. CSF/serum albumin ratio did not significantly associate with $A\beta_{42/40}$ or p-tau levels in the CSF and plasma (**Supplemental Table 2**).

We further explored the impact of BBB integrity on the relationship between CSF and plasma AD biomarkers. Considering the entire population, we observed that plasma $A\beta_{42/40}$ ratio did not significantly associate neither with CSF $A\beta_{42/40}$ or Aβ-PET ($\beta = 0.14$, $P = 0.19$, **Fig. 2A** and $\beta = -0.02$, $P = 0.86$, **Fig. 2D**). When we divided the population into individuals with low and high CSF/serum albumin ratio (BBB permeability), we observed that plasma $A\beta_{42/40}$ was significantly associated with CSF $A\beta_{42/40}$ only in individuals with high BBB permeability ($\beta = 0.75$, $P = 0.00028$, **Fig. 2B** and $\beta = -0.11$, $P = 0.41$, **Fig. 2C**). Additionally, CSF/serum albumin ratio significantly moderated the association of plasma $A\beta_{42/40}$ on CSF $A\beta_{42/40}$ ratio (**Table 2**). Similarly, plasma $A\beta_{42/40}$ associated with neocortical Aβ-PET in individuals with high BBB permeability ($\beta = -0.30$, $P = 0.016$, **Fig. 2E**), whereas no significant associations was observed in individuals with low BBB permeability ($\beta = 0.05$, $P = 0.73$, **Fig. 2F**). Associations segregated by groups for both plasma $A\beta_{42}$ and $A\beta_{40}$ isoforms are shown in **Supplemental Figure 1**.

P-tau presented a strong association between plasma and brain in the entire population, and it was not affected by BBB permeability (**Table 3**). Additionally, no moderation effect was observed between CSF/serum albumin ratio and plasma p-tau on CSF p-tau or Tau-PET levels (**Table 3**).

Voxel-wise analysis estimated brain regions where the association between brain and plasma Aβ was more affected by BBB permeability

We performed voxel-wise analysis to indirectly investigate the topographical associations of Aβ-PET with plasma $A\beta_{42/40}$ as a function of BBB permeability status. For the individuals with high BBB permeability, we observed significant associations between Aβ-PET SUVR and plasma $A\beta_{42/40}$ in the frontal, cingulate, and precuneus cortices (peak t-value = 6.7, $P < 0.0001$, **Fig. 3A – left**). No associations were observed in the group presenting low BBB permeability (**Fig. 3A – right**; **Table 2**). A voxel-wise moderation analysis revealed a significant moderation effect of CSF/serum albumin on the association between plasma $A\beta_{42/40}$ and Aβ-PET (peak t-value = 3.8, $P = 0.0003$, **Fig. 3B**).

High BBB permeability is associated with elevated plasma $A\beta_{42/40}$ fold change between Aβ-positive and -negative individuals

We measured the fold change between Aβ- and Aβ+ according to BBB permeability. Aβ+ individuals with low BBB permeability presented no significant differences in plasma $A\beta_{42/40}$ levels compared to Aβ- (**Fig. 4A, D**). In the high BBB permeability group, plasma $A\beta_{42/40}$ levels were significantly different between Aβ- and Aβ+, as classified by CSF $A\beta_{42/40}$ (**Fig. 4A**, $P = 0.0002$) or Aβ-PET (**Fig. 4D**, $P = 0.022$). The fold change for individuals with high BBB permeability was as great as 62%, while the low BBB permeability group was not higher than 4% (**Fig. 4B, E)**.

On the other hand, plasma p-tau concentrations distinguished CSF/PET T- from T+ individuals independently of BBB permeability (**Fig. 4G, J**). A high fold change in plasma p-tau between T- and T+ individuals was observed in either low or high BBB permeability groups (**Fig. 4H, K**).

Plasma Aβ showed better performance to identify Aβ positivity in individuals with high BBB permeability

We tested whether BBB permeability influences the discriminative performance of plasma Aβ to predict Aβ positivity measured with CSF $A\beta_{42/40}$ or Aβ-PET. In the entire study population, plasma $A\beta_{42/40}$ (accounting for age and sex) discriminated Aβ-positive from Aβ-negative individuals with an area under the curve (AUC) of 0.66 (CI = 0.53 – 0.79) and 0.633 (CI = 0.50 – 0.75) using CSF $A\beta_{42/40}$ or Aβ-PET to define groups, respectively (**Table 4**). Considering only the population with high BBB permeability, plasma $A\beta_{42/40}$ ratio reached an AUC of 0.933 for predicting CSF $A\beta_{42/40}$ (CI = 0.81 – 1) and 0.974 for Aβ-PET (CI = 0.90 – 1) positivity, which was significantly higher than the AUCs obtained from the entire population or the population with low BBB permeability [Aβ+ defined with CSF $A\beta_{42/40}$, AUC = 0.532 (CI = 0.36 – 0.71), and Aβ+ defined with Aβ-PET, AUC = 0.588 (CI = 0.41 – 0.75)] (**Table 4**; **Fig. 4C, F**).

The accuracy of plasma p-tau for detecting CSF p-tau [high BBB permeability AUC = 0.91 (CI = 0.77 – 0.99), low BBB permeability AUC = 0.82 (CI = 0.69 – 0.93)] (**Table 4**; **Fig. 4I**), or Tau-PET positivity [high BBB permeability AUC = 0.84 (CI = 0.64 – 0.99), low BBB permeability AUC = 0.75 (CI = 0.61 – 0.89)] (**Table 4**; **Fig. 4L**) was not significantly affected by the BBB permeability.

Plasma Aβ and p-tau associate with each other only in individuals with high BBB permeability

Lastly, we investigated whether the BBB influences the association between plasma $A\beta_{42/40}$ and plasma p-tau. Plasma $A\beta_{42/40}$ and plasma p-tau did not significantly associate when considering the entire population (β = -0.21, P = 0.11, **Fig. 5A**). However, a negative association was observed between plasma $A\beta_{42/40}$ and p-tau in individuals with high BBB permeability (β = -0.47, P = 0.015, **Fig. 5B**), but not in individuals with low BBB permeability (β = -0.11, P = 0.57, **Fig. 5C**).

Discussion

We showed that brain and plasma Aβ pools are only associated with one another in individuals with a high CSF/serum albumin ratio (a well-validated index of increased BBB permeability (17). Furthermore, high BBB permeability is associated with a higher fold change of plasma Aβ levels between CSF/PET Aβ positive and negative groups and a stronger association between plasma Aβ and tau pathology. These results suggest that plasma Aβ levels better represent brain Aβ levels in individuals with a high BBB permeability, which may have repercussions for future use of these biomarkers in research and clinical practice.

Decreased BBB integrity improved the performance of plasma Aβ in detecting brain Aβ pathology. Aβ presents brain as well as an important peripheral production (18,19). Aβ exchange between the brain and blood compartments is regulated mainly through BBB transporters [e.g., advanced glycosylation end product-specific receptor (RAGE) and low-density lipoprotein receptor-related protein (LRP)] with the contribution of the circulatory/lymphatic system (14,15,20). Our results may represent that an increase in BBB permeability changes the equilibrium between CSF and blood towards plasma Aβ being more

representative of CSF Aβ concentrations than peripheral Aβ production. If the disruption of this equilibrium is due to a freer exchange between compartments or impairment of transporters' function remains to be elucidated. Furthermore, the BBB-dependent association of plasma Aβ with plasma p-tau showed in our results reinforced that in individuals with high BBB permeability, plasma Aβ better represents brain AD pathophysiology.

BBB permeability did not affect the relationship between brain and plasma levels of p-tau. Tau clearance from the central nervous system is relatively less understood than Aβ clearance. However, differently from Aβ, no tau transporters through the BBB have been identified at the moment, suggesting that its clearance does not heavily occur via BBB (15). In this sense, tau clearance seems to occur mainly by lysosomal degradation, ISF bulk flow, and CSF absorption (21,22). These results corroborate previous findings indicating an association of BBB permeability with Aβ but not p-tau (30). Our findings might contribute to understanding the lower biological variability observed in plasma p-tau biomarkers compared to plasma Aβ (23,24).

We observed a prominent increase in CSF/serum albumin ratio in the elderly compared to young individuals but no difference between elderly CU and CI. Consistently with our findings, it was already demonstrated in animal (25) and human (26) studies that BBB permeability increases with normal aging. Conversely, studies investigating CSF/serum albumin ratio changes in AD individuals present conflicting results (27–31). A recent meta-analysis showed that this ratio is elevated in individuals with AD dementia but with a small effect size (7), making it challenging to observe significant differences in relatively small populations such as ours.

Contrasting with the lack of association between BBB integrity and dementia symptoms, we identified that BBB integrity influenced the association of Aβ-PET with plasma Aβ mainly in brain regions well-known to show the highest levels of Aβ and tau pathologies (e.g., precuneus, posterior cingulate)(32). Regional differences in BBB permeability were already reported in neuropathological disorders (33). Interestingly, an MRI study using a water exchange technique has recently evidenced that BBB damage is associated with CSF $A\beta_{42}$ levels in the same brain regions observed here (34). Although fluid albumin measurements are not capable of detecting the topographical distribution of BBB damage, our voxel-wise models statistically estimated regions with the highest dissociation between the variabilities of PET and plasma Aβ levels. Future imaging studies are needed to elucidate whether BBB damage overlaps with AD proteinopathies and possible mechanistic underpinnings linking pathologies.

The results presented in this study should be interpreted considering some limitations. First, although highly accepted in the literature, BBB damage in our study was indirectly determined based exclusively on CSF/serum albumin ratio. Currently, there is no gold standard for measuring BBB damage in living persons, and the available markers very likely offer complementary information regarding BBB abnormalities. The CSF/serum albumin ratio is based on the fact that albumin is a relatively large plasma protein that is produced by the liver and lacks a canonical transporter in the BBB; thus, it is detected in the CSF in large quantities only when the permeability of the BBB is increased (35). Assessing this BBB integrity using other available markers, such as brain imaging (31,34), could present different results and provide more basis for claims on the spatial localization of BBB damage. Additionally, Lin and colleagues demonstrated that changes in the BBB permeability to water detected by MRI, but not with CSF/serum albumin ratio were associated with cognitive decline (31). Thus, BBB permeability assessed with imaging can be more associated with cognition than assessed by

CSF/serum albumin ratio. Other fluid markers, such as the soluble platelet-derived growth factor receptor β (sPDGFRβ), a marker of pericyte damage, have been investigated in AD and seem to present a better association with AD-related brain damage than the CSF/serum albumin ratio (30,36,37). Thus, further investigations exploring other markers of BBB integrity are crucial to understanding the extent of BBB damage required for impacting the relationship between brain and blood Aβ levels. Additionally, plasma Aβ levels in this study were measured using the Simoa platform. It has been shown that different plasma Aβ assays present variable performances across different assays and platforms (38,39). Thus, whether the findings presented here can be generalized to other available Aβ assays remains unclear. It would be highly desirable to replicate these results in other cohorts and using different plasma Aβ assays.

Our results suggested that plasma Aβ is a better proxy of brain Aβ pathology in individuals with a more prominent BBB breakdown. These findings raise discussion on the role of BBB integrity in the performance of plasma Aβ biomarkers and might help understand the origins of some of the variability found in plasma Aβ studies.

Methods

Study population

This study included participants from the Translational Biomarkers in Aging and Dementia (TRIAD) cohort (Montreal, Canada)(40). Participants had a detailed clinical and cognitive assessment, including the Mini-Mental State Examination (MMSE) and the Clinical Dementia Rating (CDR). CU individuals had no objective cognitive deficits and a CDR = 0. Mild cognitive impairment (MCI) subjects had subjective and objective cognitive deficits, relatively preserved activities of daily living, and a CDR = 0.5. AD patients had a CDR = 0.5-2 and met the National Institute on Aging and the Alzheimer's Association criteria for probable

AD determined by a clinician (1). MCI and AD dementia individuals were grouped in a CI group. Then, elderly CU and CI individuals were divided into low and high BBB permeability groups based on their CSF/serum albumin levels. The participants were excluded if they had inadequately treated systemic conditions, active substance abuse, major head trauma or surgery, or magnetic resonance imaging/PET safety contraindication. The study was approved by the Douglas Mental Health University Institute Research Ethics Board and Montreal Neurological Instituted PET working committee, and written informed consent was obtained from the participants.

Fluid Biomarkers

CSF and plasma samples were analyzed at the Clinical Neurochemistry Laboratory, University of Gothenburg, Sweden. CSF $A\beta_{40}$ and $A\beta_{42}$ were measured using the LUMIPULSE G1200 (Fujirebio). CSF p-tau at threonine 231 was quantified using a research enzyme-linked immunosorbent assay (ELISA) assay using a cis-conformational selective monoclonal antibody (ADx253, ADx NeuroSciences). Plasma $A\beta_{40}$ and $A\beta_{42}$ were evaluated using validated commercially available Simoa assays (4,6). Serum albumin was measured using a previously developed colorimetric method (41). CSF albumin was measured by a turbidimetric method performed on the binding site optilite. To identify individuals with high BBB permeability, we divided CSF/plasma albumin ratio values in terciles. Participants in the upper tercile were classified as high BBB permeability and individuals in the lower two terciles as low BBB permeability. CSF $A\beta_{42/40}$ and p-tau_{231} cutoffs were statically defined based on young CU (42,43).

MRI/PET Biomarkers

Aβ-PET was quantified using [^{18}F]AZD4694 at 40–70 min post-injection and Tau-PET with [^{18}F]MK-6240 at 90–110 min post-injection using a Siemens High Resolution Research Tomograph. [^{18}F]AZD4694 scans were reconstructed using the ordered subset expectation maximization algorithm on a 4D volume with three frames (3 x 600 seconds) and [^{18}F]MK-6240 with four frames (4 x 300 seconds). Standardized uptake value ratio (SUVR) was calculated using the whole cerebellum gray matter for [^{18}F]AZD4694 and full cerebellum gray matter for [^{18}F]MK-6240 as reference. Neocortical [^{18}F]AZD4694 SUVR value was estimated from precuneus, prefrontal, orbitofrontal, parietal, temporal, anterior and posteriors cingulate cortices. Individuals with Aβ-PET SUVR > 1.55 were considered Aβ-positive (40). [^{18}F]MK-6240 SUVR was measured in the transentorhinal/entorhinal region (Braak I–II) (6) and tau positivity was defined as 2.5 standard deviations (SD) higher than the mean SUVR of young CU individuals.

Statistical analysis

Neuroimaging analyses were carried out using the VoxelStats toolbox (https://github.com/sulantha2006/VoxelStats), a MATLAB-based analytical framework that allows for the execution of multimodal voxel-wise neuroimaging analyses (44). Other statistical analyses were performed using the R Statistical Software Package version 3.5.3. Comparisons of demographic characteristics between groups were performed using $\chi 2$ tests for categorical and t-tests for continuous variables. The associations between biomarkers were assessed with Pearson correlation or linear regression accounting for age, clinical diagnosis, sex, and Bonferroni correction for multiple comparisons. Comparison of groups was performed using one-way ANOVA followed by Tukey post hoc test. Three individuals did not have available CSF Aβ levels and, thus, were removed from the analysis involving CSF. Plasma $A\beta_{42/40}$ discriminative performance was assessed with Receiver Operator Characteristic (ROC)

curve ROC area under the curve (AUC). AUCs were compared using DeLong test followed by false discovery rate (FDR) multiple comparison correction. To investigate the impact of BBB permeability on the relationship between plasma $A\beta_{42/40}$ and Aβ-PET, we performed a moderation analysis (45). In the voxel-based analyses, multiple comparisons correction was performed using random field theory (RFT), with a threshold of $P < 0.05$.

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Author contributions

BB and TAP created the concept and design of the study. Data acquisition was performed by CT, ALB, NJA, SS, FZL, JT, MC, JS, NR, TKK, KB, HZ, PR-N and TAP. BB, JPF-S, DTL and FZL performed data analysis. BB, JPF-S, DTL, FZL, PCLF, CT and TAP contributed to interpretation of data. BB drafted the manuscript, and all authors revised it. All authors read and approved the final manuscript.

Disclosure and competing interests statement

HZ has served at scientific advisory boards and/or as a consultant for Abbvie, Alector, Annexon, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, Pinteon Therapeutics, Red Abbey Labs, Passage Bio, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen, and Roche, and is a co-founder of Brain Biomarker Solutions in

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References

1. 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 Jul;34(7):939–44.
2. Jack CRJ, Bennett DA, Blennow K, Carrillo MC, Dunn B, Haeberlein SB, et al. NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. Alzheimers Dement. 2018 Apr;14(4):535–62.
3. Molinuevo JL, Ayton S, Batrla R, Bednar MM, Bittner T, Cummings J, et al. Current state of Alzheimer's fluid biomarkers. Acta Neuropathol. 2018 Dec;136(6):821–53.
4. Karikari TK, Pascoal TA, Ashton NJ, Janelidze S, Benedet AL, Rodriguez JL, et al. Blood phosphorylated tau 181 as a biomarker for Alzheimer's disease: a diagnostic performance and prediction modelling study using data from four prospective cohorts. Lancet Neurol. 2020 May;19(5):422–33.
5. Palmqvist S, Janelidze S, Quiroz YT, Zetterberg H, Lopera F, Stomrud E, et al. Discriminative Accuracy of Plasma Phospho-tau217 for Alzheimer Disease vs Other Neurodegenerative Disorders. JAMA [Internet]. 2020 Aug 25;324(8):772–81. Available from: https://doi.org/10.1001/jama.2020.12134
6. Ashton NJ, Pascoal TA, Karikari TK, Benedet AL, Lantero-Rodriguez J, Brinkmalm G, et al. Plasma p-tau231: a new biomarker for incipient Alzheimer's disease pathology. Acta Neuropathol. 2021 May;141(5):709–24.
7. Olsson B, Lautner R, Andreasson U, Öhrfelt A, Portelius E, Bjerke M, et al. CSF and

blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis. Lancet Neurol. 2016 Jun;15(7):673–84.

8. Chong JR, Ashton NJ, Karikari TK, Tanaka T, Schöll M, Zetterberg H, et al. Blood-based high sensitivity measurements of beta-amyloid and phosphorylated tau as biomarkers of Alzheimer's disease: a focused review on recent advances. J Neurol Neurosurg Psychiatry. 2021 Nov;92(11):1231–41.
9. Galasko D, Golde TE. Biomarkers for Alzheimer's disease in plasma, serum and blood - conceptual and practical problems. Alzheimers Res Ther. 2013;5(2):10.
10. Zenaro E, Piacentino G, Constantin G. The blood-brain barrier in Alzheimer's disease. Neurobiol Dis. 2017 Nov;107:41–56.
11. Saito Y, Buciak J, Yang J, Pardridge WM. Vector-mediated delivery of 125I-labeled beta-amyloid peptide A beta 1-40 through the blood-brain barrier and binding to Alzheimer disease amyloid of the A beta 1-40/vector complex. Proc Natl Acad Sci U S A. 1995 Oct;92(22):10227–31.
12. Mackic JB, Stins M, McComb JG, Calero M, Ghiso J, Kim KS, et al. Human blood-brain barrier receptors for Alzheimer's amyloid-beta 1- 40. Asymmetrical binding, endocytosis, and transcytosis at the apical side of brain microvascular endothelial cell monolayer. J Clin Invest. 1998 Aug;102(4):734–43.
13. Zlokovic B V, Martel CL, Matsubara E, McComb JG, Zheng G, McCluskey RT, et al. Glycoprotein 330/megalin: probable role in receptor-mediated transport of apolipoprotein J alone and in a complex with Alzheimer disease amyloid beta at the blood-brain and blood-cerebrospinal fluid barriers. Proc Natl Acad Sci U S A. 1996 Apr;93(9):4229–34.
14. Shibata M, Yamada S, Kumar SR, Calero M, Bading J, Frangione B, et al. Clearance of Alzheimer's amyloid-ss(1-40) peptide from brain by LDL receptor-related protein-

1 at the blood-brain barrier. J Clin Invest. 2000 Dec;106(12):1489–99.

15. Tarasoff-Conway JM, Carare RO, Osorio RS, Glodzik L, Butler T, Fieremans E, et al. Clearance systems in the brain-implications for Alzheimer disease. Nat Rev Neurol. 2015 Aug;11(8):457–70.

16. Roberts KF, Elbert DL, Kasten TP, Patterson BW, Sigurdson WC, Connors RE, et al. Amyloid-β efflux from the central nervous system into the plasma. Ann Neurol. 2014 Dec;76(6):837–44.

17. Reiber H, Peter JB. Cerebrospinal fluid analysis: disease-related data patterns and evaluation programs. J Neurol Sci. 2001 Mar;184(2):101–22.

18. Roher AE, Esh CL, Kokjohn TA, Castaño EM, Van Vickle GD, Kalback WM, et al. Amyloid beta peptides in human plasma and tissues and their significance for Alzheimer's disease. Alzheimers Dement. 2009 Jan;5(1):18–29.

19. Seubert P, Vigo-Pelfrey C, Esch F, Lee M, Dovey H, Davis D, et al. Isolation and quantification of soluble Alzheimer's beta-peptide from biological fluids. Nature. 1992 Sep;359(6393):325–7.

20. Iliff JJ, Wang M, Liao Y, Plogg BA, Peng W, Gundersen GA, et al. A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid β. Sci Transl Med. 2012 Aug;4(147):147ra111.

21. Iliff JJ, Chen MJ, Plog BA, Zeppenfeld DM, Soltero M, Yang L, et al. Impairment of glymphatic pathway function promotes tau pathology after traumatic brain injury. J Neurosci. 2014 Dec;34(49):16180–93.

22. Wang Y, Martinez-Vicente M, Krüger U, Kaushik S, Wong E, Mandelkow E-M, et al. Tau fragmentation, aggregation and clearance: the dual role of lysosomal processing. Hum Mol Genet. 2009 Nov;18(21):4153–70.

23. Karikari TK, Benedet AL, Ashton NJ, Lantero Rodriguez J, Snellman A, Suárez-

Calvet M, et al. Diagnostic performance and prediction of clinical progression of plasma phospho-tau181 in the Alzheimer's Disease Neuroimaging Initiative. Mol Psychiatry. 2021 Feb;26(2):429–42.

24. Mattsson-Carlgren N, Palmqvist S, Blennow K, Hansson O. Increasing the reproducibility of fluid biomarker studies in neurodegenerative studies. Nat Commun [Internet]. 2020;11(1):6252. Available from: https://doi.org/10.1038/s41467-020-19957-6

25. Yang AC, Stevens MY, Chen MB, Lee DP, Stähli D, Gate D, et al. Physiological blood–brain transport is impaired with age by a shift in transcytosis. Nature [Internet]. 2020;583(7816):425–30. Available from: https://doi.org/10.1038/s41586-020-2453-z

26. Farrall AJ, Wardlaw JM. Blood-brain barrier: ageing and microvascular disease--systematic review and meta-analysis. Neurobiol Aging. 2009 Mar;30(3):337–52.

27. Chalbot S, Zetterberg H, Blennow K, Fladby T, Andreasen N, Grundke-Iqbal I, et al. Blood-cerebrospinal fluid barrier permeability in Alzheimer's disease. J Alzheimers Dis. 2011;25(3):505–15.

28. Janelidze S, Hertze J, Nägga K, Nilsson K, Nilsson C, Wennström M, et al. Increased blood-brain barrier permeability is associated with dementia and diabetes but not amyloid pathology or APOE genotype. Neurobiol Aging. 2017 Mar;51:104–12.

29. Musaeus CS, Gleerup HS, Høgh P, Waldemar G, Hasselbalch SG, Simonsen AH. Cerebrospinal Fluid/Plasma Albumin Ratio as a Biomarker for Blood-Brain Barrier Impairment Across Neurodegenerative Dementias. J Alzheimers Dis. 2020;75(2):429–36.

30. Miners JS, Kehoe PG, Love S, Zetterberg H, Blennow K. CSF evidence of pericyte damage in Alzheimer's disease is associated with markers of blood-brain barrier dysfunction and disease pathology. Alzheimers Res Ther. 2019 Sep;11(1):81.

31. Lin Z, Sur S, Liu P, Li Y, Jiang D, Hou X, et al. Blood-Brain Barrier Breakdown in Relationship to Alzheimer and Vascular Disease. Ann Neurol. 2021 Aug;90(2):227–38.

32. Pascoal TA, Therriault J, Benedet AL, Savard M, Lussier FZ, Chamoun M, et al. 18F-MK-6240 PET for early and late detection of neurofibrillary tangles. Brain. 2020 Sep;143(9):2818–30.

33. Villaseñor R, Kuennecke B, Ozmen L, Ammann M, Kugler C, Grüninger F, et al. Region-specific permeability of the blood-brain barrier upon pericyte loss. J Cereb blood flow Metab Off J Int Soc Cereb Blood Flow Metab. 2017 Dec;37(12):3683–94.

34. Gold BT, Shao X, Sudduth TL, Jicha GA, Wilcock DM, Seago ER, et al. Water exchange rate across the blood-brain barrier is associated with CSF amyloid-β 42 in healthy older adults. Alzheimers Dement. 2021 Dec;17(12):2020–9.

35. Pardridge WM, Eisenberg J, Cefalu WT. Absence of albumin receptor on brain capillaries in vivo or in vitro. Am J Physiol. 1985 Sep;249(3 Pt 1):E264-7.

36. De Kort AM, Kuiperij HB, Kersten I, Versleijen AAM, Schreuder FHBM, Van Nostrand WE, et al. Normal cerebrospinal fluid concentrations of PDGFRβ in patients with cerebral amyloid angiopathy and Alzheimer's disease. Alzheimers Dement. 2021 Dec;

37. Wang J, Fan D-Y, Li H-Y, He C-Y, Shen Y-Y, Zeng G-H, et al. Dynamic changes of CSF sPDGFRβ during ageing and AD progression and associations with CSF ATN biomarkers. Mol Neurodegener [Internet]. 2022;17(1):9. Available from: https://doi.org/10.1186/s13024-021-00512-w

38. Janelidze S, Teunissen CE, Zetterberg H, Allué JA, Sarasa L, Eichenlaub U, et al. Head-to-Head Comparison of 8 Plasma Amyloid-β 42/40 Assays in Alzheimer Disease. JAMA Neurol [Internet]. 2021 Nov 1;78(11):1375–82. Available from:

https://doi.org/10.1001/jamaneurol.2021.3180

39. Pannee J, Shaw LM, Korecka M, Waligorska T, Teunissen CE, Stoops E, et al. The global Alzheimer's Association round robin study on plasma amyloid β methods. Alzheimer's Dement (Amsterdam, Netherlands). 2021;13(1):e12242.

40. Therriault J, Benedet AL, Pascoal TA, Savard M, Ashton NJ, Chamoun M, et al. Determining Amyloid-β Positivity Using (18)F-AZD4694 PET Imaging. J Nucl Med. 2021 Feb;62(2):247–52.

41. Doumas BT, Watson WA, Biggs HG. Albumin standards and the measurement of serum albumin with bromcresol green. Clin Chim Acta. 1971 Jan;31(1):87–96.

42. Tissot C, Therriault J, Kunach P, L Benedet A, Pascoal TA, Ashton NJ, et al. Comparing tau status determined via plasma pTau181, pTau231 and [(18)F]MK6240 tau-PET. EBioMedicine. 2022 Feb;76:103837.

43. Benedet AL, Milà-Alomà M, Vrillon A, Ashton NJ, Pascoal TA, Lussier F, et al. Differences Between Plasma and Cerebrospinal Fluid Glial Fibrillary Acidic Protein Levels Across the Alzheimer Disease Continuum. JAMA Neurol. 2021 Oct;

44. Mathotaarachchi S, Wang S, Shin M, Pascoal TA, Benedet AL, Kang MS, et al. VoxelStats: A MATLAB Package for Multi-Modal Voxel-Wise Brain Image Analysis [Internet]. Vol. 10, Frontiers in Neuroinformatics . 2016. Available from: https://www.frontiersin.org/article/10.3389/fninf.2016.00020

45. Montoya AK. Moderation analysis in two-instance repeated measures designs: Probing methods and multiple moderator models. Behav Res Methods. 2019 Feb;51(1):61–82.

Tables

Table 1. Demographics and key characteristics of participants.

	Low BBB permeability	High BBB permeability	P-value
No.	49	25	
Age, mean (SD)	69.6 (8.46)	69.9 (8.0)	0.85
Sex, % female	60.8	36	0.074
APOEε4 carriers, No. (%)	21 (41.2%)	8 (32%)	0.601
Cognitive status, No. CI. (%)	17 (33.3)	9 (36)	0.99
Plasma $A\beta_{42/40}$, mean (SD)	0.038 (0.013)	0.039 (0.01)	0.829
CSF $A\beta_{42/40}$, mean (SD)	0.069 (0.023)	0.070 (0.025)	0.877
Neocortical Aβ-PET SUVR, mean (SD)	1.69 (0.569)	1.65 (0.5)	0.773
Plasma p-tau231, mean pg/ml (SD)	18.9 (11.4)	20.2 (11.1)	0.624
Tau-PET SUVR, mean (SD)	1.19 (0.74)	1.17 (0.48)	0.869
CSF p-tau231, mean pg/ml (SD)	457 (406)	435 (238)	0.773

Abbreviations: Aβ: Amyloid-β; APOEε4: apolipoprotein ε4; BBB: blood-brain barrier; CSF: cerebrospinal fluid; CI: cognitively impaired; PET: positron emission tomography; SD: standard deviation; SUVR: standard uptake value ratio.

Table 2. Moderation effect of CSF/serum albumin ratio in the relationship between plasma $A\beta_{42/40}$ and CSF/PET Aβ.

	β (95% CI)	T-value	P-value
Moderation effect of CSF/serum albumin between plasma Aβ isoforms and CSF $A\beta_{42/40}$			
Plasma $A\beta_{42/40}$	0.38 (0.13– 0.62)	3.08	**0.0031***
Plasma $A\beta_{42}$	0.17 (-0.1 – 0.44)	1.28	0.205
Plasma $A\beta_{40}$	-0.21 (-0.44 – 0.02)	-1.82	0.073
Moderation effect of CSF/serum albumin between plasma Aβ isoforms and Aβ-PET SUVR			

Plasma $A\beta_{42/40}$	-0.18 (-0.43 – 0.07)	-1.44	0.156
Plasma $A\beta_{42}$	-0.14 (-0.36 – 0.15)	-1.08	0.285
Plasma $A\beta_{40}$	0.03 (-0.20 – 0.25)	0.226	0.822

Abbreviations: Aβ: Amyloid-β; CSF: cerebrospinal fluid; PET: positron emission tomography; SUVR: standard uptake value ratio. *Significant after Bonferroni correction.

Table 3. Associations between CSF and plasma p-tau according to BBB permeability and moderation effect of CSF/serum albumin ratio in the relationship between plasma and CSF p-tau.

Study population	β (95% CI)	T-value	P-value
Model: CSF p-tau ~ plasma p-tau + clinical diagnosis + age + sex			
All population	0.41 (0.19 – 0.63)	3.71	**0.0004***
Low BBB permeability	0.42 (0.13 – 0.73)	2.85	**0.007***
High BBB permeability	0.44 (0.05 – 0.84)	2.39	**0.031***
Moderation effect of CSF/serum albumin between plasma and CSF p-tau			
All population	0.07 (-0.13 – 0.27)	0.712	0.479

Abbreviations: CSF: cerebrospinal fluid; CI: confidence interval; PET: positron emission tomography; p-tau: phosphorylated tau; SD: standard deviation; SUVR: standard uptake value ratio.

Table 4. Discriminative performance of plasma biomarkers as a function of BBB permeability.

Discrimination	AUC (95% CI)		
	All population	High BBB permeability	Low BBB permeability
CSF $A\beta_{42/40}$ positivity	0.660 (0.53 – 0.79)	0.933 (0.81 – 1)[b,c]	0.532 (0.36 – 0.71)
Aβ-PET positivity	0.633 (0.50 – 0.75)	0.974 (0.90 –1)[a,b]	0.588 (0.41 – 0.75)
CSF p-tau positivity	0.818 (0.71 – 0.92)	0.912 (0.77 – 0.99)	0.82 (0.69 – 0.93)
Tau-PET positivity	0.721 (0.59 – 0.83)	0.844 (0.62 – 0.99)	0.757 (0.62 – 0.88)

Abbreviations: Aβ: Amyloid-β; AUC: area under the curve; BBB: blood-brain barrier; CSF: cerebrospinal fluid; PET: positron emission tomography; ROC: receiver operating characteristic. AUC differences were tested using the DeLong test followed by false discovery rate multiple comparison correction. a: P< 0.0001 compared to all population; b: P< 0.0001 compared to low BBB permeability; c: P = 0.0019 compared to all population.

Figures

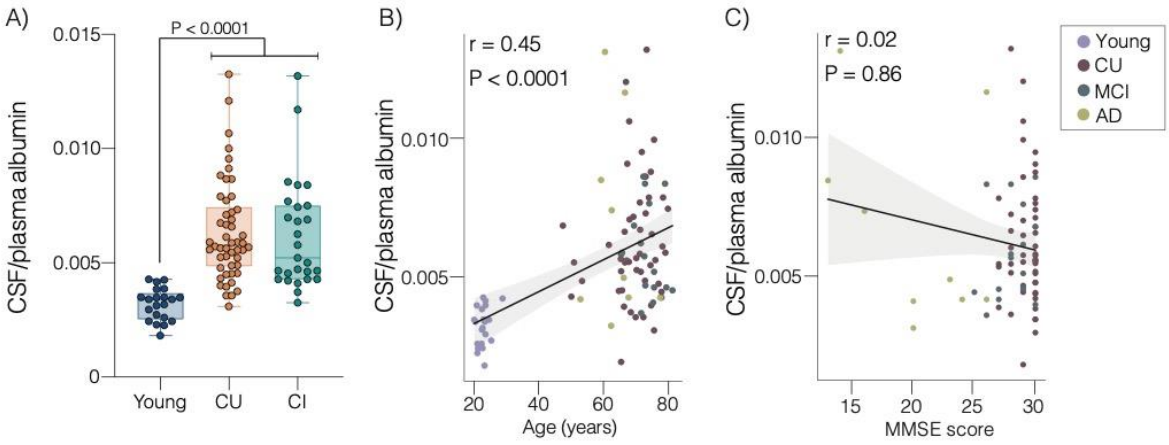


Figure 1. CSF/serum albumin ratio across the aging and AD clinical spectrum. Higher levels of CSF/serum albumin represent higher BBB permeability. **(A)** ANOVA followed by two-tailed Tukey showed that BBB permeability was reduced in young CU compared to older CU adults but was similar between cognitively unimpaired (CU) and cognitively impaired (CI) individuals. **(B)** Pearson correlations showed a correlation of CSF/serum albumin ratio with age but not **(C)** with cognitive performance measured by MMSE score.

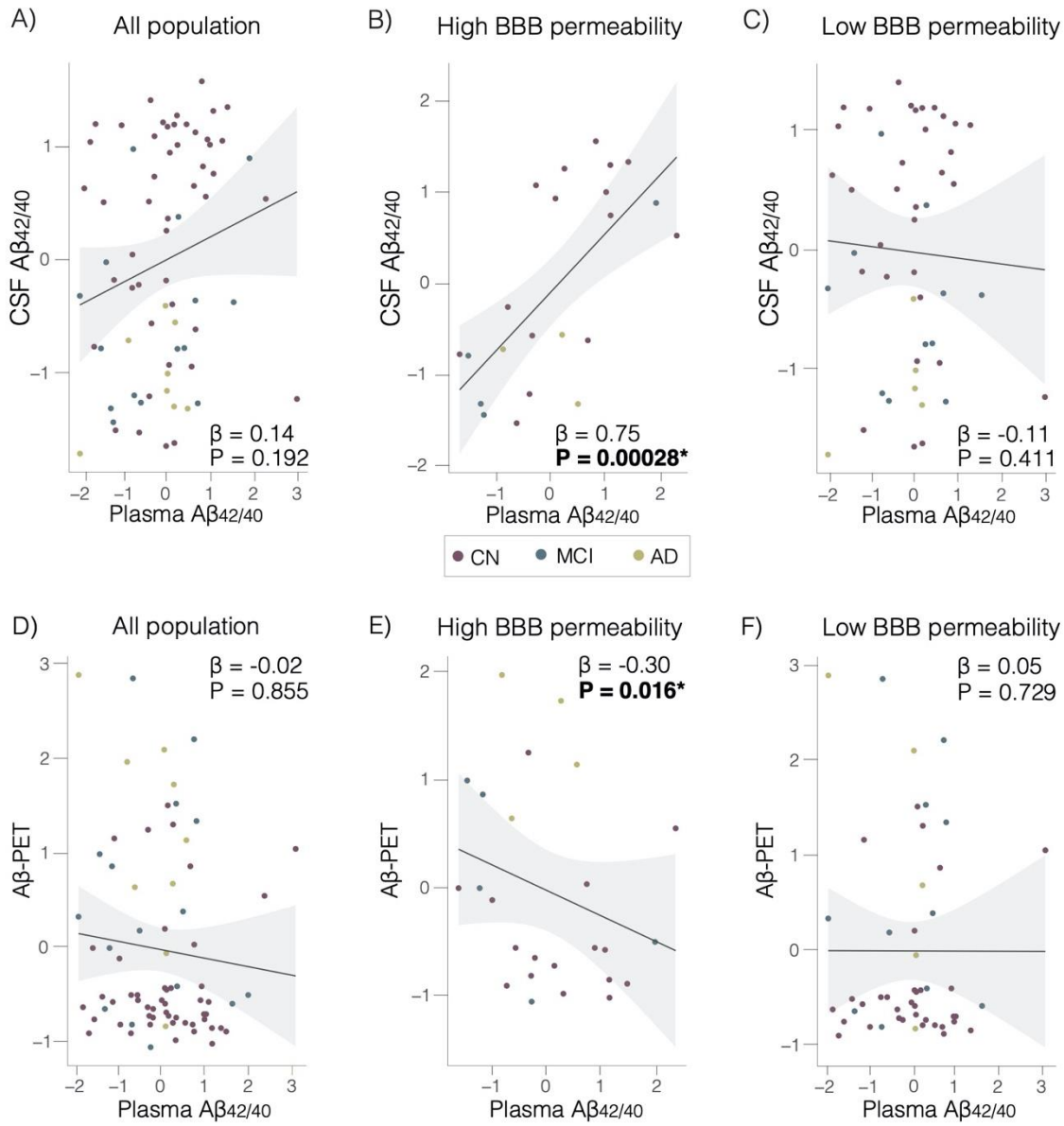


Figure 2. The associations of plasma $A\beta_{42/40}$ with CSF $A\beta_{42/40}$ or Aβ-PET depended on BBB permeability. Scatter plots show the associations between plasma $A\beta_{42/40}$ with CSF $A\beta_{42/40}$ in **(A)** all study population, **(B)** individuals with high BBB permeability, and **(C)** individuals with low BBB permeability. In addition, scatter plots show the associations between plasma $A\beta_{42/40}$ with neocortical Aβ-PET SUVR in **(D)** all study population, **(E)** individuals with high BBB permeability, and **(F)** individuals with low BBB permeability. Lines indicate regression line with their respective 95% confidence intervals. P-values were

computed using linear regression models adjusted by age, sex, and clinical diagnosis. *Represents statistical significance after Bonferroni correction for multiple comparison.

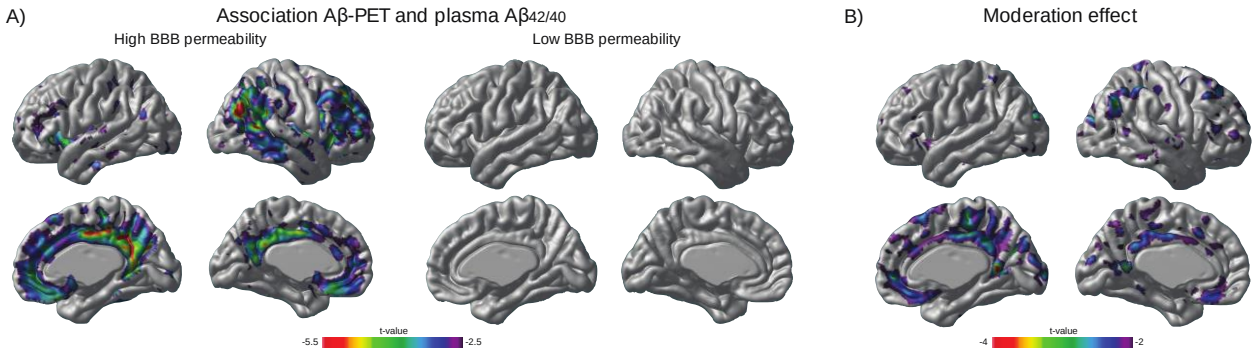


Figure 3. Plasma $A\beta_{42/40}$ associates with Aβ-PET deposition in individuals with high BBB permeability in typical AD brain regions. Regions showing voxel-wise association between plasma $A\beta_{42/40}$ and Aβ-PET in **(A)** individuals with high (left) and low (right) BBB permeability. **(B)** Regions showing significant voxel-wise moderation effects of CSF/albumin ratio in the relationship between plasma $A\beta_{42/40}$ on Aβ-PET.

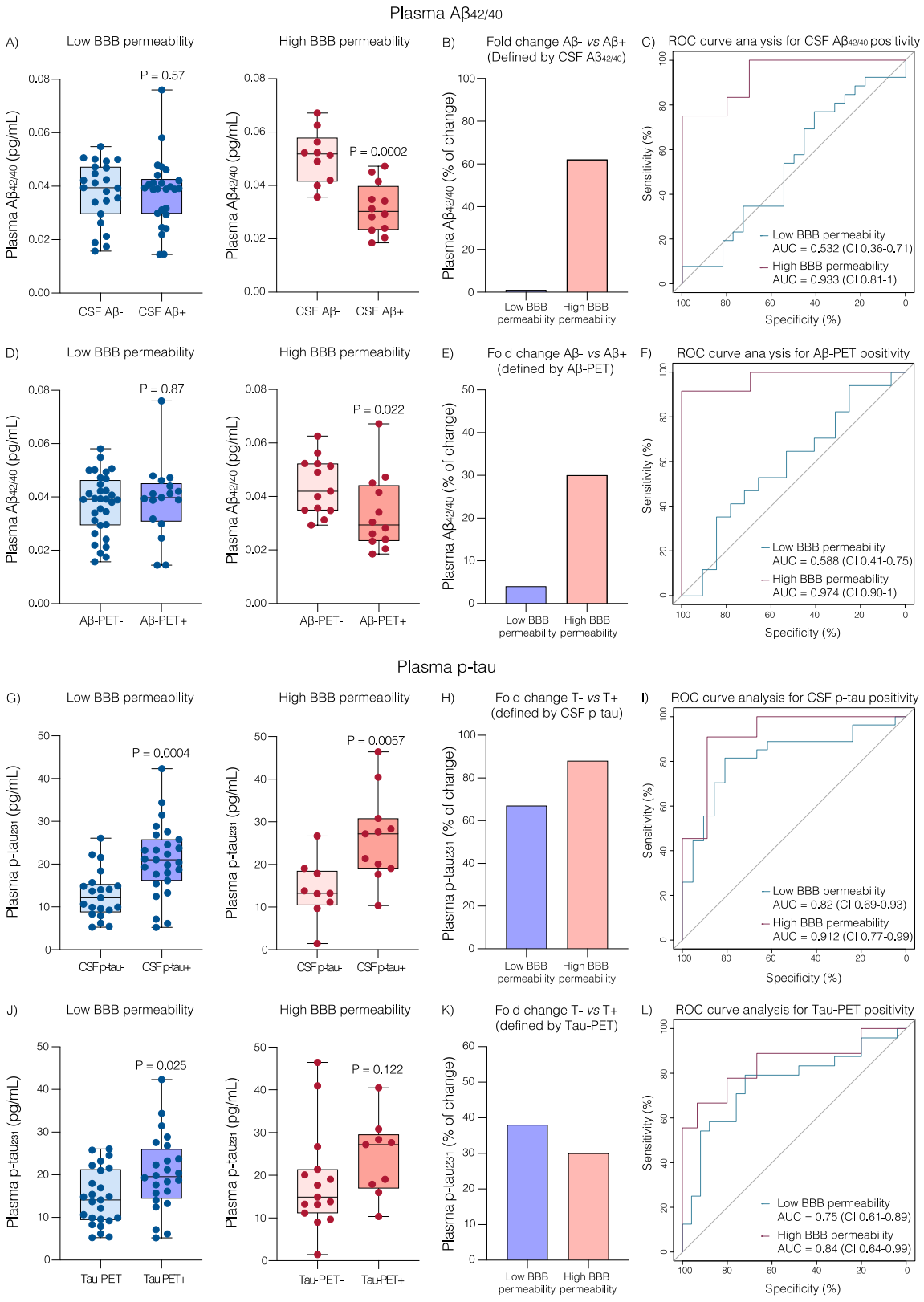


Figure 4. Fold change and discriminative accuracy of plasma Aβ42/40 and p-tau for brain AD pathology as a function of BBB permeability. Plasma Aβ42/40 levels according to Aβ-positivity determined defined by **(A)** CSF Aβ42/40 and **(D)** Aβ-PET in individuals with low and

high BBB permeability. Fold change between Aβ- and Aβ+ individuals, classified by **(B)** CSF $A\beta_{42/40}$ and **(E)** Aβ-PET according to BBB permeability. Plasma $A\beta_{42/40}$ AUC for **(C)** CSF $A\beta_{42/40}$ and **(F)** Aβ-PET positivity. Plasma p-tau levels according to Tau positivity as determined by **(G)** CSF p-tau and **(J)** Tau-PET in individuals with low and high BBB permeability. Fold change between T- and T+ individuals, classified using **(H)** CSF p-tau and **(K)** Tau-PET according to BBB permeability. Plasma p-tau AUC for **(I)** CSF p-tau and **(L)** Tau-PET positivity differentiating groups with high and low BBB permeability. Group comparisons were assessed using a non-parametric Student's t-test.

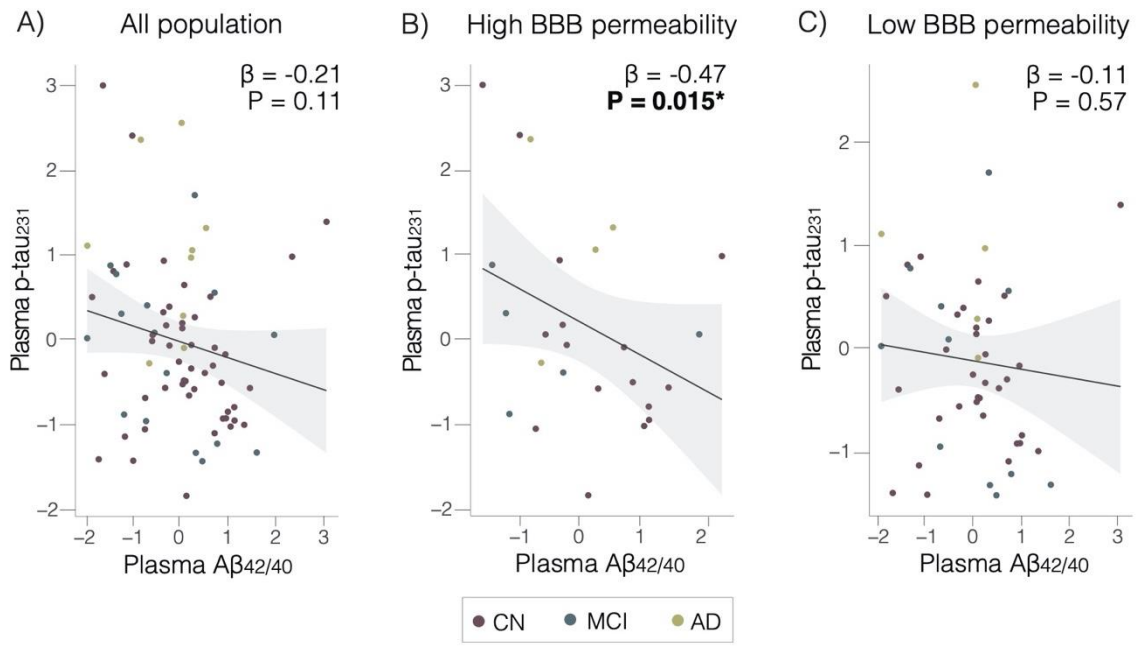


Figure 5. BBB permeability influenced the association between plasma Aβ and p-tau biomarkers. Scatter plots showing the associations between plasma $A\beta_{42/40}$ and plasma p-tau_{231} in **(A)** all study population, **(B)** individuals with high BBB permeability, and **(C)** individuals with low BBB permeability.

Supplemental Tables

Supplemental table 1. Demographics and key characteristics of participants by age.

	Elderly	Young	P-value
No.	74	21	
Age, mean (SD)	69.7 (8.2)	23.6 (2.83)	< 0.001
Sex, % female	52.7	55.6	0.794
APOEε4 carriers, No. (%)	28 (37.8)	7 (30)	0.99
Cognitive status, No. CI. (%)	25 (33.8)	0 (0)	0.005
Plasma $A\beta_{42/40}$, mean pg/ml (SD)	0.038 (0.013)	0.047 (0.013)	0.011
CSF $A\beta_{42/40}$, mean pg/ml (SD)	0.070 (0.024)	0.09 (0.006)	< 0.001
Neocortical Aβ-PET SUVR, mean (SD)	1.66 (0.543)	1.21 (0.073)	< 0.001
Plasma p-tau_{231}, mean pg/ml (SD)	18.4 (9.3)	9.67 (6.62)	< 0.001
CSF p-tau_{231}, mean pg/ml (SD)	415 (222)	138 (74.6)	< 0.001

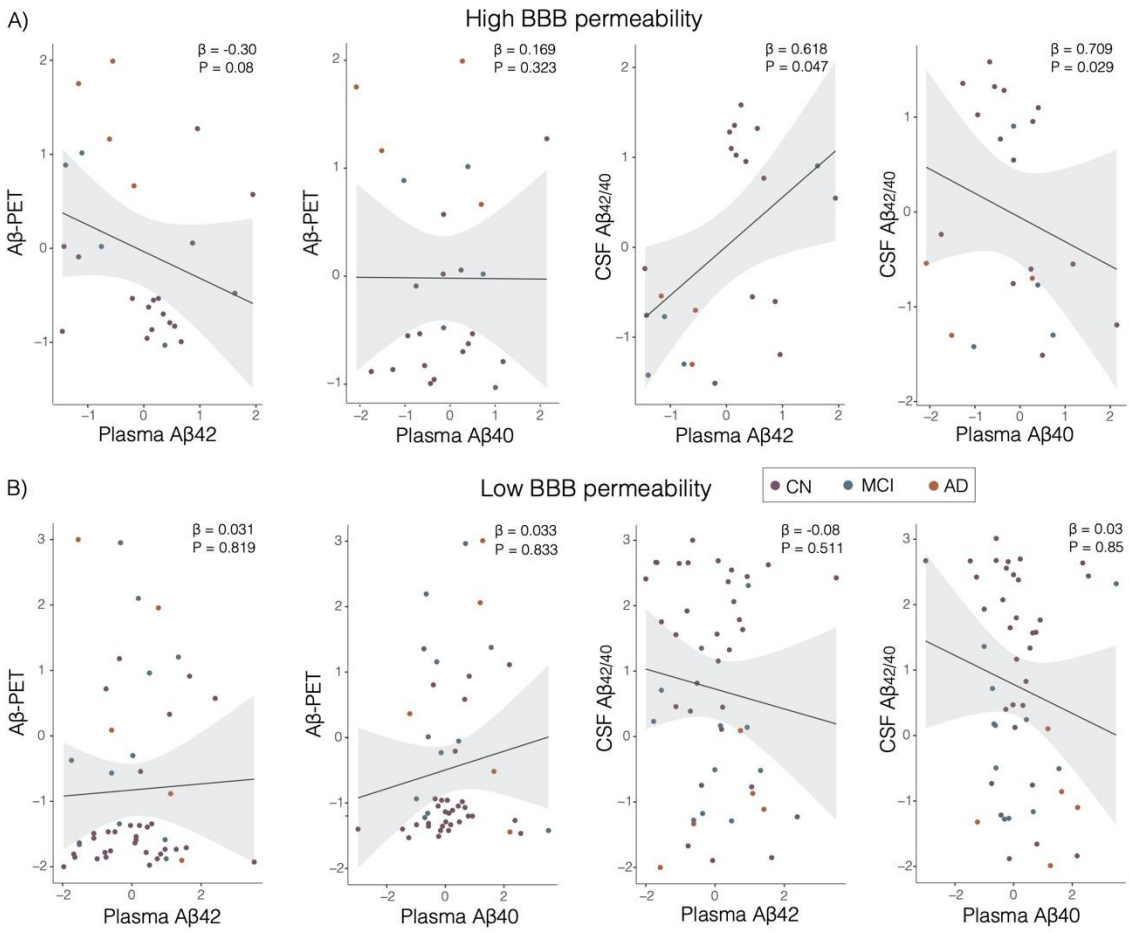
Abbreviations: Aβ: Amyloid-β; APOEε4: apolipoprotein ε4; BBB: blood-brain barrier; CSF: cerebrospinal fluid; CI: cognitively impaired; PET: positron emission tomography; SD: standard deviation; SUVR: standard uptake value ratio.

Supplemental Table 2. Associations of CSF and plasma Alzheimer's disease core biomarkers with CSF/serum albumin ratio.

	β (95% CI)	T-value	P-value
Model: CSF Biomarker ~ CSF/serum albumin ratio + clinical diagnosis + age + sex			
CSF $A\beta_{42/40}$	0.03 (-0.31 – 0.57)	0.28	0.78
CSF p-tau	0.05 (-0.16 – 0.26)	0.46	0.65
Model: Plasma biomarker ~ CSF/serum albumin ratio + clinical diagnosis + age + sex			
Plasma $A\beta_{42/40}$	0.01 (-0.23 – 0.25)	0.09	0.93
Plasma p-tau	0.11 (-0.11 – 0.33)	0.99	0.32

Abbreviations: Aβ: Amyloid-β; APOEε4: apolipoprotein ε4; CSF: cerebrospinal fluid; CI: confidence interval; PET: positron emission tomography; p-tau: phosphorylated tau; SD: standard deviation.

Supplemental Figures



Supplemental Figure 1. Associations of plasma with CSF/PET Aβ in individuals with low and high BBB permeability. Scatter plots show the associations of CSF $A\beta_{42/40}$ and Aβ-PET with plasma $A\beta_{42}$ and $A\beta_{40}$ in individuals with **(A)** high BBB permeability, and **(B)** low BBB permeability. Lines indicate the regression line with a 95% confidence interval. P-values were computed with linear regression models adjusted by age, sex, and clinical diagnosis.