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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/PET Aβ, p-tau, and albumin measures.

RESULTS: Plasma $A\beta_{42/40}$ better identified CSF $A\beta_{42/40}$ and Aβ-PET positivity in individuals with high BBB permeability. An interaction between plasma $A\beta_{42/40}$ and BBB permeability on CSF $A\beta_{42/40}$ was observed.Voxel-wise models estimated that the association of 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.

Keywords

Amyloid-β; Blood biomarkers; Blood-brain barrier; Alzheimer's disease; Confounding factors

Introduction

High accuracy in detecting brain levels of Alzheimer's disease (AD) pathophysiology *in vivo* is achieved by measuring the hallmark proteinopathies – amyloid-β (Aβ) and tau proteins – in the cerebrospinal fluid (CSF) and using positron emission tomography (PET), making it possible to diagnose and monitor AD pathological changes in living individuals[1]. Although reliable, their use in clinical practice and trials is a challenge due to their invasiveness, limited availability, and, for PET, its prohibitive costs[2].

While robust results have been reported using plasma Aβ and phosphorylated tau (p-tau) for detecting brain AD pathophysiology[3–5], significant variability in biomarkers performance is observed among cohorts[5–8]. Biological factors may account for this variability observed between individuals and, therefore, limit the performance of AD plasma biomarkers[9, 10]. One source of variability is the expression of these proteins in peripheral tissues that would eventually reflect in the same pool of blood as the brain-derived fraction. Since the brain is not in direct contact with the periphery, the blood-brain barrier (BBB), which separates these two compartments, is a key factor that likely influences the relationship between the brain and peripheral sources of biomarkers.

The BBB is a highly-specialized structure that maintain the brain's chemical composition by regulating the exchange of nutrients, inflammatory mediators, and other proteins between the brain and blood[11]. Under pathological conditions, BBB integrity is compromised potentially leading to an increase in its permeability/leakiness to several proteins, allowing some proteins to exchange more freely between peripheral and CSF compartments, without active regulation by transporters. Studies revealed a major role of the BBB in determining concentrations of proteins in biofluids by regulating their transport[12–14]. The transport across the BBB represents up to 75% of the exchange of Aβ isoforms exchange between the brain and circulatory system[15–17], while the clearance of tau is believed to occur mostly through the interstitial fluid bulk flow into the CSF[16]. Altogether, these studies suggest that the loss of BBB integrity can potentially create a lack of balance in the transport of proteins (especially Aβ) between the brain and blood, affecting the relationship between biomarkers derived from these compartments.

Here, we evaluated the impact of BBB permeability, as measured by the widely applied CSF/serum albumin ratio (qAlb), on the associations between plasma, CSF, and imaging 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.

Methods

Study population

This study included participants from two centers. The Translational Biomarkers in Aging and Dementia (TRIAD) cohort (Montreal,Canada) comprised participants that had a detailed clinical and cognitive assessment. The study was approved by the Douglas Mental Health University Institute Research Ethics Board and Montreal Neurological Institute PET working committee. The BIODEGMAR cohort is an observational study that enrolls patients visiting the Cognitive Decline and Movement Disorders Unit of Hospital del Mar (Barcelona,Spain) and donated a blood sample, underwent a detailed neurological and neuropsychological evaluation, a brain MRI, and a lumbar puncture. BIODEGMAR cohort was approved by the local ethics committee.

The same criteria were used for clinical diagnosis in both cohorts. Cognitively unimpaired (CU) individuals had no objective cognitive deficits and a Clinical Dementia Rating (CDR)=0. Mild cognitive impairment (MCI) individuals 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 cognitively impaired (CI) group.

Fluid Biomarkers

CSF $A\beta_{40}$ and $A\beta_{42}$ were measured using the LUMIPULSE G1200 (Fujirebio) at the Clinical Neurochemistry Laboratory, University of Gothenburg, Sweden (TRIAD) and at Laboratori de Referència de Catalunya (BIODEGMAR). CSF p-tau_{181} was evaluated using in-house Simoa method for TRIAD and the LUMIPULSE G1200 (Fujirebio) for BIODEGMAR. For both cohorts, plasma $A\beta_{40}$ and $A\beta_{42}$ were evaluated using validated commercially available Simoa assay (Quanterix), and plasma p-tau181 was measured using in-house Simoa method[4] at the Clinical Neurochemistry Laboratory, University of Gothenburg, Sweden. Serum albumin was measured using a previously developed colorimetric method[18]. CSF albumin was measured by a turbidimetric method performed on the binding site optilite. The permeability of the BBB is a widely used metrics applied to assess BBB integrity. To identify individuals with high BBB permeability, we divided qAlb values from each cohort in terciles. Participants in the upper tercile were classified as high BBB permeability (qAlb>6.24 for TRIAD and qAlb>6.89 for BIODEGMAR), and individuals in the lower two terciles as low BBB permeability. Alternatively, we divided the entire population (the two cohorts combined) in terciles which generated a slightly different cutpoint (qAlb>6.33). For TRIAD, CSF $A\beta_{42/40}$ and p-tau181 cutoffs (for Aβ- and tau-positivity, respectively) were defined based on young CU individuals [±2.5 standard deviations (SD)][19]. For BIODEGMAR, individuals were classified as Aβ-negative using CSF $A\beta_{42/40}$ following a previously calculated cutoff[20].

MRI/PET Biomarkers

PET imaging was available only for the TRIAD cohort. Aβ-PET was quantified using [^{18}F]AZD4694 and Tau-PET with [^{18}F]MK-6240 using a Siemens High Resolution Research Tomograph. Standardized uptake value ratio (SUVR) was calculated using the whole cerebellum gray matter for [^{18}F]AZD4694 and [^{18}F]MK-6240 as reference. Neocortical [^{18}F]AZD4694 SUVR value was estimated from precuneus, prefrontal, orbitofrontal, parietal, temporal, anterior, and posterior cingulate cortices. Individuals with Aβ-PET SUVR>1.55 were considered Aβ-positive[21]. [^{18}F]MK-6240 SUVR was extracted from the temporal meta-ROI, a composite mask used as a summary measure of tau-PET[22]. Tau-positivity was defined as 2.5 SD higher than the mean SUVR of young individuals.

Statistical analysis

Neuroimaging analyses were conducted using the VoxelStats toolbox (https://github.com/sulantha2006/VoxelStats), a MATLAB-based analytical framework that for multimodal voxel-wise neuroimaging analyses. For voxel-based analyses, multiple comparisons correction was performed using random field theory (RFT), with a threshold of $p<0.05$. 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-square and t-tests. Associations between biomarkers were assessed with Spearman

correlation or linear regression accounting for age, clinical diagnosis, sex, and Bonferroni correction for multiple comparisons in the discovery cohort. The interaction term plasma $A\beta_{42/40}$×qAlb status was also added to each model. Group comparisons were performed using one-way ANOVA followed by Tukey post hoc test or two-tailed Students' t-test. Three individuals from the TRIAD cohort did not have available CSF Aβ levels and, thus, were removed from the analysis involving CSF. In BIODEGMAR, 14 participants did not have APOEε4 genotyping. Plasma $A\beta_{42/40}$ discriminative performance was assessed with Receiver Operator Characteristic (ROC) area under the curve (AUC). AUCs were compared using DeLong test followed by false discovery rate (FDR) multiple comparison correction.

Results

Participants

In the discovery research cohort (TRIAD), we studied 24 young (age=22.9±1.8), 45 CU (age=69.5±8.4), and 27 CI (age=70.7±6.1). In the validation clinical cohort (BIODEGMAR), 113 CI (age=73.1±5.2) and 15 CU (age=73±5.2). The older CU and CI individuals for both cohorts were divided into low (lower two terciles) and high (uppermost tercile) BBB permeability based on their qAlb. Demographic and clinical characteristics of the older participants are summarized in Table 1. Demographic characteristics of the young individuals from the TRIAD cohort are presented in Supporting information Table 1. No significant differences in *APOE* ε4 carrier status, fluid, imaging Aβ and tau levels, and cognitive status were observed between individuals with low and high BBB permeability in the discovery research cohort (Table 1). In the validation clinical cohort, individuals with high BBB permeability presented higher CSF $A\beta_{42/40}$ and lower p-tau levels compared to low BBB permeability participants.

BBB permeability increases with age but not according to cognitive status

We found a significant increase in the qAlb in the older (both CU and CI) compared to the young individuals ($p<0.0001$, Supporting information Fig.1A) in the discovery cohort, but no difference between CU older and CI participants (Supporting information Fig.1A). Consistently, in the validation cohort no differences in qAlb were observed between CU and CI individuals (Supporting information Fig.1C). No correlation between qAlb and age was observed in the older individuals in both cohorts (Supporting information Fig.1B,D).

BBB permeability affects the association between brain and plasma Aβ levels

We first tested the direct association of qAlb with AD core biomarkers. qAlb did not associate with $A\beta_{42/40}$ or p-tau levels in the CSF and plasma in both cohorts (Supporting information Table 2).

We further explored the impact of BBB integrity on the relationship between CSF and plasma AD biomarkers. In the research cohort, when we divided the population into individuals with low and high qAlb (BBB permeability), we observed that plasma $A\beta_{42/40}$ was significantly associated with CSF $A\beta_{42/40}$ in individuals with high BBB permeability ($\beta=0.66$, $p=0.0003$,Fig.1A) but not in individuals with low BBB permeability ($\beta=-0.15$, $p=0.28$,Fig.1A). To test for group difference in the associations, we added

to the model an interaction term for qAlb status. We observed a significant interaction between plasma $A\beta_{42/40}$ and qAlb on CSF $A\beta_{42/40}$ (β=0.65, p=0.0022, Fig.1A;Table 2). Similarly, plasma $A\beta_{42/40}$ was associated with increased neocortical uptake in Aβ-PET in individuals with high BBB permeability (β=−0.25, p=0.037,Fig.1B), whereas no significant associations were observed in individuals with low BBB permeability (β=0.08, p=0.61,Fig.1B). However, no significant interaction between plasma $A\beta_{42/40}$ and qAlb on Aβ-PET was observed (β=−0.15, p=0.478,Fig.1B;Table 2). Accordingly, in the validation clinical cohort, we observed that plasma $A\beta_{42/40}$ was significantly associated with CSF $A\beta_{42/40}$ in individuals with high BBB permeability (β=0.49, p=0.0027,Fig.1C) but not in individuals with low BBB permeability (β=0.14, p=0.104,Fig.1A). Additionally, a significant interaction between plasma $A\beta_{42/40}$ and qAlb on CSF $A\beta_{42/40}$ was observed (β=0.35, p=0.041,Fig.1C;Table 2). Using the qAlb cutoff generated using the entire population the results remain unchanged (Supporting information Table 3). When we merged the two cohorts, we still observed a significant interaction between plasma $A\beta_{42/40}$ and qAlb on CSF $A\beta_{42/40}$ (β=0.36, p=0.0069, Supporting information Table 4) which seems to be independent of clinical diagnosis. Finally, because studies evidenced that man presents higher qAlb levels than women[23, 24], we explored whether sex plays a role in the associations observed here. For this, we added to the model an interaction term for sex. No significant interaction between plasma $A\beta_{42/40}$ and sex on CSF $A\beta_{42/40}$ was observed in both cohorts (Supporting information Table 5). A significant interaction between plasma $A\beta_{42/40}$ and sex on Aβ-PET was observed in TRIAD when using the entire population (β=0.48, p=0.03). In the analyses stratified by BBB permeability (high and low), the interaction was not significant in any group (Supporting information Table 5).

Plasma p-tau presented a strong association with CSF p-tau (discovery and validation cohorts) and with Tau-PET (discovery cohort) that was not affected by BBB permeability (Supporting information Table 6). No significant interaction between qAlb and plasma p-tau on CSF p-tau or Tau-PET levels was observed in both cohorts. Finally, because plasma p-tau biomarkers have shown a stronger correlation with Aβ than Tau pathology in some stages of the disease[25], we evaluated whether the association between plasma p-tau and brain Aβ levels was affected by BBB permeability. However, no significant interaction between qAlb and plasma p-tau on CSF $A\beta_{42/40}$ or Aβ-PET levels was observed in both cohorts (Supporting information Table 7).

Voxel-wise analysis estimates the brain regions where the association between brain and plasma Aβ is most 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.2A–left). No associations were observed in the group presenting low BBB permeability (Fig.2A–right). Voxel-wise interaction analysis across older individuals with both high and low BBB permeability revealed a significant interaction effect of qAlb on the association between plasma $A\beta_{42/40}$ and Aβ-PET (peak t-value=3.8, p=0.0003,Fig.2B).

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

For TRIAD, Aβ+ individuals with low BBB permeability presented no significant differences in plasma $A\beta_{42/40}$ levels compared to Aβ- participants (Fig.3A). In the high BBB permeability group, plasma $A\beta_{42/40}$ was significantly different between Aβ- and Aβ+ individuals, as classified by CSF $A\beta_{42/40}$ (p=0.0002,Fig.3A) or Aβ-PET (p=0.034,Fig.3C). The fold change in plasma $A\beta_{42/40}$ for individuals with high BBB permeability was as great as 62% (when Aβ-positivity was defined by CSF), while the low BBB permeability group was not higher than 4% (when Aβ-positivity was defined by PET;Fig.3A,C). For BIODEGMAR, significantly different plasma $A\beta_{42/40}$ levels were observed between CSF Aβ- and CSF Aβ+ individuals in both high and low BBB permeability groups (Fig.3E), with a numerically higher fold change in individuals with high BBB permeability group (15% in high versus 9% in low BBB permeability;Supporting information Table 8). On the other hand, plasma p-tau concentrations distinguished CSF/PET T- from T+ individuals independently of BBB permeability in both cohorts (Supporting information Fig.2).

Plasma Aβ shows better performance in identifying Aβ-positivity in individuals with high BBB permeability

We tested whether BBB permeability influences the discriminative performance of plasma $A\beta_{42/40}$ to predict Aβ-positivity measured with CSF $A\beta_{42/40}$ or Aβ-PET. For the discovery cohort, in the entire population, plasma $A\beta_{42/40}$ (accounting for age and sex) discriminated Aβ- from Aβ+ individuals with an area under the curve (AUC) of 0.655 (95%CI=0.51–0.77) and 0.585 (95%CI=0.45–0.71) using CSF $A\beta_{42/40}$ or Aβ-PET to define groups, respectively (Table 3). Considering only the population with high BBB permeability, plasma $A\beta_{42/40}$ reached an AUC of 0.99 for predicting CSF $A\beta_{42/40}$ (95%CI=0.95–1) and 0.98 for Aβ-PET (95%CI=0.94–0.99) positivity, which was significantly higher than the AUCs obtained from the population with low BBB permeability [Aβ+ defined with CSF $A\beta_{42/40}$ AUC=0.59 (95%CI=0.42–0.76), and Aβ+ defined with Aβ-PET, AUC=0.61 (95%CI=0.45–0.78)] and was significantly higher than the AUCs obtained from the entire population (Table 3;Fig.3B,D). In the validation cohort, plasma $A\beta_{42/40}$ discriminated Aβ- from Aβ+ individuals in the high BBB permeability group with an AUC of 0.84 (95%CI=0.71–0.95; Fig.3F). For the low BBB permeability group, a lower AUC for predicting Aβ-positivity was observed; however, this difference did not reach statistical significance (Table 3).

The accuracy of plasma p-tau for detecting CSF p-tau [high BBB permeability AUC=0.80 (95%CI=0.60–0.96), low BBB permeability AUC=0.83 (95%CI=0.70–0.94); Supporting information Fig.2B)] or Tau-PET positivity [high BBB permeability AUC=0.82 (95%CI=0.59–0.98), low BBB permeability AUC=0.77 (95%CI=0.64–0.90); Supporting information Fig.2D)] in the TRIAD and BIODEGMAR cohort [high BBB permeability AUC=0.80 (95%CI=0.66–0.92), low BBB permeability AUC=0.75 (95%CI=0.62–0.85); Supporting information Fig.2F] was not significantly affected by the BBB permeability.

Discussion

In this study, where we validated our initial results in an independent memory clinic cohort, we showed that brain and plasma Aβ pools only significantly associate with one another in individuals with a high qAlb[26]. Additionally, the significant interaction between plasma $A\beta_{42/40}$ and qAlb status modified the association between brain and plasma Aβ. Furthermore, high BBB permeability was associated with a higher fold change of plasma $A\beta_{42/40}$ levels between brain Aβ- and Aβ+ groups (defined either by CSF or PET). These results suggest that plasma Aβ better represents brain Aβ levels in individuals with a high BBB permeability, which may have important implications for the future use of these biomarkers in research and clinical practice.

Rapid advances in sensitive and precise plasma biomarker assays promise a change in the diagnosis and prognosis of AD. However, moving from research settings to clinical practice brings new challenges. For instance, patients from memory clinics will likely present a diverse range of comorbidities that are avoided in the selection of participants in research cohorts. In fact, it has been recently shown that the performance of plasma p-tau biomarkers is affected by factors such as chronic kidney disease, stroke, and myocardial infarction[10]. The validation of our findings in a clinical cohort highlights the relevance of BBB permeability for plasma biomarkers' performance despite the presence of additional confounding factors associated with a large methodological heterogeneity presented in clinical settings. However, other comorbidities not considered in our study might be impacting the performance of plasma $A\beta_{42/40}$ in the clinical, but not in the research cohort.

Decreased BBB integrity improved the performance of plasma $A\beta_{42/40}$ in detecting brain Aβ pathology. Although the brain is considered the source of Aβ in AD, other peripheral tissues considerably contribute to Aβ production[27, 28]. 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)][15, 16, 29]. Our results may indicate that an increase in BBB permeability changes the equilibrium between the central nervous system and blood towards plasma $A\beta_{42/40}$ being more representative of brain Aβ concentrations than peripheral Aβ production. Whether the disruption of this equilibrium is due to a freer passive exchange between compartments or impairment of the function of active transporters remains to be elucidated.

BBB permeability did not significantly affect the relationship between brain and plasma levels of p-tau. Tau clearance from the central nervous system is less well understood than Aβ[16]. However, in contrast to Aβ, no tau transporters through the BBB have been identified at the moment, suggesting that its clearance does not heavily occur via BBB[16]. In this sense, tau clearance seems to occur mainly by lysosomal degradation, interstitial fluid bulk flow, and CSF absorption[30, 31]. These results corroborate previous findings indicating a lack of association of BBB permeability with CSF p-tau[32]. Our findings might contribute to understanding the lower biological variability observed in plasma p-tau biomarkers compared to plasma Aβ[33, 34].

We observed a prominent increase in qAlb in the older compared to young individuals but no difference between clinical diagnosis. Consistently with our findings, it has already been demonstrated that BBB permeability increases with normal aging[35, 36]. Conversely, studies investigating qAlb changes in AD individuals present conflicting results[37–41]. A meta-analysis showed that qAlb is elevated in individuals with AD dementia but with a small effect size[6], making it challenging to observe significant differences in small populations. Additionally, despite influencing the relationship between brain and plasma Aβ levels, qAlb levels did not correlate with plasma $A\beta_{42/40}$. The lack of a direct correlation might demonstrate that the permeability in the BBB must reach a point where proteins can cross and freely exchange between compartments.

We identified that BBB permeability 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)[42]. Regional differences in BBB permeability have already been reported in other neuropathological disorders[43]. Interestingly, an MRI study using a water exchange technique recently showed that BBB permeability is associated with CSF $A\beta_{42}$ levels in the same brain regions observed here[32]. Although fluid albumin measurements are not capable of detecting the topographical distribution of BBB permeability, our voxel-wise models estimated regions with the highest dissociation between the variabilities of PET and plasma Aβ levels. Future imaging studies are needed to elucidate whether BBB integrity overlaps with AD proteinopathies and possible mechanistic underpinnings linking pathologies.

Although highly accepted in the literature, BBB permeability was indirectly determined based only on qAlb in our study. Currently, there is no gold standard for measuring BBB integrity in living persons, and the available markers likely offer complementary information regarding BBB's properties. Albumin is a relatively large plasma protein (~67 kDa) and lacks a canonical transporter in the BBB, thus, large quantities are detected in the CSF only when the BBB permeability is increased[44]. Assessing BBB integrity using other markers, such as brain imaging[32, 40], could present different results and provide more information regarding the spatial localization of BBB permeability. Additionally, Lin and colleagues demonstrated that changes in the BBB permeability to water detected by MRI, but not with qAlb, were associated with cognitive decline[40]. Other fluid markers, such as the CSF soluble platelet-derived growth factor receptor β, a marker of pericyte damage, have been investigated in AD and seem to present a better association with AD-related brain damage than the qAlb[39, 45]. Thus, further investigations exploring other markers of BBB integrity are crucial to understanding the extent of BBB permeability required for impacting the relationship between brain and blood Aβ levels.

The results presented in this study should be interpreted considering limitations. For both cohorts, while plasma $A\beta_{42/40}$ was measured with Simoa, CSF $A\beta_{42/40}$ was measured with the LUMIPULSE platform (ELISA-based method). CSF Aβ was measured with LUMIPULSE platform since this technique is the most widely applied and has been used as reference to determine the performance of plasma biomarkers quantified using multiple assays and platforms[5, 46–48]. ELISA- and Simoa-based plasma Aβ assay presented equivalent performances to detect cerebral amyloidosis[48]. However, it has been shown

that mass spectrometry-based plasma Aβ methods perform better than immunoassays to identify Aβ[5, 8, 49]. Thus, it would be highly desirable to replicate these results using different plasma Aβ assays.

Our results suggest that plasma Aβ may be a better proxy of brain Aβ pathology in individuals with a more prominent BBB breakdown. These findings raise questions about the role of BBB integrity in the performance of plasma Aβ biomarkers and might help elucidate the origins of some of the variability found in plasma Aβ studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Conflicts of interest and Disclosure 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 Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). KB has served as a consultant, at advisory boards, or at data monitoring committees for Abcam, Axon, Biogen, JOMDD/Shimadzu. Julius Clinical, Lilly, MagQu, Novartis, Prothena, Roche Diagnostics, and

Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program, all unrelated to the work presented in this paper. SG has served as a scientific advisor to Cerveau Therapeutics. ERZ served at scientific advisory boards and/or as a consultant for Nintx and Cristalia. MSC has served as a consultant and at advisory boards for Roche Diagnostics International Ltd and has given lectures in symposia sponsored by Roche Diagnostics, S.L.U and Roche Farma, S.A. The other authors declare that they have no conflict of interest.

Abbreviations

Aβ	amyloid-β
AD	Alzheimer's disease
AUC	area under the curve
BBB	blood-brain barrier
CDR	Clinical Dementia Rating
CI	cognitively impaired
CSF	cerebrospinal fluid
CU	cognitively unimpaired
FDR	false discovery rate
LRP	low-density lipoprotein receptor-related protein
MCI	Mild cognitive impairment
MMSE	Mini-Mental State Examination
PET	positron emission tomography
p-tau	phosphorylated tau
qAlb	CSF/serum albumin ratio
RAGE	advanced glycosylation end product-specific receptor
RFT	random field theory
ROC	Receiver Operator Characteristic

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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;

Research in context

Systematic review:

The authors reviewed the literature using traditional sources.It was recently demonstrated that the performance of plasma biomarkers is affected by biological factors unrelated to AD pathophysiology.Because the blood-brain barrier (BBB) regulates the transport of proteins between cerebrospinal fluid (CSF) and blood, altered BBB permeability likely impacts the association between brain and blood levels of AD biomarkers.

Interpretation:

Our study highlights that BBB integrity influences the association between plasma and CSF/PET amyloid-β (Aβ), but not phosphorylated-tau (p-tau) levels. Specifically, individuals with an increased BBB permeability present a better accuracy in detecting brain Aβ pathology.These findings highlight the need for exploring confounding factors that might affect the clinical performance of plasma biomarkers.

Future directions:

Our findings were obtained using Aβ values measured by the most widely used immunoassay currently available. However, it has been shown that mass spectrometry-based plasma Aβ methods perform better than immunoassays.Thus further studies exploring different plasma Aβ assays are desired.

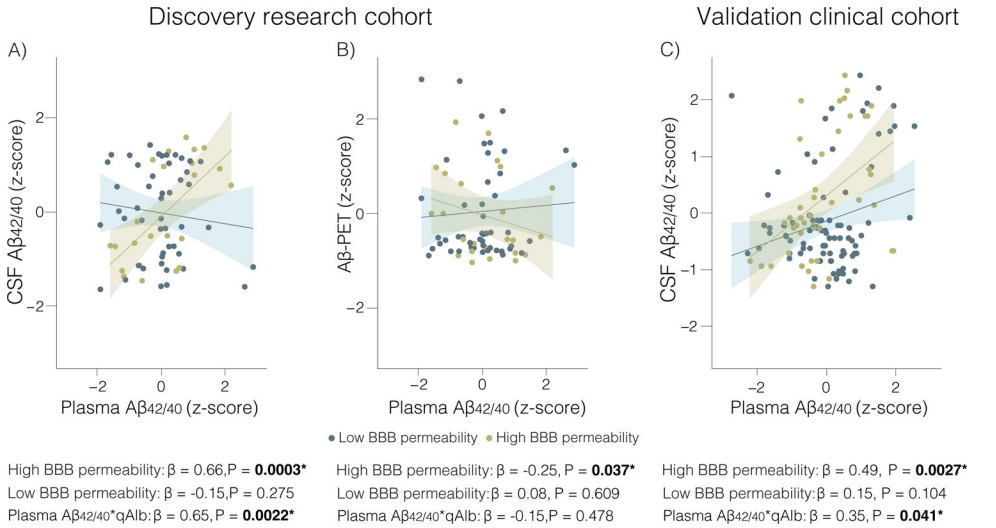

Figure 1. Increased BBB permeability modifies the associations of plasma $A\beta_{42/40}$ ratio with brain levels of Aβ pathology.

Scatter plots show the associations between plasma $A\beta_{42/40}$ with **(A)** CSF $A\beta_{42/40}$ ratio and with **(B)** neocortical Aβ-PET SUVR in the high and low BBB permeability groups in the TRIAD cohort. **(C)** Scatter plots show the associations between plasma $A\beta_{42/40}$ ratio with CSF $A\beta_{42/40}$ ratio in the BIODEGMAR cohort. 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. In addition, the plasma $A\beta_{42/40}$ × qAlb status on CSF/PET Aβ status interaction term was computed. CSF $A\beta_{40}$ and $A\beta_{42}$ were measured using the LUMIPULSE platform for both cohorts. Plasma measures were performed using the Simoa platform for both cohorts.

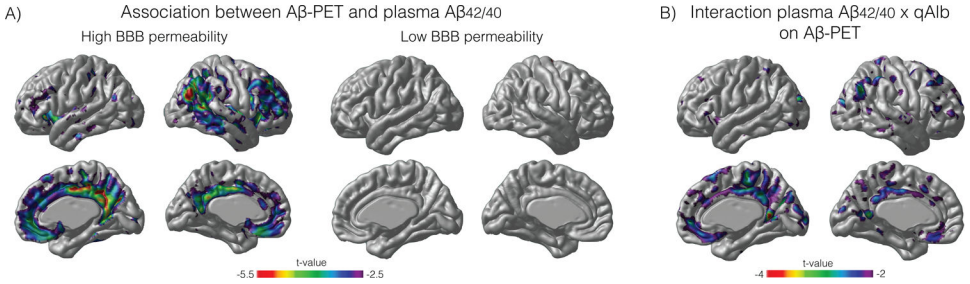

Figure 2. Plasma $A\beta_{42/40}$ only associates with Aβ-PET deposition in individuals with high BBB permeability within typical AD brain regions.

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 interaction of CSF/albumin ratio (qAlb) in the relationship between plasma $A\beta_{42/40}$ ratio on Aβ-PET. Multiple comparisons correction was performed using random field theory (RFT) with a threshold of $p<0.05$.

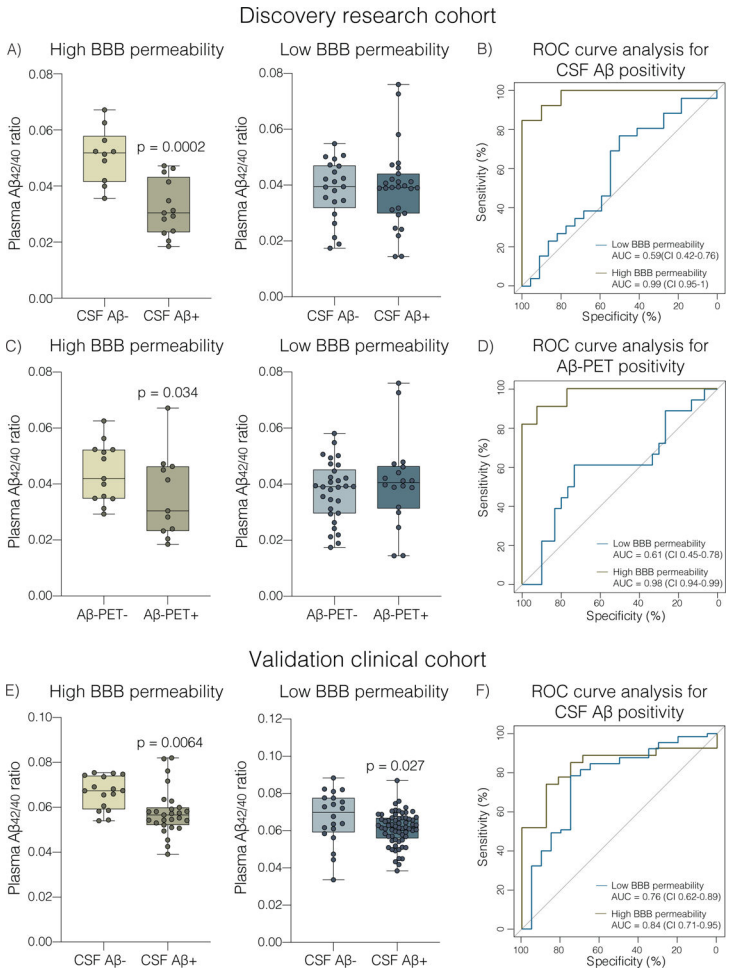

Figure 3. Discriminative accuracy of plasma $A\beta_{42/40}$ for brain AD pathology as a function of BBB permeability.

Plasma $A\beta_{42/40}$ levels according to Aβ-positivity defined by **(A)** CSF $A\beta_{42/40}$ ratio and **(C)** Aβ-PET in individuals with low and high BBB permeability in the TRIAD cohort. Plasma $A\beta_{42/40}$ AUC for **(B)** CSF $A\beta_{42/40}$ and **(D)** Aβ-PET positivity in the TRIAD cohort. Plasma $A\beta_{42/40}$ levels according to Aβ-positivity defined by **(E)** CSF $A\beta_{42/40}$ in individuals with low and high BBB permeability in the BIODEGMAR cohort. Plasma $A\beta_{42/40}$ AUC for **(F)** CSF $A\beta_{42/40}$ positivity in the BIODEGMAR cohort. Group comparisons were assessed using a non-parametric Student's t-test.

Table 1.

Demographics and key characteristics of participants.

	Discovery research cohort (TRIAD)		Validation clinical cohort (BIODEGMAR)	
	Low BBB permeability	**High BBB permeability**	**Low BBB permeability**	**High BBB permeability**
No.	48	24	85	43
Age, mean (SD)	69.7 (7.4)	70.4 (6.8)	72.9 (5.5)	73.4 (4.6)
Sex, % female	60.4	33.3	75.3	18.6
APOEε4 carriers, No. (%)	19 (39.6)	8 (33.3)	42(49.4)	14 (32.6)
Cognitive status, No. CI. (%)	17 (35.4)	10 (41.7)	79 (92.9)	34 (79.1)*
Plasma $A\beta_{42/40}$, mean (SD)	0.039 (0.013)	0.040 (0.013)	0.063 (0.011)	0.062 (0.01)
CSF $A\beta_{42/40}$, mean (SD)	0.069 (0.023)	0.069 (0.025)	0.053 (0.021)	0.062 (0.024)*
Neocortical Aβ-PET SUVR, mean (SD)	1.69 (0.58)	1.65 (0.5)	-	-
Plasma p-tau, mean pg/ml (SD)	13.4 (7.4)	12.9 (6.37)	15.2 (3.7)	14.9 (3.88)
Temporal Meta-ROI Tau-PET SUVR, mean (SD)	1.13 (0.59)	1.19 (0.78)	-	-
CSF p-tau, mean pg/ml (SD)	532 (527)	543 (350)	109 (58.8)	70.1 (36.2)*

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: standardized uptake value ratio. CSF Aβ42 and Aβ42 were measured using the LUMIPULSE platform for both cohorts. Plasma measures were performed using the Simoa platform for both cohorts.

Table 2.

Interaction effect of qAlb and plasma $A\beta_{42/40}$ on CSF/PET Aβ.

	β (95% CI)	T-value (df)	p-value
Interaction effect of qAlb and plasma $A\beta_{42/40}$ on CSF $A\beta_{42/40}$			
Plasma $A\beta_{42/40}$ (TRIAD)	0.66 (0.24– 1.07)	3.19 (64)	0.0022
Plasma $A\beta_{42/40}$ (BIODEGMAR)	0.35 (0.02 – 0.69)	2.06 (120)	0.041
Interaction effect of qAlb and plasma $A\beta_{42/40}$ on Aβ-PET SUVR			
Plasma $A\beta_{42/40}$ (TRIAD)	−0.15 (−0.59 – 0.28)	−0.72 (65)	0.478

Abbreviations: Aβ: Amyloid-β; CSF: cerebrospinal fluid; PET: positron emission tomography; SUVR: standardized uptake value ratio. CSF Aβ42 and Aβ42 were measured using the LUMIPULSE platform for both cohorts. Plasma measures were performed using the Simoa platform for both cohorts.

Table 3.

Discriminative performance of plasma $A\beta_{42/40}$ ratio as a function of BBB permeability.

	AUC (95% CI)		
	Entire sample	**High BBB permeability**	**Low BBB permeability**
CSF Aβ positivity (TRIAD)	0.655 (0.51 – 0.77)	0.992 (0.95 – 1)[a,b]	0.591 (0.42 – 0.76)
Aβ-PET positivity (TRIAD)	0.585 (0.45 – 0.71)	0.984 (0.94 –1)[a,b]	0.607 (0.45 – 0.78)
CSF Aβ positivity (BIODEGMAR)	0.779 (0.69 – 0.86)	0.836 (0.71 – 0.95)	0.76 (0.62 – 0.89)

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 entire sample;

b: P< 0.0001 compared to low BBB permeability.