

FEATURED ARTICLE

Confounding factors of Alzheimer's disease plasma biomarkers and their impact on clinical performance

Alexa Pichet Binette¹  | Shorena Janelidze¹ | Nicholas Cullen¹ | Jeffrey L. Dage² |
 Randall J. Bateman³ | Henrik Zetterberg^{4,5,6,7,8} | Kaj Blennow^{4,5} | Erik Stomrud^{1,9} |
 Niklas Mattsson-Carlgren^{1,10,11} | Oskar Hansson^{1,9}

¹Clinical Memory Research Unit, Faculty of Medicine, Lund University, Lund, Sweden

²Department of Neurology, Indiana University School of Medicine, Indianapolis, Indiana, USA

³Department of Neurology, Washington University School of Medicine, St Louis, Missouri, USA

⁴Department of Psychiatry and Neurochemistry, The Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden

⁵Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden

⁶Department of Neurodegenerative Disease, UCL Institute of Neurology, Queen Square, London, UK

⁷UK Dementia Research Institute at UCL, London, UK

⁸Hong Kong Center for Neurodegenerative Diseases, Hong Kong, China

⁹Memory Clinic, Skåne University Hospital, Malmö, Sweden

¹⁰Department of Neurology, Skåne University Hospital, Lund University, Lund, Sweden

¹¹Wallenberg Center for Molecular Medicine, Lund University, Lund, Sweden

Correspondence

Alexa Pichet Binette and Oskar Hansson, Clinical Memory Research Unit, Memory Clinic, Skåne University Hospital, SE-20502 Malmö, Sweden.

Email: alexa.pichet_binette@med.lu.se; oskar.hansson@med.lu.se

Abstract

Introduction: Plasma biomarkers will likely revolutionize the diagnostic work-up of Alzheimer's disease (AD) globally. Before widespread use, we need to determine if confounding factors affect the levels of these biomarkers, and their clinical utility.

Methods: Participants with plasma and CSF biomarkers, creatinine, body mass index (BMI), and medical history data were included (BioFINDER-1: $n = 748$, BioFINDER-2: $n = 421$). We measured beta-amyloid (A β 42, A β 40), phosphorylated tau (p-tau217, p-tau181), neurofilament light (NfL), and glial fibrillary acidic protein (GFAP).

Results: In both cohorts, creatinine and BMI were the main factors associated with NfL, GFAP, and to a lesser extent with p-tau. However, adjustment for BMI and creatinine had only minor effects in models predicting either the corresponding levels in CSF or subsequent development of dementia.

Discussion: Creatinine and BMI are related to certain plasma biomarkers levels, but they do not have clinically relevant confounding effects for the vast majority of individuals.

KEYWORDS

amyloid, cerebrospinal fluid, dementia, glial fibrillary acidic protein, neurofilament light, p-tau

Highlights

- Creatinine and body mass index (BMI) are related to certain plasma biomarker levels.
- Adjusting for creatinine and BMI has minor influence on plasma-cerebrospinal fluid (CSF) associations.
- Adjusting for creatinine and BMI has minor influence on prediction of dementia using plasma biomarkers.

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1 | BACKGROUND

Plasma biomarkers of pathologic events in Alzheimer's disease (AD) and other neurodegenerative diseases are becoming more prevalent and increasingly accessible.¹ Plasma levels of beta-amyloid (A β) and phosphorylated tau (p-tau) reflect the two key pathological hallmarks of AD – brain amyloidosis and tau pathology, respectively – while plasma neurofilament light (NfL) is a marker of axonal degeneration that is elevated in many different neurodegenerative diseases.²⁻⁸ Plasma glial fibrillary acidic protein (GFAP), a marker of glial activation, is also elevated early in the course of AD and has been increasingly studied.^{9,10} Blood-based biomarkers can for instance discriminate controls from patients, and cognitively stable participants from those who convert to dementia, and they are related to the same markers measured in cerebrospinal fluid (CSF) or in the brain.¹¹⁻¹⁵ They are currently mainly available in research settings, but a lot of efforts are being devoted to making them more widely available, with potential use to screen participants for clinical trials and/or inform clinical practice in the near future.^{1,16} However, determining if confounding factors affect the levels, and maybe even clinical utility, of these blood biomarkers is necessary before their widespread implementation. In particular, recent evidence suggests that reduced kidney function might be associated with increased plasma biomarkers concentrations,^{17,18} and higher body mass index (BMI) with lower blood NfL levels.^{18,19} In this study, we set out to study the effects of potential confounding factors on several state-of-the-art blood-based biomarkers for AD and neurodegeneration, including p-tau217, p-tau181, and GFAP, as well as A β 42/A β 40 and NfL, in two independent cohorts. We first investigated associations between blood-based biomarkers levels and common comorbidities and medication use, as well as factors that are proxies to blood volume (BMI) and kidney function (plasma creatinine). This allowed us to identify key potential confounding factors of plasma biomarkers concentrations. More importantly, we studied whether the performance of the blood-based biomarkers was improved when adjusting for potential confounding factors to determine whether such adjustments are needed in future clinical settings. For this, we studied whether the potential confounding factors either influenced (1) the associations between individual plasma biomarkers and their CSF counterparts, or (2) the ability of the plasma biomarkers to predict conversion to AD dementia or all-cause dementia in non-demented individuals.

2 | METHODS

2.1 | Participants

Participants from the two independent prospective Swedish BioFINDER-1(NCT01208675) and BioFINDER-2(NCT03174938) cohort studies were included. All participants were recruited at Skåne University Hospital and the Hospital of Ängelholm, Sweden and cover the full spectrum of AD, ranging from older adults with intact cognition or subjective cognitive decline, mild cognitive impairment (MCI), and dementia. Both studies were approved by the Regional Ethics Commit-

RESEARCH IN CONTEXT

- 1. Systematic review:** We reviewed the literature on Alzheimer's disease (AD) plasma biomarkers and confounding factors of associations between plasma biomarkers and AD using PubMed. While a recent study, appropriately cited, suggests the importance of chronic kidney disease, no study has looked at the effect of confounding factors on clinical outcomes, nor included a comprehensive assessment of all state-of-the-art plasma biomarkers currently available.
- 2. Interpretation:** Creatinine and body mass index (BMI) were related to plasma biomarkers concentrations (plasma neurofilament light [NfL], glial fibrillary acidic protein [GFAP], p-tau) across two large cohorts. However, when adding these two factors in models assessing biomarker performance for different clinical outcomes (relation to cerebrospinal fluid [CSF] markers and conversion to dementia), their impact was modest.
- 3. Future directions:** Assessing how potential confounding factors might affect plasma biomarkers level in longitudinal settings will be important, especially in individuals within the abnormal range of such confounding factors. Similar investigations in populations with more diverse presentation of comorbidities is needed.

tee in Lund, Sweden. All participants gave written informed consent to participate. BioFINDER-1 participants were enrolled between January 2010 and December 2014 and were followed longitudinally for up to 8 years. Baseline data and conversion status to AD dementia or any dementia was used from BioFINDER-1. BioFINDER-2 is an ongoing longitudinal study that was launched in 2017, which does not overlap with BioFINDER-1. For the current study, baseline data was included from participants recruited between April 2017 and April 2021.

The main inclusion criteria were to be 60 years or older in BioFINDER-1 or 40 years and older in BioFINDER-2, being fluent in Swedish, having Mini-Mental State Examination (MMSE) score between 27 and 30 for cognitively normal participants and between 24 and 30 for MCI. Exclusion criteria were having significant unstable systemic illness, neurological or psychiatric illness, significant alcohol or substance misuse, or refusing lumbar puncture or neuroimaging. MCI diagnostic differed slightly between studies. In BioFINDER-1, MCI status was determined after an extensive neuropsychological assessment if participant performed worse than one to two standard deviations on various tests.²⁰ In BioFINDER-2, MCI diagnosis was established if participants performed below 1.5 standard deviation from norms on at least one domain from an extensive neuropsychological battery examining verbal, episodic memory, visuospatial ability, and attention/executive domains.¹⁴ Dementia diagnosis was determined by consensus of memory clinic physicians and neuropsychologist. For

AD dementia, diagnosis was based on the criteria from the Diagnostic and Statistical Manual of Mental Disorders Fifth Edition and if positive on A β biomarkers based on the updated National Institute on Aging and Alzheimer's Association (NIA-AA) criteria for AD.²¹ BioFINDER-2 further included a cohort of non-AD dementias and neurodegenerative disorders in which fulfilment of criteria for dementia or major neurocognitive disorder could be due to frontotemporal dementia, Parkinson's disease, vascular dementia, dementia with Lewy bodies, progressive supranuclear palsy, multiple system atrophy, corticobasal syndrome, or primary progressive aphasia.

Greater detail of both studies has been described previously.^{7,14,22}

2.2 | Medical history

In both cohorts, information on medical history and medications were recorded at the baseline visit. The information was retrieved from both the medical records of each participant and questionnaires answered by the participant and/or his/her informant.

2.3 | Creatinine and BMI measurements

All participants had data available for plasma creatinine and BMI. Creatinine levels were measured in plasma taken from a blood draw at the baseline visit. Blood was sampled in the morning (not after fasting) and each sample was analyzed at Skåne University Hospital. The CREP2 Cobas 501 (2016-12, V13.0) or CREP2 Cobas 701 (2018-03, V10.0) analytical unit from Roche Diagnostics were used to measure creatinine (reported in $\mu\text{mol/l}$). In BioFINDER-1, the average time between the blood draw at baseline to measure creatinine and the blood draw/lumbar puncture to measure biomarkers was 12 days \pm 36 days, with six participants (<1%) exceeding a 6-month difference. In BioFINDER-2, it was 51 days \pm 53 days, with no one exceeding 6-month difference. To calculate BMI, height and weight were taken at baseline visit for every participant. As complementary analyses, we also calculated two common measures of creatinine clearance from the Cockcroft-Gault equation²³ (that includes creatinine, age, sex, weight, and body surface area) and the the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation²⁴ (that includes only creatinine, age, and sex).

2.4 | Plasma and cerebrospinal fluid biomarkers

Biomarkers analyzed in plasma and CSF included A β 42, A β 40, p-tau217, p-tau181, NfL, and GFAP. All biomarker concentrations were measured from blood and CSF samples taken the same day in the morning, not after fasting.

2.4.1 | A β 42 and A β 40

In BioFINDER-1, plasma A β 42 and A β 40 were measured using Elecsys immunoassays on a Cobas e 601 module (Roche Diagnostics)

as described previously.³ In BioFINDER-2, plasma A β 42 and A β 40 were measured using the liquid chromatography mass-spectrometry based technology from Araclon Biotech, as described recently.²⁵ In both cohorts, CSF A β 42 and A β 40 were measured using the Elecsys immunoassays (Roche Diagnostics).²⁶

For a subset of BioFINDER-1 participants ($n = 635$), plasma A β 42 and A β 40 was also measured using the immunoprecipitation-coupled mass spectrometry developed at Washington University (WashU-IP-MS), as described previously.⁵ In head-to-head comparisons with eight other plasma A β assays, the IP-MS-WashU assay was recently shown as the best one to identify participants classified as being A β -positive on CSF and on PET.²⁷ We validated the main plasma A β findings with the WashU-IP-MS assays, and all results can be found in Supplementary material.

2.4.2 | p-tau217 and p-tau181

In both BioFINDER-1 and BioFINDER-2, plasma as well as CSF p-tau217 was measured using immunoassays on the Meso-Scale Discovery platform developed by Lilly Research Laboratories as described previously.^{20,28} The calibration of the assays differed between cohorts, which explains the different range of values. In BioFINDER-1 the assays were calibrated with a synthetic p-tau217 peptide, while in BioFINDER-2 a recombinant tau protein phosphorylated in vitro was used. All details have been described recently.²⁵

For p-tau181, plasma assays differed between cohorts. In BioFINDER-1 the assay from Lilly Research Laboratories based on the Meso-Scale Discovery platform was used, as described recently⁷, and was available for 570 participants out of 748. In BioFINDER-2, the Simoa assay developed at the University of Gothenburg was used.² CSF p-tau181 was measured using the Elecsys immunoassays (Roche Diagnostics)^{26,29} in both cohorts.

In both cohorts, some participants had plasma p-tau217 and p-tau181 levels below the detection level of the assay (56 participants [7.5%] in BioFINDER-1 and 52 participants [12%] in BioFINDER-2 for p-tau217; 44 participants [7.7%] in BioFINDER-1 and 10 participants [2.4%] in BioFINDER-2 for p-tau181). In those cases, values were imputed by the lowest detection level of the assay. We also repeated the analyses excluding the participants with very low values and all results remained the same.

2.4.3 | NfL

In both cohorts, plasma NfL concentrations were measured using the commercially available Single molecule array (Simoa),^{30,31} and CSF NfL was measured using the Elecsys immunoassay (Roche Diagnostics).

2.4.4 | GFAP

In BioFINDER-2, plasma GFAP was measured with Simoa GFAP Discovery kits for SR-X, as previously described.³² For plasma GFAP in

BioFINDER-1 and CSF GFAP in both cohorts, the Elecsys immunoassay (Roche Diagnostics) was used.¹⁰

2.5 | Statistical analysis

The two cohorts were analyzed separately. First, we used linear regression models (including age and sex as covariates) to investigate the associations of each plasma marker (dependent variable) with creatinine, BMI, comorbidities, and medication use separately, to identify the most important potential confounders for further analyses. For associations that were significant, we further adjusted the model for global A β -PET SUVR (flutemetamol)¹⁴ to evaluate if the associations between plasma biomarkers and potential confounding factors were independent of existing pathology (e.g., prodromal AD has been shown to be directly associated with lower BMI, which is likely to be a consequence of the disease³³). Second, we wanted to evaluate if the identified factors influenced the associations between each biomarker measured in plasma and its CSF counterpart. For that, we compared the plasma coefficient between two regression models. The first model only included basic co-variates (CSF level ~ plasma level + age + sex). The second model further included the potential confounders as covariates (e.g., CSF level ~ plasma level + age + sex + creatinine + BMI). To determine whether the plasma coefficients were significantly different between the two models, we used a non-parametric procedure: we bootstrapped each model over 10,000 iterations and subtracted the plasma coefficients from all iterations between the two models. We then used the 95% confidence interval (CI) of this difference to assess if plasma estimates differed between models. Third, we wanted to evaluate whether the key confounders influenced the plasma estimate in assessing conversion to either AD dementia or all-cause dementia over a 4-year period,²⁰ using logistic regression. This was only done in BioFINDER-1, where longitudinal data was available. Non-dementia converters with less than 4 years of follow-up were censored. For analyses related to conversion to AD dementia, individuals who converted to another type of dementia were excluded. We used a similar approach as for the plasma-CSF comparisons, with a bootstrap procedure to compare odds ratio of plasma biomarkers from logistic models that discriminated between participants who remained stable versus those who converted to dementia. The base logistic regression model included plasma biomarker, age, and sex, and the second model further included key confounders as covariates. Standardized coefficients are reported for all analyses, so that different models can be compared. For data visualization, the raw biomarkers values are shown, to display accurately the range of values of the different assays used. Note that the raw biomarkers values were used in the main analyses, but results were unchanged when using log-transformed values (Supporting Information Figure S4 and Tables S5 and S6, which are available online). To further investigate the effect of adjusting for potential confounding factors at the individual level, we also calculated the relative change (in %) in predicting the dependent variable from the base model versus the model including confounding factors for each participant (more details and results in Supporting Information Figure S3).

All analyses were performed on R version 4.0.5 using the packages ABA (Automated Biomarker Analyses) version 1.0.0¹⁵ for linear regressions, Boot version 1.3-27 for bootstrapping and pROC version 1.17.0.1 for logistic regression.

3 | RESULTS

3.1 | Participants

Two-thirds of BioFINDER-1 participants were cognitively normal and the remaining third were MCI participants (Table 1). Diagnoses were more varied in BioFINDER-2, where 56%, 23%, and 21% were diagnosed as cognitively normal, MCI, or AD dementia or non-AD neurodegenerative diseases (52 with AD, 3 with Parkinson's disease, 6 with progressive supranuclear palsy, 6 with Lewy body dementia, 1 with corticobasal syndrome, 7 with behavioral variant frontotemporal dementia, 3 with multiple system atrophy, 3 with primary progressive aphasia, 7 with vascular dementia, 1 with unspecified cause of dementia), respectively. Both cohorts were balanced between men and women and creatinine levels and BMI were similar between cohorts. Generally, both cohorts presented similar frequency of comorbidities and medication use, with the exception that more participants in BioFINDER-1 had hypertension, hyperlipidemia, and a previous stroke (Table 1). Since all plasma assays apart from NfL differed between cohorts, each cohort was analyzed separately (see Supporting Information Table S1 for average plasma and CSF biomarkers levels). The main biomarkers of interest were the A β 42/A β 40 ratio, p-tau217, NfL, and GFAP. Results related to A β 42 and A β 40 used individually, and p-tau181 can be found in Supporting Information material.

3.2 | Associations of creatinine, BMI, and comorbidities with plasma biomarkers

Of all evaluated variables, creatinine and BMI were the main factors associated with different plasma biomarkers concentrations (Figure 1). In both cohorts, creatinine level was positively associated with NfL and GFAP levels. In BioFINDER-1 only, higher creatinine was also associated with higher p-tau217 and p-tau181 (Supporting Information Figure S1). In both cohorts, BMI was negatively associated with NfL, GFAP, p-tau217, and p-tau181 levels. Further, creatinine and BMI were associated with A β 42 and A β 40 alone (Supporting Information Figure S1), but not with the A β 42/A β 40 ratio. Across both cohorts, there were no consistent effect of different comorbidities and medication use on plasma levels of A β 42/40, p-tau217, p-tau181, NfL, or GFAP (Figure 1 and Supporting Information Figure S1). Rather, comorbidities or medication use were more related to levels of A β 42 and A β 40 alone, but not their ratio. Hypertension, diabetes, taking anti-hypertensive/cardioprotective medication or lipid lowering medication were related to both higher plasma A β 42 and A β 40 levels (Supporting Information Figure S1), as reported previously.³⁴ In BioFINDER-1, a subset of participants had plasma A β levels measured

TABLE 1 Demographics of BioFINDER-1 and BioFINDER-2 cohorts

	BioFINDER-1 (n = 748)	BioFINDER-2 (n = 421)
Diagnostic CN:MCI:Dementia	503:245:0	235:97:89
Age	71.7 ± 5.5	65.7 ± 13.3
Sex F:M (%F)	359:389 (48%)	210:211 (50%)
Creatinine (μmol/l)	78.6 ± 18.4	78.2 ± 18.4
Body mass index (kg/m ²)	26.0 ± 4.1	26.2 ± 4.3
Conversion to AD dementia, n (%)	92/563 (16.3%)	-
Conversion to any dementia, n (%)	149/619 (24.1%)	-
Comorbidities		
Hypertension, n (%)	276 (36.9%)	113 (26.8%)
Hyperlipidemia, n (%)	174 (23.3%)	45 (10.7%)
Diabetes, n (%)	72 (9.6%)	46 (10.9%)
Ischemic heart disease, n (%)	83 (11.1%)	34 (8.1%)
Stroke, n (%)	62 (8.3%)	14 (3.3%)
Medication use		
Anti-hypertensive/cardioprotective drugs, n (%)	348 (46.5%)	175 (41.6%)
Lipid lowering drugs, n (%)	230 (30.7%)	129 (30.6%)
Platelet aggregation inhibitors, n (%)	200 (26.7%)	83 (19.7%)

Note: Data presented as mean ± standard deviation unless specified otherwise.
Abbreviations: CN, cognitively normal; MCI, mild cognitive impairment.

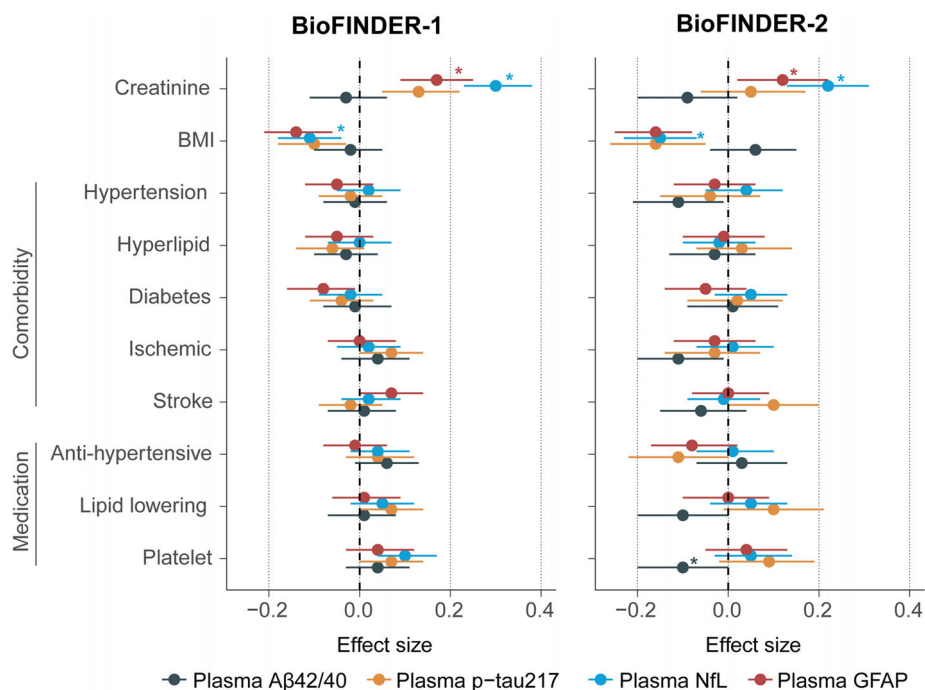


FIGURE 1 Associations between creatinine, body mass index, comorbidities, and medication use with plasma biomarkers. Standardized beta coefficients and 95% confidence interval from linear regression models adjusted for age and sex with plasma biomarkers in BioFINDER-1 (left) and BioFINDER-2 (right). The A β 42/40 ratio was reversed so that the effect size moves in the same direction for each biomarker. As such, higher value on each biomarker is more abnormal (towards AD). Stars indicate that the association remained significant when further adjusting for flutemetamol global A β -PET SUVR. In BioFINDER-2, all associations also remained significant if adding temporal meta-ROI tau-PET SUVR from [18F]RO948 as a covariate. A β , beta-amyloid; BMI, body mass index; CSF, cerebrospinal fluid; GFAP, glial fibrillary acidic protein; NfL, neurofilament light; p-tau217, phosphorylated tau 217

with the WashU-IP-MS assay ($n = 635$), in which the same effects on A β 42 and A β 40 alone, but none on the A β 42/A β 40 ratio, were corroborated (Supporting Information Figure S2). Results were similar using measures of creatinine clearance (Supporting Information Figure S3) or log-transformed plasma values (Supporting Information Figure S4).

We further examined if the significant associations between potential confounders and plasma biomarkers were independent of brain pathology by adding global A β -PET SUVR as a covariate. The associations of creatinine with NfL and GFAP were unchanged. However, BMI was no longer associated with plasma biomarkers, except for plasma NfL (Figure 1). All associations with A β 42 and A β 40 alone remained unchanged (Supporting Information Figure S1). These results suggest that the association of plasma biomarker concentrations with creatinine are likely due to a confounding effect on plasma concentrations (i.e., lower kidney function resulting in somewhat higher biomarker concentrations especially for NfL and GFAP). On the other hand, associations with BMI might be due partly to blood volume diluting plasma biomarkers concentrations in the case of NfL but to general disease progression for other markers (i.e., a lower BMI in individuals with prodromal AD induced by the disease^{33,35}). Overall, given that the strongest and consistent associations with plasma biomarkers were with creatinine and BMI, subsequent analyses focused on these two confounders.

3.3 | Effect of creatinine and BMI on plasma-to-CSF associations of AD biomarkers

Next, we investigated whether accounting for creatinine and BMI influenced the associations between plasma and CSF concentrations of each biomarker. Generally, p-tau217, followed by NfL showed the greatest correlations between the concentrations in plasma and the concentrations in CSF of the same biomarker (Figure 2 and Table 2).

In both cohorts, NfL was the only biomarker for which accounting for creatinine and BMI consistently improved the plasma coefficient (by 6% to 10%) in relation to CSF when using plasma concentration as independent variable (Figure 2C, Table 2). When inverting the associations (using CSF concentration as the independent variable), no improvements were seen in CSF estimates when accounting for creatinine and BMI, showing that these two confounders only affect plasma, and not CSF, concentrations. At the individual level, we evaluated the relative change in percentage in predicting CSF NfL in the basic model versus the models further including creatinine and BMI as covariates. Generally, the plasma NfL model adjusted for creatinine improved the predictions of CSF NfL concentrations in participants with high creatinine values, but worsened the predictions in subjects with low creatinine values ($R = 0.44$ (BioFINDER-1) and $R = 0.28$ (BioFINDER-2) from associations between relative change between models and creatinine; Supporting Information Figure S5), while there was no or only minor effect of BMI (Supporting Information Figure S3). However, in general, the relative change between models across participants was modest, with an average of 0% and standard deviation of 13%. The

number of participants where the adjusted plasma NfL model improved the prediction of the CSF NfL concentrations was just slightly higher (50% in BioFINDER-1 and 51% in BF-2) compared to the number of cases where the model worsened the prediction (49% in BioFINDER-1 and 44% in BioFINDER-2) when compared to the basic plasma NfL model.

Plasma GFAP estimates were improved when the CSF measure was accounted for creatinine and BMI in BioFINDER-1 by 6%, but no effect was seen in BioFINDER-2 (Figure 2D, Table 2). Regarding the associations in A β biomarkers, the strongest effects of adjusting for creatinine and BMI were seen on A β 42 and A β 40 alone (Supporting Information Table S2 and Supporting Information Figure S6, Table S3 for WashU-IP-MS assays), while effects on the A β 42/40 ratio were more limited, with improvements between 2% and 3% (Figure 2A, Table 2).

3.4 | Effect of creatinine and BMI on plasma biomarkers to estimate subsequent conversion to dementia in non-demented participants

In BioFINDER-1, long-term longitudinal data were available, and participants had follow-up visits. In this cohort, we assessed whether accounting for creatinine and BMI influenced the plasma estimate to discriminate participants who remained stable versus those who converted to dementia within a 4-year period. Receiver operating characteristic (ROC) curves from models assessing conversion to AD dementia using plasma A β 42/40, p-tau217 and GFAP, and conversion to all-cause dementia using NfL are shown in Figure 3, and results from all models are shown in Table 3. When further including creatinine and BMI in models, plasma p-tau217 odds ratio in discriminating AD dementia converters from stable participants, and plasma NfL odds ratio in discriminating all-cause dementia converters from stable participants were improved by 4.5% (Table 3). Similar results as with p-tau217 was also observed with p-tau181 (Supporting Information Table S4). Although plasma estimates were significantly improved when accounting for creatinine and BMI, the discriminative accuracies between models were virtually the same, with a maximum change in area under the ROC curve (AUC) of 0.01. Looking at the individual level, the relative changes between basic model versus when including creatinine and BMI for p-tau217 and NfL was lower than the changes seen in the plasma-CSF associations, with a standard deviation of 5%. Again, slightly more participants had better outcome prediction when including creatinine and BMI in logistic regressions (44% and 48% for p-tau217 and NfL, respectively) compared to the number of cases where the model adjusted for confounding factors was worse than the basic model (33% and 40% for p-tau217 and NfL respectively; Supporting Information Figure S7). Here, improvements in the models adjusted for creatinine and BMI were seen in participants with higher creatinine levels and higher BMI for both biomarkers ($R = 0.33$ and 0.36 for associations between relative change between models and creatinine, and $R = 0.29$ for same associations with BMI, Supporting Information Figure S7). All previous analyses related to progression to dementia or associations with CSF are unchanged if using log-transformed values

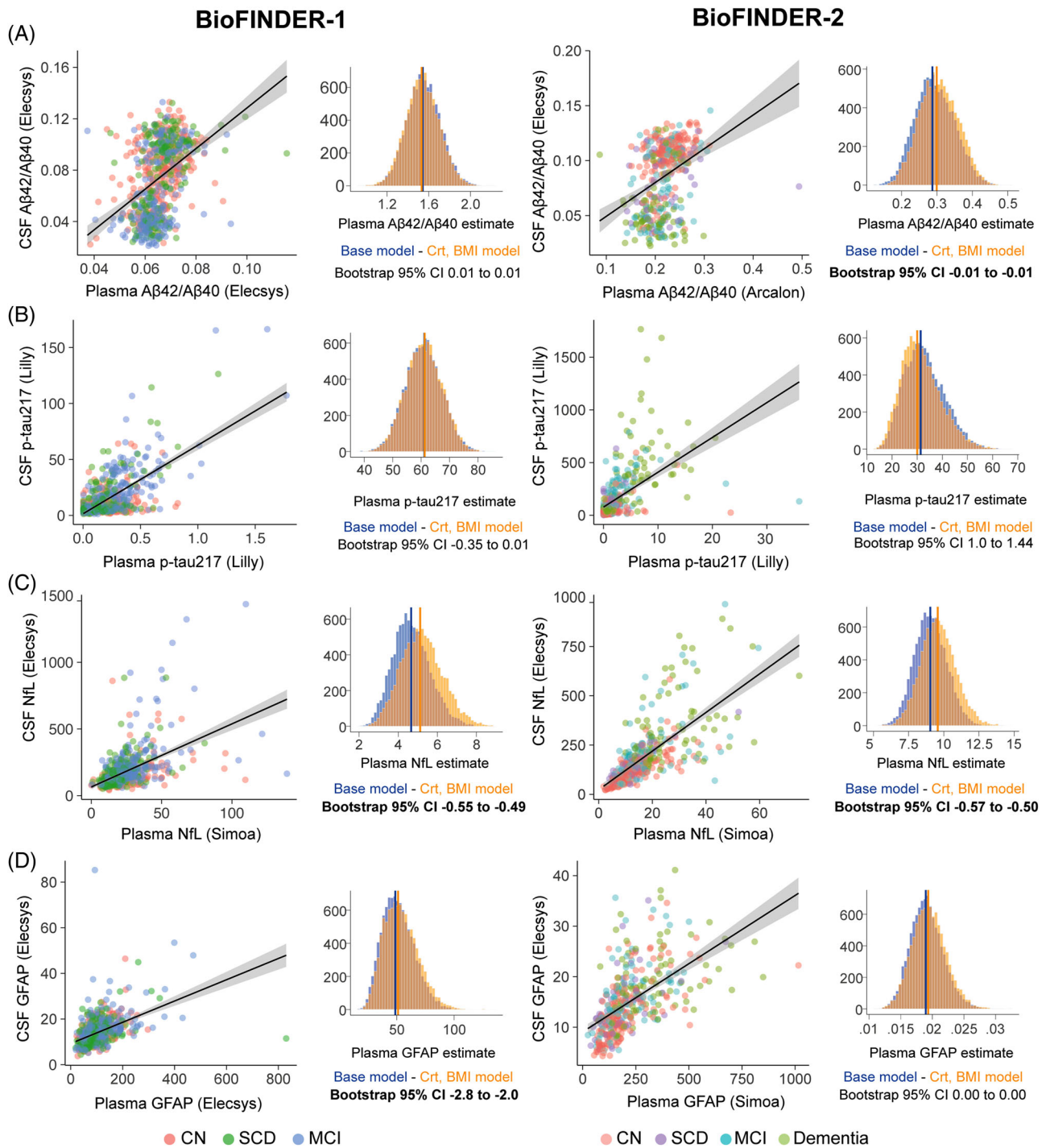


FIGURE 2 Associations between plasma and corresponding cerebrospinal fluid biomarkers. Scatter plots depict bivariate associations between plasma and CSF levels for A β 42/40 ratio (A), p-tau217 (B), NfL (C), and GFAP (D) in BioFINDER-1 on the left-hand side and BioFINDER-2 on the right-hand side. To compare plasma coefficients between the base model and the one including creatinine and BMI as covariates, we generated 10,000 bootstrap samples of both models, shown in the histograms. Significant difference between models was based on the 95% confidence interval difference of the difference of plasma estimates between models. A β , beta-amyloid; BMI, body mass index; Crt, creatinine; CSF, cerebrospinal fluid; GFAP, glial fibrillary acidic protein; NfL, neurofilament light; p-tau217, phosphorylated tau 217

TABLE 2 Plasma biomarker coefficients related to corresponding cerebrospinal fluid biomarker levels

	A β 42/40		p-tau217		NfL		GFAP		
	β	R ²	β	R ²	β	R ²	β	R ²	
BioFINDER-1									
Base model	0.41	0.18	0.66	0.43	0.49	0.27	0.31	0.13	
Model including creatinine and BMI	0.41	0.19	0.66	0.45	0.54	0.29	0.33	0.13	
BioFINDER-2									
Base model	0.34	0.26	0.51	0.33	0.64	0.49	0.40	0.43	
Model including creatinine and BMI	0.35	0.31	0.49	0.36	0.68	0.50	0.41	0.43	

Note: Standardized beta coefficients of plasma biomarkers from models assessing CSF (dependent variable) and plasma biomarkers levels adjusted for age and sex ("Base model"), or from models further including creatinine and BMI as covariates. The assays used for each biomarker are detailed on Figure 2. Adjusted R² of models are also reported. Bolded values indicate when significant improvement in plasma estimate was seen in models including creatinine and BMI based on bootstrapping. Significance of all plasma coefficients and of all models is $P < .001$.

Abbreviations: A β , beta-amyloid; BMI, body mass index; GFAP, glial fibrillary acidic protein; NfL, neurofilament light; p-tau217, phosphorylated tau 217.

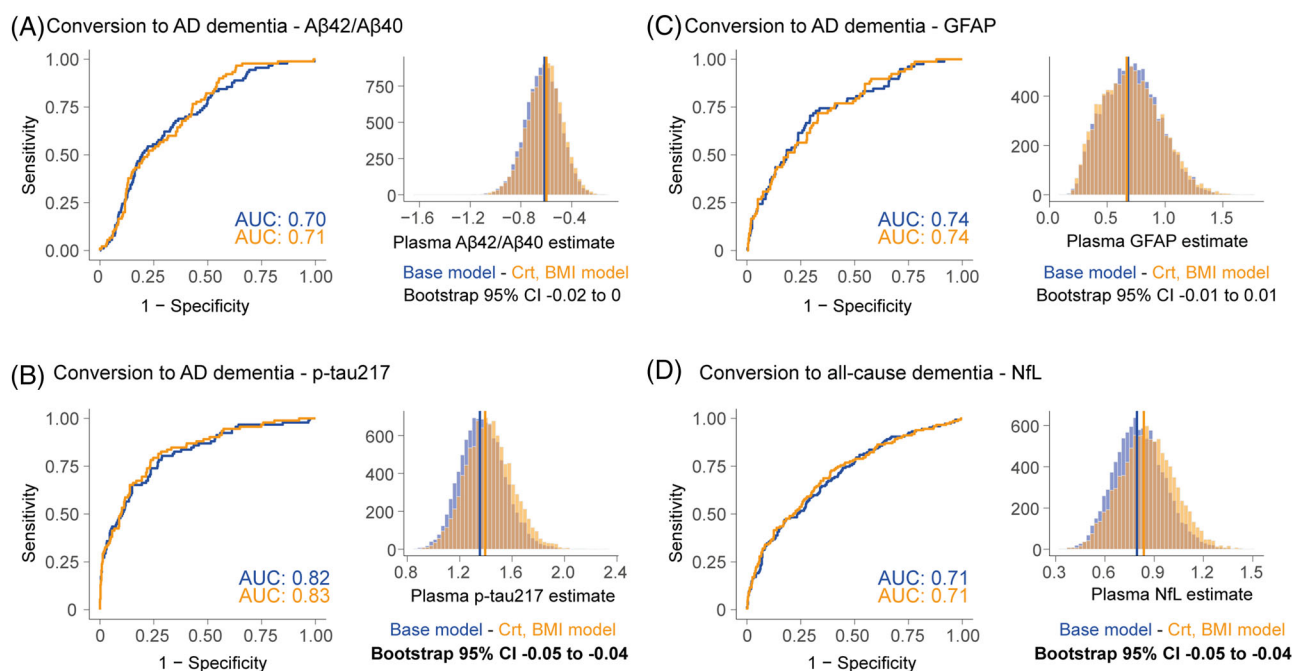


FIGURE 3 Plasma biomarkers on assessing conversion to AD or all-cause dementia in non-demented participants. ROC curves showing accuracy to discriminate between BioFINDER-1 participants who remained cognitively normal versus those who progressed to AD dementia based on plasma A β 42/A β 40 ratio (A), p-tau217 (B) and GFAP (C), and to discriminate participants who remained cognitively normal versus those who progressed to all-cause dementia based on plasma NfL (D). Results from logistic regression including plasma levels, age and sex are shown in the blue curve and models including creatinine and BMI as additional covariates in the orange curve. Plasma estimates between both models were then compared using bootstrapping as shown in the histograms and as done previously. A β , beta-amyloid; BMI, body mass index; Crt, creatinine; CSF, cerebrospinal fluid; GFAP, glial fibrillary acidic protein; NfL, neurofilament light; p-tau217, phosphorylated tau 217

or measures of creatinine clearance (Supporting Information Tables S5 and S6).

4 | DISCUSSION

We investigated whether comorbidities, medication use, plasma creatinine, and BMI – measures routinely assessed in clinical settings – influenced key plasma AD markers in two large and independent

cohorts. We studied whether such measures affected plasma markers levels, and whether they influenced the associations between plasma and CSF markers and the ability of plasma markers to predict future dementia. Our focus was on A β 42/A β 40 ratio, p-tau217, NfL, and GFAP, and supplementary analyses included A β 42, A β 40, and p-tau181. Creatinine and BMI were related to concentrations of plasma NfL, GFAP, and to a lesser extent p-tau217 and 181, but not the A β 42/A β 40 ratio. However, including creatinine and BMI in models aimed toward clinical outcomes provided only very modest effect in improving plasma

TABLE 3 Plasma estimates related to conversion to dementia from logistic regressions in BioFINDER-1

	A β 42/40 (Elecys)		p-tau217		NfL		GFAP	
	OR	AUC	OR	AUC	OR	AUC	OR	AUC
Conversion to AD-dementia within 4 years								
Base model	0.54	0.70	3.87	0.82	1.66	0.67	1.98	0.74
Model including creatine and BMI	0.55	0.71	4.03	0.83	1.69	0.67	1.95	0.74
Conversion to all-cause dementia within 4 years								
Base model	0.65	0.65	2.48	0.73	2.21	0.71	1.79	0.70
Model including creatine and BMI	0.65	0.67	2.46	0.74	2.31	0.71	1.78	0.71

Note: Odds ratio of plasma biomarkers from logistic regression to discriminate participants who remained stable versus those who converted to AD dementia or all-cause dementia. Odds ratio represents the increased odds of converting to dementia for each increase in standard deviation biomarker value. Models included age and sex as covariates. Bolded values indicate when significant improvement in plasma estimate was seen in models including creatinine and BMI based on bootstrapping. Significance of all plasma odds ratio is $P < .001$.

Abbreviations: AUC, area under the curve; A β , beta-amyloid; BMI, body mass index; GFAP, glial fibrillary acidic protein; NfL, neurofilament light; OR, odds ratio.; p-tau217, phosphorylated tau 217.

estimates when predicting either (1) the corresponding concentrations in CSF or (2) subsequent development of dementia in non-demented individuals.

We investigated a comprehensive array of comorbidities and medication use, but creatinine and BMI were the two measures being consistently associated with plasma concentrations of several AD-related biomarkers. Higher creatinine levels and lower BMI were related to greater plasma concentrations of certain markers (especially plasma NfL and GFAP, and to a lesser extent p-tau). The directionality of such results might be explained by lower blood filtration (and thus higher creatinine) being associated with greater levels of biomarkers, and that greater blood volume, due to higher BMI, dilutes biomarkers levels in plasma. Furthermore, most associations between creatinine and plasma biomarkers seem to be independent of pathology in the brain as measured by PET, which was not the case for most associations with BMI. Apart from NfL, the relations between lower BMI and more abnormal plasma biomarkers concentrations are likely due to loss of weight associated with prodromal AD.^{33,36} Consequently, plasma creatinine is likely to have confounding effect on plasma biomarkers related to blood filtration, but the effect of BMI might not solely be a result of a confounding effect of higher blood volume in subjects with higher BMI, except for NfL which was associated with BMI even when adjusting for A β -PET. Our results are in line with previous studies that showed associations between creatinine, kidney function or BMI and plasma NfL and A β .^{18,19,37} We also found associations with these confounding factors and plasma A β 42 or A β 40 alone, but not with the ratio A β 42/A β 40 which was consistent in two cohorts studied here using three different A β assays. Generally, all associations detected were consistent with either A β 42 or A β 40, but were not present with the A β 42/40 ratio. Our interpretation is that the effects on A β 42 or A β 40 were essentially cancelled out using the ratio, further highlighting the ratio as a measure of choice less affected by confounding factors.

An important novelty of the study resides in extending analysis to evaluate to which extent the main confounding factors (i.e., kidney function and BMI) affected the clinical performance of the blood-based biomarkers, that is, their ability to predict (1) their corresponding con-

centrations in CSF (which should be more closely related to the levels in the brain) and (2) clinical progression to dementia (which is a very clinically relevant outcome). First, we reported correlations between the concentrations in plasma and CSF for each marker, which were especially strong for NfL and p-tau217.^{16,38} Given that the highest effect of creatinine and BMI were seen on plasma NfL concentrations, it is perhaps not surprising that creatinine and BMI improved the plasma NfL estimates related to NfL concentrations in the CSF, which was consistent in both cohorts. We note, however, that the improvement in the magnitudes of plasma estimates and R2 values were very modest, when assessed at the group level (Figure 2C and Table 2). Furthermore, when assessed at the individual level very few participants exhibited clearly improved prediction of the CSF NfL concentration when plasma NfL was adjusted for either plasma creatinine or BMI (Supporting Information Figure S3). Several studies have shown that plasma biomarkers can be used to predict subsequent development of AD dementia as well as all-cause dementia,^{7,15,20} however, no study has yet determined whether adjustment for kidney function or BMI might improve such predictions. We found that estimates for p-tau217 (when predicting AD dementia) and NfL (when predicting all-cause dementia) were improved when creatinine and BMI were considered. However, those improvements were even smaller than those seen in the plasma-CSF associations. The accuracies to differentiate stable participants from the ones who converted to dementia were virtually unchanged by including creatinine and BMI, with a change of only 0.01 in AUCs between models (Figure 3 and Table 3). Similarly, at the individual level very few participants exhibited improved prediction of dementia when adjusting for creatinine or BMI (Supporting Information Figure S5). We therefore propose that BMI and creatinine play a minor role in predictive value of plasma biomarkers for the vast majority of individuals assessed. Still, the classification accuracy of plasma biomarkers and basic demographics alone to predict clinical progression is in line with other studies, with p-tau217 being the best marker for AD dementia.^{20,39}

The study has various strengths and limitations. We relied on two large, independent, and well-characterized cohorts. While the assays

were not always the same between the two cohorts, most of the results were nevertheless consistent across the two cohorts. As the plasma A β assays relied on different technology between cohorts (immunoassays and mass spectrometry), we validated the main results using the currently best mass spectrometry assays available in a large subset of participants.²⁷ Results are reflective of two cohorts in which the majority of participants have BMI or creatinine levels within normal range. For instance, across both cohorts, around 15% of participants have BMI above 30, and 6% of women had creatinine levels above 90 $\mu\text{mol/L}$ and 11% of men had creatinine levels above 105 $\mu\text{mol/L}$, taken as general cut-points for high values. Participants did not present with severe kidney diseases, which in itself had been related to greater risk of cognitive impairment⁴⁰ and shown to affect plasma biomarkers levels.¹⁸ Given that we only studied baseline levels of plasma biomarkers, it will be important to evaluate how fluctuations in plasma creatinine levels might influence longitudinal changes in plasma biomarker concentrations, because when used as outcomes in trials rather small changes in biomarker concentrations over time (e.g., 10%–15%) might be considered relevant and thereby more susceptible to minor variations. Also, our current focus was on using measures that are routinely assessed clinically, like plasma creatinine. Future studies may also include other proxies of kidney function, such as cystatin-C, which may allow a more precise estimate of the glomerular filtration rate.^{41,42}

Taken together, we showed that plasma creatinine levels and BMI were related to axonal degeneration marker NfL in plasma, and to a lesser extent to GFAP and p-tau proteins. The current study suggests that creatinine and BMI only have minor effects on improving the performance of AD-relevant plasma biomarkers. Consequently, diagnostic and prognostic algorithms for AD based on plasma biomarkers generally do not need to be adjusted for creatinine levels or BMI, even though these two factors have been the two most relevant confounding variables for plasma AD biomarkers identified until now.

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CONFLICTS OF INTEREST

O.H. has acquired research support (for the institution) from ADx, AVID Radiopharmaceuticals, Biogen, Eli Lilly, Eisai, Fujirebio, GE Healthcare, Pfizer, and Roche. In the past 2 years, he has received consultancy/speaker fees from Amylyx, Alzpath, BioArctic, Biogen, Cerveau, Fujirebio, Genentech, Novartis, Roche, and Siemens. J.L.D. is an inventor on patents or patent applications of Eli Lilly and Company relating to the assays, methods, reagents and/or compositions of matter used in this work. J.L.D. has served as a consultant for Genotix Biotechnologies Inc, Gates Ventures, Karuna Therapeutics, AlzPath Inc, and received research support from ADx Neurosciences, Roche Diagnostics and Eli Lilly and Company in the past 2 years. H.Z. has served at scientific advisory boards and/or as a consultant for Alector, Eisai, Denali, Roche Diagnostics, Wave, Samumed, Siemens Healthineers, Pinteon Therapeutics, Nervgen, AZTherapies, CogRx and Red Abbey Labs, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure and Biogen. K.B. 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. H.Z. and K.B. are co-founders of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program. RJB cofounded C2N Diagnostics. Washington University and RJB have equity ownership interest in C2N Diagnostics and may receive income based on technology (stable isotope labeling kinetics and blood plasma assay) licensed by Washington University to C2N Diagnostics. R.J.B. receives income from C2N Diagnostics for serving on the scientific advisory board. R.J.B. has received honoraria as a speaker, consultant, or advisory board member from Amgen and Roche. All other authors

declare no conflict of interest. Author disclosures are available in the supporting information.

ORCID

Alexa Pichet Binette  <https://orcid.org/0000-0001-5218-3337>

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SUPPORTING INFORMATION

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