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Original Article

# Impact of cardiovascular risk factors on plasma biomarkers in prediction of Alzheimer's and cerebrovascular neuropathology

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ABSTRACT

*Background:* Plasma biomarkers for Alzheimer's disease and neurodegeneration have shown accurate prediction of underlying neuropathology. However, chronic cardiovascular risk factors such as diabetes and hypertension are associated with plasma biomarker levels and can influence the accurate prediction of underlying neuropathologic changes.

*Objective:* To understand the interaction between plasma biomarkers of Alzheimer's disease and neurodegeneration with cardiovascular risk factors in relation to neuropathologic change in a heterogenous population to ascertain a more accurate utilization of these biomarkers.

*Design:* Retrospective, case-control study.

*Setting:* Population-based, Olmstead county, Minnesota, USA.

*Participants:* Three-hundred and fifty-one participants (aged 87.4 ± 7.5 years) with brain autopsy and antemortem plasma biomarker testing.

*Measurements:* Plasma biomarker testing for A$\beta$42/40, p-tau181, GFAP, and NfL using Quanterix Simoa assays. Cardiovascular risk factors were quantified by a composite score of cardiovascular metabolic conditions (CMC) consisting of a binary history of diabetes, congestive heart failure, stroke, coronary artery disease, atrial fibrillation, hypertension, or dyslipidemia. Plasma biomarkers and cardiovascular metabolic conditions score were Z-scored and neuropathologic scales were binarized into high and low categories. Outcomes included elevated microvascular (Kalaria) and macrovascular (Strozyk) neuropathologic scales as well as Alzheimer's disease neuropathologic change (ADNC), Thal phase, Braak stage, and neuritic plaque score. Multivariate logistic regression models incorporated interaction terms between plasma biomarkers and CMC while controlling for age, sex, cognitive impairment, and BMI.

*Results:* We observed that at higher cardiovascular metabolic conditions score, the association between GFAP and overall ADNC (OR = 0.61 [0.42, 0.89]), Thal phase (OR = 0.48 [0.33, 0.71]), and Braak Stage (OR = 0.56 [0.37, 0.84]), became weaker, while the association with Strozyk score (OR = 1.65 [1.11, 2.46]) was stronger with higher CMC. Meanwhile, at higher CMC A$\beta$42/40 became more strongly negative with high Braak stage (OR = 0.63 [0.47, 0.85]), neuritic plaque score (OR 0.70 [0.52, 0.95]), Kalaria score (OR = 0.71 [0.57, 0.88]), and Strozyk score (OR = 0.60 [0.43, 0.83]). The association between p-tau181 and Thal phase (OR = 1.43 [1.00, 2.04]) was stronger at higher CMC while the association between p-tau181 and Strozyk score (OR = 0.47 [0.31,

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0.71]) was weaker at higher CMC. There was no interaction between NfL and CMC score for any metric of neuropathologic change.

*Conclusion:* Understanding how cardiovascular risk factors can modulate plasma biomarkers is important for their interpretation with respect to underlying pathology and their clinical application in screening, diagnosis, and prognosis of neurodegenerative diseases.

## 1. Introduction

Plasma biomarkers have shown great promise as emerging tools in early diagnosis of Alzheimer's Disease (AD) and other dementias [1,2] to discriminate those cognitively unimpaired individuals and those with mild cognitive impairment (MCI) or dementia due to AD [3–10]. Plasma biomarkers include amyloid-$\beta$ 42, amyloid-$\beta$ 40, amyloid-$\beta$ 42/40 ratio (A$\beta$42/40), total tau, several phosphorylated tau proteins (e.g. p-tau181, p-tau217, and p-tau231), glial fibrillary acidic protein (GFAP), and neurofilament light chain (NfL). Recent work has also shown that subsets of these plasma biomarkers have comparable performance to CSF biomarkers and amyloid PET when predicting underlying AD neuropathologic change (ADNC) [5,7,11–18]. Quantifying the antemortem burden of amyloid, tau, and neurodegeneration (as proposed by the A/T/N framework) [19] and an overall measure of ADNC can establish the effect of neuropathologic change with clinical symptoms [20]. Ideally, plasma biomarkers would serve as tools for early diagnosis to benefit patients through counseling, therapy eligibility, and clinical trial candidacy.

Despite these promising applications, there are limited data examining the effect of cardiovascular risk factors (CVRF) on plasma biomarker concentrations, which may be an impediment towards generalizability and implementation. Studies showed altered plasma levels of A$\beta$42/40, NfL, GFAP and total tau in participants with medical comorbidities like elevated body mass index (BMI), tobacco use, dyslipidemia, hypertension, and diabetes [6,21,22]. Subgroup analyses in these studies have shown differences in prediction of cognitive status based on presence of single specific comorbidities, but there is a lack of studies aiming to predict early AD or cerebrovascular neuropathologic changes while considering the effect of multiple chronic metabolic conditions, which tend to co-occur. Similarly, studies have shown associations between plasma levels of NfL and A$\beta$42/40 with cerebrovascular disease pathology [23], or total tau and degree of arteriosclerosis [24], but the modification of cardiovascular comorbidities on the association between plasma measurements and neuropathologic change was not examined. It is therefore unclear if cerebrovascular comorbidities act as direct cardiovascular risk factors versus having an indirect physiological effect in plasma biomarker measurement.

In the current study, our goal was to examine the interaction between plasma biomarkers of AD and neurodegeneration with CVRF in relation to neuropathology. To this end, we investigated the interaction between a summed score of seven chronic metabolic conditions and commercially available plasma biomarkers using the SIMOA Quanterix platform (i.e. A$\beta$42/40 ratio, p-tau181, GFAP, and NfL) to predict ADNC and vascular neuropathologic change.

## 2. Methods

### 2.1. Participants

Participants were enrolled in the Mayo Clinic Study of Aging (MCSA) at Mayo Clinic Rochester, a population-based prospective study of residents of Olmsted County, Minnesota. During each visit, participants underwent a physician exam, cognitive testing, and a blood draw for plasma biomarkers on the same day. MCSA participants who had undergone autopsy with antemortem plasma biomarkers within 5 years were included in this study. Clinical diagnoses of cognitively unimpaired (CU), MCI, or dementia were ascertained employing existing consensus criteria in a panel composed of a physician, a study coordinator, and a neuropsychologist [25,26]. The Institutional Review Boards of Mayo Clinic and Olmsted Medical Center approved this study. All participants provided written informed consent.

### 2.2. Cardiovascular and metabolic conditions

We used the Rochester Epidemiology Project (REP) medical records-linkage system [27,28], to ascertain CVRF from health care medical records. We leveraged the REP to capture International Classification of Diseases, Ninth Revision (ICD-9, through September 2015) codes and Tenth Revision (ICD-10, from October 2015) codes in a 5-year window prior to the MCSA visit. We then pooled the ICD codes into 7 cardiovascular and metabolic conditions (CMC) including hypertension, hyperlipidemia, cardiac arrhythmias, coronary artery disease, congestive heart failure, diabetes mellitus, and stroke, which were proposed indicators of vascular health by the U.S. Department of Health and Human Services in 2010 [29,30]. We then computed a composite CMC score, which represents the summation of the presence or absence of each of these conditions [31,32].

### 2.3. Plasma assays

Participant plasma was collected in EDTA after overnight fasting. After centrifuging, 0.5 mL of plasma were aliquoted into polypropylene tubes and stored at −80 °C until testing. We used the Neurology 4-Plex E Advantage kit (N4PE, item #103,670) to measure Plasma A$\beta$ 1–40, A$\beta$ 1–42, GFAP, and NfL; we used the Simoa® p-tau181 Advantage V2 kit (item #103,714) to measure phospho-Tau 181 (p-tau181). Both kits were used per manufacturer's instructions on a Quanterix HD-X analyzer (Quanterix, Lexington, MA, United States). This consisted of centrifuging thawed samples for 5 min x 4000 g then diluting 1:4 using the instrument's dilution protocol onboard. Samples were tested in singlet. The N4PE test used eight-point calibration curves with $1/y^2$ weighting; a 4-parameter logistic fitting algorithm was used for NfL and GFAP, while a 5-parameter logistic fitting algorithm was used for A$\beta$ 1–40 and A$\beta$ 1–42. We used a weighting factor of $1/y^2$ and a 4-parameter logistic curve fitting algorithm for p-tau181 to determine a seven-point calibration curve and sample concentrations using the Simoa® HD-X Analyzer software. Duplicates of quality control material in two levels were run following assay calibrators. Below are the inter-assay imprecision expressed as a % coefficient of variation for the quality control material: A$\beta$ 1–40, 5 % and 3 % at approximate concentrations of 16 and 117 pg/mL; A$\beta$ 1–42, 4 % and 7 % at approximate concentrations of 5.5 and 31 pg/mL; GFAP, 7 % and 7 % at approximate concentrations of 181 and 3702 pg/mL; NfL, 12 % and 14 % at approximate concentrations of 21 and 432 pg/mL; p-tau181, 6 % and 5 % at approximate concentrations of 3.7 and 119 pg/mL.

### 2.4. Alzheimer's disease neuropathologic assessments

We followed the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) [33] for neuropathologic sampling. Tissue sections were formalin-fixed and paraffin-embedded prior to performing immunohistochemistry on 5 $\mu$m thick sections. We used a Thermo Fisher Lab Vision 480S autostainer using 3,3-diaminobenzidine (DAB) as the chromogen, with primary antibodies being A$\beta$ (mouse monoclonal (6F/3D), DAKO M0872) and phospho-tau (mouse monoclonal, AT8; Thermo Fisher MN1020). The neuropathologic evaluations were done

as part of the Neuropathology Core of the Mayo Clinic Alzheimer's Disease Research Center (P30 AG062677).

According to the National Institute on Aging Alzheimer's Association (NIA-AA) criteria [20,34], we ascertained ADNC by describing (A) Thal amyloid phase for the distribution of A$\beta$ plaques [35], (B) Braak tangle stage [36], and (C) CERAD neuritic plaque score [33]. We recategorized Thal amyloid phase and Braak tau tangle stage into 4-point scales (absent, low, intermediate, high) to evaluate the probability of greater A$\beta$ and tau deposition in a comparable scale. According to NIA-AA criteria, ABC scores were subsequently combined into a 4-point scale.

In order to facilitate logistic regression modelling between plasma biomarker levels and the presence of underlying pathology, each neuropathologic scale was binarized into a "high" and "low" category: Thal amyloid phase 0–2 were placed in the "low" category and Thal amyloid phase 3–5 were placed in the "high" category; Braak staging I-III were considered to be in the "low" category and IV-VI were "high"; and for neuritic plaque, a score of "none" or "sparse" was placed in the "low" category and scores of "moderate" or "frequent" placed in the "high" category. The overall ADNC was binarized by placing "none" or "low" evidence of neuropathologic change as the "low" category, and "intermediate" or "high" ADNC was placed in the "high" category.

### 2.5. Neuropathologic assessments of cerebrovascular disease

Hematoxylin and eosin-stained sections were used to evaluate cerebrovascular disease pathology for all participants. We then quantify microvascular pathology and macrovascular pathology with two published scales, modified Kalaria and Strozyk scale, respectively. The modified Kalaria scale for microvascular pathology consists of 0–6 points from neocortical regions and 0–4 points from the basal ganglia for a total of 10 points [37,38]. To evaluate cerebrovascular disease severity, points were attributed based on the presence of cerebral amyloid angiopathy, arteriolosclerosis, microscopic infarcts, large infarcts, perivascular tissue rarefaction, and perivascular hemosiderin deposition on both neocortical and basal ganglia sections [38]. The Strozyk scale for macrovascular pathology consists of 0–2 points depending on the severity of three types of macrovascular lesions including large infarcts, lacunar infarcts, and white matter hyperintensities for a total of up to 6 points [39].

Again, each cerebrovascular disease scale was also binarized into a "high" and "low" category to facilitate logistic regression modelling between plasma biomarker levels and the presence of underlying pathology. Kalaria scores of 0–3 were placed in the category of "low" and scores of 5–10 were placed in the "high" category. Similarly, Strozyk scores of 0–2 were placed in the "low" category and scores of 3–6 were placed in the "high" category.

### 2.6. Statistical analyses

We performed a retrospective, cohort study to assess the interaction effects of cardiovascular comorbidities on plasma biomarkers when predicting neuropathologic change. Antemortem plasma biomarker acquisition was done during routine visits. CMC was calculated at the time of biomarker acquisition using ICD codes from the last 5 years. Basic descriptive statistics were computed to compare groups: comparisons between continuous variables used Wilcoxon rank-sum tests and categorical variables used chi-square tests when comparing subgroups of CU versus MCI and CU versus dementia. In our multivariable logistic regression models, all four plasma biomarkers and CMC scores were used to predict change in underlying neuropathology as inferred by elevated neuropathologic scales, while allowing for interaction terms between each plasma biomarker and CMC score. P-tau181, GFAP and NfL were log-transformed due to skewed distributions. All models also included age, sex, BMI, and binary cognitive impairment (cognitively unimpaired vs MCI/dementia) as covariates. Plasma markers, age, BMI, and CMC were Z-scored relative to the entire cohort sample to compare coefficients by subtracting the mean and dividing by the standard deviation (SD). Results are presented in odds ratio (OR) for change of one SD in predictor variables. Participants with missing demographic or neuropathologic data were not included in the model. An alpha level of 0.05 was employed for statistical significance. Due to variable timing between plasma sample and autopsy, each observation was weighed by the inverse of the time difference between plasma sample and autopsy in years. To avoid an outlier effect on the weights, these were capped at one week and five years. In our models, each participant has a single independent observation. Our logistic regression models have 13 predictor terms (including interactions) and all of the neuropathologic scales have approximately 160 to 170 events in the elevated category, except Kalaria, thus satisfying at least 10 events per predictor included in the model. Lastly, we verified there was no multicollinearity amongst the predictors using variance inflation factor <5. All analyses were completed using MATLAB version 23.2.0 (R2023b) (Mathworks. Natick, MA).

## 3. Results

### 3.1. Participant characteristics

The demographic characteristics of our cohort are shown in Table 1. The overall cohort included 351 participants, 345 of which had full ADNC assessment and all had cerebrovascular disease scoring. A subset of 344 participants had full cognitive testing, 193 (56.1 %) of which were cognitively unimpaired (CU), 97 (28.2 %) had mild cognitive impairment (MCI), and 54 (15.7 %) had a diagnosis of dementia at the time of plasma biomarker testing. At the time of the measurement of plasma biomarkers, the median age and interquartile range (IQR) in the overall cohort was 87.4 (7.5) years, 142 (40.5 %) participants were female, and the median BMI was 26.9 (6.3). The median CMC score was 3 (3) and the median time between plasma sample to autopsy was 1.27 (1.37) years. There was no significant difference between the cognitive subgroups with regards to age, sex, BMI, or CMC score. The MCI group had a longer time between plasma biomarkers to autopsy when compared to the CU group (1.56 vs 1.20 years, $p = 0.003$). GFAP was significantly elevated in the MCI (214.2 vs 177.1, $p = 0.002$) and dementia subgroups (230.6 vs 177.1, $p < 0.001$) relative to those CU. We did note a higher quantitative value in p-tau181 between the dementia subgroup and the CU subgroup that did not reach significance (3.68 vs 3.17, $p = 0.100$). With regards to elevated neuropathologic scales, there was a significantly higher proportion of participants with elevated ADNC in the MCI group compared to the CU group ($p < 0.05$). MCI participants also had a larger proportion of higher Kalaria scores compared to CU participants (55.7 % vs 42.0 %, $p = 0.03$), but the dementia subgroup did not differ from CU (38.9 % vs 42.0 %, $p = 0.68$). There was no difference in elevated Strozyk scales between subgroups.

### 3.2. Alzheimer's disease neuropathologic scales

All plasma biomarkers were independently associated with advanced ADNC at the average CMC value including elevated p-tau181 (OR: 1.56 [1.09 2.25], $p = 0.016$), elevated GFAP (OR: 1.84 [1.26, 2.68, $p = 0.002$), lower NfL (OR: 0.40 [0.28, 0.58], $p < 0.001$), and lower A$\beta$42/40 (OR: 0.69 [0.53, 0.90], $p = 0.006$) (Table 2). Of the demographic variables, each SD increase in age (OR: 2.06 [1.42, 2.99], $p < 0.001$),cognitive impairment (OR: 9.43 [5.27, 16.87], $p < 0.001$), CMC (OR: 1.54 [1.15, 2.06], $p = 0.004$) at average plasma marker values and male sex (OR: 2.03 [1.11, 3.71], $p = 0.021$), were independently associated with higher ADNC, but not, BMI (OR: 0.94 [0.69, 1.30], $p = 0.724$). Regarding interactions between plasma biomarkers and CMC score, we observed an inverse interaction effect between CMC score and GFAP levels (Interaction OR: 0.61 [0.42, 0.89], $p = 0.011$), meaning that GFAP is less associated with elevated ADNC at higher CMC scores (Table 2). To illustrate this, we show the odds ratio for each plasma biomarker at three CMC levels: 1 SD below average (i.e. CMC score of 1.8), average CMC

**Table 1**
Participant characteristics.

| Participants | Overall ($N$ = 351) | CU ($N$ = 193) | MCI ($N$ = 97) | Dementia ($N$ = 54) |
|---|---|---|---|---|
| **Demographics** | | | | |
| Age (years) | 87.4 (7.5) | 87.1 (8.20) | 87.7 (6.89) | 88.0 (5.67) |
| Sex ( % Female) | 142 (40.5 %) | 79 (40.9 %) | 42 (43.3 %) | 19 (35.2 %) |
| BMI (Kg/m$^2$) | 26.9 (6.25) | 27.0 (6.06) | 27.1 (7.26) | 25.7 (5.41) |
| CMC Score | 3.0 (3.0) | 4.0 (3.0) | 4.0 (3.0) | 3.0 (3.0) |
| Time from plasma to autopsy (Years) | 1.27 (1.37) | 1.20 (1.14) | 1.56 (1.64)* | 1.30 (1.26) |
| **Plasma Biomarkers** | | | | |
| P-tau181 (pg/mL) | 3.42 (2.90) | 3.17 (2.61) | 3.53 (2.86) | 3.68 (2.60) |
| GFAP (pg/mL) | 197.7 (127.7) | 177.1 (117.5) | 214.2 (123.3)* | 230.6 (179.0)* |
| NfL (pg/mL) | 51.8 (36.2) | 50.0 (35.2) | 55.3 (34.1) | 49.2 (52.4) |
| A$\beta$42/40 ratio | 0.062 (0.018) | 0.062 (0.019) | 0.062 (0.019) | 0.062 (0.014) |
| **Neuropathologic scales** | | | | |
| Elevated ADNC (Intermediate or High) | 164 (47.5 %) | 71 (37.0 %) | 50 (53.2 %)* | 40 (76.9 %)* |
| Elevated Thal Phase (Phase 3 or greater) | 168 (52.8 %) | 80 (44.2 %) | 50 (58.8 %)* | 35 (74.5 %)* |
| Elevated Braak Stage (IV-VI) | 165 (47.3 %) | 65 (34.0 %) | 57 (58.8 %)* | 40 (74.1 %)* |
| Elevated Neuritic plaque score (Moderate or Frequent) | 164 (46.9 %) | 73 (37.8 %) | 51 (53.1 %)* | 36 (66.7 %)* |
| Elevated Kalaria Score (5 or greater) | 160 (45.6 %) | 81 (42.0 %) | 54 (55.7 %)* | 21 (38.9 %) |
| Elevated Strozyk Score (3 or greater) | 79 (22.5 %) | 36 (18.7 %) | 24 (24.7 %) | 16 (29.6 %) |

Median (IQR) listed for continuous variables and count ( %) for categorical variables. Note all 351 participants had cerebrovascular disease scoring but a subset of 345 participants had full ADNC grading and the remaining had at least one component missing. Fourty-seven participants did not have cognitive testing available. Seven participants did not have available cognitive testing. CU: Cognitively unimpaired, MCI: Mild cognitive impairment, BMI: Body mass index, CMC: cardiovascular and metabolic conditions, p-tau181: phosphorylated tau 181, NfL: Neurofilament light, GFAP: glial fibrillary acidic protein, ADNC: Alzheimer's disease neuropathologic change. Wilcoxon rank-sum tests and categorical variables used chi-square tests were performed to compare MCI vs CU and Dementia vs CU.

* indicates $p < 0.05$.

(i.e. CMC score of 3.4), and 1 SD above average (i.e. CMC score of 5.0), assuming all other predictors are held at average (Fig. 1). Fig. 1 shows that the OR between GFAP and ADNC decreases from 3.0 [1.9, 4.7] at below average CMC to 1.8 [1.3, 2.7] at average CMC and 1.1 [0.7, 1.8] at above average CMC, thus weakening the association between GFAP and ADNC at higher CMC. There were no significant interactions between CMC and the remaining plasma biomarkers (Table 2).

Next, we analyzed the interactions between CMC and plasma biomarkers when predicting individual contributing components of ADNC. First, prediction of elevated Thal phase showed an independent association with all plasma biomarkers at average CMC: p-tau181 (OR: 1.58 [1.12, 2.24], $p = 0.010$), GFAP (OR: 1.90 [1.30, 2.77], $p < 0.001$), NfL (OR: 0.57 [0.39, 0.82], $p = 0.003$), and A$\beta$42/40 (OR: 0.58 [0.45, 0.75], $p < 0.001$). Higher age (OR 1.89 [1.33, 2.67], $p < 0.001$), and cognitive impairment (OR: 4.69 [2.57, 8.58], $p < 0.001$) were independently associated with Thal phase, but not sex (OR 1.50 [0.83, 2.71], $p = 0.179$), BMI (OR 1.33 [0.99, 1.80], $p = 0.061$) or CMC score (OR 0.88 [0.66, 1.18], $p = 0.387$) for those with average plasma marker values. Again, GFAP was less associated with Thal phase at higher CMC scores (Interaction OR 0.48 [0.33, 0.71], $p < 0.001$) (Table 2), with the association decreasing from 3.9 [2.5, 6.1] at low CMC, to 1.9 [1.3, 2.8] at average CMC, to 0.9 [0.6, 1.4] and high CMC (Fig. 1). Additionally, there was a significant interaction between p-tau181 and CMC such that the association between p-tau181 and Thal phase was stronger with a higher CMC score (Interaction OR 1.43 [1.00, 2.04], $p = 0.050$) with the association increasing from 1.1 [0.7, 1.8] at low CMC, to 1.6 [1.1, 2.2] at average CMC, to 2.3 [1.4, 3.7] at high CMC (Fig. 1). There were no interactions between NfL or A$\beta$42/40 with CMC to predict Thal phase.

Prediction of elevated Braak stage showed independent associations with GFAP (OR: 2.65 [1.76, 3.98], $p < 0.001$) and NfL (0.25 [0.17, 0.39], $p < 0.001$), but not p-tau181 (OR 1.44 [0.99, 2.10], $p = 0.059$) or A$\beta$42/40 (OR: 0.80 [0.61, 1.06] $p = 0.118$) at average level of CMC. Demographic variables independently associated with Braak stage included advanced age (OR 1.90 [1.30, 2.78], $p < 0.001$), CMC (OR 1.91 [1.40, 2.59], $p < 0.001$), and cognitive impairment (OR: 11.5 [6.31, 21.01], $p < 0.001$) but not sex (OR 1.15 [0.62, 2.14], $p = 0.649$) or BMI (OR 0.85 [0.60, 1.19], $p = 0.334$). There were significant interactions for both GFAP (Interaction OR: 0.56 [0.37, 0.84], $p = 0.005$) and A$\beta$42/40 (Interaction OR 0.63 [0.47, 0.85], $p = 0.002$) with CMC for predicting Braak stage such that the association between Braak and GFAP was reduced with higher CMC burden decreasing from 4.7 [2.9, 7.8] at low CMC to 2.6 [1.8, 4.0] at average CMC and to 1.5 [0.9, 2.4] at high CMC (Fig. 1). The negative association between Braak and A$\beta$42/40 was stronger at higher CMC going from 1.3 [0.9, 1.8] at low CMC to 0.8 [0.6, 1.1] at average CMC to 0.5 [0.3, 0.7] at high CMC (Fig. 1). There was no significant interaction between CMC and p-tau181 or NfL when predicting Braak stage (Table 2).

Lastly, GFAP (OR: 3.08 [2.05, 4.61], $p < 0.001$), NfL (OR: 0.47 [0.32, 0.68], $p < 0.001$), and A$\beta$42/40 (OR: 0.60 [0.46, 0.78], $p < 0.001$) but not p-tau181 (OR: 1.26 [0.89, 1.78], $p = 0.197$) were associated with an elevated neuritic plaque score at the average CMC level. The only demographic variables independently associated with elevated neuritic plaque score were advanced age (OR 1.51 [1.06, 2.16] $p = 0.023$) and cognitive impairment (OR: 3.94 [2.28, 6.79], $p < 0.001$), but not sex (OR 1.41 [0.77, 2.56], $p = 0.262$), BMI (OR 1.15 [0.85, 1.57], $p = 0.363$), or CMC score (OR 1.11 [0.84, 1.48], $p = 0.454$) at average plasma biomarker levels. There was a significant interaction between A$\beta$42/40 and CMC (Interaction OR 0.70 [0.52, 0.95], $p = 0.020$) such that the association between A$\beta$42/40 and neuritic plaque score became stronger in the inverse direction at higher CMC going from 0.9 [0.6, 1.2] at low CMC to 0.6 [0.5, 0.8] at average CMC and to 0.4 [0.3, 0.6] at high CMC. (Fig. 1). There was no significant interaction between CMC and p-tau181, GFAP or NfL when predicting neuritic plaque score (Table 2).

### 3.3. Cerebrovascular disease neuropathologic scales

Next, we examined interactions between CMC score and plasma biomarkers for predicting microvascular and macrovascular cerebrovascular disease neuropathologic scales (Fig. 2P-tau181 (OR: 0.67 [0.50, 0.89], $p = 0.005$) and A$\beta$42/40 (OR: 1.47, [1.19, 1.82], $p < 0.001$) showed independent associations with Kalaria score at the average CMC level (Table 3). There were no associations between these pathological measures and GFAP or NfL. Younger age (OR 0.74 [0.57, 0.96], $p = 0.022$), lower BMI (OR 0.48 [0.37, 0.63], $p < 0.001$), being cognitively unimpaired (OR: 0.60 [0.37, 0.98], $p = 0.039$), and higher CMC score (OR 1.69 [1.31, 2.18], $p < 0.001$) for those with average plasma

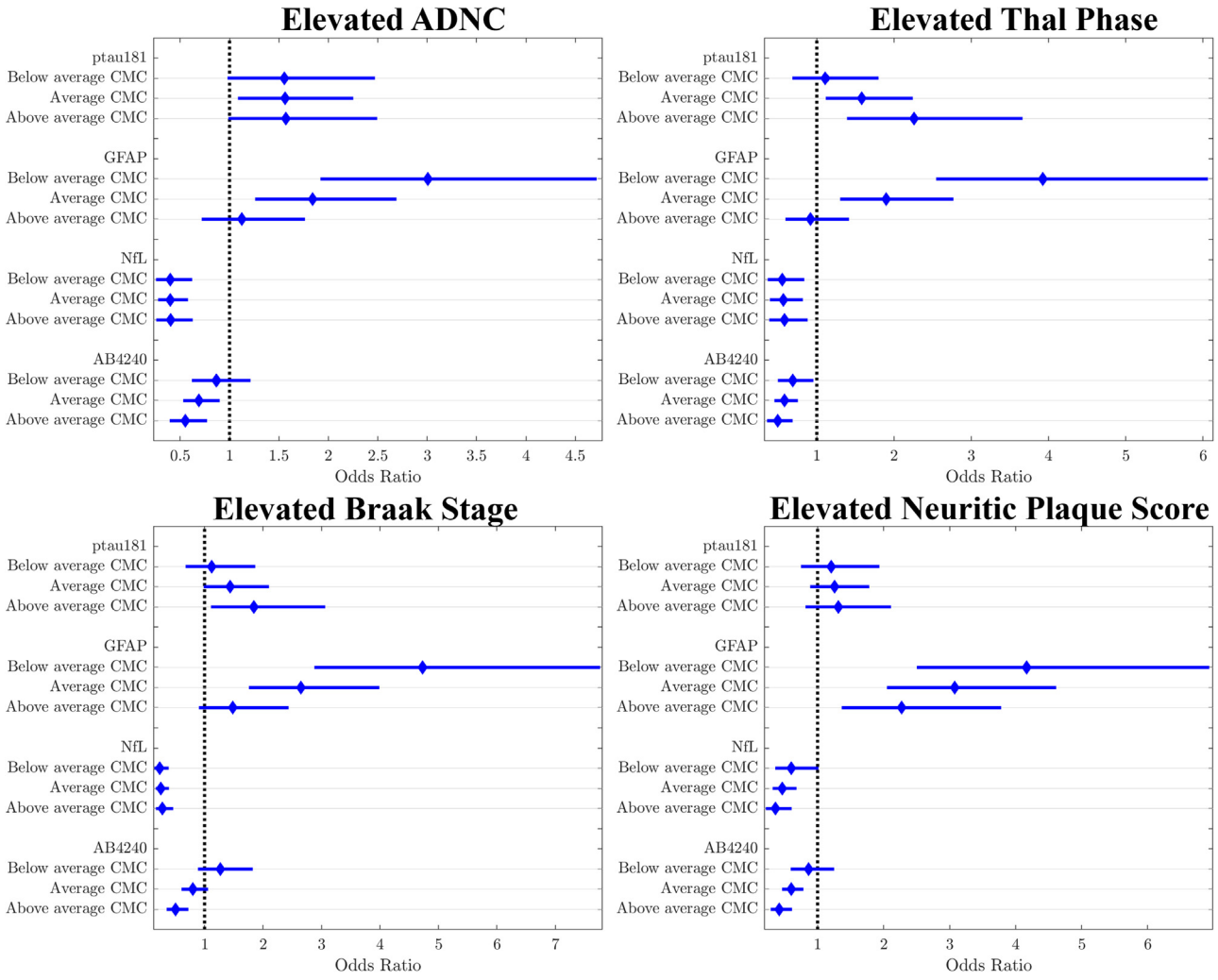


**Fig. 1.** Interaction effects between plasma biomarkers and the cardiovascular metabolic conditions (CMC) score when predicting Alzheimer's disease neuropathologic change (ADNC, top left) and its components including Thal phase (top right), Braak stage (bottom left) and neuritic plaque score (bottom right) using logistic regression. Above and below average CMC are values at one Z-score above (CMC = 5.0) and below (CMC = 1.8) the average CMC score (CMC = 3.4).

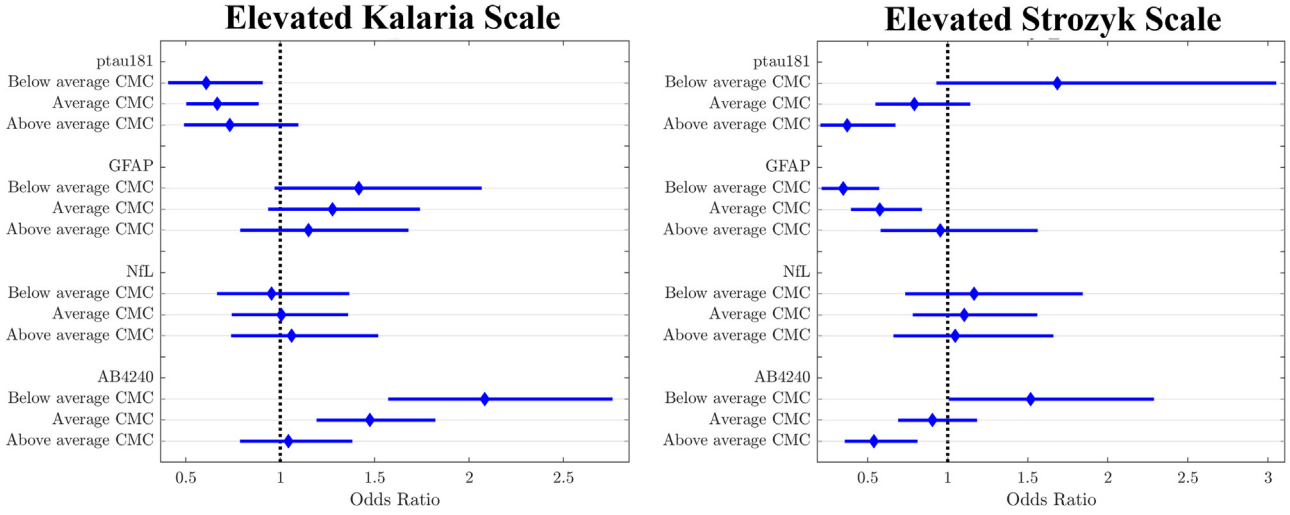


**Fig. 2.** Interaction effects between plasma biomarkers and the cardiovascular metabolic conditions (CMC) score when predicting cerebrovascular disease scales for microvascular (Kalaria, left) and macrovascular (Strozyk, right) changes. Above and below average CMC are values at one Z-score above (CMC = 5.0) and below (CMC = 1.8) the average CMC score (CMC = 3.4).

**Table 2**
Interaction effects in the prediction of AD neuropathologic change (ADNC) and its components using logistic regression.

| Variable | OR | 95 % CI | p-value |
|---|---|---|---|
| **ADNC** | | | |
| p-tau181 | 1.56 | [1.09, 2.25] | **0.016** |
| GFAP | 1.84 | [1.26, 2.68] | **0.002** |
| NfL | 0.40 | [0.28, 0.58] | **<0.001** |
| Aβ42/40 | 0.69 | [0.53, 0.90] | **0.006** |
| CMC | 1.54 | [1.15, 2.06] | **0.004** |
| p-tau181 * CMC | 1.00 | [0.70, 1.44] | 0.980 |
| GFAP * CMC | 0.61 | [0.42, 0.89] | **0.011** |
| NfL * CMC | 1.00 | [0.72, 1.40] | 0.978 |
| Aβ42/40 * CMC | 0.80 | [0.60, 1.07] | 0.128 |
| **Thal Phase** | | | |
| p-tau181 | 1.58 | [1.12, 2.24] | **0.010** |
| GFAP | 1.90 | [1.30, 2.77] | **<0.001** |
| NfL | 0.57 | [0.39, 0.82] | **0.003** |
| Aβ42/40 | 0.58 | [0.45, 0.75] | **<0.001** |
| CMC | 0.88 | [0.66, 1.18] | 0.387 |
| p-tau181 * CMC | 1.43 | [1.00, 2.04] | **0.050** |
| GFAP * CMC | 0.48 | [0.33, 0.71] | **<0.001** |
| NfL * CMC | 1.03 | [0.74, 1.42] | 0.878 |
| Aβ42/40 * CMC | 0.85 | [0.64, 1.12] | 0.241 |
| **Braak Stage** | | | |
| p-tau181 | 1.44 | [0.99, 2.10] | 0.059 |
| GFAP | 2.65 | [1.76, 3.98] | **<0.001** |
| NfL | 0.25 | [0.17, 0.39] | **<0.001** |
| Aβ42/40 | 0.80 | [0.61, 1.06] | 0.118 |
| CMC | 1.91 | [1.40, 2.59] | **<0.001** |
| p-tau181 * CMC | 1.28 | [0.88, 1.87] | 0.200 |
| GFAP * CMC | 0.56 | [0.37, 0.84] | **0.005** |
| NfL * CMC | 1.10 | [0.76, 1.58] | 0.629 |
| Aβ42/40 * CMC | 0.63 | [0.47, 0.85] | **0.002** |
| **Neuritic Plaque Score** | | | |
| p-tau181 | 1.26 | [0.89, 1.78] | 0.197 |
| GFAP | 3.08 | [2.05, 4.61] | **<0.001** |
| NfL | 0.47 | [0.32, 0.68] | **<0.001** |
| Aβ42/40 | 0.60 | [0.46, 0.78] | **<0.001** |
| CMC | 1.11 | [0.84, 1.48] | 0.454 |
| p-tau181 * CMC | 1.04 | [0.73, 1.49] | 0.810 |
| GFAP * CMC | 0.74 | [0.50, 1.09] | 0.126 |
| NfL * CMC | 0.77 | [0.54, 1.11] | 0.164 |
| Aβ42/40 * CMC | 0.70 | [0.52, 0.95] | **0.020** |

Models were adjusted for age, sex, BMI, and cognitive impairment and included interaction terms for CMC with plasma markers. Individual plasma marker results correspond to those at average CMC. The CMC result corresponds to those at average levels of all plasma markers. Odds ratios correspond to one standard deviation changes. P-tau181, GFAP, and NfL were log-transformed. OR: Odds ratio. CI: Confidence Interval, BMI: Body mass index, CMC: cardiovascular metabolic conditions, p-tau181: phosphorylated tau 181, NfL: Neurofilament light, GFAP: glial fibrillary acidic protein, ADNC: Alzheimer's disease neuropathologic change.

marker values, were independently associated with elevated Kalaria scores, but not male sex (Table 3). There was an interaction between Aβ42/40 and CMC (Interaction OR:0.71 [0.57, 0.88], $p = 0.001$) such that the association between Aβ42/40 and Kalaria score became weaker at higher CMC (Fig. 2) going from 2.1 [1.6, 2.8] at low CMC, to 1.5 [1.2, 1.8] at average CMC and to 1.0 [0.8, 1.4] at high CMC. There were no other significant interactions between CMC and p-tau181, NfL, or GFAP when predicting Kalaria score (Table 3).

GFAP (OR: 0.58 [0.40, 0.84], $p = 0.004$) was independently associated with Strozyk score but not the other plasma biomarkers at average CMC (Table 2). Advanced age (OR 1.46 [1.05, 2.04], $p = 0.025$) and lower BMI (OR 0.51 [0.35, 0.73], $p < 0.001$) were also independently associated with elevated Strozyk scale, but sex (OR 1.72 [0.92, 3.22], $p = 0.090$), cognitive impairment (OR 1.48 [0.84, 2.59], $p = 0.172$) and CMC (OR 1.10 [0.81, 1.49], $p = 0.558$) score were not. There was an interaction between three of the four plasma biomarkers and CMC when predicting Strozyk scale, such that the association between p-tau181 and Strozyk became stronger in the inverse direction at higher CMC (Interaction OR: 0.47 [0.31, 0.71], $p < 0.001$) going from 1.7 [0.9, 3.1] at low CMC to 0.8 [0.5, 1.1] at average CMC and 0.4 [0.2, 0.7] ant high CMC. The association between Aβ42/40 and Strozyk also became stronger in the inverse direction at higher CMC (Interaction OR: 0.60 [0.43, 0.83], $p = 0.002$) going from 1.5 [1.0, 2.3] at low CMC to 0.9 [0.7, 1.2] at average CMC and 0.5 [0.4, 0.8] at high CMC.Meanwhile the association between GFAP and Strozyk scale became weakened from the inverse direction towards no association at high CMC (Interaction OR: 1.65 [1.11, 2.46], $p = 0.013$) going from 0.3 [0.2, 0.6] at low CMC to 0.6 [0.4, 0.8] at average CMC and 1.0 [0.6, 1.6] at high CMC (Fig. 2, Table 3).

**Table 3**
Interaction effects in the prediction of cerebrovascular disease scales for microvascular (Kalaria) and macrovascular (Strozyk) changes using logistic regression.

| Variable | OR | 95 % CI | p-value |
|---|---|---|---|
| **Kalaria** | | | |
| p-tau181 | 0.67 | [0.50, 0.89] | **0.005** |
| GFAP | 1.28 | [0.94, 1.74] | 0.121 |
| NfL | 1.01 | [0.74, 1.36] | 0.974 |
| Aβ42/40 | 1.47 | [1.19, 1.82] | **<0.001** |
| CMC | 1.69 | [1.31, 2.18] | **<0.001** |
| p-tau181 * CMC | 1.10 | [0.83, 1.46] | 0.512 |
| GFAP * CMC | 0.90 | [0.67, 1.21] | 0.488 |
| NfL * CMC | 1.05 | [0.81, 1.37] | 0.688 |
| Aβ42/40 * CMC | 0.71 | [0.57, 0.88] | **0.001** |
| **Strozyk** | | | |
| p-tau181 | 0.79 | [0.55, 1.14] | 0.208 |
| GFAP | 0.58 | [0.40, 0.84] | **0.004** |
| NfL | 1.10 | [0.78, 1.56] | 0.573 |
| Aβ42/40 | 0.90 | [0.69, 1.18] | 0.461 |
| CMC | 1.10 | [0.81, 1.49] | 0.558 |
| p-tau181 * CMC | 0.47 | [0.31, 0.71] | **<0.001** |
| GFAP * CMC | 1.65 | [1.11, 2.46] | **0.013** |
| NfL * CMC | 0.95 | [0.66, 1.36] | 0.776 |
| Aβ42/40 * CMC | 0.60 | [0.43, 0.83] | **0.002** |

Models were adjusted for age, sex, BMI, and cognitive impairment and included interaction terms for CMC with plasma markers. Individual plasma marker results correspond to those at average CMC. The CMC result corresponds to those at average levels of all plasma markers. Odds ratios correspond to one standard deviation changes. P-tau181, GFAP, and NfL were log-transformed OR: Odds ratio. CI: Confidence Interval, BMI: Body mass index, CMC: cardiovascular metabolic conditions, p-tau181: phosphorylated tau 181, NfL: Neurofilament light, GFAP: glial fibrillary acidic protein, ADNC: Alzheimer's disease neuropathologic change.

## 4. Discussion

This study investigated the interaction between CVRF and plasma biomarkers of AD and neurodegeneration when predicting underlying neuropathologic change. A key finding was that higher burden of cardiovascular comorbidities can modulate the association between plasma biomarkers like GFAP, p-tau181, and Aβ42/40 and underlying AD and cerebrovascular disease. Despite substantial evidence suggesting a link between vascular disease and the development of cognitive impairment, there is limited evidence on the effect of these comorbidities on plasma biomarkers that help predict neuropathologic change and cognitive decline. Considering cardiovascular comorbidities may improve the validity and accuracy of plasma biomarkers for a specific patient population.

### *4.1. Higher CMC results in a weaker association between GFAP and ADNC and a stronger association with macroavscular pathology*

When predicting ADNC and its components, we observed the strongest interaction effect between GFAP and CMC score. Specifically, we observed that increases in CMC result in a weaker association between GFAP and elevated ADNC, suggesting that at high enough levels the predictive capabilities of some biomarkers may be altered. This was again true in the prediction of Thal phase and Braak stage, but not neuritic plaque score. This interaction between plasma GFAP and CMC score

may be secondary to increased activity by reactive astrocytes in participants with cerebrovascular disease-related inflammation, as observed after ischemic stroke [40].

With regards to cerebrovascular disease neuropathologic change, GFAP showed an independent negative association with Strozyk but not Kalaria scores at the average CMC level. Conversely, CMC had a significant positive interaction with GFAP when predicting Strozyk but not the Kalaria score. Previous work has shown associations between white matter hyperintensity burden and plasma GFAP positivity [41], but a direct association between plasma GFAP and cerebrovascular disease neuropathologic change has limited evidence. Here we show that GFAP is strongly modulated by the presence of cardiovascular comorbidities when predicting macrovascular neuropathologic change. Our models included age, sex, BMI, cognitive impairment, and CMC, which may capture some of the variance in macrovascular changes, possibly explaining the lack of significant independent association of GFAP and Strozyk and a positive modulation by CMC score. Interestingly, BMI had an independent negative association with both Kalaria and Strozyk metrics. This may be due to the J-shaped distribution of BMI against all-cause mortality, where lower BMI at a late age may be associated with worse cerebrovascular disease neuropathologic change [42]. Overall, although GFAP has shown utility regarding some aspects of cerebrovascular disease, its utility seems less certain for chronic macro- and microvascular changes.

### 4.2. Higher CMC results in a stronger association between Aβ42/40 and Braak stage, neuritic plaque score, and cerebrovascular disease score

Lower levels of Aβ42/40 ratio were shown to be associated with early changes associated with AD and increased risk of progression to cognitive decline [43]. In this work, we observed that CMC modulates the association between Aβ42/40 and both Braak stage and neuritic plaque score: increasing CMC score resulted in a stronger negative association between Aβ42/40 and higher tau and Aβ neuropathologic change. It is important to note that Aβ42/40 was independently associated with neuropathologic markers that involve Aβ as well, including overall ADNC, Thal phase, and neuritic plaque score. Although not typically associated with tau metrics like Braak stage, our study suggests that Aβ42/40 may become more strongly associated in those with a higher comorbidity burden. Whether this is due to an indirect effect causing a false positive in plasma biomarker levels or a direct effect of CVRF on tau pathology requires further study.

With regards to cerebrovascular disease, we found an independent positive association between Aβ42/40 and Kalaria scores but not Strozyk. However, both metrics of cerebrovascular disease showed an inverse interaction effect between Aβ42/40 and CMC score. Previous work showed a weak independent positive association between Aβ42/40 and both Strozyk and Kalaria but did not take into account cardiovascular comorbidities [23]. It is possible that because of the advanced age of our cohort, which is predominantly cognitively unimpaired, the burden of cerebrovascular disease outweighs Aβ deposition, thus reflecting an inverse relationship with plasma Aβ42/40. Moreover, even when controlling for cognitive impairment, we found an inverse association with elevated Strozyk scales. However, we do note that increasing CMC score is more likely to lead to a stronger negative association between Aβ42/40 ratio and both metrics. We also noted a direct association between CMC and Kalaria score, but not Strozyk. CMC was independently linked to both ADNC and Braak stage, but not to Thal phase or neuritic plaque score. This suggests a stronger relationship between tau neuropathologic changes and small vessel cerebrovascular disease, akin to findings showing an interaction effect between the Framingham cardiovascular risk score amyloid PET positivity with regards to tau accumulation on PET [44]. Given that there was no direct association between CMC and Aβ neuropathologic metrics, the interaction between CMC and plasma biomarkers may suggest both an indirect effect on the measured plasma levels due to metabolic conditions as well as a direct effect on cerebrovascular neuropathology that subsequently may lead to higher Aβ deposition reflected in lower Aβ42/40 ratio in those with higher CMC scores.

### 4.3. NfL and p-tau181 are minimally affected by CMC score

NfL is largely considered a non-specific biomarker of neurodegeneration [45]. In our analyses, which included demographics, all plasma biomarkers, CMC, and interaction terms, we observed an independent negative association between NfL and ADNC, Thal phase, and neuritic plaque score. Additionally, NfL was not modulated by CMC score when predicting any of our neuropathologic metrics. Previous work has shown that NfL is susceptible to comorbidities like stroke, cancer, and CKD, which tend to increase NfL levels, while higher BMI tends to decrease it[6]. In our analyses, NfL showed a weak association with these amyloid neuropathologic scales which was reversed in the modeling process when covariates and weights were included. It is possible that by considering variables like BMI and CMC in the model, as well as including more specific plasma biomarkers for ADNC that can better account for the variance, the relationship between NfL and ADNC is weakened. Lastly, prior work has shown that NfL has poor predictive performance in terms of AUC during a prediction task of underlying neuropathology [18].

The relationship between p-tau181 and neuropathologic metrics showed an independent positive association with overall ADNC and Thal phaseand a negative association with Kalaria score. Plasma p-tau181 was only modulated by CMC scores with regards to predicting Thal phase but no other components of ADNC. With cerebrovascular pathology, we observed that a higher burden of cardiovascular comorbidities strengthened in the negative direction the association between p-tau181 and Strozyk scales but not the Kalaria scale. Together with the negative independent association between p-tau181 and Kalaria scores, this suggests that p-tau181 plasma biomarkers may be inversely associated with cerebrovascular neuropathology late in life, which may be confounded by survival bias in this population. Overall, p-tau181 has shown promising results as a predictive biomarker of underlying neuropathology and cognitive decline [9,10,18,46,47]. In this work, we show that it is also the least susceptible to interaction effects from cardiovascular comorbidities. As newer and more accurate tau-based biomarkers like p-tau217 become available, it will be important to continue to validate its independence from cardiovascular comorbidities. With regards to cerebrovascular pathology, previous work has shown that an elevated p-tau181 to Aβ42/40 ratio served as an accurate discriminator between Alzheimer's disease and vascular dementia [48]. In this work we show that indeed p-tau181 is inversely associated with neuropathologic change of small vessel disease without being modulated by preexisting CVRF. This combination of biomarkers may indeed be a cost-effective and noninvasive metric for cognitive aging associated with small-vessel disease separate from AD.

### 4.4. Limitations and future directions

This study presented the interaction effects between CVRF and plasma biomarkers when predicting neuropathologic change associated with AD and cerebrovascular disease in a large community-based cohort. However, there are some important limitations to this work. One key limitation is the time difference between plasma biomarker sampling and autopsy evaluation, which in this cohort was a median of 1.3 years. Ideally, plasma biomarkers would be used for early evaluation for preclinical diagnosis, prognostication, and treatment. Here, we demonstrated an important relationship between cardiovascular and metabolic comorbidities, which tend to accumulate with age, and plasma biomarkers, but the advanced age of the participants likely overestimates these associations for those who reached advanced age without severe complications of cardiovascular disease. Although we account for this limitation by weighing the observations by the inverse of the time difference,

this does not account for non-linear effects of aging in a younger cohort, which may affect the generalizability of these results. Now that plasma biomarkers have been available for several years, long-term follow up studies may better illustrate the utility of plasma biomarkers at an early age.

The assay used for plasma biomarkers and its scope present another limitation to this work. Head-to-head comparisons of the Quanterix assay used here, particularly plasma biomarkers of AD such as A$\beta$42/40 ratio and p-tau181, have shown relatively low diagnostic accuracy compared to newer isoforms of phosphorylated tau like p-tau217 and p-tau231 [10,11,49]. Many of the observations of this work will require repeat validation with future plasma biomarker assays. Similarly, the representation of CVRF used in this work through the CMC score presents its own limitations. The score is composed of binary presence or absence of cardiovascular and metabolic conditions, but the severity of each of these is not taken into account. Moreover, a limitation of using an overall burden scale is that some subcomponents can have different effects on the biomarker levels. A more detailed study looking at the individual contributions of each component in a continuous fashion like diabetes, heart failure, stroke, dyslipidemia, coronary artery disease, atrial fibrillation, and chronic kidney disease, as well as those not included in this study such as tobacco use and physical activity, may better discriminate the influence of each risk factor that affects either measurement of the assay or directly influences neuropathologic change. There are other demographic variables that warrant further study due to their association with cardiovascular health such as race, socioeconomic status, geographic location, medications, or prior brain injuries. Similarly, in this work we binarized the outcome neuropathologic scales to model the data using logistic regression as ordinal logistic regression would divide up our data too much relative to the number of predictor terms included. Additionally, this facilitates comparison between neuropathologic scales with different range and allows for an interpretation that is easily accessible to clinicians. . Lastly, the MCSA is representative of southeast Minnesota but further evaluation in populations with a greater vascular risk factor dynamic range such as the stroke belt would be important to fully capture the impact of CVRF on biomarker prediction of neuropathology.

### 4.5. Conclusions

Overall, this work showed that plasma biomarkers of AD and neurodegeneration are not immune to the effects of cardiovascular and metabolic comorbidities extending beyond age and BMI. The interactions between CMC and plasma biomarkers observed here may be independent of the effect of cardiovascular comorbidities on brain pathology, as CMC did not have a significant independent association with elevated Thal phase or neuritic plaque score. Interestingly, CMC did have an independent association with Braak stage and overall ADNC. It is possible that CVRF may have a direct effect on tau over A$\beta$ deposition that ultimately leads to multifactorial cognitive decline. In this case, different biomarker thresholds may be necessary for individuals with high burden of comorbidities or early referral for advanced testing, such as amyloid PET, may be necessary when blood biomarkers cannot provide reliable information. An ideal plasma biomarker would be a pure representation of early neuropathologic change, without being affected by the presence of preexisting medical comorbidities. As new biomarkers become available, then rigorous studies are necessary to interpret plasma biomarkers in the appropriate clinical context.

## Declaration of generative AI and AI-assisted technologies in the writing process

No generative AI or AI-assisted technologies were used in any part of the production of this manuscript.

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The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Jonathan Graff-Radford reports financial support was provided by National Institutes of Health. Melissa E. Murray reports financial support was provided by National Institutes of Health. Melissa M. Mielke reports financial support was provided by National Institutes of Health. Ronald C. Petersen reports financial support was provided by National Institutes of Health. Alicia Algeciras-Schimnich reports a relationship with Roche Diagnostics that includes: board membership and consulting or advisory. Alicia Algeciras-Schimnich reports a relationship with Fujirebio Diagnostics Inc that includes: board membership and consulting or advisory. Jonathan Graff-Radford reports a relationship with StrokeNET that includes: funding grants. Melissa E. Murray reports a relationship with Alzheimer's Association that includes: funding grants. Melissa E. Murray reports a relationship with Rainwater Charitable Foundation that includes: funding grants. Melissa E. Murray reports a relationship with Eli Lilly and Company that includes: funding grants. Melissa E. Murray reports a relationship with Biogen Inc that includes: consulting or advisory. Michelle M. Mielke reports a relationship with US Department of Defense that includes: funding grants. Michelle M. Mielke reports a relationship with Alzheimer's Association that includes: funding grants. Michelle M. Mielke reports a relationship with Davos Alzheimer's Collaborative that includes: funding grants. Michelle M. Mielke reports a relationship with Althira that includes: consulting or advisory. Michelle M. Mielke reports a relationship with Biogen Inc that includes: consulting or advisory. Michelle M. Mielke reports a relationship with Eisai Inc that includes: consulting or advisory. Michelle M. Mielke reports a relationship with Eli Lilly and Company that includes: consulting or advisory. Michelle M. Mielke reports a relationship with Merck & Co Inc that includes: consulting or advisory. Michelle M. Mielke reports a relationship with Novo Nordisk Inc that includes: consulting or advisory. Michelle M. Mielke reports a relationship with Neurogen Corp that includes: consulting or advisory. Michelle M. Mielke reports a relationship with Siemens Healthcare Diagnostics Inc that includes: consulting or advisory. Ronald C. Petersen reports a relationship with GHR Foundation that includes: funding grants. Ronald C. Petersen reports a relationship with The Alexander Family Foundation that includes: funding grants. Ronald C. Petersen reports a relationship with Roche that includes: consulting or advisory. Ronald C. Petersen reports a relationship with Genentech Inc that includes: consulting or advisory. Michelle M. Mielke reports a relationship with Roche that includes: consulting or advisory. Ronald C. Petersen reports a relationship with Eli Lilly and Company that includes: consulting or advisory. Ronald C. Petersen reports a relationship with Novo Nordisk Inc that includes: consulting or advisory. Ronald C. Petersen reports a relationship with Novartis that includes: consulting or advisory. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The remaining authors including Camilo Bermudez, Jeremy A. Syrjanen, Nikki H. Stricker, Naomi Kouri, Walker K. Kremers, Clifford R. Jack, Jr., David S. Knopman, Dennis W. Dickson, Darren M. Rothberg, Christina M. Moloney, Baayla D.C. Boon, Aivi T. Nguyen, R. Ross Reichard, and Prashanthi Vemuri have nothing to declare.

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