Antemortem plasma phosphorylated tau (181) predicts Alzheimer's disease neuropathology and regional tau at autopsy

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Running title: Plasma p-tau and Alzheimer's disease

Abstract

Blood-based biomarkers such as tau phosphorylated at threonine 181 (phosphorylated-tau$_{181}$) represent an accessible, cost-effective, and scalable approach for the *in vivo* detection of Alzheimer's disease pathophysiology. Plasma-pathological correlation studies are needed to validate plasma phosphorylated-tau$_{181}$ as an accurate and reliable biomarker of Alzheimer's disease neuropathologic changes.

This plasma-to-autopsy correlation study included participants from the Boston University Alzheimer's Disease Research Center who had a plasma sample analyzed for phosphorylated-tau$_{181}$ between 2008-2018 and donated their brain for neuropathological examination. Plasma phosphorelated-tau$_{181}$ was measured with single molecule array technology.

Of 103 participants, 62 (60.2%) had autopsy-confirmed Alzheimer's disease. Average time between blood draw and death was 5.6 years (SD=3.1 years). Multivariable analyses showed higher plasma phosphorylated-tau$_{181}$ concentrations were associated with increased odds for having autopsy-confirmed Alzheimer's disease (AUC=0.82, OR=1.07, 95% CI=1.03-1.11, p<0.01; phosphorylated-tau standardized [z-transformed]: OR=2.98, 95% CI=1.50-5.93, p<0.01). Higher plasma phosphorylated-tau$_{181}$ levels were associated with increased odds for having a higher Braak stage (OR=1.06, 95% CI=1.02-1.09, p<0.001) and more severe phosphorylated-tau across six cortical and subcortical brain regions (ORs=1.03-1.06, p<0.05). The association between plasma phosphorylated-tau$_{181}$ and Alzheimer's disease was strongest in those who were demented at time of blood draw (OR=1.25, 95%CI=1.02-1.53), but an effect existed among the non-demented (OR=1.05, 95% CI=1.01-1.10). There was higher discrimination accuracy for Alzheimer's disease when blood draw occurred in years closer to death, however, higher plasma

phosphorylated-tau$_{181}$ levels were associated with Alzheimer's disease even when blood draw occurred >5 years from death.

Antemortem plasma phosphorylated-tau$_{181}$ concentrations were associated with Alzheimer's disease neuropathology and accurately differentiated brain donors with and without autopsy-confirmed Alzheimer's disease. These findings support plasma phosphorylated-tau$_{181}$ as a scalable biomarker for the detection of Alzheimer's disease.

Keywords: plasma p-tau$_{181}$; Alzheimer's disease; autopsy; biomarkers; tau

Abbreviations: BU ADRC = Boston University Alzheimer's Disease Research Center; BUMC IRB = BU Medical Campus Institutional Review Board; CERAD = Consortium to Establish a Registry for Alzheimer's Disease; CSF = cerebrospinal fluid; FTLD = frontotemporal lobar degeneration; LP = lumbar puncture; MCI = mild cognitive impairment; p-tau$_{181}$ = phosphorylated-tau$_{181}$; NACC = National Alzheimer's Coordinating Center

Introduction

Alzheimer's disease is characterized by the extracellular accumulation of the amyloid-β (Aβ) peptide and intracellular aggregation of hyper-phosphorylated tau (p-tau) protein.[1] In the National Institute on Aging and Alzheimer's Association framework,[2] it is possible to detect preclinical Alzheimer's disease neuropathological changes using *in vivo* biomarkers, allowing for early disease detection and timely therapeutic intervention.[2] Lumbar puncture for analysis of cerebrospinal fluid and positron emission tomography ligands for Aβ and p-tau have revolutionized our ability to detect Alzheimer's disease pathology.[2] However, lumbar puncture is viewed as invasive and positron emission tomography scans are expensive, not covered by medical insurance, and involve exposure to radiation. They have limited scalability and often unavailable in non-specialized clinics and in low- and middle-income countries.

It is now possible to detect low abundant proteins associated with Alzheimer's disease neuropathology in the blood, including p-tau.[3] Recent studies demonstrate plasma p-tau$_{181}$ is associated with cerebrospinal fluid levels of p-tau$_{181}$ and tau and amyloid uptake on PET.[4–7] Higher plasma p-tau concentrations (including at 181 and 217 phosphorylation sites) can accurately differentiate mild cognitive impairment and Alzheimer's disease dementia participants from those with normal cognition.[4–6,8] Research on the validity of plasma p-tau in Alzheimer's disease is nascent and the extent to which proteins in the blood reflect the central nervous system environment is emerging.

Clinical-pathological correlation studies are the gold standard for the development and validation of *in vivo* biomarkers.[9–15] There have been a few plasma-to-autopsy correlation studies in Alzheimer's disease. Brickman *et al.*[4] showed higher antemortem plasma p-tau$_{217}$ and p-tau$_{181}$ in 33 brain donors with high Alzheimer's disease neuropathologic changes compared to 80 donors

who had low Alzheimer's disease. Among 115 individuals with longitudinal blood samples, plasma p-tau$_{181}$ accurately discriminated Alzheimer's disease from non-Alzheimer's disease neuropathological diagnoses as long as 8 years before death (AUC=0.97).[16] Smirnov et. al.[17] also demonstrated strong sensitivity and specificity of plasma p-tau$_{181}$ in predicting Alzheimer's disease neuropathology among 312 brain donors (AUC=0.856). Furthermore, plasma p-tau$_{181}$ accurately discriminated (AUC=0.88) 15 participants with autopsy-confirmed cases of Alzheimer's disease from 67 brain donors with frontotemporal lobar degeneration.[7] A recent study found plasma p-tau$_{181}$ accurately discriminated (AUC=0.91) 14 cases of autopsy-confirmed Alzheimer's disease from Aβ-negative controls, as well as Alzheimer's disease from non-Alzheimer's disease autopsy cases (n = 4).[18] Plasma p-tau$_{181}$ levels also correlated with Braak stage and neuritic amyloid plaque scores.[7,16,18]

Additional large scale plasma-pathological correlation studies are needed to validate plasma p-tau$_{181}$ as an accurate and reliable biomarker of Alzheimer's disease neuropathologic changes. In addition, no study has examined the association between plasma p-tau$_{181}$ and regional p-tau aggregation, which is an important validation step as it will provide insight on the association between plasma p-tau$_{181}$ and tau in regions classically affected by Alzheimer's disease (e.g., hippocampus). This study examined the ability of antemortem plasma p-tau$_{181}$ levels to accurately differentiate brain donors with and without autopsy-confirmed Alzheimer's disease. We tested the association between antemortem plasma p-tau$_{181}$ and p-tau aggregation across six cortical and subcortical brain regions. We hypothesized that antemortem p-tau$_{181}$ levels would accurately discriminate between brain donors with and without Alzheimer's disease neuropathology and be associated with p-tau severity at autopsy.

Materials and methods

Study Design and Brain Donors

This study included participants from the National Institute on Aging-funded Boston University Alzheimer's Disease Research Center (BU ADRC) Clinical Core who donated their brain to the BU ADRC Neuropathology Core for neuropathological examination. The BU ADRC is one of more than 30 centers funded by the National Institute on Aging that provides standardized data to the National Alzheimer's Coordinating Center to promote collaborative research on Alzheimer's disease and related dementias. The BU ADRC follows older adults with and without cognitive impairment from the Boston neighborhoods surrounding Boston Medical Center and the Greater Boston area. All participants are English-speaking older adults with adequate visual acuity and hearing. Participants are excluded for a history of a serious mental illness (*e.g.*, bipolar disorder, schizophrenia, etc.), non-Alzheimer's disease or related dementias neurological disorders (*e.g.*, brain tumor, multiple sclerosis), or medical conditions that preclude study participation. The BU ADRC protocol involves an annual National Alzheimer's Coordinating Center Uniform Data Set evaluation that includes neurological examination, a clinical and medical interview, neuropsychological testing, and other procedures. Participants are asked to donate their brain following death to the BU ADRC brain bank for comprehensive neuropathological processing and examination.

Beginning in 2008, voluntary annual blood draws were initiated at the BU ADRC. Blood samples collected through 2018 were analyzed for plasma p-tau$_{181}$ as part of a separate published study that examined the ability of plasma p-tau$_{181}$ to discriminate participants with cognitive impairment from normal cognition.[19] We leveraged p-tau$_{181}$ data from that study. We included participants from that sample who had p-tau$_{181}$ and who donated their brain for

neuropathological examination. If multiple blood draws were performed, the most recent was used. Because p-tau$_{181}$ data were acquired from a study focused on clinical outcomes, the visit of the plasma sample did not necessarily correspond to the visit proximate to death. This resulted in a sample size of 103 after exclusion for missing data on primary study variables and exclusion of one brain donor with p-tau concentration level below the lower limit of quantification (eFigure 1). Procedures including brain donation were approved by the BU Medical Campus Institutional Review Board. Participants (or their Legally Authorized Representatives) provided written informed consent prior to participation in the BU ADRC protocol. Approval for neuropathological evaluation was obtained through the Boston University Medical Campus Institutional Review Board. Next of kin provided written informed consent if written informed consent from the participant was obtained more than three years prior to death.

Plasma Biomarker Collection and Analysis

Blood collection, processing, and storage followed standard operating procedures that adhere to those set forth by the National Centralized Repository for Alzheimer's Disease and Related Dementias. Non-fasting blood samples were collected into plastic dipotassium EDTA tubes and processed with plasma aliquoted and frozen at -80°C. Frozen plasma aliquots were shipped on dry ice to the University of Gothenburg (Sweden) for batch analysis. Plasma p-tau$_{181}$ concentration was measured using an in-house single molecule array method on an HD-X analyzer (Quanterix, Billerica, Massachusetts), as previously described in detail.[20] The lower limit of quantification (LLoQ) was 1.0 pg/mL, with a dynamic range of 1.0-128.0 pg/mL. The measurements were performed in one round of experiments, using one batch of reagents. Intra-assay coefficients of variation were below 10%.

Neuropathological Evaluation

Neuropathological processing and evaluation were conducted using published methodology[21,22] and following procedures described in the National Alzheimer's Coordinating Center standardized Neuropathology Form and Coding Guidebook.[23–26] Ratings of Thal phase were added later to the BU ADRC and available on 56 of 103 brain donors. The NIA-Reagan Institute criteria utilizing Braak stage and CERAD scores were thus used for the neuropathological diagnosis of Alzheimer's disease.[27] Brain donors who had no or low Alzheimer's disease neuropathologic changes were combined (non-Alzheimer's disease). The Alzheimer's disease group included brain donors who had intermediate or high likelihood of Alzheimer's disease. Established criteria were used for other neuropathological diagnosis of neurodegenerative diseases.[28–32] Semi-quantitative scales (0 [none]– 3 [severe]) were used to rate severity of cerebral amyloid angiopathy, atherosclerosis, and arteriolosclerosis.

The Consortium to Establish a Registry for Alzheimer's Disease (CERAD) score was used to rate the presence and severity of neuritic Aß plaques.[33] Braak staging of neurofibrillary degeneration was rated on a scale from 0 (no degeneration) to VI (widespread degeneration that has spread to the neocortex) based on Bielchowsky silver staining.[34] Independent assessments of the density of AT8-positive p-tau pathology were performed by study neuropathologists using semi-quantitative rating scales (0-3 scale; 0=none, 3=severe) in various cortical and subcortical brain regions. AT8-immunostained, 10 µm thick paraffin-embedded sections of the following regions were examined in this study: inferior parietal cortex, superior temporal cortex, CA1-hippocampus, CA2-hippocampus, entorhinal cortex, and the amygdala. These regions were *a priori* selected due to their involvement in Alzheimer's disease.[34]

Dementia Severity

Dementia severity was rated using the global score from the Clinical Dementia Rating (CDR®) Dementia Staging Instrument.[35,36] An algorithm is used to calculate a global severity rating designated as: 0 (no dementia), 0.5 (mild cognitive impairment), 1.0 (mild dementia), 2.0 (moderate dementia), and 3.0 (severe dementia). Global CDR score at the time of blood draw was included in statistical models.

Statistical Analytic Plan

All analyses were conducted using SPSS statistical software version 27. A p-value<0.05 was considered statistically significant. Plasma p-tau$_{181}$ served as the independent variable. Three binary logistic regression models were performed to examine the association between plasma p-tau$_{181}$ and Alzheimer's disease neuropathological diagnosis: (1) Model 1: unadjusted (*i.e.*, plasma p-tau$_{181}$ alone), (2) Model 2: controlling for age at death, years between last blood draw and death, sex (1=female, 0=male), and *APOE* ε4 status (1=ε4 carrier, 0=non-carrier), and (3) Model 3: Model 2 covariates in addition to global CDR score at the time of blood draw to account for differences in disease severity[7]. CDR scores were stratified by <1 and 1 or higher (*i.e.*, dementia vs no dementia). For each model, discrimination accuracy for Alzheimer's disease neuropathological diagnosis was evaluated using the area under the receiver operating curve (AUC) statistic. AUC statistic was calculated based on p-tau$_{181}$ alone (Model 1) and using predicted probabilities from the multivariable logistic regression that included the aforementioned covariates (Models 2 and 3). Note that AUC statistic was also calculated for a covariate only model (i.e., Model 2 without plasma p-tau$_{181}$) as reference for Models 1-3. Discrimination accuracy was categorized based on guidelines suggested in Hosmer and

Lemeshow (AUC =0.50: no discrimination; AUC=0.70-0.80: acceptable discrimination; AUC=0.80–0.90: excellent discrimination; AUC≥0.90: outstanding discrimination).[37]

In the entire sample, multivariable ordinal logistic regressions tested the associations between plasma p-tau$_{181}$ and Braak NFT stage (stage 0, I/II, III/IV, V/VI), CERAD neuritic plaque score, and semi-quantitative ratings of p-tau severity for the inferior parietal cortex, superior temporal cortex, entorhinal cortex, amygdala, CA1-hippocampus, and CA2-hippocampus. Sample size for the semi-quantitative ratings of regional p-tau severity was reduced to 90 due to missingness. Covariates included age at death, years between last blood draw and death, sex, and *APOE* ε4 status. Due to the number of analyses performed for the semi-quantitative ratings of regional p-tau severity (six total outcomes), p-values were false discovery rate-adjusted using the Benjamini-Hochberg procedure.

As sensitivity analyses, the logistic regression was repeated with a p-tau$_{181}$ x CDR score (at time of blood draw) (Model 3 repeated) and a p-tau$_{181}$ x years between blood draw and death interaction term included (Model 2 repeated), in separate models. These models tested whether (1) dementia severity and (2) the time between blood draw and neuropathological examination moderated the association between plasma p-tau$_{181}$ levels and Alzheimer's disease neuropathological diagnosis. We examined the accuracy of plasma p-tau$_{181}$ in discriminating Alzheimer's disease and non-Alzheimer's disease brain donors, using the AUC statistic, stratified by CDR scores (<1 and 1 or higher) and by who those who had a blood draw greater than or equal to and less than 5 years prior to death.

Data availability

All uniform and neuropathology data set evaluation data are shared with the National Alzheimer's Coordinating Center and are publicly available. Data is also available upon reasonable request to the BU ADRC.

Results

Sample Characteristics

Table 1-2 presents sample characteristics of the 103 brain donors. The mean (standard deviation) time between blood draw and death was 5.6 (3.1) years with a median of 5.0 and range of 0.0 (blood draw done same month of death)-12.0 years. Sixty-two (60.2%) had Alzheimer's disease at autopsy. Compared to those without autopsy-confirmed Alzheimer's disease, those with Alzheimer's disease were more likely to have an *APOE* ε4 allele (p=0.06) and a higher global CDR score at time of death and blood draw (p<0.01). There were no statistically significant differences between the donors with and without Alzheimer's disease in terms of age at death, race, ethnicity, sex, years between blood draw and death, or self-reported vascular risk factors. Donors with Alzheimer's disease had more severe ratings of cerebral amyloid angiopathy and regional p-tau than the non-Alzheimer's disease donors (p-values<0.01). There were no statistically significant differences between Alzheimer's disease and non-Alzheimer's disease on neuropathological diagnosis of Lewy body disease, frontotemporal lobar degeneration, arteriolosclerosis, or atherosclerosis.

Plasma P-tau$_{181}$ Associations with Alzheimer's disease Neuropathology and P-tau

Statistical models are summarized in Table 3. Figure 1 shows the distribution of plasma p-tau$_{181}$ concentrations by Alzheimer's disease status. A covariate only model (i.e., age at death, years between last blood draw and death, sex, and *APOE* ε4 status) had an AUC of 0.65 (95% CI = 0.54-0.76) for discriminating between brain donors with and without Alzheimer's disease. In a plasma p-tau$_{181}$ only model, higher plasma p-tau$_{181}$ concentrations were associated with increased odds of having Alzheimer's disease neuropathologic changes (OR=1.05, 95% CI=1.02-1.09) with an AUC of 0.73 (95% CI=0.63-0.83). This association remained after controlling for age at death, years between blood draw and death, sex, and *APOE* ε4 status (OR=1.06, 95% CI=1.02-1.10) with an AUC of 0.76 (95% CI=0.67-0.86), as well as when global CDR score was included as a covariate (OR=1.07, 95% CI=1.03-1.11). The full multivariable model that included global CDR discriminated Alzheimer's disease from non-Alzheimer's disease with excellent accuracy (AUC=0.82, 95% CI=0.74-0.91). Figure 2 shows the ROC curves for each model. We repeated the fully adjusted model 3 with p-tau$_{181}$ standardized (z-transformed) to facilitate interpretation of its association with Alzheimer's disease status in this sample. The OR for the association between standardized p-tau$_{181}$ levels and Alzheimer's disease status at autopsy was 2.98 (95% CI=1.50-5.93, p<0.01).

Higher levels of plasma p-tau$_{181}$ were associated with Braak stage (OR=1.06, 95% CI=1.02-1.09) and CERAD neuritic plaque score (OR=1.05, 95% CI=1.02-1.08). Higher plasma p-tau$_{181}$ concentrations corresponded to higher odds for having more severe p-tau in the superior temporal cortex, inferior parietal cortex, entorhinal cortex, amygdala, CA1-hippocampus, and

CA2-hippocampus (OR=1.03-1.06, FDR-adjusted p-values<0.05) (Table 3). Figure 3 shows the associations.

Stratified by Global CDR Score at Blood Draw

Corresponding to global CDR scores at time of blood draw of 0, 0.5, 1.0, 2.0, and 3.0, there were 39 (37.9%), 18 (17.5%), 25 (24.3%), 12 (11.7%), and 9 (8.7%) participants, respectively. See eTable 1 for sample characteristics by CDR score. Figure 1 also shows distribution of plasma $ptau_{181}$ concentrations by CDR score. Those who had a higher CDR score were more likely to have Alzheimer's disease neuropathology and have higher p-tau severity ratings. There was a significant plasma p-tau_{181} x CDR interaction effect on Alzheimer's disease neuropathological diagnosis (OR=1.22, 95% CI=1.01-1.48, p=0.04). Plasma p-tau_{181} levels had better discrimination accuracy among those with high CDR scores compared with low. Discrimination for Alzheimer's disease neuropathological diagnosis was good for both the participants with CDR scores ≥1.0 (AUC=0.89, 95% CI=0.78-0.99, p<0.01) and those who had a CDR score <1.0 (AUC=0.78, 95% CI=0.65-0.91, p<0.01), for models that included p-tau_{181}, age at death, years between last blood draw and death, sex, and *APOE* ε4 status (Figure 2).

Stratified by Blood Draw Greater and Less than 5 Years Before Death

In the sample stratified by donors who had a blood draw <5 (n=45) or ≥5 years (n=58) prior to death, 29 (64.4%) and 33 (56.9%) had autopsy-confirmed Alzheimer's disease, respectively. Those who had a blood draw within 5 years were older at the time of blood draw by approximately 4 years (p=0.01), had a higher global CDR score (p<0.001), and had a higher Braak stage (p<0.01). There were no other differences between the groups (ps>0.05; eTable 2). As shown in Table 4 and eFigure 2, all three models had excellent discrimination accuracy

among brain donors who had a blood draw <5 years from death with AUCs ranging from 0.83-0.91 (ps<0.01). Discrimination accuracy for plasma p-tau$_{181}$ was worse in brain donors who had a blood draw ≥5 years from death, particularly for plasma p-tau$_{181}$ alone (AUC=0.65, p=0.049). Discrimination accuracy for the adjusted models remained acceptable with an AUC of 0.71 (p=0.006) for Model 2 and AUC of 0.77 (p<0.001) for Model 3. There was a statistical trend for plasma p-tau$_{181}$ x years between blood draw and death interaction effect on Alzheimer's disease neuropathological diagnosis in Model 3 (p=0.099).

Discussion

In this sample of 103 brain donors (62 with autopsy-confirmed Alzheimer's disease), antemortem plasma p-tau$_{181}$ concentrations were associated with Alzheimer's disease neuropathologic changes at autopsy, including National Institute on Aging-Reagan Alzheimer's disease neuropathological diagnosis, Braak stage, CERAD neuritic plaque score, and semi-quantitative ratings of cortical and subcortical p-tau severity. Higher plasma p-tau$_{181}$ levels accurately differentiated donors with and without autopsy-confirmed Alzheimer's disease, including among a subgroup who were cognitively unimpaired or had MCI at the time of blood sampling. Discrimination accuracy across all models was superior when plasma p-tau$_{181}$ was examined jointly with demographics, *APOE* ε4 status, and global CDR score. Discrimination accuracy was optimal when blood draw was within 5 years of death, however, plasma p-tau$_{181}$ levels from ≥5 years before death also accurately discriminated—albeit to a lesser extent—between Alzheimer's disease and non-Alzheimer's disease neuropathological diagnoses. These findings support plasma p-tau$_{181}$ as a biomarker for the accurate and early detection of underlying Alzheimer's disease neuropathology.

The development and validation of plasma biomarkers for Alzheimer's disease and related dementias has been the focus of research in recent years.[38] Clinical-pathological correlation studies are the gold standard but there are few plasma-to-autopsy studies and existing ones are limited by smaller sample sizes. The present findings are consistent with previous studies that show an association between antemortem plasma p-tau$_{181}$ and Alzheimer's disease neuropathological changes at autopsy. Jointly published results from two independent neuropathology cohorts replicated and cross-validated the finding that plasma p-tau$_{181}$ differentiates autopsy-proven Alzheimer's disease in small samples (n=15 and n=16, respectively[5,7]). This compares with a similarly sized sample (n=14) from the Alzheimer's Disease Neuroimaging Initiative that associated cerebrospinal fluid levels of p-tau$_{181}$ with autopsy-confirmed Alzheimer's disease neuropathological changes.[18] Plasma p-tau$_{181}$ correlated with cerebrospinal fluid p-tau$_{181}$ levels in that study, and higher plasma p-tau$_{181}$ levels had similar pathologic specificity as cerebrospinal fluid p-tau$_{181}$ for Braak stage and neuritic Aβ plaques.[18]

Recent studies and the present one support the utility of plasma p-tau$_{181}$ in larger samples. A UK clinical registry cohort associated elevated plasma p-tau$_{181}$, measured by single molecular array, with Alzheimer's disease neuropathological diagnosis and higher Braak stage among 111 brain donors (67 with autopsy-confirmed Alzheimer's disease).[16] Similar results associating elevated plasma p-tau$_{181}$ with neuropathological diagnosis of Alzheimer's disease were demonstrated in a sample of 312 brain donors.[17] In the present sample of 103 brain donors (62 with Alzheimer's disease), we observed similar associations and, for the first time, show that plasma p-tau$_{181}$ levels signaled regional p-tau aggregation in areas such as the entorhinal cortex, hippocampus, amygdala, inferior parietal cortex, and superior temporal cortex. These are important regions of

neuropathological changes in Alzheimer's disease and are affected early in the disease.[2,34,39] This finding highlights the utility of plasma p-tau$_{181}$ for early disease detection, which is necessary if blood-based biomarkers are to be used in clinical trials for primary prevention of Alzheimer's disease.

Additional data from the present study and others suggest that plasma p-tau$_{181}$ has potential use as a biomarker for the early detection of Alzheimer's disease.[4,5,7,15–18,40] As many studies show that amyloid deposits precede tauopathy in the brain for Alzheimer's disease,[2,41] our study shows that plasma p-tau$_{181}$ was associated CERAD neuritic plaque scores. Plasma p-tau$_{181}$ discriminated Alzheimer's disease from non-Alzheimer's disease in participants who were either cognitively unimpaired or were rated as having mild cognitive impairment based on global CDR score at the time of the blood draw. Plasma p-tau$_{181}$ prediction was superior in those who had a CDR of 1 or higher at the time of blood draw. However, AUC for those with a low CDR was still of acceptable discrimination. Supporting these findings, the Washington Heights-Inwood Columbia Aging Project showed that higher plasma p-tau$_{181}$ values improved prediction of future clinical Alzheimer's disease among participants without dementia at the time of first blood draw.[4] Mielke et al.[6] associated plasma p-tau$_{181}$ with tau (on positron emission tomography) in cognitively unimpaired participants or participants with only mild cognitive impairment. Plasma p-tau$_{181}$ was recently shown to accurately discriminate Alzheimer's disease from non-Alzheimer's disease pathology from blood drawn 7.9 years prior to autopsy (mean±SD 7.9±1.2, range 6.3-9.4).[16] Biomarker levels in that study increased across time points from 8 to 4 years before death, providing information on the longitudinal trajectory of plasma p-tau$_{181}$ levels and demonstrating how the biomarker could be used to track progression of Alzheimer's disease. In our sensitivity analysis, discrimination accuracy of p-tau$_{181}$ for Alzheimer's disease pathology

was acceptable among brain donors who had a blood draw greater than 5 years before death, although there was higher discrimination accuracy among participants with blood draw less than 5 years from death and this might have been because these individuals had a higher CDR.

The present findings add to the literature for plasma p-tau$_{181}$ as a putative risk biomarker to screen for Alzheimer's disease and to enrich clinical trials for participants at high risk for Alzheimer's disease. A potential use of biomarkers is to select for and enroll clinical trial participants that have no or subtle symptoms and are at a stage before pathology has advanced, where an early intervention may be more effective. In our models that included age, sex, *APOE* ε4 status, and global CDR rating score, which are commonly collected and measured in clinic, plasma p-tau$_{181}$ measurement greatly improved prediction of Alzheimer's disease. This observation underscores the potential utility of measuring a putative Alzheimer's disease blood biomarker, both for clinical trials and in the clinic, to better estimate risk.

There are limitations to the present findings. We did not explore trends in plasma p-tau$_{181}$ levels longitudinally. Although plasma p-tau$_{181}$ accurately detects Alzheimer's disease at autopsy, the clinical meaning of a unit increase in raw pg/mL values of plasma p-tau$_{181}$ is unclear. When plasma p-tau$_{181}$ was standardized, the odds ratio for Alzheimer's disease status substantially increased (OR=2.98). Although standardizing plasma p-tau$_{181}$ can facilitate interpretation and clarify the true association in this sample, raw plasma p-tau$_{181}$ values were of the primary focus to facilitate comparison across studies and generalizability to the clinic. Non-fasting blood samples were collected. At this time, there are not formal recommendations to require fasting blood samples for plasma biomarker analysis of neurodegenerative disease proteins given the insufficient evidence to support its superiority. Additional research is needed to compared fasting and non-fasting samples on plasma biomarker assay analysis. The findings are limited to

participants from a single clinical cohort, which introduces the potential for selection bias. The present sample is from a National Institute on Aging-funded ADRC and is most representative of individuals who present to a clinic with concerns regarding their cognitive functioning. This population allows for development and validation of biomarkers, but inferences regarding risk and screening for Alzheimer's disease in the general population cannot be made. The sample was demographically homogenous and a majority identified as white. Prospective population-based studies are needed to address these knowledge gaps and identify generalizable cutoff values that optimize sensitivity and specificity for the detection of Alzheimer's disease.

CONCLUSION

Results of this study show an association between plasma p-tau$_{181}$ levels and Alzheimer's disease neuropathological changes at autopsy. With millions of individuals living with or at risk of developing Alzheimer's disease, an increased understanding of accessible, cost-effective tools for evaluating disease diagnosis, including plasma p-tau$_{181}$, is essential.

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Competing interests

Kaj Blennow has served as a consultant, at advisory boards, or at data monitoring committees for Abcam, Axon, BioArctic, Biogen, JOMDD/Shimadzu. Julius Clinical, Lilly, MagQu, Novartis, Pharmatrophix, Prothena, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program, outside the work presented in this paper. Henrik Zetterberg has served at scientific advisory boards and/or as a consultant for Abbvie, Alector, Annexon, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Pinteon Therapeutics, Red Abbey Labs, Passage Bio, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen, and Roche, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program. Andrew E. Budson has served on a consultant or on advisory boards for Sage Pharmaceuticals and Cognito Therapeutics and has received grant monies from Biogen, Bristol Myers Squibb, and Cyclerion. He receives publishing royalties from Elsevier and Oxford University Press. Rhoda Au serves on the scientific advisory board of Signant Health, as consultant to Biogen and has given a lecture in a symposia sponsored by Eisai. Robert A. Stern has served as a consultant to Biogen and Lundbeck. He receives royalties for published neuropsychological tests from Psychological Assessment Resources, Inc.

Supplementary material

Supplementary material is available at *Brain* online.

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Figure legends

Figure 1. Distribution of Plasma P-tau$_{181}$ Concentrations Between Brain Donors with and without Autopsy-confirmed Alzheimer's Disease. National Institute on Aging-Reagan Institute criteria were used for the neuropathological diagnosis of Alzheimer's disease (AD). Analyses were done in the entire sample (n=62 AD vs 41 non-AD) and stratified by CDR score greater than or equal to 1 (n=37 AD vs 9 non-AD) and CDR score < 1 (n=25 AD vs 32 non-AD). Figure shows the median (bar) and interquartile range (whiskers) as well as the individual data points. Results of the binary logistic regression models that tested the association between plasma p-tau$_{181}$ and Alzheimer's disease status in the entire sample and stratified by CDR are shown in Table 3.

Figure 2. Accuracy of Plasma P-tau$_{181}$ in Discriminating Brain Donors with and without Autopsy-confirmed Alzheimer's Disease. National Institute on Aging-Reagan Institute criteria were used for the neuropathological diagnosis of Alzheimer's disease (AD). Analyses were done in the entire sample and stratified by CDR score greater than or equal to 1 and CDR score < 1. AUC statistic was calculated based on p-tau$_{181}$ alone (Model 1) and using predicted probabilities from multivariable binary logistic regression that included age at death, years between last blood draw and death, sex (1=female, 0=male), and *APOE* ε4 status (1=ε4 carrier, 0=non-carrier) (Model 2). For the entire sample, a third model was done that included Model 2 covariates in addition to inclusion of global CDR score at the time of blood draw (Model 3). This Model was not done in those stratified by CDR score.

Figure 3. Distribution of Plasma P-tau$_{181}$ by P-tau Severity Ratings at Autopsy. Figure shows the median (bar) and interquartile range (whiskers) as well as the individual data points for plasma p-tau$_{181}$ levels by Braak staging of neurofibrillary tangles, as well as hyper-phosphorylated tau severity across six cortical and subcortical brain regions rated at autopsy using a 0 (none) – 3 (severe) scale. As shown in Table 3, ordinal logistic regression controlling for age at death, years between last blood draw and death, sex, and *APOE* ε4 status showed higher plasma p-tau$_{181}$ levels were associated with increased odds for more severe Braak stage and p-tau severity across all of the regions (ps < 0.05).

Table 1 Sample Characteristics

	Total Sample (N=103)	Alzheimer's Disease Pathology (N = 62)	Non-AD Pathology (N = 41)	P-value (effect size)
Demographics				
Sex, n (%) women	47 (45.6)	28 (45.2)	19 (46.3)	0.91
Age at blood draw, mean (SD)	78.77 (8.21)	77.97 (8.46)	79.98 (7.76)	0.23
Age at death, mean (SD)	84.40 (8.25)	83.27 (8.26)	86.10 (8.02)	0.09
Race, n (%)				0.19
American Indian/Alaska Native	2 (1.9)	2 (3.2)	0	
Asian	1 (1.0)	0	1 (2.4)	
Black or African American	4 (3.9)	1 (1.6)	3 (7.3)	
White	95 (92.2)	59 (95.2)	36 (87.8)	
Other	1 (1.0)	0	1 (2.4)	
Ethnicity, n (%)				–
Hispanic	0	0	0	
Diagnosis at Death, n (%)				<0.01 (OR = 7.53)
Normal cognition	10 (9.7)	0 (0)	10 (24.4)	
MCI/non-MCI cognitively impaired	20 (19.4)	7 (11.3)	13 (31.7)	
Dementia	73 (70.9)	55 (88.7)	18 (43.9)	
Dementia Severity				
Global CDR score at death, mean (SD)	1.22 (1.13)	1.59 (1.14)	0.66 (0.85)	<0.01 (d = 0.90)
Global CDR score at death, n (%)				<0.01 (OR = 4.91)
<1	55 (53.4)	24 (38.7)	31 (75.6)	
≥1	48 (46.6)	38 (61.3)	10 (24.4)	
Global CDR score at blood draw, mean (SD)	0.83 (0.93)	1.11 (0.97)	0.39 (0.68)	<0.01 (d = 0.84)
Global CDR score at blood draw, n (%)				<0.01 (OR = 5.26)
<1	57 (55.3)	25 (40.3)	32 (78.0)	
≥1	46 (44.7)	37 (59.7)	9 (22.0)	
Vascular Risk Factors, n (%)				
Hypertension	61 (59.2)	34 (54.8)	27 (65.8)	0.27
Diabetes	13 (12.6)	8 (12.9)	5 (12.2)	0.92
Obstructive sleep apnea	9 (8.7)	3 (4.8)	6 (14.6)	0.10
Genetic				
APOE ε4 allele status, n (%) carrier	47 (45.6)	33 (53.2)	14 (34.1)	0.06 (OR = 2.20)
Plasma biomarker				
P-tau$_{181}$, mean (SD)/range pg/mL	27.19 (16.52)/3–95	31.28 (15.67)/9–90	20.99 (16.00)/3–95	<0.01 (d = 0.65)

The 1997 NIA Reagan criteria were used for the neuropathological diagnosis of Alzheimer's disease and those with sparse neuritic plaques and Braak stage 5 or 6 were classified as Alzheimer's disease. Binary logistic regression was used to compare donors with and without autopsy-confirmed Alzheimer's disease (AD) on binary outcomes; independent samples t-test was used for continuous outcomes. For race, white and non-white were compared and coded as 1 (white) and 0 (non-white). Sex was coded as 0 (male) and 1 (female). Sample size for ethnicity was 101 as two were unknown. Abbreviations: AD = Alzheimer's disease; CDR = Clinical Dementia Rating (CDR®) Dementia Staging Instrument; MCI = mild cognitive impairment; OR = odds ratio; d = Cohen's d

Table 2 Neuropathology Characteristics

	Total Sample (N=103)	Alzheimer's Disease Pathology (N = 62)	Non-AD Pathology (N = 41)	P-value (effect size)
Braak stage, n (%)				--
Stage 0	4 (3.9)	0 (0)	4 (9.8)	
Stage I/II	15 (14.6)	0 (0)	15 (36.6)	
Stage III/IV	30 (29.1)	8 (12.9)	22 (53.7)	
Stage V/VI	54 (52.4)	54 (87.1)	0 (0)	
Semi-quantitative ratings of regional p-tau severity, n (%) moderate-severe				
Inferior parietal cortex	48 (53.3)	48 (88.9)	0	–
Superior temporal cortex	56 (62.2)	50 (92.6)	6 (16.7)	<0.01 (OR = 62.50)
Entorhinal cortex	74 (82.2)	52 (96.3)	22 (61.1)	<0.01 (OR = 16.55)
Amygdala	63 (70.0)	51 (94.4)	12 (33.3)	<0.01 (OR = 34.00)
CA1-hippocampus	67 (74.0)	50 (92.6)	17 (47.2)	<0.01 (OR = 13.97)
CA2-hippocampus	53 (58.9)	40 (74.1)	13 (36.1)	<0.01 (OR = 5.06)
CERAD neuritic plaque score, n (%)				–
None	23 (22.3)	0 (0)	23 (56.1)	
Sparse	20 (19.4)	6 (9.7)	14 (34.1)	
Moderate	27 (26.2)	23 (37.1)	4 (9.8)	
Frequent	33 (32.0)	33 (53.2)	0	
Lewy Body Disease, n (%)				0.16
Brainstem predominant	4 (4.0)	3 (4.8)	1 (2.4)	
Limbic (transitional)	8 (8.1)	4 (6.5)	4 (9.8)	
Neocortical (diffuse)	18 (18.2)	11 (17.7)	7 (17.1)	
Amygdala predominant	4 (4.0)	4 (6.5)	0 (0)	
Olfactory bulb	3 (3.0)	3 (4.8)	0 (0)	
Frontotemporal lobar degeneration, n (%)	11 (10.7)	4 (6.5)	7 (17.1)	0.10
Chronic traumatic encephalopathy, n (%)	2 (2.0)	2 (3.4)	0	–
Cerebral amyloid angiopathy, n (%) moderate-severe	45 (43.7)	35 (56.5)	10 (24.4)	<0.01 (OR = 4.02)
Arteriosclerosis, n (%) moderate-severe	84 (81.6)	51 (82.3)	33 (80.5)	0.82
Atherosclerosis, n (%) moderate-severe	38 (36.9)	23 (37.1)	15 (36.6)	0.96

The 1997 NIA Reagan criteria were used for the neuropathological diagnosis of Alzheimer's disease and those with sparse neuritic plaques and Braak stage 5 or 6 were classified as Alzheimer's disease. Binary logistic regression was used to compare donors with and without autopsy-confirmed Alzheimer's disease on all outcomes. Braak and CERAD were not compared because they were used to define the Alzheimer's disease groups. Note analyses were not performed for those with insufficient cell sizes. For semi-quantitative ratings of regional p-tau, cerebral amyloid angiopathy, arteriolosclerosis, and atherosclerosis, donors with moderate to severe ratings were grouped compared with donors who had no or mild severity ratings. Lewy body disease was examined as absent/present. Sample size for Lewy body disease was 99 because it was not assessed for four donors. Three brain donors had missingness for chronic traumatic encephalopathy. Sample sizes for the semi-quantitative ratings of p-tau severity was 90 (sample restricted to donors who had complete ratings for all regions). Abbreviations: AD = Alzheimer's disease; CERAD = Consortium to Establish a Registry for Alzheimer's Disease

Table 3 Association Between Plasma P-tau$_{181}$, Alzheimer's Disease Neuropathology, and Regional P-tau Severity

	OR	95% CI	P-value	AUC (95% CI), P-value
Autopsy-confirmed Alzheimer's disease (n=62 vs 41 non-AD)				
Model 1	1.05	1.02–1.09	<0.01	0.73 (0.63–0.83), <0.01
Model 2	1.06	1.02–1.10	<0.01	0.76 (0.67–0.86), <0.01
Model 3	1.07	1.03–1.11	<0.01	0.82 (0.74–0.91), <0.01
Autopsy-confirmed Alzheimer's disease, CDR <1.0 (n=25 AD vs 32 non-AD)				
Model 1	1.04	0.99–1.08	0.05	0.71 (0.57–0.84), <0.01
Model 2	1.05	1.01–1.10	0.02	0.78 (0.65–0.91), <0.01
Autopsy-confirmed AD, CDR ≥1.0 (n=37 AD vs 9 non-AD)				
Model 1	1.23	1.05–1.45	0.01	0.85 (0.73–0.97), <0.01
Model 2	1.25	1.02–1.53	0.03	0.89 (0.78–0.99), <0.01
Braak stage (n=103)	1.06	1.02–1.09	<0.01	–
CERAD neuritic plaque score (n=103)	1.05	1.02–1.08	<0.01	–
Regional p-tau severity (n=90)				
Superior temporal cortex	1.06	1.02–1.09	<0.01	–
Inferior parietal cortex	1.04	1.01–1.07	<0.01	–
Entorhinal cortex	1.06	1.02–1.10	<0.01	–
Amygdala	1.04	1.01–1.07	0.03	–
CA1-hippocampus	1.06	1.02–1.10	<0.01	–
CA2-hippocampus	1.03	1.01-1.06	0.02	–

Binary logistic regression examined the association between plasma p-tau$_{181}$ levels and Alzheimer's disease neuropathologic changes (per NIA-Reagan criteria). Model 1 examined plasma p-tau$_{181}$ alone. Model 2 controlled for age at death, years between last blood draw and death, sex, and *APOE* ε4 status. Model 3 controlled for Model 2 covariates in addition to global CDR (<1 and 1 or higher) score at time of blood draw. The AUC statistics for Models 2 and 3 were calculated using predicted probabilities from the binary logistic regression. P-values that examined the semi-quantitative ratings of regional p-tau severity as outcomes (six total outcomes) were false discovery rate (FDR)-adjusted using the Benjamini-Hochberg procedure and covariates included age at death, years between last blood draw and death, sex, and *APOE* ε4 status. Abbreviations: AD = Alzheimer's disease; CDR = Clinical Dementia Rating (CDR®) Dementia Staging Instrument; OR = odds ratio; CI = confidence interval; CERAD = Consortium to Establish a Registry for Alzheimer's Disease

Table 4 Accuracy of Plasma P-tau$_{181}$ in Discriminating Donors with and without Autopsy-Confirmed Alzheimer's Disease Stratified by Blood Draw Greater and Less than 5 Years Before Death

	AUC	95% CI	p-value
Blood draw less than 5 years before death (n = 45)			
Autopsy-confirmed Alzheimer's disease			
Model 1	0.83	0.71–0.95	<0.01
Model 2	0.85	0.74–0.97	<0.01
Model 3	0.91	0.83–1.00	<0.01
Blood draw greater than 5 years before death, n = 58			
Autopsy-confirmed Alzheimer's disease			
Model 1	0.65	0.51–0.80	0.049
Model 2	0.71	0.58–0.85	<0.01
Model 3	0.77	0.65–0.90	<0.01

Model 1 examined plasma p-tau$_{181}$ alone. Model 2 was based on predicted probabilities from binary logistic regression that included plasma p-tau$_{181}$, age at death, years between last blood draw and death, sex, and *APOE* ε4 status. Model 3 was also based on predicted probabilities from binary logistic regression that included Model 2 covariates in addition to global CDR score at time of blood draw.

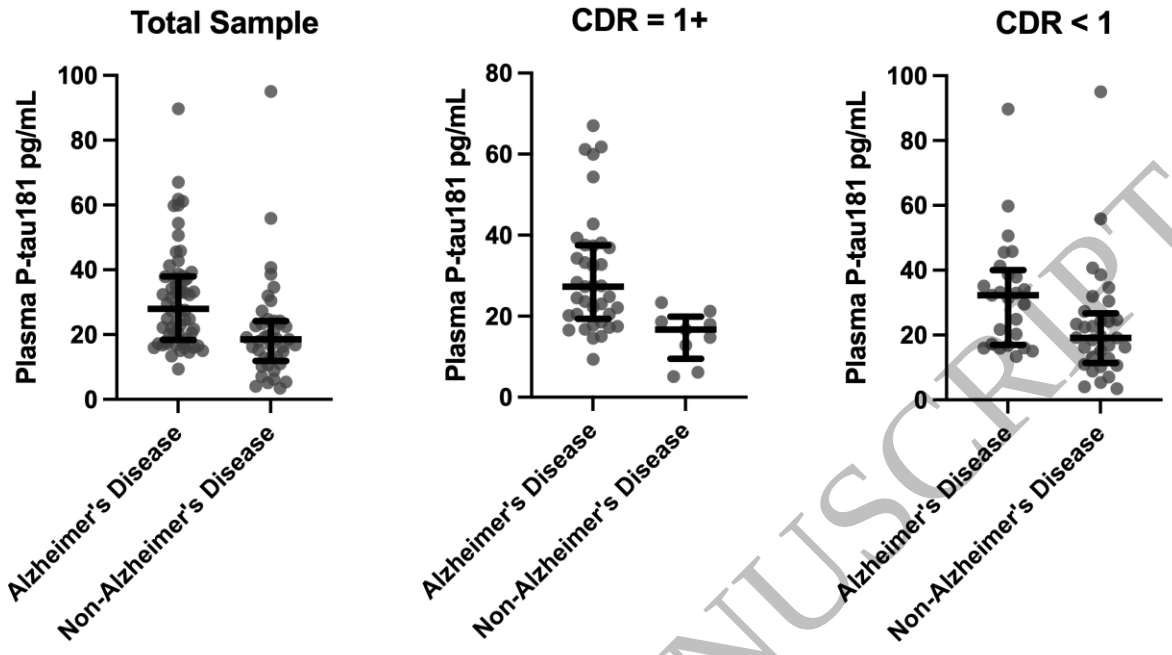

Figure 1
165x91 mm (1.3 x DPI)

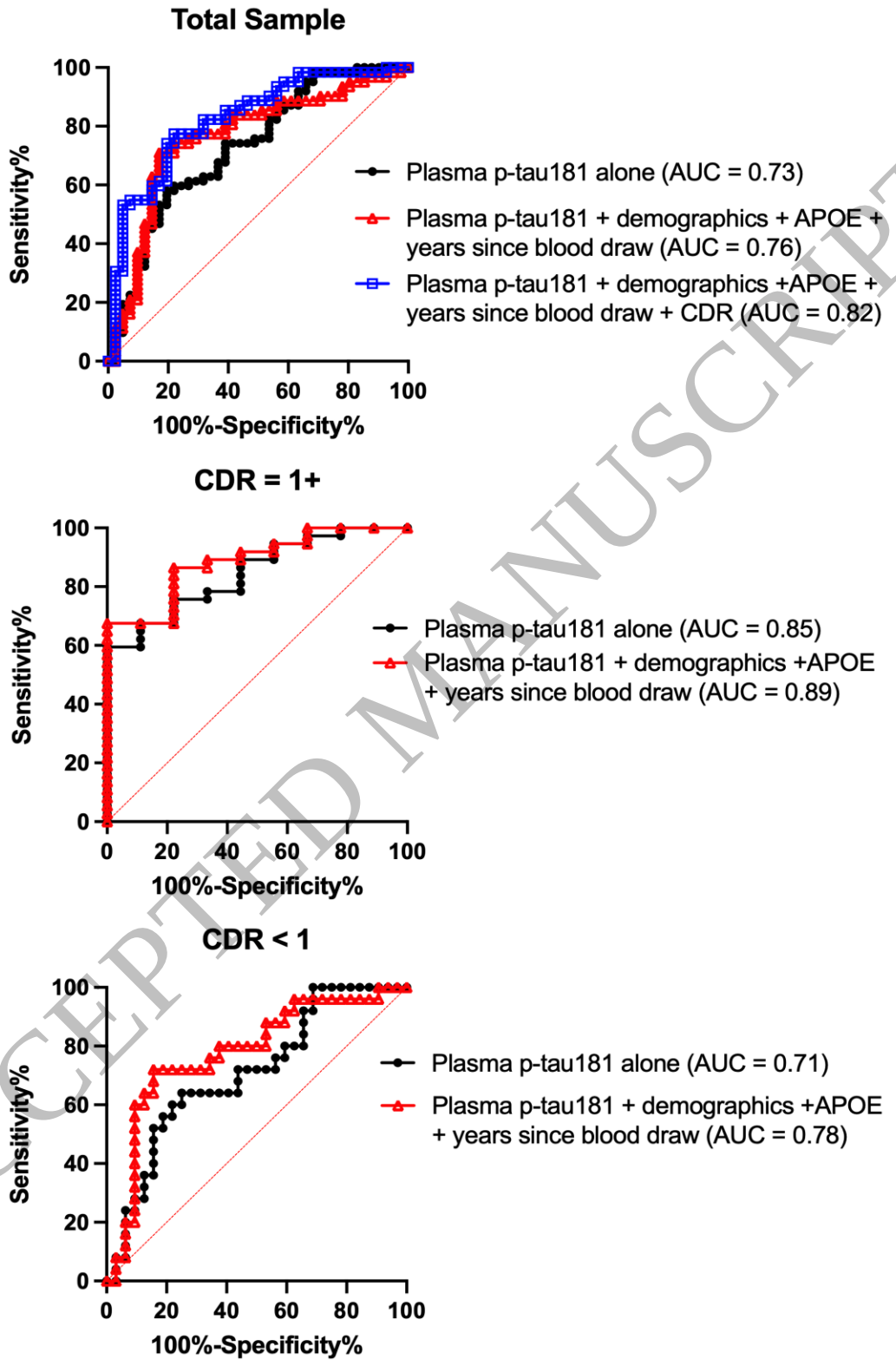

Figure 2
148x229 mm (1.3 x DPI)

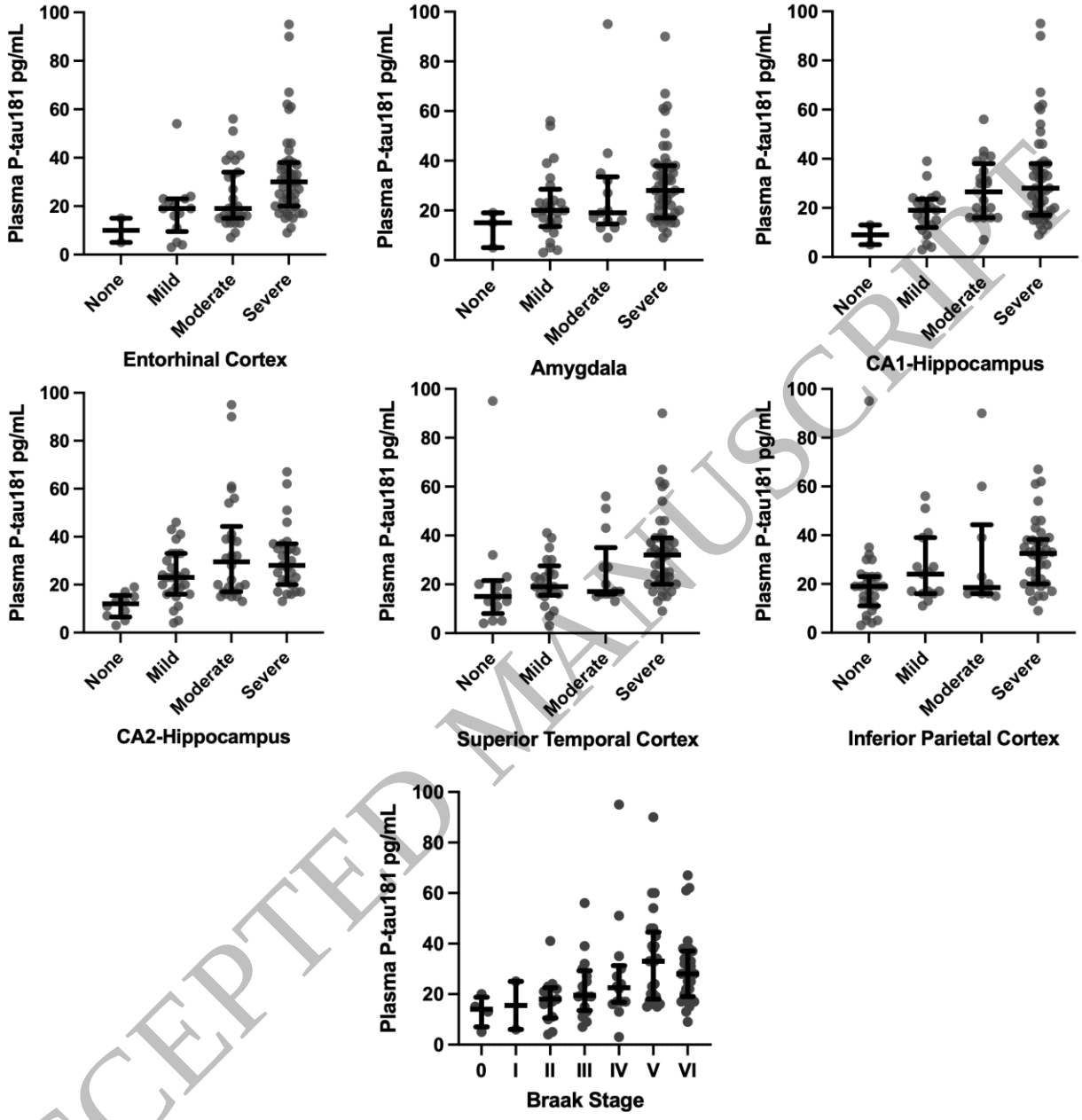

Figure 3
165x173 mm (1.3 x DPI)