

## FEATURED ARTICLE

## Plasma p-tau181, p-tau217, and other blood-based Alzheimer's disease biomarkers in a multi-ethnic, community study

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## Abstract

**Introduction:** Blood-based Alzheimer's disease (AD) biomarkers provide opportunities for community studies and across ethnic groups. We investigated blood biomarker concentrations in the Washington Heights-Inwood Columbia Aging Project (WHICAP), a multi-ethnic community study of aging and dementia.

**Methods:** We measured plasma amyloid beta (A $\beta$ )40, A $\beta$ 42, total tau (t-tau), phosphorylated tau (p-tau)181, and p-tau217, and neurofilament light chain (NfL) in 113 autopsied participants (29% with high AD neuropathological changes) and in 300 clinically evaluated individuals (42% with clinical AD). Receiver operating characteristics were used to evaluate each biomarker. We also investigated biomarkers as predictors of incident clinical AD.

**Results:** P-tau181, p-tau217, and NfL concentrations were elevated in pathologically and clinically diagnosed AD. Decreased A $\beta$ 42/A $\beta$ 40 ratio and increased p-tau217 and p-tau181 were associated with subsequent AD diagnosis.

**Discussion:** Blood-based AD biomarker concentrations are associated with pathological and clinical diagnoses and can predict future development of clinical AD, providing evidence that they can be incorporated into multi-ethnic, community-based studies.

## KEYWORDS

Alzheimer's disease, amyloid, blood-based biomarkers, neurofilament light chain, tau

## 1 | INTRODUCTION

Alzheimer's disease (AD) is the leading cause of dementia.<sup>1</sup> In 1984,<sup>2</sup> the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) diagnostic criteria for AD, categorized three diagnostic levels: definite AD (neuropathological diagnosis), probable AD (clinical diagnosis), and possible AD (clinical diagnosis with comorbidities).

The sensitivity and specificity of the clinical criteria for probable AD compared to the *post mortem* diagnosis was of 81% and 70%.<sup>3</sup> In 2011,<sup>3</sup> updated criteria recognized that the pathological process begins before the onset of clinical symptoms. The use of magnetic resonance imaging (MRI), positron emission tomography (PET) imaging, and cerebrospinal fluid (CSF) assays were included more systematically into diagnosis. Additional sets of criteria, one for preclinical AD<sup>4</sup> and for mild cognitive impairment (MCI) due to AD,<sup>4</sup> were introduced

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that explicitly incorporated biomarkers (MRI, PET, and CSF) into diagnoses. By 2018, a diagnostic scheme<sup>5</sup> was recommended based on biomarker evidence of amyloid ("A"), tau ("T"), and neurodegeneration ("N").<sup>6</sup>

In the clinical setting, incorporating biomarkers into the diagnosis of AD is somewhat easier compared to incorporating them into observational studies. The widespread use of PET and CSF biomarkers is difficult because of limitations in access to radiopharmaceuticals and performing lumbar punctures. Recent developments of AD blood-based biomarkers<sup>7-10</sup> may overcome these issues, providing an opportunity to improve diagnostic accuracy in observational research.

Here we used stored plasma to measure blood-based biomarkers in the Washington Heights-Inwood Columbia Aging Project (WHICAP) cohort, a multi-ethnic community-based study, in which diagnosis of AD was defined either clinically or neuropathologically. Our focus was on the current state-of-the-art AD-related plasma biomarkers, including amyloid beta (A $\beta$ )<sub>40</sub> and A $\beta$ <sub>42</sub> as markers of amyloid pathology, total tau (t-tau) and neurofilament light (NfL) chain as markers of neurodegeneration, and phosphorylated tau (p-tau) 181 and 217 as markers of tau pathology. We compared plasma biomarker concentrations between clinically and pathologically defined diagnostic groups and examined differences by race/ethnicity groups. A subset had undergone florbetaben PET to assess cortical A $\beta$  plaque burden.

## 2 | METHODS

### 2.1 | Participants

We selected all individuals (n = 113) from WHICAP who had brain autopsy with pathological examination and stored plasma. We also selected 300 individuals from the clinical cohort for analysis; the goal was to include equal numbers of participants from each of the three major race/ethnicity groups represented in WHICAP with similar numbers of individuals characterized as having clinical AD at their last available diagnostic visit. Race/ethnicity was self-reported<sup>11</sup> and included non-Hispanic White (White), Hispanic, and non-Hispanic Black/African American. Participants were considered for inclusion if they had stored plasma and had been assessed clinically more than once. All participants received neuropsychological testing, structured medical and neurological examinations, and blood sampling at study entry and at 18- to 24-month intervals. An independent consensus committee derived diagnoses of clinical AD,<sup>3</sup> control, or other forms of dementia. The diagnosis of clinical AD included individuals with frank dementia and those with a Clinical Dementia Rating (CDR<sup>12</sup>) of 0.5 deemed by the consensus committee as having a syndrome consistent with very early or mild AD. For primary analyses in the clinical sample, we compared individuals with and without clinical AD at the time of the blood draw used to derive biomarker concentrations. For those without AD, we compared individuals who subsequently developed clinical AD to those who remained unimpaired. A subset of the clinical cohort (n = 40) had received florbetaben PET scanning. Informed consent was obtained from all participants.

### RESEARCH IN CONTEXT

- 1. Systematic review.** The literature on blood-based biomarkers for Alzheimer's disease (AD) has included White, non-Hispanic individuals and has been primarily clinic-based. Here we used the state-of-the-art AD biomarkers in a multi-ethnic community in northern Manhattan.
- 2. Interpretation.** Plasma phosphorylated tau (p-tau)<sub>217</sub>, was strongly associated with *post mortem* diagnosis of AD, and cerebral amyloidosis on positron emission tomography (PET). P-tau<sub>217</sub> was also the most accurate biomarker to identify clinically diagnosed AD. Reduced amyloid beta (A $\beta$ )<sub>42</sub>/A $\beta$ <sub>40</sub> ratio and increased p-tau<sub>217</sub> were associated with incident AD.
- 3. Future directions.** These results underscore the precision gained by including blood-based biomarkers in observational studies of AD in which autopsy is infrequent and where amyloid PET and the acquisition of spinal fluid are limited. The need for increased sample sizes and more diversity is critical. Further development of the risk assessment of these biomarkers will be a priority.

### 2.2 | Plasma A $\beta$ <sub>42</sub> and A $\beta$ <sub>40</sub>, t-tau, and NfL

Centrifuged plasma aliquoted in polypropylene tubes and stored at  $-80^{\circ}\text{C}$  was used to measure A $\beta$ <sub>42</sub>, A $\beta$ <sub>40</sub>, and t-tau using SIMOA technology (Quanterix). The multiplex Neuro 3-plex A kit (#101995), and NfL kit (#103400) were used on 96-well plates. Rapid-thawed plasma (25  $\mu\text{L}$  diluted 4-fold in buffer) was added to kit beads (100  $\mu\text{L}$ ) by pipette in each well, the plate was incubated for 15 minutes at  $30^{\circ}\text{C}$ , centrifuged at 10,000xg for 10 min, magnetic-washed 3x for 5 minutes total, subjected to addition of SBG reagent (100  $\mu\text{L}$ ), followed by another incubation for 10 minutes at  $30^{\circ}\text{C}$ , centrifugation at 10,000xg for 10 min, wash again x 5 for 7 minutes total, and reading on the SIMOA SR-X machine. Each plate assays (in duplicate) 34 samples, 8 calibrators, and 2 controls. We considered the ratio of A $\beta$ <sub>42</sub> to A $\beta$ <sub>40</sub> (A $\beta$ <sub>42</sub>/A $\beta$ <sub>40</sub>) as the primary amyloid biomarker.

### 2.3 | Plasma p-tau<sub>181</sub> and p-tau<sub>217</sub>

The p-tau assays were optimized to measure disease-related differences through the selection of monoclonal antibodies. Selection of the monoclonal antibody pair provided a unique combination of sensitivity and selectivity for the tau forms in plasma that differ between AD and healthy control participants. The p-tau<sub>181</sub> assay was modified from that published previously<sup>8</sup> to improve the assay and more directly compare between phosphorylation sites. The assays were performed on a streptavidin small spot plate using the Meso Scale

Discovery (MSD) platform. For the p-tau181 assay, Biotinylated-AT270 was used as a capture antibody (anti-p-tau181 antibody) and SULFO-TAG-Ru-4G10-E2 (anti-tau monoclonal antibody) for the detector. The assay was calibrated using a synthetic p-tau181 peptide. For the p-tau217 assay, Biotinylated-IBA493 was used as a capture antibody (anti-p-tau217 antibody) and SULFO-TAG-Ru-4G10-E2 (anti-tau monoclonal antibody) for the detector. The assay was calibrated using a synthetic P-tau217 peptide. Additional detailed methods for the p-tau217 and p-tau181 assays are provided in the supporting information.

## 2.4 | Autopsy

Cases were classified according to National Institute on Aging–Alzheimer's Association guidelines for the neuropathological assessment of AD,<sup>13</sup> which characterizes likelihood of AD according to an "ABC" staging. In this scheme, amyloid plaques ("A") are rated according to the method of Thal et al.,<sup>14</sup> neurofibrillary tangles are rated according to Braak et al. ("B"),<sup>15,16</sup> and neuritic plaques are rated according to Consortium to Establish a Registry for Alzheimer's Disease (CERAD; "C") criteria.<sup>17</sup> ABC staging yields a rating for each case, corresponding to one of four categories: not AD, low AD, intermediate AD, or high AD neuropathological change (ADNC). In this study, similar to others that examined plasma biomarkers,<sup>8,18</sup> our primary pathological grouping compared those classified as "high ADNC" with all other groups. Systematic Thal ratings for amyloid plaques were added later in the study and available for  $n = 38$  cases in the current sample, so our primary ADNC classification reflected a CERAD neuritic plaque rating of 2 (moderate) or 3 (severe), and a Braak rating of V or VI without incorporation of Thal ratings. In secondary analyses, we considered biomarker levels across the four degrees of AD neuropathological change among the  $n = 38$  participants with complete ADNC ratings (Thal, Braak, and CERAD).

## 2.5 | Amyloid PET

A subset of participants from the clinical sample had undergone amyloid PET scanning with [<sup>18</sup>F]Florbetaben (8.1 mCi target dose). The standard uptake value ratio (SUVR) was calculated with data 50 to 70 minutes post-injection and an inferior cerebellar gray matter reference region in native space. FreeSurfer<sup>19</sup> was used to parcellate the brain and derive regions of interest (ROIs) from T1-weighted MRI. A global composite SUVR was calculated as an average of frontal, temporal, and parietal cortex SUVRs, based on the FreeSurfer-defined ROIs. An SUVR cut-score of 1.25 was additionally used as an amyloid positivity threshold.

## 2.6 | Analyses

We examined differences in each individual biomarker between pathological cases characterized as high ADNC and all other groups, between

those diagnosed clinically with AD patients and controls, and between PET amyloid positive and negative participants by t-tests. For autopsy data, we compared biomarker concentrations between those defined as high ADNC and all other groups with t-tests and examined receiver operating characteristics (ROC) with associated areas under the curve (AUCs) and Aikake and Bayesian information criteria (AIC/BIC) for diagnostic classification. Differences in AUCs were determined with the method of DeLong et al.<sup>20–22</sup> We used logistic regression analyses to test how well the biomarkers classified those with high ADNC with and without age as a covariate. For these analyses we chose the p-tau217 concentrations as the p-tau biomarker. We used general linear models to examine differences in biomarker concentration across pathological ABC staging (not AD, low ADNC, intermediate ADNC, and high ADNC). In the clinical group, we examined associations between biomarker concentrations and demographic features (age, sex, apolipoprotein E [APOE]  $\epsilon$ 4 status) and amyloid PET SUVR with Pearson correlations and t-tests. We used logistic regression analysis with biomarker concentrations entered simultaneously to calculate the model AUCs with respect to diagnostic and amyloid status, and we assessed the additional contribution of body mass index (BMI)<sup>23</sup> and age as covariates to the base biomarker model by likelihood ratio test (LRT) and by testing for a change in AUC. Prior to statistical analysis, frequency distributions were inspected for outliers, which were removed from subsequent analyses. Three outliers were identified in the clinical group only: one participant with t-tau concentration value of 472.3, one with a p-tau181 concentration of 29.5, and one with NfL concentration of 628.5; these values are greater than 10 times the 90% quantile level of all other values and thus physiologically unlikely. The logistic regression analyses in the clinical sample were repeated with the outlier values included to examine whether they notably affected results.

Finally, we used Cox regression analyses to examine the relationship between biomarker concentrations and clinical diagnosis of AD at the last available clinical follow-up visit among individuals classified as controls at time of the blood draw, using individuals who remained controls at last visit as reference. These analyses included biomarker concentrations entered simultaneously and the period between the initial blood draw and the last clinical diagnosis as the time-to-event adjusted for age at blood draw, sex, APOE  $\epsilon$ 4, and race/ethnicity. We ran separate models for p-tau181 and p-tau217. We repeated these analyses after inclusion of outlier values and with BMI as an additional covariate.

## 3 | RESULTS

### 3.1 | Participant characteristics

#### 3.1.1 | Autopsy group

Average age at death and at last clinical follow-up were 88.63 (standard deviation [SD] = 6.80) and 85.64 (SD = 7.13) years, respectively (time interval between last evaluation/blood draw and autopsy, 2.99 [SD = 2.77] years). Thirty-four (30%) had a clinical diagnosis of AD

at their last WHICAP follow-up visit. Thirty-three (29%) had an ABC score indicating high ADNC. Table 1 displays demographic characteristics of the autopsy group. Cases with high ADNC were similar in age and race/ethnicity distribution but had a higher proportion of women and APOE  $\epsilon$ 4 carriers than those with lower ADNC.

### 3.1.2 | Clinical group

Demographic characteristics at the time of the blood draw used to derive biomarker concentrations of participants included in the clinical cohort are in Table 1. Compared to controls, those with clinical AD were similar for sex distribution, race/ethnicity distribution, and APOE  $\epsilon$ 4 allele frequency, but were slightly older.

Of those characterized as controls at time of blood draw, 71 subsequently had a clinical diagnosis of AD by the last assessment. They were similar in age ( $t = 0.52, P = .46$ ), sex ( $\chi^2 < 0.01, P = .99$ ), APOE  $\epsilon$ 4 allele frequency ( $\chi^2 = 0.52, P = .46$ ), and race/ethnicity distribution ( $\chi^2 = 1.72, P = .42$ ) as those who remained controls. The last available diagnosis took place an average of 4.29 (SD = 3.04) years after the blood draw used to derive biomarker concentrations.

### 3.1.3 | Amyloid PET subgroup

We compared demographic features between amyloid positive and negative participants (see Table 1). Amyloid positive individuals were more likely to be APOE  $\epsilon$ 4 allele carriers, but were similar to amyloid negative participants in age, sex, and race/ethnicity distribution. Fifty percent of amyloid positive individuals ( $n = 4$ ) were considered to have clinical AD at the diagnostic visit closest to the blood draw whereas 25% ( $n = 8$ ) amyloid negative individuals met clinical criteria for AD ( $\chi^2 = 1.90, P = .16$ ). PET scans were completed 3.79 (SD = 20.8) months on average after the blood draw used to derive biomarker concentrations.

## 3.2 | Biomarker concentration and neuropathological diagnosis

Individuals with high ADNC had higher concentrations of p-tau181 and p-tau217, but similar A $\beta$ 42/A $\beta$ 40, t-tau, and NfL concentrations compared to those with lower ADNC (see Table 1). P-tau217 and p-tau181 showed good diagnostic classification, but concentrations for the other biomarkers did not (see Table 2 and Figure 1A-D). Although sample sizes were small and confidence intervals relatively wide, classification accuracy for p-tau181 and particularly p-tau217 concentrations was numerically better in non-Hispanic Black and Hispanic cases relative to Whites (see Table 2 and Figure 1). When we considered classification accuracy of all biomarkers together in a logistic regression analysis in the overall sample, the resulting ROC area was 0.925. Adding age at death to the model increased the ROC area to 0.932. Although age improved model fit by LRT ( $P = .019$ ), the increase in ROC area was not

statistically significant ( $P = .597$ ). There was a monotonic increase in p-tau181 ( $F = 5.04, P = .005$ ) and p-tau217 ( $F = 6.27, P = .002$ ) but not the other biomarkers ( $F$  values range = 0.12–0.25,  $P$ -values range = 0.70–0.94) across neuropathological classification groups (see Figure 2).

## 3.3 | Biomarker concentration and clinical characteristics

### 3.3.1 | Demographic features

Increased age was associated with t-tau ( $r = 0.149, P = .01$ ), p-tau181 ( $r = 0.214, P < .001$ ), p-tau217 ( $r = 0.192, P = .001$ ), and NfL ( $r = 0.286, P < .001$ ), but not A $\beta$ 42/A $\beta$ 40 ( $r = -0.099, P = .08$ ) concentrations. Biomarker concentrations did not differ between men and women ( $t$  value range = 0.14–1.12,  $P$  value range = 0.25–0.88). Compared to non-carriers, APOE  $\epsilon$ 4 allele carriers had higher concentrations of p-tau181 (mean  $\pm$  SD =  $1.20 \pm 0.95$  vs.  $0.96 \pm 0.91, t = 2.03, P = .04$ ) and p-tau217 (mean  $\pm$  SD =  $0.31 \pm 0.23$  vs.  $0.22 \pm 0.26, t = 2.78, P = .006$ ), but were similar for all other biomarker concentration levels ( $t$  value range 0.16–1.30,  $P$  value range 0.86–0.19). Increased BMI was associated with lower p-tau181 ( $r = -0.20, P = .001$ ), p-tau217 ( $r = -0.20, P = .001$ ), and NfL ( $r = -0.15, P = .02$ ) concentrations but was unrelated to A $\beta$ 42/A $\beta$ 40 ( $r = -0.06, P = .32$ ) and t-tau concentrations ( $r = -0.07, P = .28$ ) concentrations. Biomarker concentrations were similar across the three race/ethnicity groups ( $F$  value range 0.61–2.15,  $P$  value range 0.54–0.11).

### 3.3.2 | Clinical diagnosis at time of blood draw

We examined the differences in biomarker concentrations between participants with clinically diagnosed AD and controls (Table 1 and Figure 3). Participants with AD had higher concentrations of p-tau181, p-tau217, and NfL, and similar A $\beta$ 42/A $\beta$ 40 and t-tau levels compared to controls. We constructed ROCs for each biomarker as a function of diagnostic status (Figure 4), which revealed the best diagnostic classification for p-tau concentrations (Table 2). A logistic regression model with all biomarkers resulted in an ROC area of 0.663. Inclusion of three outliers did not reveal any striking changes to this model comparing coefficients or ROC area (0.668). Comparing models after adding age and BMI covariates did not significantly improve the model fit or prediction (LRT  $P = .251$ , ROC comparison  $P = .235$ ). When stratified by race/ethnicity, classifications were comparable among non-Hispanic White and Black participants but AUCs among Hispanic participants were relatively low for all biomarkers measured (Table 2 and Figure 4).

### 3.3.3 | Amyloid PET

Compared to amyloid negative individuals the amyloid positive individuals had higher levels of p-tau217 and p-tau181 (Table 2). Total tau concentrations trended higher in amyloid positive individuals, but

**TABLE 1** Demographic and biomarker concentration characteristics in participants classified pathologically, clinically, and according to amyloid PET status

	Pathological status			Clinical status			PET amyloid status			Statistic	Total	Statistic	Total	Statistic
	Controls		high AD	Controls		AD	-		+					
	n	(%)	n	(%)	n	(%)	n	(%)	n					
N	80	33	113		169	131	300	32	8	40	-			
Age, mean (SD) years	84.93 (7.45)	87.38 (6.04)	85.64 (7.13)	t = 1.67, P = 0.09	81.01 (6.31)	82.99 (6.49)	81.87 (6.46)	t = 2.66, P = 0.008	82.16 (5.19)	84.25 (4.55)	82.58 (5.08)	t = 1.04, P = 0.30		
Sex, n (%) women	41 (51%)	28 (85%)	69 (61%)	X <sup>2</sup> = 11.09, P = 0.001	109 (64%)	91 (69%)	200 (67%)	X <sup>2</sup> = 0.82, P = 0.36	19 (59%)	5 (63%)	24 (60%)	X <sup>2</sup> = 0.03, P = 0.87		
APOE, n (%) ε4	16 (20%)	13 (41%)	32 (29%)	X <sup>2</sup> = 4.89, P = 0.02	46 (27%)	38 (29%)	84 (28%)	X <sup>2</sup> = 0.11, P = 0.73	11 (34%)	6 (75%)	17 (43%)	X <sup>2</sup> = 4.32, P = 0.04		
Race/ethnicity, n (%)														
White	37 (46%)	15 (45%)	52 (46%)	X <sup>2</sup> = 1.90, P = 0.38	63 (37%)	37 (28%)	100 (33%)	X <sup>2</sup> = 2.71, P = 0.25	11 (34%)	4 (50%)	15 (38%)	X <sup>2</sup> = 2.15, P = 0.34		
Black	25 (31%)	7 (21%)	32 (28%)		53 (31%)	47 (36%)	100 (33%)		17 (53%)	2 (25%)	19 (48%)			
Hispanic	18 (25%)	11 (33%)	29 (26%)		53 (31%)	47 (36%)	100 (33%)		4 (13%)	2 (25%)	6 (15%)			
Plasma biomarker concentration, mean (SD) pg/mL														
Aβ42/Aβ40	0.06 (0.04)	0.05 (0.01)	0.06 (0.03)	t = 1.24, P = 0.21	0.05 (0.01)	0.06 (0.01)	0.05 (0.01)	t = 0.57, P = 0.56	0.06 (0.01)	0.05 (0.005)	0.06 (0.009)	t = 0.83, P = 0.40		
T-tau	4.30 (3.22)	4.12 (2.37)	4.25 (2.98)	t = 0.29, P = 0.76	4.94 (2.13)	4.90 (2.06)	4.92 (2.09)	t = 0.15, P = 0.88	4.55 (2.13)	5.64 (1.43)	4.77 (2.04)	t = 1.36, P = 0.18		
P-tau181	1.06 (0.81)	1.93 (1.14)	1.32 (1.00)	t = 4.54, P < 0.001	0.86 (0.73)	1.24 (1.09)	1.02 (0.92)	t = 3.55, P < 0.001	0.73 (0.61)	1.35 (0.54)	0.86 (0.64)	t = 2.59, P = 0.01		
P-tau217	0.19 (0.16)	0.51 (0.40)	0.28 (0.29)	t = 5.99, P < 0.001	0.18 (0.17)	0.32 (0.32)	0.25 (0.26)	t = 4.80, P < 0.001	0.15 (0.14)	0.39 (0.18)	0.20 (0.18)	t = 3.86, P < 0.001		
NfL	51.45 (51.11)	34.41 (25.35)	46.43 (45.63)	t = 1.82, P = 0.07	31.10 (28.96)	36.55 (24.63)	33.48 (27.25)	t = 1.76, P = 0.08	30.29 (20.73)	30.31 (14.15)	30.30 (19.43)	t < 0.01, P = 0.99		

Notes: For the autopsy group, "high AD" refers to ADNC classification of high degrees of AD neuropathological change and controls include all other groups. For the clinical group, AD diagnosis is based on standardized clinical classification without consideration of biomarker status. For the PET subgroup, amyloid positivity was determined according to an SUVR threshold of 1.25.

Abbreviations: AD, Alzheimer's disease; ADNC, AD neuropathological change; PET, positron emission tomography; SD, standard deviation; SUVR, standardized uptake value ratio.

**TABLE 2** AUC statistics for each biomarker in the total sample and stratified by race/ethnicity, together with 95% confidence intervals, and P-values

Table 2 A (autopsy)	N	AUC	95% CI	P	AIC/BIC	A $\beta$ 42/40	Tau	P-tau181	P-tau217	NfL
Whole sample	113					Statistical comparison between ROC curves, P-value				
A $\beta$ 42/40	112	0.58	0.47 to 0.68	0.1769	138/143					
Tau	112	0.48	0.37 to 0.60	0.7614	140/145	0.3183				
P-tau181	112	0.77	0.67 to 0.87	<0.0001	120/126	0.0039	0.0017			
P-tau217	112	0.84	0.75 to 0.92	<0.0001	108/113	0.0001	<0.0001	0.0116		
NfL	112	0.59	0.48 to 0.70	0.0396	136/141	0.8387	0.1185	0.0345	0.0017	
<b>Non-Hispanic White</b>										
A $\beta$ 42/40	52	0.60	0.44 to 0.77	0.1769	65/69					
Tau	52	0.56	0.39 to 0.73	0.4007	66/70	0.7328				
P-tau181	52	0.65	0.48 to 0.83	0.2058	65/69	0.6377	0.5378			
P-tau217	52	0.75	0.61 to 0.89	0.0156	61/65	0.1444	0.1805	0.0480		
NfL	52	0.64	0.49 to 0.79	0.0103	60/64	0.7167	0.4227	0.9216	0.3471	
<b>Non-Hispanic Black</b>										
A $\beta$ 42/40	31	0.53	0.31 to 0.75	0.9436	38/40					
Tau	31	0.58	0.37 to 0.80	0.4466	37/39	0.5973				
P-tau181	31	0.94	0.86 to 1.00	0.0001	28/31	0.0020	0.0061			
P-tau217	31	0.96	0.90 to 1.00	<0.0001	21/23	0.0005	0.0020	0.4304		
NfL	31	0.54	0.27 to 0.82	0.5863	37/40	0.9340	0.7570	0.0138	0.0078	
<b>Hispanic</b>										
A $\beta$ 42/40	29	0.62	0.42 to 0.82	0.4671	42/45					
Tau	29	0.67	0.45 to 0.90	0.2676	42/44	0.6928				
P-tau181	29	0.82	0.66 to 0.98	0.0029	34/36	0.1008	0.1998			
P-tau217	29	0.85	0.69 to 1.00	0.0001	28/31	0.0754	0.1671	0.5223		
NfL	29	0.44	0.20 to 0.67	0.7539	43/45	0.2516	0.0579	0.0026	0.0024	
Table 2 B (clinical)	N	AUC	95% CI	P	AIC/BIC	A $\beta$ 42/40	Tau	P-tau181	P-tau217	NfL
Whole sample						Statistical comparison between AUC curves, P-value				
A $\beta$ 42/40	297	0.49	0.43 to 0.56	0.4951	411/418					
Tau	297	0.49	0.43 to 0.56	0.8586	411/418	0.9763				
P-tau181	297	0.61	0.54 to 0.67	0.0003	398/405	0.0245	0.0348			
P-tau217	297	0.63	0.57 to 0.70	<0.0001	384/391	0.0076	0.0079	0.1208		
NfL	297	0.59	0.52 to 0.66	0.0800	408/415	0.0517	0.0943	0.5684	0.2638	
<b>Non-Hispanic White</b>										
A $\beta$ 42/40	99	0.50	0.39 to 0.63	0.9423	135/140					
Tau	99	0.53	0.42 to 0.65	0.8990	135/140	0.7510				
P-tau181	100	0.69	0.59 to 0.80	0.0011	125/130	0.0261	0.0392			
P-tau217	100	0.71	0.61 to 0.82	<0.0001	119/124	0.0114	0.0298	0.4363		
NfL	99	0.65	0.54 to 0.77	0.1383	133/138	0.1074	0.0853	0.5661	0.4325	

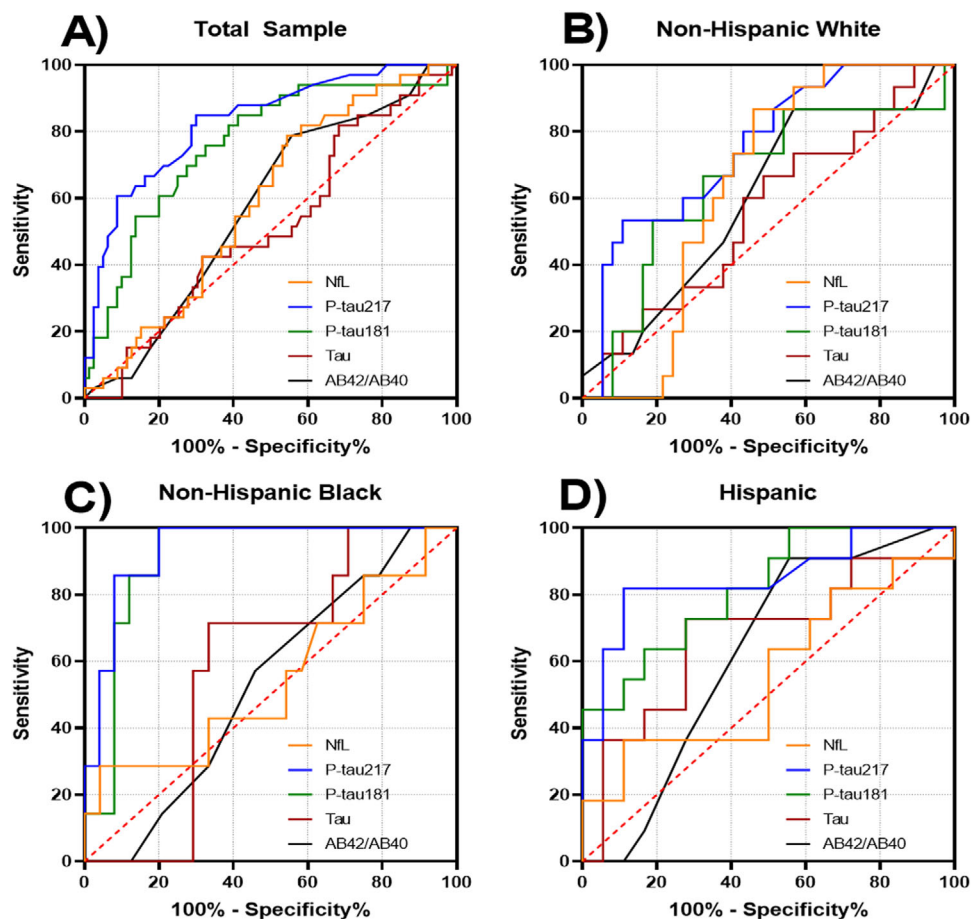
(Continues)

**TABLE 2** (Continued)

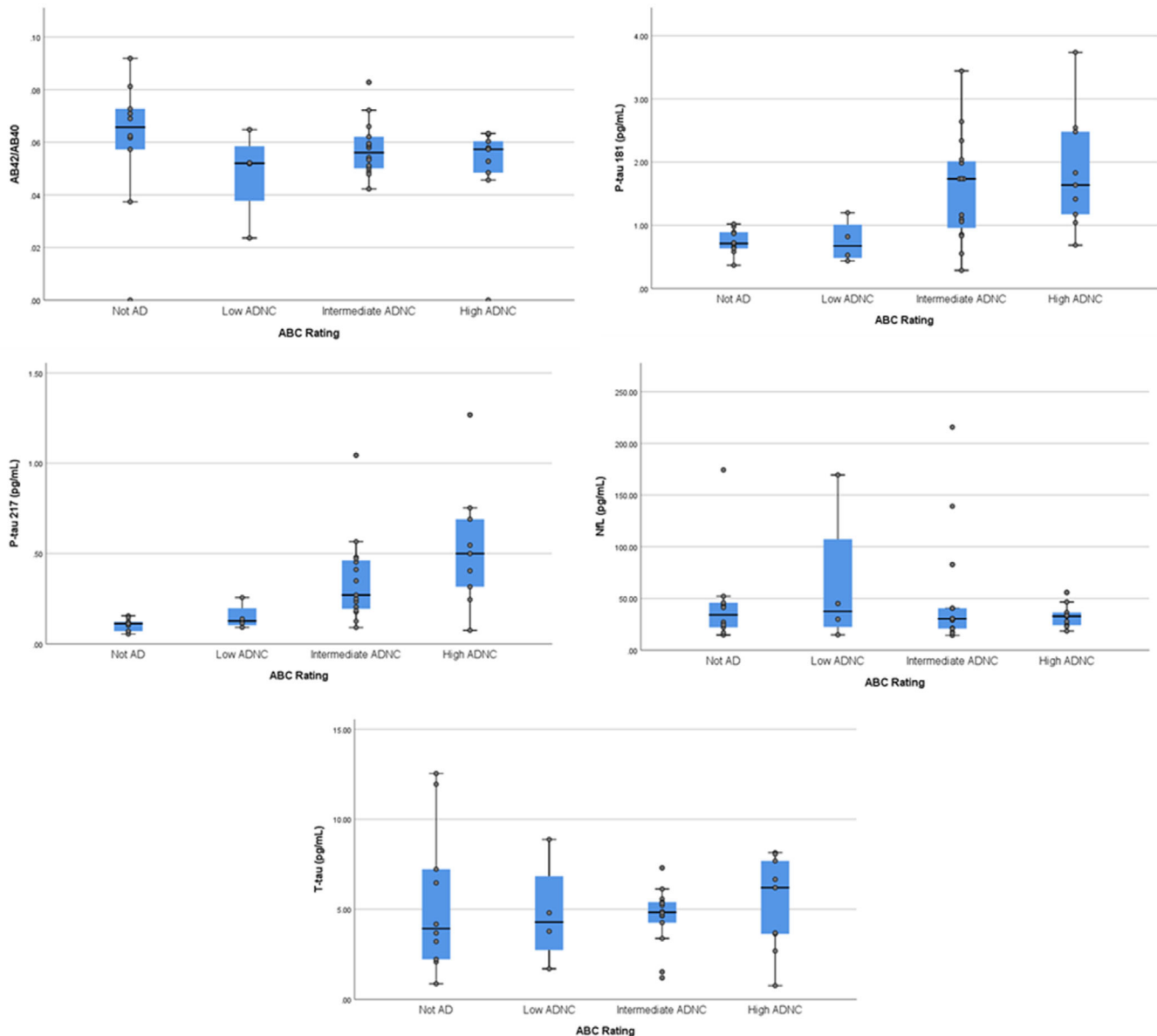
Table 2 B (clinical)	N	AUC	95% CI	P	AIC/BIC	A $\beta$ 42/40	Tau	P-tau181	P-tau217	NfL
<b>Non-Hispanic Black</b>										
A $\beta$ 42/40	98	0.50	0.39 to 0.61	0.3616	139/144					
Tau	98	0.56	0.44 to 0.67	0.3041	138/144	0.5543				
P-tau181	98	0.63	0.51 to 0.74	0.1093	137/142	0.1758	0.2455			
P-tau217	98	0.68	0.57 to 0.78	<b>0.0030</b>	131/136	<b>0.0539</b>	<b>0.0690</b>	<b>0.0455</b>		
NfL	98	0.64	0.53 to 0.76	<b>0.0040</b>	131/136	0.0797	0.1453	0.7547	0.5945	
<b>Hispanic</b>										
A $\beta$ 42/40	100	0.45	0.35 to 0.57	0.9405	142/147					
Tau	100	0.58	0.47 to 0.70	0.1528	140/145	0.1264				
P-tau181	100	0.51	0.40 to 0.64	0.0672	139/144	0.5267	0.4876			
P-tau217	100	0.52	0.40 to 0.64	<b>0.0344</b>	138/143	0.5163	0.4787	0.9224		
NfL	100	0.48	0.37 to 0.6	0.4999	142/147	0.6974	0.1470	0.7688	0.7443	

Notes: Fit statistics are presented as AIC and BIC. P-values for statistical comparisons among AUCs are presented. Table 2A displays results from the autopsy sample. Table 2B presents results from the clinical sample. Bolded values are statistically significant.

Abbreviations: A $\beta$ , amyloid beta; AIC, Akaike information criterion; AUC, area under the receiver operating characteristic curve; BIC, Bayesian information criterion; CI, confidence interval; NfL, neurofilament light.



**FIGURE 1** Receiver operating curves for classification of *post mortem* diagnosis of Alzheimer's disease. A, Total sample. B, Non-Hispanic Whites. C, Non-Hispanic Blacks. D, Hispanics



**FIGURE 2** Biomarker concentrations across pathological "ABC" ratings. Midline represents median, box represents 25th and 75th percentile, and T-bars represent 95% confidence interval. Individual subject data points are superimposed. AD, Alzheimer's disease; ADNC, AD neuropathological change;

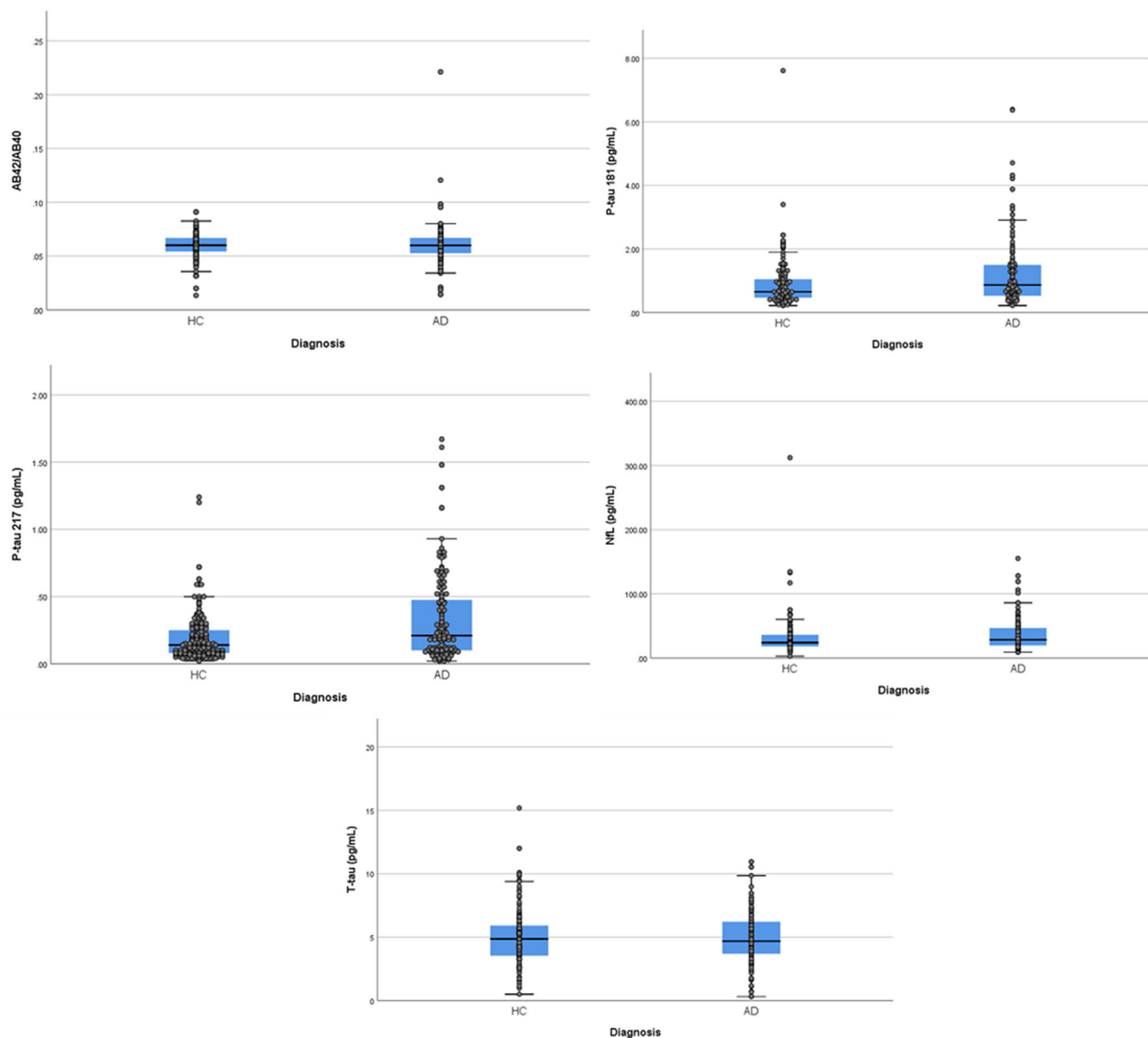
A $\beta$ 42/A $\beta$ 40 and NFL levels were quite similar between the groups. There were strong correlations of amyloid SUVR with p-tau217 ( $r = 0.48$ ,  $P = .002$ ) and p-tau181 ( $r = 0.36$ ,  $P = .02$ ), more modest associations with t-tau ( $r = 0.23$ ,  $P = .15$ ) and A $\beta$ 42/A $\beta$ 40 ( $r = -0.16$ ,  $P = .30$ ), and trivial correlations with the other biomarkers ( $r$  value range = 0.01–0.07,  $P$  value range = 0.93–0.65). When we examined classification of amyloid positivity, the findings paralleled the other results: AUCs were greatest for p-tau217 (AUC: 0.84, 95% confidence interval [CI]: 0.68–0.99) and p-tau181 (AUC: 0.82, 95%CI: 0.65–0.99); AUCs for the other biomarkers ranged from 0.40 (95%CI: 0.22–0.59, A $\beta$ 42/A $\beta$ 40) to 0.56 (95%CI: 0.34–0.79, NFL). A multiple logistic regression model with all biomarkers resulted in an ROC area of 0.840. Comparing models after adding age and BMI covariates did not improve the

model fit or prediction (LRT  $P = .868$ , ROC comparison  $P = .732$ ). We did not examine differences across race/ethnicity groups because of the reduced sample size and low number of amyloid positive participants.

### 3.4 | Risk of subsequent clinical AD among those without dementia at the first blood draw

We used the median value of A $\beta$ 42/A $\beta$ 40, p-tau181, and p-tau217 to estimate the risk of developing AD. The overall effect of biomarker concentration on subsequent development of clinical AD was similar for the model that included p-tau181 ( $\chi^2 = 26.27$ ,  $P = .002$ ), and p-tau217



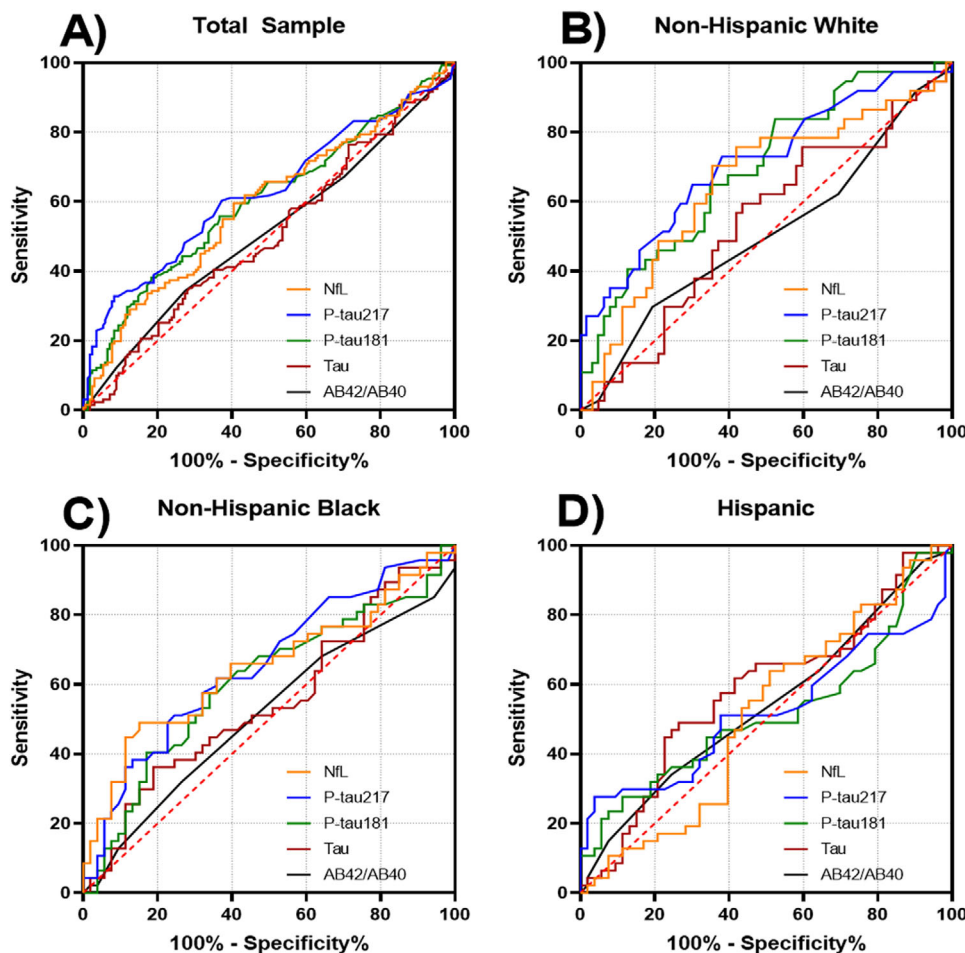


**FIGURE 3** Differences between clinically diagnosed patients with Alzheimer's disease (AD) and healthy controls (HC) in absolute concentrations of each plasma biomarker. Midline represents median, box represents 25th and 75th percentile, and T-bars represent 95% confidence interval. Individual subject data points are superimposed

( $\chi^2 = 21.98$ ,  $P = .009$ ) in independent analyses. Including outlier values did not notably affect the model that included P-tau181 ( $\chi^2 = 29.08$ ,  $P < .001$ ) and had no impact on the model that examined p-tau217. Similarly, including BMI as an additional covariate did not markedly affect either the model that examined p-tau181 ( $\chi^2 = 25.04$ ,  $P = .005$ ) or p-tau217 ( $\chi^2 = 26.26$ ,  $P = .004$ ); BMI was not predictive of outcome in either model ( $B = 0.002$ ,  $P = .95$  and  $B = 0.006$ ,  $P = .83$ ). We found reduced A $\beta$ 42/A $\beta$ 40 ratio and an increase in either p-tau217 or p-tau181 to be associated with increased risk of developing a clinical diagnosis of AD (Table 3). None of the other biomarker concentrations were associated with increased risk, but APOE  $\epsilon$ 4 allele was associated with a slightly higher risk.

## 4 | DISCUSSION

We found that the plasma biomarker concentrations of phosphorylated tau, particularly p-tau217, were strongly associated with autopsy-confirmed AD. As expected, this observation did not completely translate to clinically diagnosed participants due to known limitations of clinical diagnosis for AD. Nonetheless, at the time of blood draw those diagnosed clinically with AD had similar plasma biomarker profiles to the participants who were autopsy-confirmed AD, including higher p-tau217 and NFL concentrations and classification accuracy was generally quite good. P-tau biomarkers were also associated with amyloid pathology on PET, more so than other



**FIGURE 4** Receiver operating curves for classification of clinical diagnosis of Alzheimer's disease. A, Total sample. B, Non-Hispanic Whites. C, Non-Hispanic Blacks. D, Hispanics

plasma biomarkers, including  $A\beta_{42}/A\beta_{40}$ . Among individuals classified as controls at time of blood draw, lower  $A\beta_{42}/A\beta_{40}$  ratio, and higher p-tau217 or p-tau181 concentrations, were associated with increased risk of subsequent AD diagnosis.

Plasma levels of p-tau217 outperformed all other biomarkers across analyses, achieving AUCs of 0.84 overall, and 0.85 and 0.96 in Hispanic and Black participants with pathological diagnosis. However, in the analysis of those who developed subsequent AD dementia, both p-tau181 and p-tau217 as well as the  $A\beta_{42}/A\beta_{40}$  ratio were reliable predictors, consistent with previous observations.<sup>18</sup> It is clear that among the currently available plasma biomarkers, p-tau217 concentrations reflect underlying tau pathology with the greatest fidelity and are useful to aid the clinical diagnosis of AD. As expected, biomarker concentrations were somewhat age-dependent and varied with respect to APOE  $\epsilon 4$  status. Concentrations did not differ across race/ethnicity or sex, although larger studies will be necessary to understand fully the potentially moderating effects of these demographic variables.

The results we observed for p-tau181 are similar with those of Janelidze et al.<sup>7</sup> In addition, p-tau217 concentrations were numerically more strongly associated with *post mortem* and amyloid PET outcomes, consistent with Palmqvist et al., who showed p-tau217

was superior to p-tau181 for determination of pathology by PET or neuropathology.<sup>10,18</sup> Our p-tau217 results are similar to Palmqvist et al.,<sup>18</sup> although we did not observe large differences between p-tau217 and p-tau181, possibly attributable to differences in assay design that incorporated the 4G10E2 antibody in place of the LRL antibody for the p-tau181 assay. The slight differences may partially be attributed to the older ages in this study and higher likelihood of comorbidities.<sup>24</sup> Additionally, Janelidze et al.<sup>7</sup> separated AD from other forms of dementia, aiding in the specificity of p-tau. For this study, we used strict criteria to select individuals with clinical AD and did not include other dementias.

The WHICAP cohort is racially/ethnically diverse and community-based. Most previous studies that incorporated plasma biomarkers included clinic-based samples with minimal racial and ethnic diversity. Here, we provide evidence that plasma biomarker data can be incorporated successfully into community-based research, correspond with neuropathological changes seen in AD, and perform equally well or better in racial/ethnic groups typically under-represented in aging research.

While the observations shown in this study highlight the promise and potential of plasma-based biomarkers in identification of AD

**TABLE 3** The association between plasma biomarker concentrations derived at blood draw and the subsequent clinical AD diagnosis ≈4 years later

Table 3 A	B	P	OR (95% CI)
P-tau181 (pg/mL)	1.01	0.001	2.74 (1.54–4.86)
Aβ42/Aβ40	0.60	0.02	1.82 (1.09–3.03)
T-tau (pg/mL)	-0.095	0.06	0.90 (0.82–1.00)
NfL (pg/mL)	0.005	0.18	1.00 (0.99–1.01)
Age	0.06	0.03	1.06 (1.00–1.12)
APOE e4 allele	-0.25	0.38	0.77 (0.43–1.37)
Race/ethnicity			
Non-Hispanic White	1.0		reference
Black	-0.19	0.57	0.82 (0.43–1.59)
Hispanic	0.09	0.77	1.09 (0.58–2.04)
Sex	0.40	0.14	1.49 (0.87–2.58)
Table 3 B	B	P	OR (95% CI)
P-tau217 (pg/mL)	0.82	0.003	2.27 (1.31–3.93)
Aβ42/Aβ40	0.57	0.03	1.78 (1.05–3.00)
T-tau (pg/mL)	-0.04	0.41	0.96 (0.87–1.05)
NfL (pg/mL)	0.007	0.05	1.00 (1.00–1.01)
Age	-0.027	0.31	0.97 (0.92–1.02)
APOE e4 allele	-0.38	0.19	0.67 (0.37–1.21)
Race/ethnicity			
Non-Hispanic White	1.0	-	Reference
Black	-0.21	0.52	0.80 (0.42–1.54)
Hispanic	-0.41	0.22	0.66 (0.34–1.29)
Sex	0.27	0.32	1.31 (0.76–2.25)

Notes: We used Cox regression in which the time to event was calculated as the period between the blood draw and last diagnostic assessment. Biomarker predictors were dichotomized according to their median (Aβ42/Aβ40 the higher value was the reference; P-tau181 and P-tau217 the lower values were the references); outcome was coded as 1 = healthy control, 2 = incident AD and we adjusted for age at last visit, sex (reference group is male sex), ethnic group, and APOE-ε4; Table 3A shows the adjusted results using p-tau181, while Table 3B displays the adjusted results using p-tau217.

Abbreviations: Aβ, amyloid beta; AD, Alzheimer's disease; APOE, apolipoprotein E; CI, confidence interval; NfL, neurofilament light; OR, odds ratio.

pathology and risk of developing AD, they fall short as stand-alone diagnostics. Based on the findings here, p-tau181 or p-tau217 would augment the clinical diagnostic accuracy of AD as well as the presence of pathology preceding detectable symptoms.

Although the plasma biomarkers showed modest associations with clinical dementia, the p-tau associations improved when confirmed with PET or *post mortem* evidence of high ADNC. Pathological and PET diagnoses are more definitive than clinical diagnoses. Clinical AD is a heterogeneous condition, determined by multiple pathologies that may vary across individuals.<sup>25</sup> While our study was not designed to

test the specificity of the plasma biomarkers in distinguishing AD from other dementias, future research will need to consider other forms of dementia. Admittedly, the small number of amyloid positive individuals may increase the likelihood of a Type 1 statistical error, although the p-tau181 and p-tau217 observations were consistent, validating observations with autopsy and clinical data.

Prior research on AD biomarkers across racial/ethnic groups has been challenged by small sample sizes and selection biases and has mostly focused on comparing non-Hispanic Whites to Blacks, while inclusion of Hispanics is less frequent.<sup>26–30</sup> Large population studies are required to provide key information as to the role race/ethnicity has in disease prevalence or the socioeconomic factors that contribute to dementia. This study provides evidence that p-tau217 may be useful as an indicator of pathology that will aid in the evaluation of other biomarkers as well as understanding race/ethnicity and socioeconomic factors in the context of large community-based studies.

Taken together the results here provide encouraging data for the use of blood-based biomarkers in diverse cohorts across clinical settings and in observational, epidemiological studies. Establishing a universal cut-point for p-tau181 or p-tau217 should be a priority in future studies of these biomarkers.

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#### CONFLICTS OF INTEREST

The following authors have no conflicts of interest to report: Adam M. Brickman, Jennifer J. Manly, Lawrence S. Honig, Danurys Sanchez, Dolly Reyes-Dumeyer, Rafael A. Lantigua, Patrick J. Lao, Yaakov Stern, Jean Paul Vonsattel, Andrew F. Teich and Richard Mayeux. David C. Airey, Nicholas Kyle Proctor, and Jeffrey L. Dage are employees and stock holders of Eli Lilly and Company.

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#### SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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