

RESEARCH ARTICLE

Plasma and CSF concentrations of N-terminal tau fragments associate with in vivo neurofibrillary tangle burden

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Abstract

INTRODUCTION: Fluid biomarkers capable of specifically tracking tau tangle pathology in vivo are greatly needed.

METHODS: We measured cerebrospinal fluid (CSF) and plasma concentrations of N-terminal tau fragments (NTA-tau), using a novel immunoassay (NTA) in the TRIAD cohort, consisting of 272 individuals assessed with amyloid beta (A β) positron

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emission tomography (PET), tau PET, magnetic resonance imaging (MRI) and cognitive assessments.

RESULTS: CSF and plasma NTA-tau concentrations were specifically increased in cognitively impaired A β -positive groups. CSF and plasma NTA-tau concentrations displayed stronger correlations with tau PET than with A β PET and MRI, both in global uptake and at the voxel level. Regression models demonstrated that both CSF and plasma NTA-tau are preferentially associated with tau pathology. Moreover, plasma NTA-tau was associated with longitudinal tau PET accumulation across the aging and Alzheimer's disease (AD) spectrum.

DISCUSSION: NTA-tau is a biomarker closely associated with in vivo tau deposition in the AD *continuum* and has potential as a tau tangle biomarker in clinical settings and trials.

KEYWORDS

Alzheimer's disease, biomarkers, cerebrospinal fluid, NTA, plasma, tau

HIGHLIGHTS

- An assay for detecting N-terminal tau fragments (NTA-tau) in plasma and CSF was evaluated.
- NTA-tau is more closely associated with tau PET than amyloid PET or neurodegeneration.
- NTA-tau can successfully track in vivo tau deposition across the AD *continuum*.
- Plasma NTA-tau increased over time only in cognitively impaired amyloid- β positive individuals.

1 | BACKGROUND

Neuropathological examination is the gold standard for definitive diagnosis of Alzheimer's disease (AD) through *post mortem* confirmation of amyloid beta (A β) plaques and neurofibrillary tangles (NFTs).^{1,2} In 2018, the National Institute on Aging and the Alzheimer's Associations (NIA-AA) Research Framework established AD as a biological construct defined in vivo based on cerebrospinal fluid (CSF) and imaging biomarkers, the A/T/(N) framework, where "A" stands for A β pathology, "T" for NFT pathology, and "N" for neurodegeneration.³

Recently, blood-based biomarkers have shown great promise to identify AD pathophysiological changes.⁴ Novel assays targeting blood p-tau are highly AD specific; however, accumulating evidence suggests that the neuropathological changes inducing increases in soluble p-tau are not explained only by NFT pathology.^{5,6} First, p-tau concentrations increase early during preclinical AD before tau positron emission tomography (PET) positivity, and demonstrate an early association with A β pathology.⁷ In later symptomatic stages, p-tau presents high

association with both A β and tau,^{5,8,9} but is often more closely linked to A β compared to NFT accumulation in the brain measured by PET.¹⁰ In addition, plasma p-tau has been found to increase in amyotrophic lateral sclerosis and has been linked to spinal cord neuronal loss.¹¹ Thus, it is difficult to determine the specificity of soluble p-tau as a marker of AD-related "T" in the A/T/(N) scheme.¹² The spatiotemporal accumulation of NFTs in AD correlates more strongly with clinical symptoms and cognitive decline than A β plaque depositions; thus, there is still a need for a blood biomarker capable of specifically tracking tangle pathology.

Previously, we reported a novel immunoassay targeting N-terminal tau fragments in CSF and plasma, referred to as NTA,¹³ using a single molecule array (Simoa) platform. Here, we aimed to investigate the biomarker potential for tangle pathology of NTA-tau in a well-characterized cohort including participants across the AD *continuum*, non-AD neurodegenerative diseases, and healthy controls. Throughout this paper the abbreviation NTA is used for the novel immunoassay, whereas NTA-tau is used to describe the N-terminal tau fragments detected by the NTA assay.

2 | METHODS

2.1 | Participants

We included individuals from the TRIAD cohort (McGill University,¹⁴ $n = 272$), with data obtained from December 2017 to May 2021. Details of the information gathered from participants can be found elsewhere (<https://triad.tnl-mcgill.com/>). All participants underwent a full neuropsychological evaluation, magnetic resonance imaging (MRI), [¹⁸F]AZD4694 A β PET, [¹⁸F]MK6240 tau PET and plasma NTA assessment within 6 months. A first subset of participants ($n = 154$) had quantification of CSF NTA-tau. A second subset ($n = 127$) had a follow-up visit for plasma, MRI, A β PET, and tau PET up to 3 years after baseline (mean follow-up time of 1.86 [standard deviation (SD) 0.61] years).

Diagnosis was determined using Mini-Mental State Examination (MMSE), and Clinical Dementia Rating (CDR) scores and the NIA-AA criteria.³ Cognitively unimpaired (CU) had no objective impairment, an MMSE score of ≥ 26 , and CDR score of 0.¹⁵ Mild cognitive impaired (MCI) individuals had objective cognitive impairment, relatively preserved activities of daily life, an MMSE score of ≥ 26 , and a CDR score of 0.5.¹⁶ Diagnosis of AD dementia was assessed with an MMSE score of < 26 and a CDR of ≥ 0.5 , and met the NIA-AA criteria for probable AD determined by a dementia specialist.¹⁷ Individuals diagnosed with suspected non-AD neurodegenerative diseases were AD biomarker negative based on visual assessment of tau PET and A β PET scans and met clinical criteria for frontotemporal dementia ($n = 11$), progressive supranuclear palsy ($n = 3$), corticobasal degeneration ($n = 2$), or Duchenne muscular dystrophy ($n = 1$). Non-AD individuals were categorized by a consensus panel of neurologists based on clinical symptoms and brain images. No participant met the criteria for another neurological or major neuropsychiatric disorder.

2.2 | CSF and plasma biomarker measurements

CSF and plasma NTA-tau levels were quantified using an in-house developed Simoa immunoassay using a Simoa HD-X platform (Quanterix) at the Clinical Neurochemistry Laboratory (Mölndal, Sweden). Development and validation of the NTA assay has been previously described.¹³ In brief, the NTA assay comprises a mouse monoclonal antibody with epitope 159-163aa (HT7, Thermo Scientific) conjugated to paramagnetic beads and used as capture antibody. A mouse monoclonal antibody with epitope 6-18aa (Tau12, BioLegend) is biotinylated and used as detector antibody. Recombinant non-phosphorylated 2N4R tau was used as calibrator (SignalChem).

For CSF NTA, randomized samples were allowed to thaw at room temperature for 45 minutes, vortexed (30 seconds, 2000 rpm), plated and diluted 1:4 using Tau 2.0 assay diluent (Quanterix). An eight-point calibrator curve was run in duplicate. For plasma NTA, randomized samples were allowed to thaw at room temperature for 45 minutes, vortexed (30 seconds, 2000 rpm), and subsequently centrifuged (10

RESEARCH IN CONTEXT

- 1. Systematic Review:** The authors reviewed the available scientific literature on biofluid markers in Alzheimer's disease (AD) and tau pathology using PubMed. Several publications report phosphorylated tau (p-tau), for example, p-tau181, p-tau217, or p-tau231 as highly specific for AD and associated with both amyloid- β (A β) and tau pathology. Yet, no plasma biomarker can specifically track tau deposition in the brain.
- 2. Interpretation:** In this study, we included cross-sectional and longitudinal cerebrospinal fluid (CSF) and plasma samples, and measured N-terminal tau fragments (NTA-tau) using a novel in-house Simoa assay. Our findings demonstrate that NTA-tau is a biomarker closely associated with tau positron emission tomography (PET) accumulation in the brain, and capable of tracking tau tangle pathology progression across the AD continuum.
- 3. Future Directions:** Validation studies will be needed to further confirm the relationship between plasma and CSF NTA-tau concentrations and tau deposition in the brain. NTA-tau should also be explored in further neurodegenerative diseases characterized by tau pathology.

minutes, 4000 \times g). Subsequently, samples were plated and diluted 1:2 using Tau 2.0 assay diluent (Quanterix). An eight-point calibrator curve was run in duplicate. For both CSF and plasma measurements, two internal quality control (iQC) samples were run in duplicate before and after the analyzed TRIAD samples. The repeatability and intermediate precision for TRIAD CSF measurements were 5.0% and 9.0%, respectively, and for plasma measurements 6.1% and 8.5%, respectively (Table S1 in supporting information). Eight out of 531 samples (1.5%) were under the calculated limit of detection (0.032 pg/mL).

2.3 | Image processing

Detailed description concerning acquisition and processing of A β PET, tau PET and MRI can be found in the supporting information. Briefly, [¹⁸F]MK6240 images were acquired 90 to 110 minutes post-injection and used the inferior cerebellar gray (CG) as the reference region.^{18,19} [¹⁸F]AZD4694 images were acquired 40 to 70 minutes post-injection, using the CG as the reference region.²⁰ Global A β standardized uptake value ratio (SUVR) was determined using an average of A β PET SUVR in the precuneus, prefrontal, orbitofrontal, parietal, temporal, anterior, and posterior cingulate cortices. Individuals were categorized as A β - or A β + based on a threshold of 1.55 SUVR.²¹ In vivo classification of PET-based Braak stages was done following Pascoal et al.,²² with cut-offs assessed as 2.5 SD above the mean of CU young adults. Temporal meta-ROI SUVR of tau PET was acquired from

a composite mask including the entorhinal, amygdala, fusiform, inferior, and middle temporal cortices, which capture changes associated with AD.^{23,24} Finally, neurodegeneration was assessed using voxel-based morphometry (VBM) to obtain gray matter volume in an AD signature mask containing regions related to neurodegeneration in AD (entorhinal, inferior temporal, middle temporal cortices, and fusiform gyrus).²⁵

2.4 | Statistical analyses

Non-imaging statistical analyses were performed with R statistical software (version 4.0.0). Analysis of variance (ANOVA) tests were conducted for continuous variables and Fisher tests for categorical variables for demographic information. ANOVA with Tukey's multiple comparison test compared plasma and CSF NTA-tau concentrations across diagnostic groups. Non-AD and MCI A β - cases were only included in statistical analyses when comparing diagnostic groups but removed from further analyses. Spearman's rank correlations (R) assessed the relationship between NTA-tau concentrations and AD pathophysiological hallmarks. Linear regression models, adjusted for diagnosis, age, and sex, tested the effect of different predictors: neocortical A β PET (A), temporal meta-ROI tau PET (T), temporal neurodegeneration (N), A β PET, and tau PET (A+T), and all predictors (A+T+N) on plasma and CSF NTA ($LM : NTA\ tau \sim imaging + age + sex + diagnosis$). To compare these nested models, we used the Akaike information criterion (AIC) and the adjusted coefficient of determination (R^2), as measures of how well the model fits the data and of how much of the outcome variability is explained by the model. ΔAIC was calculated as the difference between two AIC values for a given biomarker, and the best model was defined as the simplest model presenting the lowest AIC value. AIC values were considered significantly different when the difference between them is higher than two (i.e., $\Delta AIC > 2$), and we further conducted likelihood ratio (LR) tests between the best fitting models. Linear mixed models assessed changes in plasma NTA-tau concentrations over time. The model included plasma NTA-tau as the dependent variable and the interaction between time and diagnostic group as the independent variable. The models' covariates were age at baseline, sex, and random intercept ($LMM : NTA\ tau \sim time * baseline\ diagnosis + baseline\ age + sex + [1|ID]$).

Voxel-wise analyses were performed on VoxelStats, a statistical toolbox implemented in MATLAB.²⁶ Linear models assessed the cross-sectional relationship between NTA-tau concentrations and A β PET, tau PET, and VBM images, correcting for age, sex, and diagnosis. Linear mixed models had brain imaging (either A β PET, tau PET, or VBM) as the dependent variable, and the interaction between time and plasma NTA-tau as the independent variable. Other predictors were age at baseline, sex, and diagnosis, and the mixed models were fitted with random intercepts on the participant level ($LMM : Imaging \sim NTA\ tau * time + baseline\ age + sex + baseline\ diagnosis + [1|ID]$). We further corrected images for multiple comparisons using random field theory (RFT) correction.²⁷

3 | RESULTS

3.1 | Demographics

We included 272 individuals categorized as CU A β -, CU A β +, MCI A β -, MCI A β +, AD A β +, and non-AD neurodegenerative condition. We observed no significant between-group differences in sex or years of education. However, there was a significant difference in terms of age, with the non-AD neurodegenerative condition group being younger than the other groups (Table 1). Demographic information on the CSF and longitudinal subsamples can be found in, respectively, Table S2 and Table S3 in supporting information.

3.2 | NTA-tau concentrations across diagnostic groups

In plasma, NTA-tau concentrations were significantly higher ($P < 0.001$) in AD A β + individuals compared to all other diagnostic groups (CU A β -, CU A β +, MCI A β -, MCI A β +, and non-AD neurodegenerative conditions; Figure 1A). NTA-tau concentrations in CSF were increased across all cognitively impaired (i.e., MCI and AD) A β + groups. First, NTA-tau was significantly increased in AD A β + individuals compared to all other groups (CU A β -, CU A β +, MCI A β -, and non-AD dementia, P -value < 0.001 for all), except MCI A β +. Moreover, MCI A β + individuals had higher CSF NTA-tau levels compared to CU A β - (P -value < 0.001), CU A β + (P -value < 0.05), MCI A β - (P -value < 0.05), and non-AD neurodegenerative conditions (P -value < 0.001 ; Figure 1B). Additionally, plasma and CSF NTA-tau measures were associated with each other ($R = 0.33$, P -value < 0.001 ; Figure S1 in supporting information).

3.3 | NTA-tau concentrations associate with global measures of neuroimaging markers of A/T(N)

Plasma NTA-tau concentrations ($n = 254$) correlated positively with A β PET SUVR ($R = 0.36$, P -value < 0.001) and tau PET SUVR ($R = 0.49$, P -value < 0.001), and negatively with temporal VBM ($R = -0.32$, P -value < 0.001 ; Figure 2A). Among the regression models with plasma NTA-tau as outcome, the highest adjusted R^2 value included the combination of A β , tau, and neurodegeneration (A+T+N: $R^2 = 0.333$), the second highest was tau only (T: $R^2 = 0.325$), and the third one included A β and tau (A+T: $R^2 = 0.322$). However, AICs of tau only and A+T+N model were similar ($\Delta AIC = 0.17$) and LR tests between T and A+T+N models did not show a significant difference (P -value = 0.112); thus, the simplest model—that is, tau only—was considered the best-fitting model (Figure 2A). We further assessed how linear models between plasma NTA-tau and AD-related measures differed based on diagnostic groups. We observed that the cognitively impaired individuals (MCI A β + and AD A β +) depicted the strongest associations, especially with [^{18}F]MK6240 temporal meta-ROI SUVR (Figure S2A in supporting information). In A β PET-positive individuals ($n = 129$), A β PET and

TABLE 1 Demographics.

	CU A β - (N = 104)	CU A β + (N = 41)	MCI A β - (N = 21)	MCI A β + (N = 44)	AD A β + (N = 44)	Non-AD (N = 18)	P-value
Age (mean [SD])	69.71 (9.0)	71.34 (10.3)	72.60 (5.2)	71.08 (5.5)	68.13 (8.6)	61.36 (8.6)	<0.001
Sex							0.197
Female	62 (59.6%)	29 (70.7%)	8 (38.1%)	26 (59.1%)	27 (61.4%)	13 (72.2%)	
Male	42 (40.4%)	12 (29.3%)	13 (61.9%)	18 (40.9%)	17 (38.6%)	5 (27.8%)	
Years of education (mean [SD])	15.54 (3.8)	14.66 (3.7)	15.00 (4.5)	15.50 (3.6)	14.62 (3.3)	13.83 (3.2)	0.314
A β PET neocortical SUVR (mean [SD])	1.29 (0.1)	2.01 (0.3)	1.31 (0.1)	2.30 (0.5)	2.53 (0.4)	1.19 (0.1)	<0.001
Tau PET temporal meta-ROI SUVR (mean [SD])	0.81 (0.1)	0.94 (0.2)	0.94 (0.5)	1.38 (0.6)	2.42 (1.0)	0.81 (0.1)	<0.001
Plasma NTA-tau (pg/mL) (mean [SD])	0.24 (0.3)	0.25 (0.2)	0.23 (0.1)	0.29 (0.2)	0.63 (0.4)	0.35 (0.4)	<0.001
APOE ϵ 4 status							<0.001
0	76 (73.1%)	30 (73.2%)	15 (71.4%)	15 (34.1%)	19 (43.2%)	16 (88.9%)	
1	26 (25.0%)	10 (24.4%)	6 (28.6%)	19 (43.2%)	18 (40.9%)	2 (11.1%)	
2	2 (1.9%)	1 (2.4%)	0 (0%)	9 (20.5%)	6 (13.6%)	0 (0%)	
Missing	0 (0%)	0 (0%)	0 (0%)	1 (2.3%)	1 (2.3%)	0 (0%)	
MMSE score	29.07 (1.0)	29.00 (1.2)	28.43 (1.4)	28.41 (1.5)	20.52 (5.3)	25.83 (5.6)	<0.001
CDR score	0.00 (0.0)	0.00 (0.0)	0.50 (0.0)	0.49 (0.1)	1.00 (0.5)	0.58 (0.3)	<0.001

Abbreviations: A β , amyloid beta; AD, Alzheimer's disease; APOE, apolipoprotein E; CU, cognitively unimpaired; CDR, Clinical Dementia Rating; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; PET, positron emission tomography; ROI, region of interest; SD, standard deviation; SUVR, standardized uptake value ratio.

temporal VBM correlations were similar, whereas tau PET R^2 improved compared to the whole sample ($R = 0.64$, P -value < 0.001; Figure S3A in supporting information).

CSF NTA-tau measures ($n = 141$) showed similar results to those of plasma, correlating with A β PET SUVR ($R = 0.46$, P -value < 0.001), tau PET SUVR ($R = 0.53$, P -value < 0.001), and temporal VBM ($R = -0.28$, P -value = 0.006; Figure 2B). Among the regression models with CSF NTA-tau as outcome, the model combining A β and tau (A+T) had the highest R^2 ($R^2 = 0.481$), followed by tau only (T: $R^2 = 0.480$) and A β , tau, and neurodegeneration (A+T+N: $R^2 = 0.477$). The smallest AIC was for the tau-only model (T: AIC = 1271.5), but this did not differ (i.e., Δ AIC > 2) from that of A+T (Δ AIC = 0.91; Figure 2B). LR tests revealed that there was no significant difference between T only and A+T models (P -value = 0.253). Conducting the linear models separating groups based on diagnosis, we observed that the strongest associations were depicted by MCI A β + and AD A β + groups, especially with [18 F]MK6240 temporal meta-ROI SUVR (Figure S2B). In A β PET-positive individuals ($n = 70$), CSF NTA-tau concentrations did not correlate with A β PET SUVR ($R = 0.17$, P -value = 0.15). On the other hand, the correlation of CSF NTA-tau with tau PET SUVR ($R = 0.57$, P -value < 0.001) and temporal VBM ($R = -0.32$, P -value = 0.0079) slightly improved (Figure S3B).

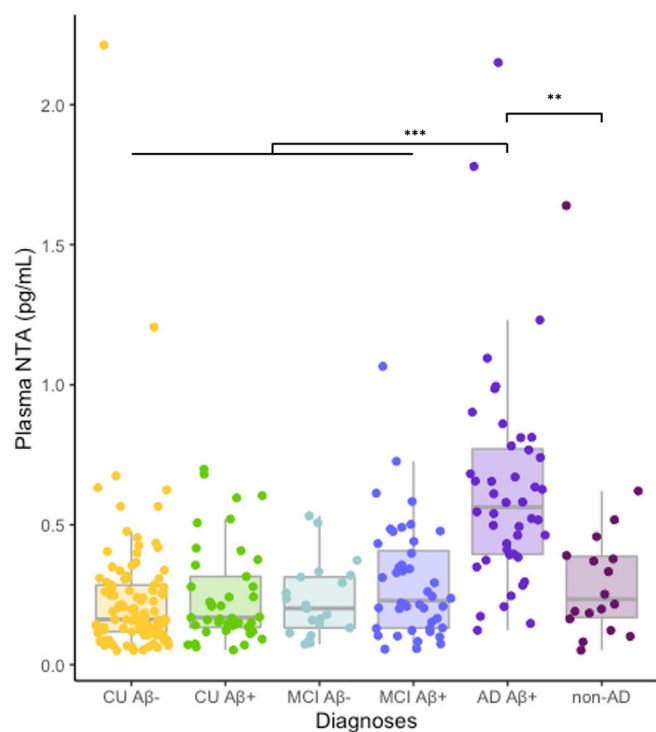
Finally, we assessed plasma and CSF NTA-tau levels based on Braak staging. We observed that individuals classified Braak stages 0 to II had significantly different plasma NTA-tau levels than Braak IV and above.

We further detected significant differences between Braak stages 0 to IV with Braak stages V and VI. Regarding CSF NTA-tau, individuals classified as Braak stage 0 had significantly lower levels than Braak stages I, IV, V, and VI. An increase in levels was then significant among individuals at Braak stage I and II with IV and above (Figure 2C). In A β PET-positive individuals, plasma and CSF NTA-tau behaved similarly. The only difference observed is that there was no significant difference in CSF NTA-tau levels between Braak stage 0 and I (Figure S3C).

3.4 | Voxel-wise association between NTA-tau concentrations with neuroimaging markers of A/T/(N)

We then conducted voxel-wise analyses between plasma and CSF NTA-tau concentrations and A β PET, tau PET, and VBM, correcting for age, sex, and diagnosis. Plasma NTA-tau was more strongly associated with tau PET signal in the precuneus, temporal, and medial frontal lobes, while no results survived RFT correction for A β PET. A small but significant association was observed with VBM in the entorhinal and lateral superior temporal cortex (Figure 3A). CSF NTA-tau concentrations associated with A β PET signal in the medial frontal and hippocampus. It strongly associated with tau PET signal throughout the medial cortex, and temporal lobes. Associations with VBM were not significant after RFT correction (Figure 3B).

(A) Plasma NTA levels based on diagnosis



(B) CSF NTA levels based on diagnosis

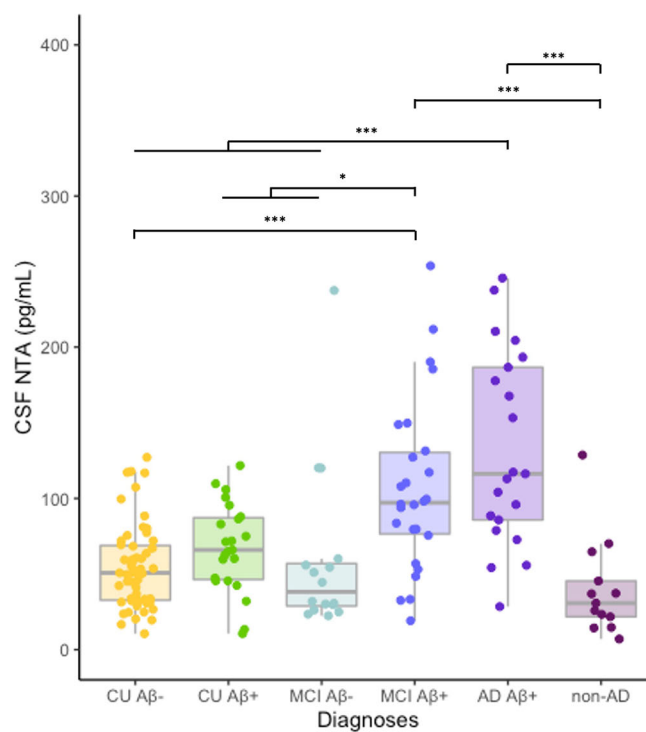


FIGURE 1 Plasma (A) and CSF (B) NTA-tau concentrations stratified by diagnosis and amyloid- β ($A\beta$) positivity. $A\beta$, amyloid beta; AD, Alzheimer's disease; CSF, cerebrospinal fluid; CU, cognitively unimpaired; MCI, mild cognitive impairment.

In $A\beta+$ individuals (Figure S4 in supporting information) significant associations with tau PET were observed for both plasma and CSF NTA-tau in similar regions. No results survived RFT correction for $A\beta$ PET. NTA-tau concentrations were also negatively associated with VBM, in the superior temporal and occipital lobe for plasma NTA-tau, and in the inferior temporal lobe for CSF NTA-tau.

3.5 | Plasma NTA-tau compared to plasma p-tau measures

We further conducted exploratory analyses in a subsample of 129 individuals that in addition to plasma NTA-tau had plasma p-tau181, p-tau217, and p-tau231 measures ($N[CU A\beta-] = 56$, $N[CU A\beta+] = 24$, $N[MCI A\beta+] = 23$, $N[AD A\beta+] = 26$). Plasma NTA-tau correlated with p-tau181 ($R = 0.50$, $P < 0.0001$), p-tau217 ($R = 0.61$, $P < 0.0001$) and p-tau231 ($R = 0.41$, $P < 0.0001$; Figure S5 in supporting information). ROI-based analyses revealed that all plasma markers were correlated with global $A\beta$ PET, temporal meta-ROI tau PET, and neurodegeneration assessed by VBM (Figure S6 in supporting information). However, plasma NTA-tau was the only biomarker more strongly associated with tau PET ($R = 0.58$, $P < 0.0001$) than $A\beta$ PET ($R = 0.44$, $P < 0.0001$). Plasma p-tau181 was similarly associated with $A\beta$ PET ($R = 0.56$, $P < 0.0001$) and tau PET ($R = 0.57$, $P < 0.0001$), whereas plasma p-tau217

and p-tau231 showed stronger associations with $A\beta$ PET (p-tau217: $R = 0.79$, $P < 0.0001$; p-tau231: $R = 0.56$, $P < 0.0001$) than tau PET (p-tau217: $R = 0.71$, $P < 0.0001$; p-tau231: $R = 0.49$, $P < 0.0001$). Plasma NTA-tau significantly correlated with temporal neurodegeneration ($R = -0.34$, $P < 0.0001$). The strength of this association was similar to plasma p-tau181 ($R = -0.31$, $P < 0.001$) and p-tau231 ($R = -0.32$, $P < 0.001$), but weaker than that of plasma p-tau217 ($R = -0.42$, $P < 0.0001$).

3.6 | Longitudinal changes in plasma NTA-tau concentrations

We observed a longitudinal increase in plasma NTA-tau concentrations in participants classified as AD $A\beta+$, and MCI $A\beta+$ at baseline (P -value < 0.001 and P -value < 0.01 , respectively) compared to CU $A\beta-$, whereas no changes were seen in the other groups (CU $A\beta+$ [P -value = 0.211], MCI $A\beta-$ [P -value = 0.393], and non-AD [P -value = 0.251]; Figure 4A). Voxel-wise linear mixed models assessed the association between longitudinal changes in plasma NTA-tau and each imaging modality. We observed no association with $A\beta$ PET; however, we found a significant positive association with tau PET, especially in the medial frontal, precuneus, and temporal lobes. Changes in plasma NTA-tau concentrations were also associated with decrease in VBM in the medial temporal lobe (Figure 4B).

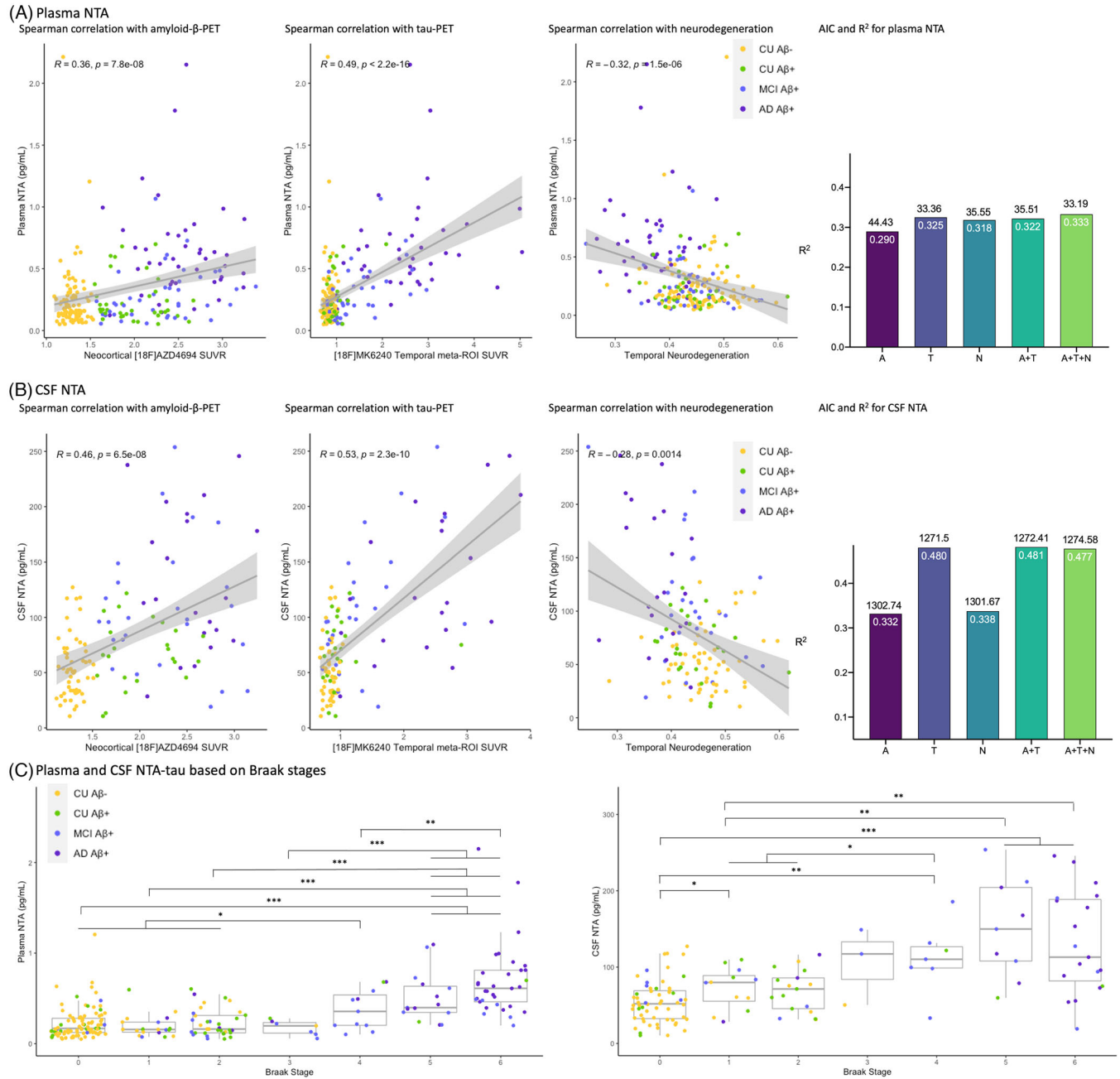


FIGURE 2 Associations of NTA-tau concentration with AD hallmarks. Correlation of plasma (A) and CSF (B) NTA-tau concentration with A β PET, tau PET, and neurodegeneration estimated by VBM. Corresponding AIC and R² AIC and R² values for each predictor were obtained from linear regression models correcting for age, sex, and diagnosis. C, Boxplots assessing plasma and CSF NTA-tau levels based on Braak stages assessed via [¹⁸F]MK6240. A β , amyloid beta; AD, Alzheimer's disease; AIC, Akaike information criterion; CSF, cerebrospinal fluid; CU, cognitively unimpaired; MCI, mild cognitive impairment; ROI, region of interest; SUVR, standardized uptake value ratio; VBM, voxel-based morphometry.

4 | DISCUSSION

Here, we report the first comprehensive characterization of the novel NTA immunoassay in plasma and CSF, using a cohort comprised of individuals across the AD *continuum* as well as non-AD cases, characterized through imaging biomarkers. Our results support that (1) NTA-tau is increased in symptomatic AD cases, (2) NTA-tau concentration is more closely associated with tau PET than with A β PET or

neurodegeneration (indexed by VBM), and (3) plasma NTA-tau is associated with longitudinal tau PET accumulation throughout the cortex, as well as neurodegeneration in medial temporal areas.

In recent years, there has been a successful development of various immunoassays measuring brain-derived biomarkers in blood and CSF.^{5,7,8} Among them, p-tau181, p-tau217, and p-tau231, have proven especially promising.^{5,8,28} Among dementia disorders, p-tau species are the most specific for AD pathology and start to increase early

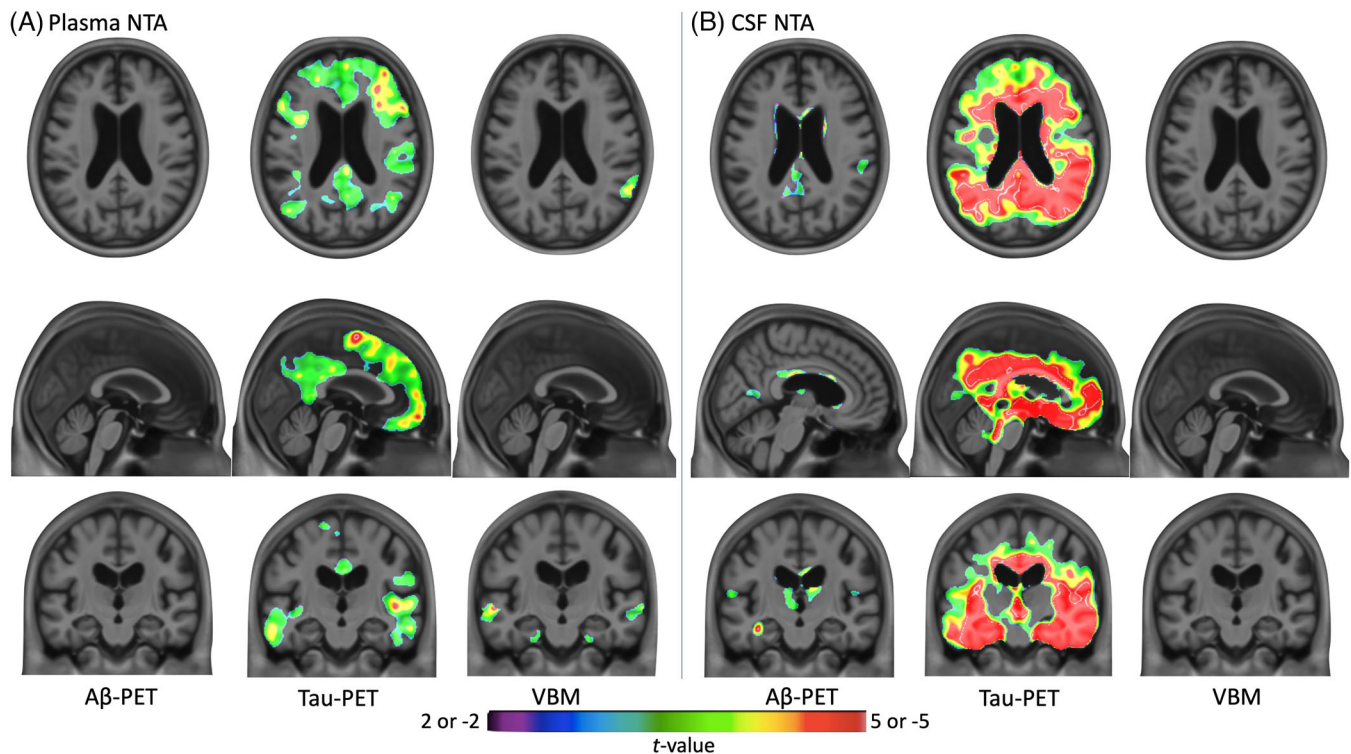


FIGURE 3 Voxel-wise associations between plasma (A) and CSF (B) NTA-tau concentrations and Aβ PET, tau PET and neurodegeneration assessed by voxel-based morphometry (VBM). Aβ, amyloid beta; CSF, cerebrospinal fluid; PET, positron emission tomography; VBM, voxel-based morphometry.

during preclinical stages, when only subtle changes in CSF Aβ are detectable and prior to tau NFT pathology being severe enough to be visualized on tau PET.⁷ Contrarily, quantification of tau fragments in blood irrespective of isoform and phosphorylation state, traditionally referred to as total tau (t-tau), has rendered mixed results. For example, blood t-tau assays have proven meaningful in acute neurological conditions, such as traumatic brain injury, and in chronic neurological diseases characterized by intense neurodegeneration such as Creutzfeldt–Jakob disease.^{29,30} However, in AD, t-tau assays show large overlaps between diagnostic groups, raising uncertainty around their potential clinical utility,^{31,32} possibly due to the presence of peripherally produced “t-tau” species. Recently, a t-tau assay referred to as NT1 and targeting tau species ranging from N-terminal to mid-region, showed promising results in blood.^{33–35} This assay was shown to be AD specific and predicted cognitive decline and neurodegeneration.^{33,35} Given the success of several N-terminal directed p-tau immunoassays in identifying early AD pathophysiological changes, the same N-terminal targeted strategy was used when developing NTA.¹³ In a previous study, CSF NTA-tau was increased in MCI Aβ+ and AD Aβ+ compared to AD biomarker-negative neurological controls, MCI Aβ–, and non-AD Aβ– individuals,¹³ and similar findings were also observed here. Interestingly, our results suggest that plasma and CSF NTA show different emergences along the AD *continuum*: CSF NTA-tau concentrations increase during preclinical AD, while plasma NTA-tau is increased in AD Aβ+ cases. It is therefore likely that this affected the correlation between the two measurements.

However, it should be noted that in a subset of 129 participants with available plasma p-tau181, p-tau217, and p-tau231 measurements, plasma NTA-tau strongly correlated with these plasma biomarkers. The different emergence of NTA-tau in CSF and plasma may be explained by the fact that NTA-tau concentrations are approximately 100-fold lower in plasma than in CSF,¹³ and tau biomarkers generally perform better and show higher fold changes when measured in CSF,³⁶ because tau protein in plasma is more exposed to degradation by proteases, kidney clearance, and liver metabolism. Altogether, this might prevent the NTA assay from successfully detecting subtle early alterations in plasma levels of N-terminal tau fragments, which are, however, detectable in CSF.

Despite p-tau being currently categorized as a tangle marker in the A/T(N) framework, accumulating evidence suggests p-tau concentrations in CSF and blood rise in response to Aβ pathology.^{37,38} Various studies support the idea that increased tau phosphorylation is an early event in the Aβ cascade.^{28,39–41} A recent study showed that p-tau abnormality is one of the first events related to AD pathogenesis, and is more closely associated with Aβ pathology, rather than NFT accumulation.¹⁰ Altogether, these studies bring to light that p-tau measures in CSF and plasma need to be used cautiously in the A/T(N) system, as they might not exclusively reflect “T,” and that fluid biomarkers reflecting tangle pathology are still needed. NTA-tau, however, seems to be more closely associated with tau accumulation compared to other AD pathophysiological processes. Both plasma and CSF NTA-tau concentrations correlated with Aβ PET accumulation and

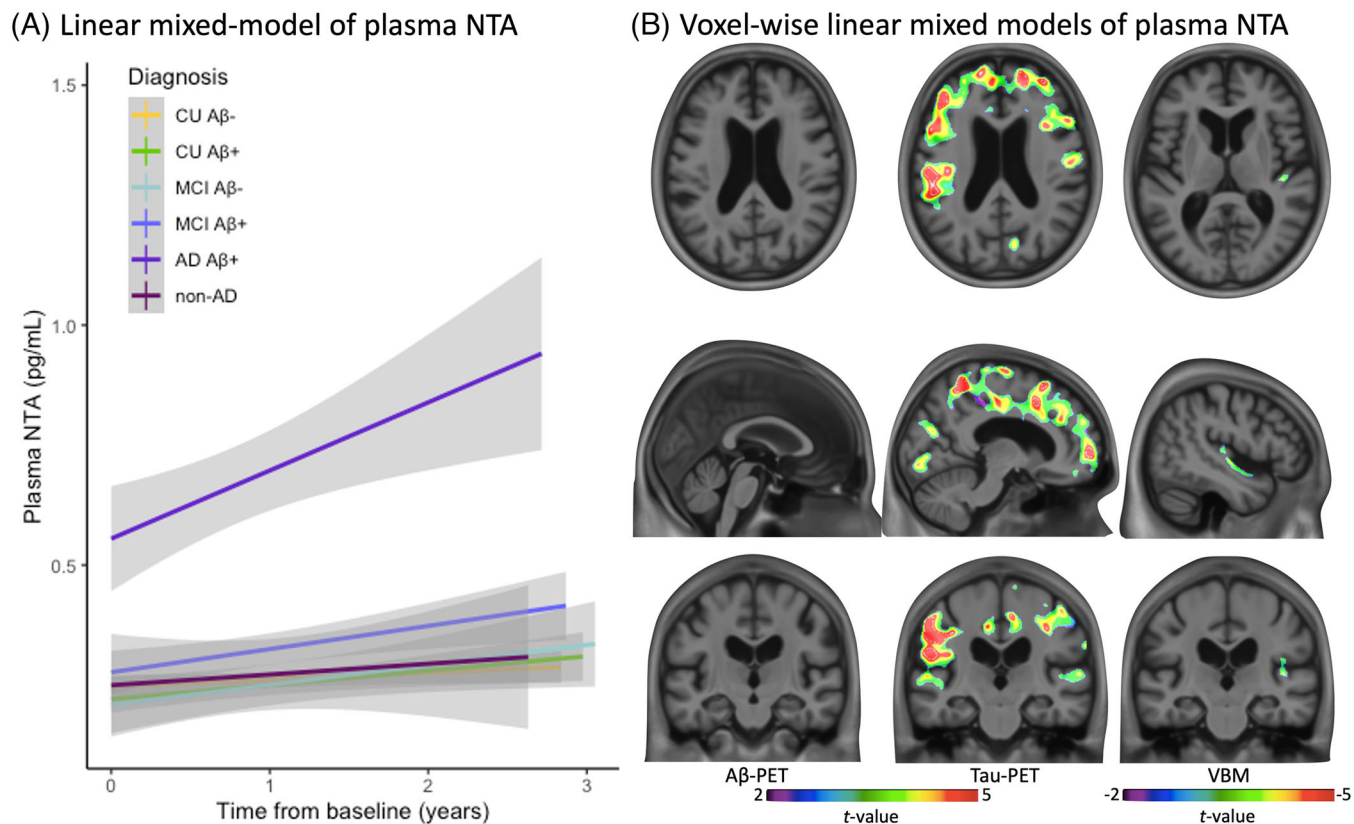


FIGURE 4 Longitudinal changes in plasma NTA-tau concentrations. A, Longitudinal changes in plasma NTA levels based on diagnosis. B, Linear mixed models presenting the association between longitudinal changes in plasma NTA-tau concentrations and in Aβ PET, tau PET, and neurodegeneration assessed by VBM, in individuals along the aging and AD spectrum. Aβ, amyloid beta; AD, Alzheimer's disease; CU, cognitively unimpaired; MCI, mild cognitive impairment; PET, positron emission tomography; VBM, voxel-based morphometry.

temporal neurodegeneration, but only at global, not voxel-level, analysis. NTA-tau levels on the other hand were associated with tau PET in both voxel-wise and ROI-based analyses. Our comparison of nested regression models showed that temporal meta-ROI measures of tau PET better explained plasma and CSF NTA-tau concentrations, with the tau-only model often presenting as the most parsimonious one. Importantly, NTA-tau changes followed the hierarchical Braak staging system.¹ CSF NTA-tau increases after tau PET positivity is detected in Braak stage I (transentorhinal), while plasma NTA-tau concentrations increase at a later stage, starting at Braak III (amygdala, parahippocampal gyrus, fusiform gyrus, and lingual gyrus). Moreover, the availability of plasma p-tau181, p-tau217, and p-tau231 measurements in a subset of 129 participants, allowed an exploratory assessment on how different plasma tau biomarkers associate with the AD pathological hallmarks. Plasma p-tau181 was similarly associated with tau PET and Aβ PET, whereas plasma p-tau217 and p-tau231 showed stronger correlations with Aβ PET than tau PET. These results aligned well with previous studies reporting a strong association between blood p-tau biomarkers and Aβ pathology.^{6,10,28,42} Contrarily, plasma NTA-tau displayed a stronger association with tau PET, further highlighting the tight link between plasma NTA-tau levels and tau deposition in the brain. Finally, while the correlation of plasma NTA-tau with neurodegeneration was similar to that of plasma p-tau181 and p-tau231, this

was weaker than that of plasma p-tau217. Altogether, these findings further corroborate the idea that CSF and plasma NTA-tau indicate different stages of NFT progression, with CSF NTA-tau increasing first.

The same analyses were repeated in Aβ-positive individuals, and comparison of goodness-of-fit metrics enforced the idea that NTA-tau concentrations are more strongly associated with tau PET rather than other AD hallmarks. However, in this subgroup, cross-sectional ROI-based and voxel-wise analyses revealed also an association between NTA-tau concentrations and neurodegeneration. As individuals presenting Aβ positivity are more advanced in disease progression, they are expected to display more neurodegeneration.

Additionally, a subset of individuals had follow-up measures of plasma NTA-tau, tau PET, Aβ PET, and MRI. First, we observed that only cognitively impaired Aβ+ individuals showed a significant increase in plasma NTA-tau concentrations over time. This suggests a potential novel ability for plasma NTA-tau to track late disease progression, as commonly studied AD plasma biomarkers such as p-tau usually start to increase at preclinical AD, but reach a plateau at advanced AD stages.⁴³ Notably, plasma NTA-tau predicted tau PET accumulation in middle to late Braak regions.¹ Comparatively, plasma p-tau markers have been related to longitudinal accumulation of Aβ, NFT, and neurodegeneration in broader brain regions.^{6,44,45} This finding corroborates the idea that plasma NTA-tau is a predictor of mid- to late-stage

tau accumulation. Plasma NTA-tau also predicted neurodegeneration in the medial temporal lobe, related to memory problems observed in AD dementia.⁴⁶ Among key AD pathophysiological changes, neurodegeneration has been observed at the latest stages.⁴⁷ Following the progression of AD pathophysiological changes, we would expect plasma NTA-tau concentrations to rise before neurodegeneration, when NFT accumulation is high enough to induce neuronal damage.

Because AD is characterized by the accumulation of A β and tau pathologies, and current fluid p-tau markers seem more closely related to A β , there is an urgent need for fluid biomarkers that can specifically track tau pathology. Because of their close association with A β and tau pathologies, it is difficult to determine to which extent NFT deposition ultimately contributes to soluble p-tau signal, and their strong association with A β is also supported by the reduction of p-tau after A β removal.⁴⁸ Thus, NTA-tau measurements could potentially be useful in clinical settings, providing a cost-effective tool capable of tracking tau pathology in vivo, and in clinical trials, as an inclusion/exclusion criterion,⁴⁹ or to potentially monitor the downstream effects of anti-A β drugs. This is especially important as N-terminal tau is thought to be closely linked with presynaptic toxicity, which is currently exploited therapeutically.^{50,51} Moreover, plasma NTA-tau could be an easily implementable tool to detect individuals at middle to late stages of AD, as well as individuals at risk of accumulating further tau. Our results suggest NTA-tau would be a suitable fluid marker for the "T" category of the A/T(N) system.^{3,52} However, studies in different cohorts in combination with other AD markers are still required.

A strength of this study is the TRIAD cohort, comprising participants across the aging and AD *continuum* and with other neurodegenerative diseases, extensively characterized using multiple state-of-the-art biomarkers. Moreover, this cohort includes follow-up blood and imaging collection, allowing for longitudinal analysis. Additionally, matching plasma and CSF samples were available. Altogether, this enabled a detailed characterization of the novel NTA assay, shedding light on the underlying pathophysiological mechanisms that induce abnormal increase of NTA-tau in biofluids. Limitations include that CSF was not available for all subjects, limiting our ability to conduct certain analyses, for example, longitudinal analysis using CSF NTA. Second, despite the consistency of our findings in plasma and CSF, a replication cohort would have further strengthened our results. Additionally, it would have been interesting to investigate the concordance between fluid NTA measurements with *post mortem* Braak staging. Finally, TRIAD is composed of individuals willing to participate in research focused on dementia, thus creating sampling and self-selection biases.

5 | CONCLUSIONS

To conclude, our study provides evidence that NTA-tau differentiates individuals in distinct diagnostic groups across aging and the AD *continuum* and is a biomarker more closely associated with NFT accumulation in AD, rather than A β and neurodegeneration. Moreover, plasma

NTA-tau is a predictor of tau PET progression in middle to late Braak stage regions.

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CONFLICT OF INTEREST STATEMENT

K.B. has served as a consultant, on advisory boards, or on data monitoring committees for Abcam, Axon, Biogen, JOMDD/Shimadzu, Julius Clinical, Lilly, MagQu, Novartis, Prothena, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program. H.Z. has served on scientific advisory boards and/or as a consultant for Abbvie, Acumen, Alector, Alzinova, ALZPath, Annexon, Apellis, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, Optoceutics, Passage Bio, Pinteon Therapeutics, Prothena, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave; has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen, and Roche; and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). J.L.R., C.T., A.S., S.S., A.L.B., N.R., L.M.G., J.T., W.S.B., J.S., F.Z.L., G.B., A.C.M., M.C., S.S.M., T.A.P., N.J.A. and P.R.N. report no

conflicts of interest. Author disclosures are available in the supporting information.

CONSENT STATEMENT

The TRIAD study was approved by the Research Ethics Board of the Douglas Mental Health Institute and the PET Working Committee. All study participants provided written informed consent.

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SUPPORTING INFORMATION

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