Diagnostic value of plasma phosphorylated tau181 in Alzheimer's disease and frontotemporal lobar degeneration

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Abstract

With the potential development of new disease-modifying Alzheimer's disease (AD) therapies, simple, widely available screening tests are needed to identify which individuals, who are experiencing symptoms of cognitive or behavioral decline, should be further evaluated for initiation of treatment. A blood-based test for AD would be a less invasive and less expensive screening tool than the currently approved cerebrospinal fluid or amyloid β positron emission tomography (PET) diagnostic tests. We examined whether plasma tau phosphorylated at residue 181 (pTau181) could differentiate between clinically diagnosed or autopsy-confirmed AD and frontotemporal lobar degeneration. Plasma pTau181 concentrations were increased by 3.5-fold in AD compared to controls and differentiated AD from both clinically diagnosed (receiver operating characteristic area under the curve of 0.894) and autopsy-confirmed frontotemporal lobar degeneration (area under the curve of 0.878). Plasma pTau181 identified individuals who were amyloid β-PET-positive regardless of clinical diagnosis and correlated with cortical tau protein deposition measured by 18F-flortaucipir PET. Plasma pTau181 may be useful to screen for tau pathology associated with AD.

Introduction

Fluid and imaging biomarkers are increasingly essential to the accurate diagnosis of dementia. Differentiation of patients with Alzheimer's disease (AD) from other related dementias, such as frontotemporal lobar degeneration (FTLD), is important for patient care and for enrollment in clinical trials of potential therapeutic agents. Evaluation of brain beta-amyloid (Aβ) deposition measured with Aβ positron emission tomography (Aβ-PET) in patients with mild cognitive impairment (MCI) or dementia is associated with meaningful changes in clinical management in ~60% of the patients.[1] Aβ and tau measurements in cerebrospinal fluid (CSF) are similarly useful to Aβ-PET imaging for AD diagnostic verification and prognosis.[2,3] Still, biomarkers are not widely used because of the invasiveness of obtaining CSF and the high costs of PET imaging, often not reimbursed by third-party payers.[1] Moreover, access to PET imaging is often restricted to specialized centers and novel PET ligands that measure tau, such as ^{18}F-Flortaucipir PET (FTP-PET), are not yet approved for clinical use. A blood-based diagnostic test for AD would provide a less invasive and less expensive alternative to CSF or PET studies.

FTLD is a common cause of dementia in younger individuals that can be difficult to differentiate from AD.[4] Brain tau pathology is present in both AD and a subset of FTLD syndromes, including approximately half of behavioral variant frontotemporal dementia (bvFTD), most nonfluent variant primary progressive aphasia (nfvPPA) and almost all progressive supranuclear palsy (PSP) patients.[5] In AD, tau pathology is associated with elevated concentrations of CSF tau species, including (total) tau and phosphorylated tau at residue 181 (pTau).[6,7] However in FTLD, CSF tau and pTau can be either elevated or decreased.[8] Insoluble tau deposition can be visualized in the brains of living individuals using FTP-PET, that binds with high affinity to mixed 3 and 4 repeat (3R/4R) tau in AD neurofibrillary tangles,[9] and can distinguish AD from other diseases.[10] However, FTP has low affinity for pure 3R- or 4R tau deposits, limiting its usefulness in FTLD.[8]

Neurofilament light chain (NfL) is a marker of axonal damage that is increasingly used in research as a marker of neurodegeneration both in AD and FTLD.[11,12] CSF, plasma and serum NfL levels are increased in FTLD and correlate with survival,[13] clinical severity and brain volume loss.[14–17] CSF and serum NfL concentrations are also elevated in AD, but less than in FTLD.[12,15,18] As in FTLD, serum NfL is predictive of cortical thinning and rate of disease progression in AD.[19,20]

Recently, a new plasma pTau assay was found to differentiate AD from healthy controls.[21] We tested the differential diagnostic ability of this plasma pTau assay as compared to plasma NfL for MCI and AD relative to a variety of clinical FTLD phenotypes. A subset of diagnoses were verified using neuropathological examination at autopsy. We compared plasma pTau and NfL levels to cortical Aβ- and FTP-PET uptake and brain atrophy measured with MRI, to evaluate whether these biomarkers provide comparable information.

Materials and Methods

Participants

This retrospective study included 309 patients from three independent cohorts (**Table 1, eTable 1**), a primary cohort of 267 cases; 248 cases from the University of California San Francisco (UCSF) Memory and Aging Center and 19 from the Advancing Research and Treatment for Frontotemporal Lobar Degeneration (ARTFL) consortium, and a secondary cohort of 42 cases from an Eli Lilly sponsored clinical trial (NCT02624778). 208 participants had previous Aβ-PET, 116 had FTP-PET (106 AD/MCI and 10 FTLD), MRIs were available from 221 participants (72 AD/MCI, 110 FTLD, 39 NC), and 74 cases had previous CSF pTau concentrations available (20 NC, 25 MCI/AD, 29 FTLD). The primary cohort consisted of 45 normal controls (NC), 39 AD per NINCDS-ADRDA criteria,[22] 40 MCI,[23] and 143 patients meeting clinical criteria for a syndrome in the FTLD spectrum: 36 corticobasal syndrome (CBS)[24], 47 PSP,[25] 46 bvFTD,[26] and 14 primary progressive aphasia (PPA).[27] These included 39 carriers of FTLD-causing mutations: 23 tau (*MAPT*), six progranulin (*GRN*) and ten chromosome 9 open reading frame 72 *(C9orf72)*. Healthy elderly controls had normal neurological examinations, neuropsychological testing and clinical dementia rating (CDR)[28] scores. Longitudinal measures of disease severity, neuropsychological testing and executive function were available at baseline and two follow visits (average n visit one = 115±16 cases, average n visit two = 40±9 cases) with an average 1.2±0.1 years between measurements. Participants provided written informed consent at the time of recruitment. The study was approved by the institutional review board of each research center from which the individual was recruited.

Clinical evaluation

Disease severity was assessed using the CDR scale sum of boxes (CDRsb),[28] and Mini-Mental State Exam (MMSE).[29] Neuropsychological measures included a Trail-Making test,[30] Color Trails

test,[31] phonemic fluency,[32] the Boston Naming Test (BNT),[33] Modified Rey Benson Figure copy and recall,[30] and Geriatric Depression Scale (GDS).[34] Disability was assessed using the Functional Activities Questionnaire (FAQ),[35] and the Schwab and England Activities of Daily Living (SEADL) scale.[36]

Fluid biomarkers and neuroimaging

Plasma pTau was measured using a streptavidin small spot plate using a Mesoscale Discovery assay as previously described, using a biotinylated-AT270 capture antibody (anti pTau181 antibody) and a SULFO-TAG-Ru-LRL (anti-tau monoclonal antibody developed by Lilly Research Laboratory) detector.[21] Plasma NfL was measured using Simoa technology using either a homebrew kit[37] or commercial kit on a Quanterix HD-1 analyzer. CSF pTau181 was measured using the INNO-BIA AlzBio3 platform.

Methods for brain MRI, FTP-PET, and Aβ-PET acquisition, pre-processing and (voxelwise) analyses are described in **Supplementary Methods**.

Statistical analysis

A two-sided $p<0.05$ was considered statistically significant. Biomarker concentrations were not normally distributed and natural log-transformed data or non-parametric statistics were used. Differences in biomarker values and in clinical and neuroimaging variables were assessed with one-way ANOVA or Kruskal-Wallis comparisons, with Bonferroni correction. Associations between pTau and NfL concentrations, FTP-PET cortical standardized uptake values (SUVRs), and clinical measures were assessed using linear regression models, corrected for false discovery rate.[38] Receiver operating characteristic (ROC) analyses determined the ability of plasma pTau and NfL to differentiate between diagnostic groups. Youden cut-off values were used for sensitivity and specificity.[39] Analyses were corrected for age, but not CDRsb because

this did not substantially alter the results. Linear mixed effect models evaluated the relationship of baseline ln pTau with changes in clinical variables. Models allowed random intercepts at the subject level and were adjusted for age, sex, time differences from specimen collection date, disease duration, and biomarker by time interaction. Statistical analyses were performed using SPSS (version 25; SPSS/IBM, Chicago, IL), Stata (Stata 14.0, StataCore LP) and R (version 3.5.1).

Results

Sample characteristics

Baseline demographics, clinical assessments imaging measures, and fluid biomarker levels are shown in **Table 1**. The control group (NC) was older than the AD, MCI, CBS, and bvFTD groups. Plasma pTau and NfL concentrations were similar in men and women. Age correlated with NfL (ρ=0.188, p=0.006), but not with pTau. CDRsb correlated with plasma pTau (β=0.184, p=0.004) and NfL (β=0.456, p<0.0001). FTP-PET binding was highest in AD cases, compared to MCI, CBS, and bvFTD. PiB-PET binding was highest in AD. 27% of controls were amyloid PET positive. CSF pTau was higher in AD compared to every diagnosis except for MCI.

Plasma pTau and NfL comparisons by diagnostic group

Plasma pTau concentrations were elevated in AD compared to all other groups (**Figure 1A, Table 1**). Plasma NfL concentrations were elevated in PSP, CBS and PPA, compared to AD and MCI as well as controls (**Figure 1B**). NfL concentrations were also elevated in bvFTD as compared to controls and MCI. The ratio of pTau/NfL was elevated in CBS, PSP and PPA compared to controls, AD and MCI patients (**Figure 1C**). An aged-adjusted plasma pTau cut-off of 7.1 pg/mL differentiated AD from the other diagnostic groups and controls with a ROC area under the curve (AUC) of 0.904 (p<0.0001, **Figure 1D**). Plasma pTau differentiated AD/MCI from FTLD with an AUC of 0.781 (**eTable 2**).

Association between plasma pTau and other fluid biomarkers

Plasma pTau and plasma NfL concentrations were correlated in the combined AD and MCI cases (β=0.66, p<0.0001, **Figure 2A**), but not in the whole patient sample**.** CSF pTau181 correlated with plasma pTau β=0.49 (p<0.0001; n=74). CSF pTau concentrations were higher in AD but could not differentiate AD from the other diagnoses (AUC=0.665, p=0.045, **Table 1, eTable 2**).

pTau and NfL in pathology-confirmed cases and mutation carriers

Neuropathological diagnosis was available in 79 cases. Due to potential effects of disease severity at blood draw, analyses were adjusted for age and CDRsb. Median plasma pTau concentrations were higher in AD (n=8, 4.5±5 pg/mL) compared to FTLD-TDP (n=16, 1.8±2 pg/mL, p=0.004) and FTLD-tau (n=53, 2.3±2 pg/mL, p=0.047). Plasma pTau differentiated autopsy-confirmed AD from the combined FTLD-TDP and FTLD-tau group (AUC=0.799, p=0.006), from FTLD-TDP alone (AUC=0.883, p=0.003) and from FTLD-tau alone (AUC=0.774, p=0.013, **eTable 2**). Plasma NfL was a poor discriminator of autopsy-confirmed diagnoses. pTau correlated with Braak stage (β=0.446, p<0.0001) and was higher in Braak stage 5-6 (n=10, 4.9±4 pg/mL) compared to Braak 0 (n=10, 2.1±2 pg/mL, p=0.018) and Braak 1-2 (n=45, 2.2±2 pg/mL, p=0.001). NfL could not differentiate Braak stages.

32 cases were symptomatic FTLD mutation carriers (21 *MAPT*, 5 *GRN*, 6 *C9orf72*). There was no difference in pTau levels between the mutation carriers or with normal controls (**eFigure 2**). There was a trend (p=0.089) towards higher pTau (5.2±3 pg/mL) in *MAPT* mutations producing combined 3R/4R tau pathology (R406W and V377M, n=7) than those producing 4R tau alone[40] (i.e. P301L and N279K, n=16; 3.2±4 pg/mL). Plasma pTau differentiated pathology-confirmed AD from pathology-confirmed FTLD and mutation carriers combined (AUC=0.796, p=0.010).

Plasma pTau and NfL concentrations with tau (FTP)-PET and Aβ-PET

There was a strong correlation between global cortical FTP uptake and plasma pTau concentrations (β=0.73, p<0.0001, **Figure 2B** and **2C**), which also reflected Aβ-PET status and clinical diagnosis. The relationship between cortical FTP SUVR and NfL was weaker (β=0.28, p=0.023). Plasma pTau differentiated between Aβ-PET positive and negative individuals (AUC 0.920, p<0.0001, **Figure 3A**). A plasma pTau cut-off for Aβ-PET positivity was 7.7 pg/mL (0.919 sensitivity and 0.824 specificity, **eTable 2**). Plasma pTau also differentiated between Aβ-PET

positive and negative cases within the controls and MCI groups separately. In controls, the AUC was 0.875 (p<0.0001, 11 Aβ-PET positive, 29 negative). Within the MCI group, the AUC was 0.942 (p<0.0001, 18 Aβ-PET positive, 21 negative, **eTable 2, eFigure 3**). When a cortical FTP-SUVR diagnostic threshold[41] was applied to divide cases in FTP-PET positive and negative, plasma pTau was also a good discriminator of FTP-PET status (AUC=0.939, p<0.0001, **Figure 3B**). In the MCI cases the AUC for FTP-PET status was 0.977 (p<0.0001, 11 FTP-PET positive, 20 negative, **eTable 2**). Similar results were obtained with the AD/MCI clinical trial replication cohort (n=42; **Supplementary Results**). Plasma NfL did not differentiate between Aβ-PET positive and negative cases (AUC = 0.559, p=0.276) or FTP-PET positive and negative cases (AUC = 0.644, p=0.064, **eTable 2**).

Plasma pTau and NfL correlations with clinical disease severity and cognitive function

pTau showed strong associations with baseline scores of CDRsb (β=0.486, p<0.0001), FAQ (β=0.541, p<0.0001) and Rey recall (β=-0.585, p<0.0001) only in the MCI/AD group but not in the control or FTLD groups. In contrast, NfL showed correlations with clinical severity and neuropsychological performance in both the MCI/AD and FTLD groups (β=0.472, p<0.0001 in AD/MCI; β=0.244, p<0.010 in FTLD; supplemental **eTable3 and eTable4**). In longitudinal analyses, higher baseline pTau was associated with faster rate of decline in MCI/AD patients in CDRsb, MMSE, Rey recall, BNT and FAQ, whereas higher baseline NfL predicted faster decline over time in FTLD patients in MMSE, phonemic fluency and trails. Similar associations were observed when pTau was used as a categorical variable with a cut-off value of 7.1 pg/mL. (Supplemental **eTables 5-7**).

Voxelwise analyses of FTP-PET and grey matter atrophy in relation to plasma pTau and NfL

pTau concentrations were strongly correlated with FTP-PET SUVR values (ρ values approaching 0.75 in peak regions) in the frontal, temporoparietal cortex, posterior cingulate cortices and precuneus regions (**Figure 4A**). Associations remained significant in the AD/MCI patients only, though with slightly lower ρ values. There were insufficient data to perform the analyses in the FTLD group. There was no association between NfL concentrations and FTP-PET uptake in the whole group. In the AD/MCI patients only there were weak correlations in the right hemisphere that did not survive multiple comparisons corrections, predominantly frontal and insular cortex, and in the right temporal horn (reaching ρ~0.6 in the insula; **Figure 4A**).

High pTau concentrations correlated with more severe grey matter atrophy in the bilateral medial temporal lobe, the posterior cingulate cortex and precuneus (ρ=0.35, p<0.001, **Figure 4B**). This association was driven by the AD/MCI cases, who showed the highest correlation coefficients in these regions (ρ=0.55, p<0.001). There was no association in FTLD cases. In the combined group there were strong negative correlations between NfL and grey matter volume in the right putamen and insula (ρ~0.5, p<0.001), and to a lesser extent medial prefrontal cortices (ρ~0.45, p<0.001). In the FTLD group, the association was maximal in the right putamen and insula (ρ~0.4, p<0.001), with lower correlations present in the frontal and lateral temporal regions, and right precuneus (**Figure 4B**).

Discussion

The main findings of this study are that plasma pTau concentrations strongly differentiated AD patients from FTLD patients and controls, in both clinically diagnosed and neuropathologically- or genetically-verified cases, and that plasma pTau levels reflected *in vivo* Aβ and tau deposition measured using Aβ- and FTP-PET. Specifically, plasma pTau concentrations correlated with FTP-PET uptake and grey matter atrophy in AD-related brain regions and increased with FTP-PET-estimated Braak stages.[10,42] In contrast, plasma concentrations of NfL, a nonspecific biomarker of neurodegeneration, were not related to AD diagnosis, Aβ- or FTP-PET signal. Instead, NfL levels correlated with measures of disease severity, cognitive function and grey matter atrophy mainly in the FTLD patients.[43] Plasma pTau levels accurately identified controls and MCI individuals who had Aβ-PET evidence of incipient AD. Together, these data suggest that plasma pTau, alone or in combination with NfL, may be a useful tool for identifying underlying AD pathology in cognitively impaired individuals or those who are at risk for cognitive decline.

These data are consistent with a previous study by Mielke *et al.*[21] that used this assay and showed a positive correlation between plasma pTau concentrations and Aβ-PET SUVR and cortical FTP-PET uptake in controls, MCI and AD. In that study, pTau was less accurate in identifying Aβ-PET positivity (AUC 0.803, 95%CI: 0.749-0.856) and there was no association of pTau with cortical thickness in AD specific regions. The better diagnostic accuracy we observed might relate to the different participants studied here or the approach to determining Aβ-PET positivity, using visual read instead of an SUVR threshold. Plasma Aβ measured on the Elecsys platform has recently been demonstrated as a promising and cost-effective tool to identify Aβ-PET positive individuals with or at risk for AD.[44] Here we show that plasma pTau is strongly linked with measures of AD tau pathology and therefore may have additional diagnostic and prognostic value.

Aβ-PET uptake has established clinical utility for differential diagnosis of AD from other dementias, is associated with worse global cognition in dementia,[45] and has been validated as a

measure of AD neuropathology.[46] We found that increased pTau concentrations strongly correlated with Aβ-PET positivity, even in cognitively normal controls. In individuals with cognitive impairment, pTau was associated with neuropsychological and functional measures, as well as underlying AD pathology at autopsy. pTau differentiated between AD and FTLD with remarkable accuracy, similar to the previously reported accuracy with Aβ-PET.[47] This suggests that the diagnostic value of plasma pTau could be comparable to Aβ-PET. Importantly, the fold change in mean plasma pTau concentration between Aβ positive and negative individuals in our study exceeded the fold change obtained using plasma Aβ42/Aβ40 ratio and the overlap was much smaller (PMIDs: 27241045; 28734653; 29420472; 31233127).

Previous work has established that CSF measures of $A\beta_{1-42}/A\beta_{1-40}$ and tau/$A\beta_{1-42}$ can distinguish AD from controls and FTLD, and these measures are sometimes used for differential diagnosis.[48] Whereas CSF tau has little diagnostic value differentiating FTLD from AD,[49] CSF pTau is able to differentiate clinically diagnosed AD from FTLD (84% sensitivity and 78% specificity),[50] which is similar to the accuracy found in this study using plasma pTau. Together, these results suggest that pTau181 reflects insoluble 3R/4R tau deposition in the brain in the form of neurofibrillary tangles that are usually associated with AD pathology, but that can also be found in rare *MAPT* mutations (R406W and V337M).[51,52] The trend towards increased plasma pTau in the small number of individuals with these mutations studied here supports this hypothesis.

Plasma pTau concentrations showed a strong correlation with regional FTP-PET uptake that is thought to reflect AD neurofibrillary tangle deposition.[10,53,54] Similar correlations with FTP-PET in these regions have also been reported with CSF pTau and plasma tau/$A\beta_{1-42}$.[53,55] As expected, we found no correlation between NfL and FTP-uptake in our combined sample. However, there was an association between plasma NfL and FTP-PET in the right frontal regions in AD/MCI. This could suggest that there is a greater rate of neurodegeneration with high levels of tau deposition in these regions. The increased pTau levels in AD and their strong association

with AD patterns of brain atrophy further supports the hypothesis that plasma pTau reflects AD-related brain pathology.

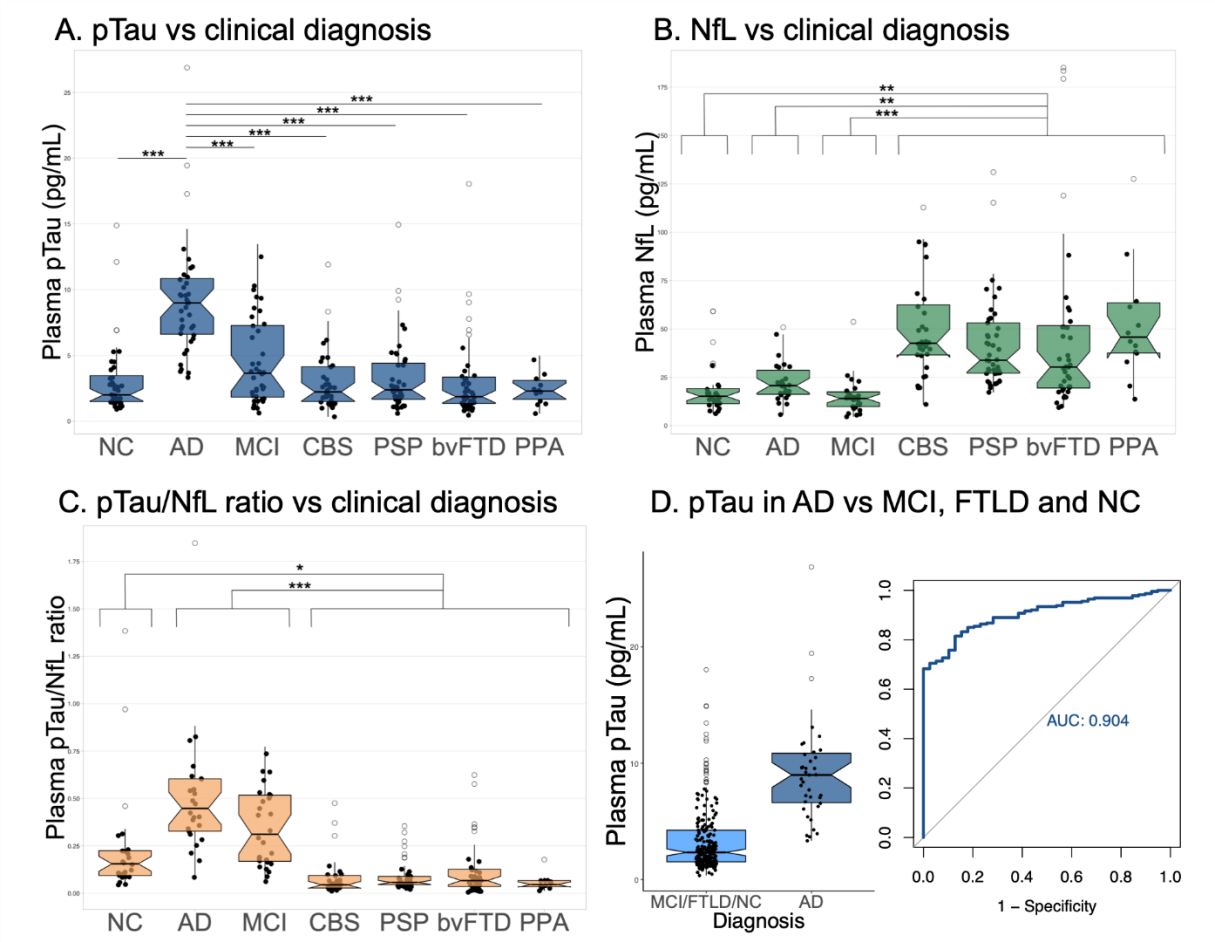
This study has a number of limitations. There were several outlier high plasma pTau values in the clinical diagnostic groups who were not expected to have elevated pTau: two controls, one in CBS, PSP and bvFTD. These findings may have reflected previously undetected brain 3R/4R tau deposition. One of the controls was Aβ-PET positive, the CBS case had unknown amyloid status and could have had AD pathology, the PSP case had autopsy data showing AD co-pathology, and the bvFTD case was an N279K mutation carrier, which is associated 4R tau pathology. Larger studies including *MAPT* mutation carriers will also be necessary to address the question of whether plasma pTau differentiates between tauopathies with predominantly 3R, 4R or mixed 3R/4R tau pathology.[56,57]

Conclusion

This study provides strong evidence that plasma pTau concentration differentiates AD from other neurodegenerative diseases and controls, and likely reflects insoluble 3R/4R tau deposition in the brain in the form of neurofibrillary tangles. Since PET scans are expensive and require specialized imaging centers, plasma pTau may be a more readily accessible tool for the differential diagnosis of dementia.

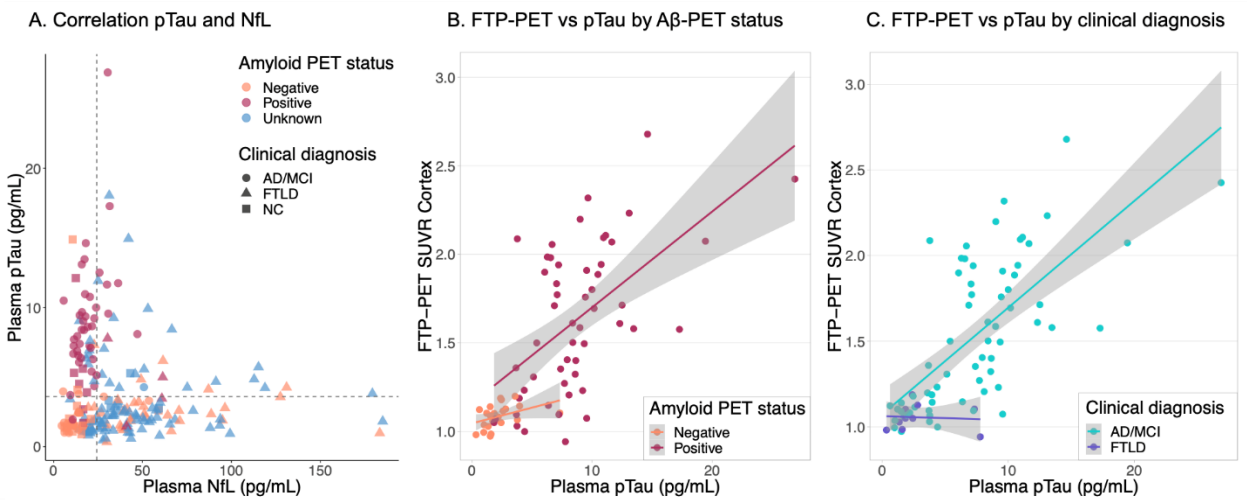
Figures

Figure 1. Plasma pTau, plasma NfL and the pTau/NfL ratio per clinical diagnosis



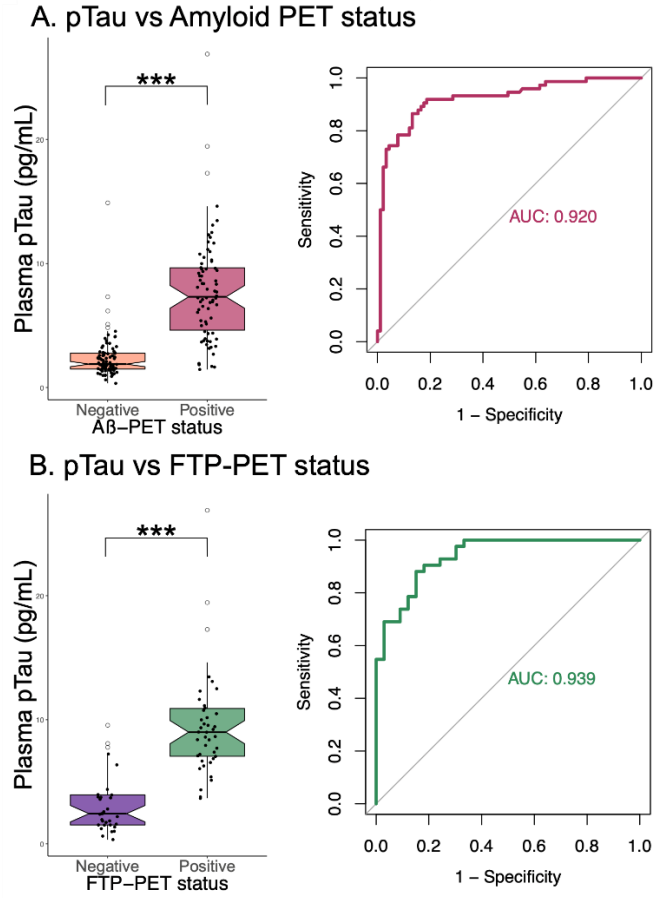
A. PTau levels were elevated in AD compared to non-AD clinical diagnoses. B. Plasma NfL were lower in controls, AD and MCI patients compared to PSP, CBS and PPA, and NfL levels in controls and MCI patients were lower than in bvFTD cases. C. The pTau/NfL ratio could differentiate control, AD and MCI patients from CBS, PSP and PPA, and was lower in bvFTD cases compared to MCI and AD. D. Plasma pTau concentrations are increased in AD cases compared to control, MCI, and FTLD clinical diagnoses and can differentiate between these groups. Notch displays the confidence interval around the median. ***p<0.0001, **p<0.01, *p<0.05

Figure 2. Association between plasma pTau and NfL, per Aβ-status and clinical diagnosis



A. Plasma pTau and plasma NfL measures are not correlated. Plasma pTau is increased in amyloid positive cases, and plasma NfL in FTLD cases. The dashed lines represent the uncorrected cut-off value for amyloid positivity (3.6 pg/mL) and the median concentration NfL (27.2 pg/mL). The color coding shows Aβ-PET status and the shape coding shows diagnostic group. B. The correlation between plasma pTau and FTP-PET standardized uptake values (SUVRs), β=0.73 p<0.0001. Color coding per Aβ-PET status C. The correlation between plasma pTau and FTP-PET SUVRs, β=0.73, p<0.0001, color coding per clinical diagnosis.

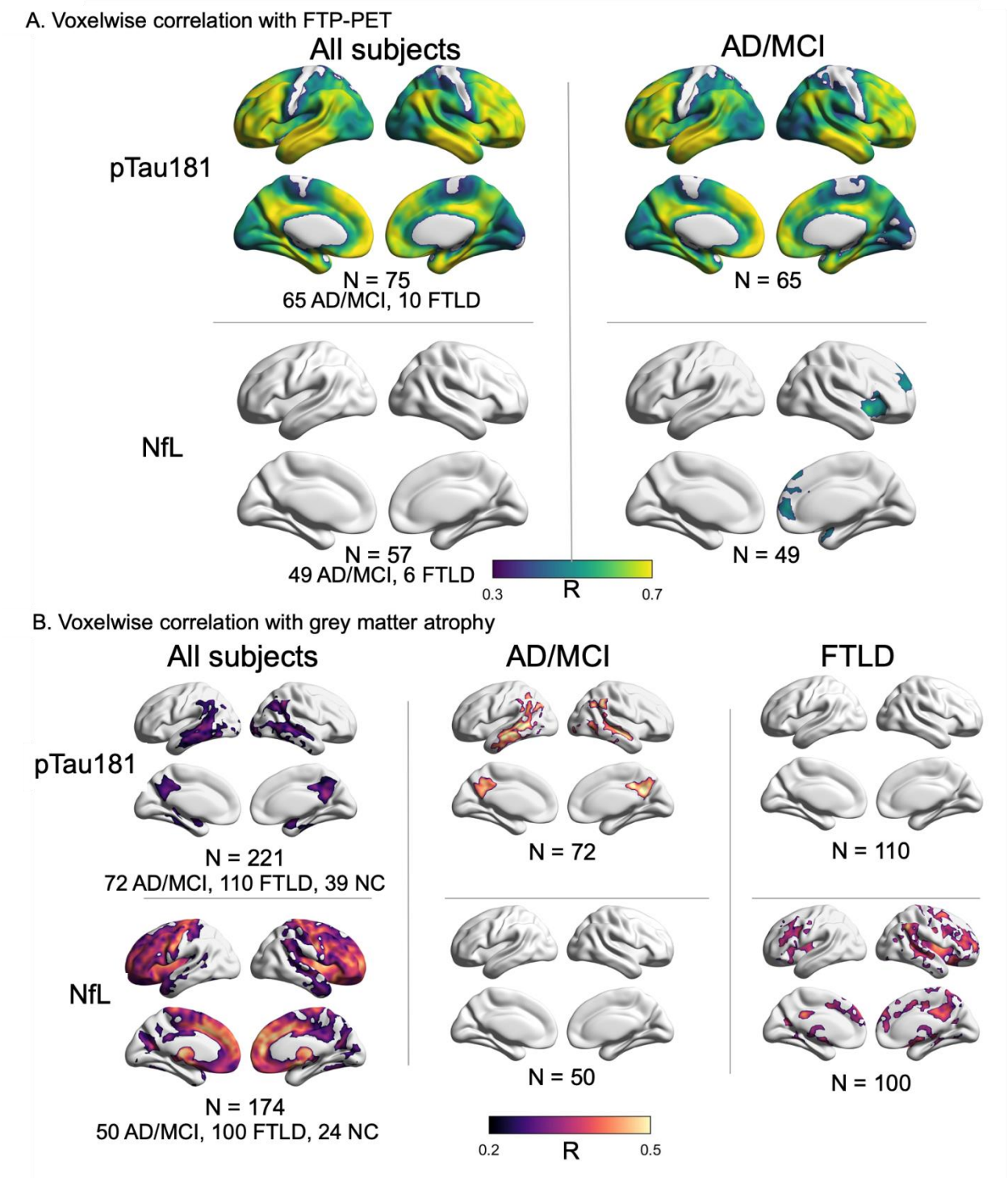
Figure 3. Receiver Operating Characteristic analyses of plasma pTau for clinical diagnosis, Aβ-PET status, and FTP-PET status



A. Plasma pTau concentrations are increased in Aβ-PET positive cases and can differentiate between Aβ-PET positive and negative cases.

B. Plasma pTau concentrations are increased in FTP-PET positive cases and can differentiate between FTP-PET positive and negative cases. Notch displays the confidence interval around the median. ****p*<0.0001

Figure 4. Voxelwise correlations of plasma pTau and plasma NfL with FTP-PET and grey matter volume loss



A. Regions of correlation between plasma pTau and FTP-PET peaked in AD specific regions; frontal and temporoparietal cortex and posterior cingulate and precuneus regions (ρ~0.75). There is no correlation with plasma NfL in the whole cohort, predominantly frontal and insular in the AD/MCI group (ρ~0.6).

B. Correlations between plasma pTau and loss of grey matter volume were highest in the bilateral temporal lobe and remained in the AD/MCI group, but no correlation was found in the FTLD group. The correlation between plasma NfL and loss of grey matter was highest in the right putamen and insular region (ρ~0.5). The association remained in the FTLD group but was not found in the AD/MCI group.

All correlations were thresholded based on uncorrected $p<0.001$ at the voxel level with family wise error-corrected $p<0.05$ at the cluster level.

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