

Title: Tau pathological activity in plasma before the onset of symptomatic Alzheimer's disease

Short running title: Tau pathological activity in plasma

Authors: Bernard J. Hanseeuw^{1-4†}, Lisa Quenon^{1-2*†}, Jean-Louis Bayart^{2,5-7}, Emilien Boyer¹⁻², Lise Colmant², Yasmine Salman², Thomas Gérard^{2,8}, Lara Huyghe², Vincent Malotiaux^{2,9}, Pascal Kienlen-Campard², Flo Blondiaux Pirson², Renaud Lhommel^{2,8}, Laurence Dricot², Adrian Ivanoiu¹⁻², Kavya Shamsundar¹⁰, Winnie Pak¹⁰, Joshua Soldo¹⁰ and Khalid Iqbal¹¹⁻¹²

†These authors contributed equally to this work (co-first authorship)

Affiliations

¹ Department of Neurology, Saint-Luc University Hospital, Brussels, Belgium

² Institute of Neuroscience, UCLouvain, Brussels, Belgium

³ WEL Research Institute, Wavre, Belgium

⁴ Department of Radiology, Massachusetts General Hospital, the Gordon Center for Medical Imaging, Boston, MA 02114, USA

⁵ Department of Laboratory Medicine, Clinique Saint-Pierre, Ottignies, Belgium

⁶ Department of Laboratory Medicine, Saint-Luc University Hospital, Brussels, Belgium

⁷ Clinical Pharmacology and Toxicology Research Unit, Namur University, Namur, Belgium

⁸ Nuclear Medicine Department, Saint-Luc University Hospital, Brussels, Belgium

⁹ Boston University, Department of Psychological & Brain Sciences, Boston, MA, USA

¹⁰ Veravas Inc., Austin, TX, & Oakdale, MN, USA

¹¹ Department of Neurochemistry, Inge Grundke-Iqbal Research Floor, New York State Institute for Basic Research in Developmental Disabilities, New York, USA

¹² Phanes Biotech, Inc., Malvern, Pennsylvania, USA

Corresponding Author:

Bernard Hanseeuw, MD PhD

Institute of Neuroscience, UCLouvain

Avenue Mounier 53, 1200, Woluwe-Saint-Lambert, Belgium

bernard.hanseeuw@uclouvain.be

Abstract

Alzheimer's disease (AD) and other tauopathies are characterized by the hyperphosphorylation of tau (pTau), leading to its aggregation in the brain, a process strongly predictive of neurodegeneration and future cognitive decline. Currently, tau positron emission tomography (PET) is the only validated method for detecting tau aggregates in vivo. However, its high cost, invasiveness, and limited accessibility restrict its use in clinical settings and preclude large-scale screening. Moreover, existing plasma biomarkers that quantify the level of pTau at specific sites (e.g., pTau217) have limited specificity for confirming AD-related tau aggregation, partly due to the heterogeneous and irregular phosphorylation patterns of pTau. Besides, the concentration of pTau is frequently elevated in the context of isolated amyloid- β pathology, which is less strongly associated with cognitive decline in the absence of aggregated tau. There is therefore an urgent need for a reliable and scalable blood-based biomarker of tau pathology. A key mechanism underlying AD tau pathology is the ability of pathologically active pTau (PA pTau) to bind to and seed normal tau, facilitating prion-like propagation of insoluble tau aggregates. Here, we assessed the diagnostic performance of the VeraBIND Tau assay, the first functional assay to detect PA pTau seeding activity in plasma.

Seventy-nine cognitively unimpaired (CU) and 66 cognitively impaired older adults underwent blood sampling, cognitive assessment, amyloid-PET or cerebrospinal fluid (CSF) analysis, and [^{18}F]-MK6240 tau-PET imaging. Plasma pTau217 concentrations were quantified using the Lumipulse platform (Fujirebio). The VeraBIND Tau assay

isolated PA pTau from plasma and evaluated its ability to bind recombinant normal tau using a tagged-tau chemiluminescent readout.

VeraBIND Tau demonstrated 94.2% sensitivity and 96.1% specificity for predicting tau-PET positivity (AUC=0.97). It outperformed plasma pTau217 in CU individuals (PPV=85.9%), regardless of the pTau217 threshold used (maximal PPV of 57.5% using the 0.256pg/mL pTau217 threshold). This higher VeraBIND Tau diagnostic accuracy was driven by early tau-PET stages (Braak-like tau-PET stages 1-3; AUC=0.96 vs. 0.74 for pTau217, $p=0.003$). Moreover, both cross-sectional values and annual changes in VeraBIND Tau were significantly correlated with cognitive performance and entorhinal tau-PET signal (all absolute Spearman $r \geq 0.23$, $p < 0.05$).

These findings highlight the strong potential of VeraBIND Tau as a scalable and accurate screening tool to detect AD tau pathology in the general population. The assay may also help enrich clinical trials with tau-PET positive CU individuals, enhance clinical diagnostic workflows and support monitoring of tau-targeted therapies. Future work should evaluate its utility in optimizing triage and early-intervention strategies.

KEYWORDS

Plasma, blood-based biomarker, screening, Alzheimer' disease, tau seeding activity, tau pathological activity, tau pathology, hyperphosphorylated tau

ABBREVIATIONS

A β = amyloid-beta

A- = amyloid negative

A+ = amyloid positive

AD = Alzheimer's disease

APOE ϵ 4 = Apolipoprotein E ϵ 4 allele

A/T = amyloid/tau

AUC = area under the curve

CI = clinically impaired

CU = clinically unimpaired

eMTBR-tau243 = endogenously cleaved microtubule-binding region containing residue 243

FTLD = fronto-temporal lobar degeneration

HAMA = human anti -mouse antibodies

MMSE = Mini-Mental State Examination

MTBR-tau243 = microtubule-binding region containing residue 243

NIA-AA = National Institute on Aging and the Alzheimer's Association

NPV = negative predictive value

NFTs = neurofibrillary tau tangles

nTau = normal tau

PA pTau = pathologically active hyperphosphorylated Tau

PART = primary age-related tauopathy

PiB = [^{11}C]Pittsburgh compound B

PPV = positive predictive values

RF = rheumatoid factor

RLU = relative luminescence unit

ROC = receiver operating characteristic

SD = standard deviation

SUVr = Standardized Uptake Value ratio

T- = Tau negative

T+ = Tau positive

VB- = VeraBIND Tau negative

VB+ = VeraBIND Tau positive

INTRODUCTION

Alzheimer's disease (AD) is biologically defined by amyloid-beta ($A\beta$) plaques and neurofibrillary tau tangles (NFTs), which can be observed *in vivo* using positron emission tomography (PET) imaging¹. These pathologies develop silently, before overt cognitive decline can be detected, offering a unique opportunity to screen for AD pathology and prevent the disease from occurring². Previous studies showed that clinical impairment is closely associated with NFTs^{3,4}. Clinically unimpaired (CU) individuals with both elevated $A\beta$ and tau-PET signals (A+T+) face a high risk of cognitive impairment within 3-5 years (risk=53-57%) compared to those with only high $A\beta$ burden (A+T-, risk=8-17%) and negative individuals (A-T-, risk=3-6%)^{5,6}. Identifying A+T+ individuals in the general population is key for prevention, but challenging as their prevalence is estimated at ~10% of CU individuals at age 75⁶. As PET imaging is invasive and not widely available, developing blood-based biomarkers reflecting the active formation of tau aggregates is critical for envisioning population screening.

Current blood-based biomarkers reflecting AD pathology include plasma levels of $A\beta$ peptides ($A\beta_{42}/A\beta_{40}$ ratio)⁷⁻⁹ and hyperphosphorylated tau (pTau) species at different sites (e.g., pTau181, pTau205, pTau212, pTau217, pTau231)¹⁰⁻¹⁷. Compelling evidence suggests that these plasma biomarkers, including pTau species, more closely reflect $A\beta$ plaque load than tau pathology, as evidenced by PET^{14,18,19}. Among them, plasma pTau217

presents the widest dynamic range and best correlates with PET imaging and post-mortem measures^{20–23}. Recent meta-analyses demonstrated excellent accuracy to predict A β and tau-PET positivity in clinically impaired (CI) individuals^{24,25}. However, the accuracy of pTau217 for detecting tau-PET positivity is low in CU individuals^{24,25}. Specifically, using high thresholds, plasma pTau217 identifies A+T+ individuals with advanced tau pathology (e.g., Braak-like tau-PET stages ≥ 4)^{26–29}, while using low thresholds detects both A+T+ and A+T- CU individuals. These observations indicate that plasma pTau217 may serve a diagnostic purpose in symptomatic individuals, as recently approved by the Food and Drug Administration (FDA), but it is insufficiently accurate as a stand-alone screening test in CU individuals to detect incipient tau pathology.

Current plasma pTau species biomarkers may lack specificity for capturing early aggregated tau pathology, as tau phosphorylation patterns are both overlapping and heterogeneous across physiological and pathological conditions³⁰. Tau contains many sites at which it is hyperphosphorylated reversibly under various stress conditions such as hypothermia. Consequently, increased levels of tau hyperphosphorylation at any one site does not necessarily result in tau aggregation. One key pathological mechanism in AD and other tauopathies involves tau seeding activity whereby specific misfolded pTau species binds to and templates normal tau, promoting prion-like aggregation and spreading of tau pathology^{31–33}. In this work, we evaluated a novel plasma assay, named VeraBIND Tau, which reflects the seeding activity of pathological pTau, using [¹⁸F]MK6240 tau-PET imaging as the *in vivo* gold standard to detect tau pathology. This assay tests the pathological seeding activity of pTau from plasma by measuring its ability to bind recombinant normal tau. The VeraBIND™ technology is first based on a purification method that enriches low-abundance peptides for their subsequent characterization. The enriched pTau peptides are then incubated with recombinant normal tau, and binding of

this recombinant normal tau by pathologically active pTau (PA pTau) is detected with an anti-recombinant tag alkaline phosphatase antibody conjugate. The resulting relative luminescence unit (RLU) is directly proportional to the amount of recombinant normal tau bound by pTau in the plasma sample. The RLU signal is converted to a ratio using a Standard run in each assay, providing a semi-quantitative result. In this study, we assessed the diagnostic performance of VeraBIND Tau to predict tau-PET positivity in a mixed sample of CU and CI participants, as well as in each of these groups separately and in low versus high Braak-like PET stages. Its diagnostic performance was compared to the several blood-based biomarkers, including the plasma concentration of pTau217 and pTau181, which are currently the main blood-based biomarkers approved for clinical use. Finally, we investigated the associations between the VeraBIND Tau semi-quantitative measure, cognition and tau-PET signal, both cross-sectionally and longitudinally.

MATERIALS AND METHODS

Participants

In total, 145 individuals aged over 45 years old were included in this study. This sample was composed of 76 patients recruited at the Memory Clinic of the Cliniques Universitaires Saint-Luc in Brussels (Belgium) and 69 volunteers who were selected from a registry established in another academic study. Volunteers' selection was enriched for carriers of the Apolipoprotein E ϵ 4 allele (*APOE* ϵ 4) to match the frequency of patients' *APOE* ϵ 4 carriership (see Supplemental Material for the *APOE* genotyping method). Recruitment and examinations were conducted between June 2019 and April 2025.

Exclusion criteria were any neurological conditions associated with long-term risk of significant cognitive impairment or dementia (e.g., multiple sclerosis, Huntington's

disease), focal brain lesions, psychiatric disorders (e.g., major depression, schizophrenia, bipolar disorder), active alcohol and drug abuses, and participation in a clinical trial of an investigational product.

This study was approved by the Ethical Committee of UCLouvain (#UCL-2016-121, Date: 13/05/2019; Eudra-CT number: 2018-003473-94) and was conducted in compliance to the Declaration of Helsinki principles. Written informed consent was obtained from all participants.

At their inclusion in the study, participants underwent blood sampling, anatomical brain magnetic resonance imaging (MRI), lumbar puncture, or an amyloid-PET, tau-PET imaging, and a comprehensive cognitive assessment.

Blood-based biomarkers

Blood drawing and plasma preparation

A standard venipuncture procedure was performed using a 21 g needle, and blood was collected in ethylenediaminetetraacetic acid (EDTA) polypropylene K2 tubes (K2-EDTA tubes, 7.5 mL S-monovette 01.1605.008, Sarstedt®). The tube was placed on ice immediately after collection and plasma isolation was performed within two hours. Blood was centrifuged at $2000 \times g$ for 10 min at 4°C. Extracted plasma was aliquoted at a volume of 500 µL into cryotubes then frozen within two hours of blood collection at -80°C until further analysis⁷. For cross-sectional analyses, we used the VeraBIND Tau scores closest in time to tau-PET (average delay= 0.4 years, SD=0.8). The delay between VeraBIND Tau scores, amyloid-PET and cognitive data was on average of 0.8 (SD=0.8) and 0.5 years (SD=0.6), respectively.

VeraBIND™ Tau assay

Measurement of PA pTau in EDTA plasma was performed at Veravas, Inc. (3510 Hopkins Place North, Oakdale, MN, 55128, USA) and Access Genetics & OralDNA Labs (7400 Flying Cloud Drive; Eden Prairie, MN, 55344, USA) using the VeraBIND™ Tau plasma assay (Fig.1; Soldo, J., Iqbal, K., Bergmann, S., & Ansari, K. Detection of Disease State Macromolecules Binding to Normal Macromolecules as a Biomarker for Disease Identification. PCT/US24/50852. October 10, 2024). Veravas, Inc. and Phanes Biotech, Inc. (271A Great Valley Parkway, Suite 500 Malvern, PA, 19355, USA) developed the new VeraBIND Tau plasma assay using the Veravas VeraBIND™ (Biomarker Isolation and eNrichment for Detection) sample transformation and biomarker purification technology, a pool of proprietary monoclonal antibody-coated capture beads to specifically capture and purify pTau from plasma, supplied by ADx Neurosciences NV, A Fujirebio Company (Technologiepark 6, 9052 Gent, Belgium). Detailed equipment and supplies to run the assay are provided in the Supplementary Methods section.

Prior to starting the VeraBIND Tau assay protocol, the clean beads, capture beads, AD wash buffer, normal tau binding buffer, and freshly diluted recombinant V5-tagged normal tau (nTau) in normal tau bind buffer are equilibrated at room temperature on a laboratory mixer, and the frozen EDTA plasma samples are thawed at 2-8°C in a cooler on a laboratory mixer for 60 minutes. 110 µL of freshly thawed 2-8°C EDTA plasma is added to 550 µL of 2-8°C assay-specific sample diluent in a 2 mL polypropylene (PP) micro tube (Sarstedt, Part No. 72.694.306) for 30 min at 2-8°C with gentle mixing to irreversibly inactivate any endogenous plasma phosphatases. 600 µL of the diluted and conditioned 2-8°C plasma sample is aspirated and dispensed into a 96 well non-sterile, PP 2mL deep well plate with round wells and round bottom (Stellar Scientific, Part No. DWP-WB-3866) with 100 µL custom VeraBIND clean beads to pre-analytically clean the sample for 30 min at 37°C and 1,000 rpm on a plate heater/shaker (Qinstruments BioShake iQ with plate-

specific thermal adapter, Part No. 1808-0506). After the clean beads are magnetically separated on a plate magnet (Alpaqua Magnum FLX® with Solid-Core™ Technology) for 15 min, 700 μ L bead-free sample supernatant is aspirated and dispensed into a new 96 well non-sterile, PP 2mL deep well plate with round wells and round bottom with 100 μ L custom VeraBIND capture beads. The capture beads and sample are mixed for 30 min at 37°C and 1,000 rpm on a plate heater/shaker for the selective capture and purification of any hyperphosphorylated tau (pTau) by the pool of monoclonal antibody-coated capture beads. The capture beads are magnetically washed 4x with 500 μ L AD wash buffer to remove the plasma matrix, and then the capture beads are buffer exchanged, and pipette mixed into 120 μ L normal tau binding buffer to facilitate ionic and hydrophobic binding of PA pTau to recombinant normal tau (nTau). Next, 100 μ L of recombinant V5-tagged full-length normal tau 1-441 (Recombinant Normal Tau-441) is added, pipette mixed and incubated static (no mixing) overnight in a 2-8°C cooler for the binding of the Recombinant Normal Tau-441 by any PA pTau, akin to PA pTau mediated normal tau aggregation observed in the brain of AD patients. The next day, normal tau wash buffer, detection buffer, and substrate are equilibrated at room temperature, the anti-V5 AP Conjugate is freshly diluted, and freshly thawed TruBlock Ultra is added to the detection buffer. After the capture beads are warmed to 37°C for 30 min and magnetically washed 4x with 500 μ L normal tau wash buffer to remove any excess or non-bound Recombinant Normal Tau-441, 100 μ L of detection buffer with Tru Block Ultra is added, pipette mixed and incubated for 5 minutes. 100 μ L of the freshly diluted AP Conjugate is added, pipette mixed and incubated static (no mixing) at 37°C for 30 min to detect any Recombinant Normal Tau-441 bound by PA pTau on the capture beads. After magnetically washing the capture beads 4x with 500 μ L normal tau wash buffer to remove any excess or non-bound AP Conjugate, the capture beads are pipette mixed with 100 μ L normal tau wash buffer, aspirated and

dispensed into a Corning® 96-well white round bottom polystyrene NBS Microplate (Corning, Part No. 3605), magnetically separated on the plate magnet, and the supernatant aspirated and discarded. 100 µL substrate is added, mixed for 60 seconds at 1000 rpm on the heater/shaker, and incubated for 60 min at 30°C static (no mixing) to generate a luminescence signal that is read by a GloMax® Discover Microplate Reader. The relative luminescence units (RLU) generated by the substrate is directly proportional to the amount of Recombinant Normal Tau-441 bound by PA pTau and serves as a representation of the PA pTau captured and purified from the plasma sample.

The semi-quantitative VeraBIND Tau assay results are reported as test result Score which is calculated using an EDTA plasma-based Standard (e.g., EDTA plasma collected from patients negative for tau pathology by [¹⁸F]MK6240 tau-PET imaging, or EDTA plasma collected from apparently healthy adults age 18-32). The Standard is run in duplicate in each assay, and each lot of Standard has a lot-specific correction factor which is used to set the assay cutoff RLU for each run. The test result Score for each sample is calculated by dividing the patient sample test result (RLU) by the assay cutoff RLU, or the test result Score = [Unknown signal response (RLU)] / [(Standard test result signal (RLU))*(Correction Factor)]. A test result Score <1.0 is a negative test result, meaning that PA pTau has not been detected in the plasma sample, while a test result Score ≥1.0 is a positive test result, indicating that PA pTau has been detected in the plasma sample. The VeraBIND Tau assay Negative Control is a pool of EDTA plasma collected from patients negative for tau pathology by [¹⁸F]MK6240 tau-PET imaging or EDTA plasma collected from apparently healthy adults age 18-32 with a test result Score <0.93. The VeraBIND Tau assay Positive Control is a pool of EDTA plasma collected from patients positive for tau pathology by [¹⁸F]MK6240 tau-PET imaging with a test result Score >1.20.

Quantification of plasma pTau217 levels

Following one hour room temperature thawing, pTau217 concentration was measured on a Lumipulse G600II analyzer, using the Lumipulse® G pTau217 Plasma RUO assay (Fujirebio, Ghent, Belgium).

Quantification of plasma pTau181, pTau231 and the Aβ42/40 ratio

Quantification of soluble pTau181, pTau231, Aβ40 and Aβ42 was performed using the SIMOA (Single-Molecular Array, Quanterix)^{7,34} pTau-181 Advantage V2.1 (REF: 104111), pTau-231 Advantage (REF: 102292), and Neurology Plex 3 A (REF:101995) kits. Each plasma sample was thawed to room temperature for one hour before being processed.

Amyloid measurement

The brain amyloid status was determined by lumbar puncture (n=44) or Aβ PET-scan (n=101) with [¹⁸F]Flutemetamol (Vizamyl™, GE Healthcare) or [¹¹C]Pittsburgh compound B (PiB).

In CSF, measurements of Aβ42 were conducted using Lumipulse automated assays.

For the [¹⁸F]Flutemetamol PET-CT, a 30-minutes list-mode acquisition was performed on a Philips Gemini PET (Philips Healthcare, Amsterdam, Netherlands) 90 minutes after intravenous injection (target activity 185±5 MBq). The images were reconstructed as a dynamic scan of 6×5 minutes frames with 2 mm isometric voxels including attenuation, scatter and decay corrections, and time-of-flight information using the manufacturer's standard reconstruction algorithm.

For the [¹¹C]PiB PET-CT, a 20 min list-mode acquisition was performed on a Philips Vereos digital PET (Philips Healthcare, Amsterdam, Netherlands) forty minutes after intravenous injection (target activity 500MBq). Images were reconstructed in 4 x 5 minutes

frames with 2 mm isometric voxels using the manufacturer's reconstruction algorithm which includes attenuation, scatter, and decay corrections, and time-of-flight information using the manufacturer's standard reconstruction algorithm.

For both radiotracers, semi-quantitative PET data were computed using PNEURO software (v4.1; PMOD LLC Technologies, Zurich, Switzerland) following the previously developed Centiloid pipeline³⁵ to return a Centiloid value for each participant.

Participants were considered amyloid positive (A+) for a Centiloid value ≥ 20 ³⁶ or CSF A β 42 levels ≤ 544 pg/mL^{37,38}.

[¹⁸F]MK6240 tau-PET

Acquisition

[¹⁸F]MK6240 (Lantheus Inc.) is an investigational drug studied as a second-generation cerebral tau tangles imaging agent. Radiosynthesis was conducted at KU Leuven (Leuven, Belgium) and delivered to our clinic in less than an hour. Ninety minutes after intravenous administration of [¹⁸F]MK6240 (target activity 185 $\pm$ 5 MBq) a 30-min dynamic list-mode acquisition was performed on a Philips Vereos digital PET-CT (Philips Healthcare, Amsterdam, Netherlands). Images were reconstructed using the manufacturer's reconstruction algorithm, which includes attenuation, scatter, and decay corrections, and time-of-flight information. Point spread function and 1 mm reslicing was also computed using the manufacturer's algorithm to obtain a better resolution recovery³⁹.

Visual Braak-like staging

Two trained nuclear physicians (R.L. and T.G.) determined a visual Braak-like stage (ranging from 0 to 6), by assigning to every participant the furthest Braak region where a significant signal was observed, excluding meningeal non-specific uptake. For some

intricate scans, MRI segmentation was used to assist visual reading. The tau-PET status was considered positive (T+) for Braak-like stages $\geq$ 0.

Regional tau burden quantification

PET images were registered on T1-weighted structural MRI images (acquired using a Signa™ Premier 3 Tesla head scanner - GE Healthcare, Chicago, USA - equipped with a 48-channel phased-array head coil, resolution: 1x1x1mm) using PetSurfer pipeline, a set of tools within FreeSurfer for end-to-end integrated MRI-PET analysis^{40,41}. Standardized Uptake Value ratio (SUVR) values were extracted for all regions from the Desikan-Killiany Atlas⁴², using the cerebellum gray matter as reference region. In this study, analyses focused on the entorhinal and inferior temporal cortex region-of-interests.

Neuropsychological assessment

All participants underwent a neuropsychological testing that evaluated global cognition using the Mini-Mental State Examination (MMSE⁴³) and four specific cognitive domains: verbal episodic memory (Free and Cued Selective Reminding Test, French version⁴⁴), language (Lexis Naming Test, Category and Letter Fluency Test for animals and letter P⁴⁵), executive functions (Trail Making Test part A and B⁴⁶ and Luria's Graphic Sequences⁴⁷), and visuospatial functions (Clock Drawing Test⁴⁸ and Praxis part of the CERAD battery⁴⁹). Z-scores were computed for each cognitive domain based on three measures within for each domain (see Ivanoiu et al., 2015, for more information about cognitive testing⁵⁰), based on an independent sample composed of 32 CU individuals who remained cognitively stable over an eight years. A cognitive domain was considered impaired if the z-score fell below -1.5. Participants were considered to have CI if at least one z-score was below this cut-off and as being CU otherwise.

Statistical analysis

Statistical analyses were performed using R (2022.07.2). The alpha statistical significance threshold was set at .05. Statistical significance was uncorrected for multiple comparisons, otherwise stated.

Participants' characteristics

Group differences in demographics, biomarkers and cognitive measures were assessed using independent-sample t-test when the assumptions of normality and homoscedasticity were satisfied, and Mann-Whitney U test otherwise. Chi-squared tests were used to examine differences in *APOE* ϵ 4 carriership, sex, and A β positivity (A+).

Performance of VeraBIND Tau to predict tau-PET status in A/T groups

We first assessed how VeraBIND Tau could detect AD tau pathology by focusing on the individuals with concordant PET results, either confirming or excluding AD tau pathology. Specifically, we calculated the sensitivity and overall accuracy of VeraBIND Tau in identifying T+ individuals in these two groups, irrespective of cognitive status, and separately in CU and CI participants. We next evaluated the proportions of VeraBIND Tau positivity in individuals with discordant PET results (A-T+ and A+T- groups) and compared them using a Fisher's Exact test.

Performance of VeraBIND Tau to predict amyloid and tau status, compared to other plasma biomarkers

The diagnostic performance of plasma VeraBIND Tau, A β 42/40 ratio, pTau217, pTau181 and pTau231 levels to predict A β and tau-PET status in the entire sample was compared using receiver operating characteristic (ROC) curve analyses and DeLong tests.

We then computed the sensitivity, specificity, overall accuracy, as well as their 95% confidence intervals, for VeraBIND Tau and plasma pTau217 (i.e., the two best performing plasma biomarkers for predicting tau-PET status), using different thresholds to define positivity on the latter. Specifically, we used the 0.142, 0.193, and 0.256 pg/mL cutoffs, which were demonstrated to provide 95% sensitivity, optimize sensitivity and specificity (~92% sensitivity/specificity) and provide 95% specificity to predict amyloid-PET status, respectively, in a large multicentric cohort of 411 individuals (partially overlapping with the current dataset) ¹⁹.

We also provided positive (PPV) and negative (NPV) predictive values for each clinical group, using the observed prevalence of tau-PET positivity in a large, recently published meta-analysis (i.e., 60% in CI individuals, 10% in CU individuals)⁶.

Moreover, we compared the performance of VeraBIND Tau to predict tau-PET positivity in low (Braak-like tau-PET stages 1-3) and high (Braak-like tau-PET stages 4-6) tau pathology stages, to the one of plasma pTau217, to test their performance since the early tau aggregation stages. In a regression model adjusted for age, sex and education, and corrected for multiple comparisons using the Bonferroni method, we first compared the respective values of both plasma assays for each two-by-two comparison among the Braak-like 0, 1-3, and 4-6 groups. Their sensitivity and area under the curve (AUC) to predict tau-PET positivity in Braak-like 1-3 versus Braak-like 0 individuals, and in Braak-like 4-6 versus Braak-like 0 participants were compared using McNemar tests and DeLong tests, respectively. In linear regression models, we also assessed the value of both assays to predict continuous values of entorhinal tau-PET signal in Braak-like stages 0-3 individuals, adjusting for age, sex, and education.

Correlational analyses

Age-adjusted Spearman's rank correlation coefficients were calculated between the VeraBIND Tau RLU ratio and the MMSE score, the episodic memory composite score (z-score), the entorhinal and inferior temporal tau-PET signal (SUVr), as well as the plasma concentrations of pTau217, pTau181 and pTau231 (pg/mL), to assess its interest as a semi-quantitative measure. Correlations were examined both in the entire sample and in analyses restricted to the CU individuals.

Longitudinal analyses

A subsample of 88 individuals (58 CU and 30 CI; 46 A-T-, 7 A+T-, 32 A+T+, and 3 A-T+) had available longitudinal blood drawings (total number of available blood samples=207). The mean follow-up duration was of 1.72 ± 0.94 years (min=0.35 – max=3.85). Participants had between two and five available longitudinal VeraBIND Tau values (Median=3.0, Q1-Q3=2.0-3.0). We first computed the annual rate of change on VeraBIND Tau for each participant using linear regression. We then examined the correlations between this annual rate of change and the entorhinal and inferior temporal tau-PET SUVr, the episodic memory z-score, the MMSE score, the plasma concentrations of pTau217, pTau181, and pTau231, using Spearman's rank coefficients. In addition, we examined the biological and cognitive profiles of individuals who converted on VeraBIND Tau over their follow-up.

RESULTS

Participants' characteristics

Table 1 provides the demographics, cognitive, and biomarker data of the 145 participants. Based on the neuropsychological assessment, 79 participants (54.5%) were classified as clinically unimpaired (CU) and 66 participants (45.5%) as clinically impaired (CI), having

either mild cognitive impairment (n=43/66, 29.6%) or dementia (n=23/66, 15.9%). CU individuals were marginally younger and more educated than CI patients. The number of A+ individuals was higher in CI (n=52/66, 78.8%) than in CU (n=21/79, 26.6%) participants. As expected, MMSE and cognitive measures were significantly lower in CI than in CU individuals, while plasma pTau species concentrations and tau-PET burden in the entorhinal cortex and inferior temporal neocortex were significantly higher in CI than in CU individuals.

VeraBIND Tau result is highly consistent with tau-PET status

In individuals with consistent PET results (i.e., A+T+, A-T-; Table 2), VeraBIND Tau was positive in 3.2% of the A-T- individuals (n=2/63, including one CU and one CI), and 96.7% of the A+T+ individuals (n=58/60, two CI patients having a negative result), achieving an overall accuracy of 96.7%. The diagnostic accuracy was relatively similar across clinical groups (CU: n=66/67, 98.5%; CI: n=53/56, 94.6%). The only A-T- CI patient with positive VeraBIND Tau result had apraxia of speech, a clinical phenotype suspected to be due to corticobasal degeneration, a primary 4R-tauopathy that is not detected by [¹⁸F]MK6240 tau-PET^{51,52}.

In individuals with discordant PET results (i.e., A+T-, A-T+; Table 2), VeraBIND Tau was negative in all but one A+T- participants (n=1/13, 7.7%), who was CI. Moreover, positive VeraBIND Tau results were observed in 77.8% (n=7/9) of the A-T+ cases, including all five A-T+ CI patients (Braak-like tau-PET stage 1-2 in four cases and one case with Braak-like tau-PET stage 4 and subthreshold amyloidosis), suspected to suffer from primary age-related tauopathy (PART) or fronto-temporal lobar degeneration (FTLD). The proportion of positive VeraBIND Tau results was higher in A-T+ cases (n=7/9, 77.8%) than in A+T-

cases ($n=1/13$, 7.7%; Fisher's Exact test $p=0.001$), indicating greater concordance between VeraBIND Tau and tau-PET status than with A β status.

Comparison of VeraBIND Tau with other plasma biomarkers

ROC curves predicting tau-PET status computed on the entire sample ($n=145$; Fig. 2.A) indicated that the largest AUC was found for VeraBIND Tau (AUC=0.97), followed by pTau217 plasma concentration (AUC=0.92). DeLong Tests indicated that VeraBIND Tau better predicted tau-PET positivity compared to all the other blood-based biomarkers (VeraBIND Tau vs. pTau217: $z=1.96$, $p=0.049$; VeraBIND Tau vs. pTau181 [missing data=3], pTau231 [missing data=27] or A β 42/A β 40 [missing data=6]: all z 's >3.68 , $p<0.001$), while the highest AUC (AUC=0.93) for predicting the A β status was found for plasma pTau217 (Fig. 2.B; DeLong Tests: pTau217 vs. VeraBIND Tau: $z=1.89$, $p=0.058$; pTau217 vs. pTau181: $z=3.72$, $p=0.0002$; pTau217 vs. pTau231: $z=4.37$, $p<0.001$; pTau217 vs. A β 42/A β 40: $z=3.03$, $p=0.002$).

We next compared the diagnostic performance of VeraBIND Tau for predicting tau-PET positivity with that of plasma pTau217 at different thresholds (the second-best performing plasma biomarker, Table 3). The sensitivity, specificity, overall accuracy, PPV and NPV for tau-PET positivity were systematically higher for VeraBIND Tau than for plasma pTau217, regardless of the threshold used. Remarkably, the VeraBIND Tau PPV was 85.9% in CU participants, while the highest PPV found for pTau217 only achieved 57.5% at the 0.256pg/mL threshold.

VeraBIND Tau outperforms plasma pTau217 to detect low tau pathology stages

To identify the participants driving the additional accuracy of VeraBIND Tau vs. pTau217 plasma concentration, we compared diagnostic performances of both plasma biomarkers in low (Braak-like 1-3) and high tau-PET stages (Braak-like 4-6; Fig. 3.A-B). In a regression model adjusted for age, sex and education, and corrected for multiple comparisons using the Bonferroni method, the VeraBIND Tau Score was elevated in both Braak-like 1-3 (Mean=1.10, SE=0.05, β =0.24, 95% CI [0.11-0.36], $p<0.001$) and Braak-like 4-6 groups (Mean=1.16, SE=0.03, β =0.29, 95% CI [0.20-0.37], $p<0.001$), compared to Braak-like 0 individuals (Mean=0.87, SE=0.02), while Braak-like 1-3 and Braak-like 4-6 groups did not differ (β =-0.05, 95% CI [-0.07-0.18], $p=0.98$). In contrast, plasma pTau217 was increased in the Braak-like 4-6 group (Mean=0.71, SE=0.04), compared to both Braak-like 0 (Mean=0.134, SE=0.04, β =0.58, 95% CI [0.44-0.72], $p<0.001$) and 1-3 groups (Mean=0.320, SE=0.08, β =0.40, 95% CI [0.18-0.61], $p<0.001$), which did not differ (β =0.19, 95% CI [-0.02-0.40], $p=0.10$).

In Braak-like 1-3 individuals, VeraBIND Tau was positive in 88.2% (n=15/17) individuals, whereas pTau217 was positive in 64.7% (n=11/17), 41.2% (n=7/11), and 23.5% (n=4/17) individuals at the 0.142, 0.193, and 0.256 thresholds, respectively (Fig. 3.A-B). McNemar tests indicated that VeraBIND Tau sensitivity was higher than pTau217 sensitivity at the 0.193 ($\chi^2=4.90$, $p=0.03$) and 0.256 cutoffs ($\chi^2=7.69$, $p=0.006$). At the 0.142 threshold, the sensitivity did not differ between VeraBIND Tau and pTau217 ($\chi^2=1.1$, $p=0.29$), but at this cutoff, pTau217 was also positive in 28.9% (n=22/76) Braak-like 0 participants (T-) against only 3.9% (n=3/76) for VeraBIND Tau. The AUC for detecting tau-PET signals in Braak-like 1-3 vs. Braak-like 0 individuals was higher for VeraBIND Tau (AUC=0.96) than for plasma pTau217 concentration (AUC=0.74, $z=2.99$, $p=0.003$). In contrast, VeraBIND Tau (n=50/52, 96.2% sensitivity, AUC=0.98) and pTau217 (0.142 and 0.193 thresholds:

n=52/52, 100% sensitivity; 0.256 threshold: n=48/52, 92.3% sensitivity; AUC=0.98) had similar sensitivity and AUC ($z=-0.43$, $p=0.66$) to distinguish individuals with Braak-like 4-6 from Braak-like 0 participants. Importantly, the high sensitivity of VeraBIND Tau for tau-PET positivity, compared with plasma pTau217, was observed as early as Braak-like stages 1-2 (Fig. 3.C-D).

In a regression model adjusted for age, sex, and education, and limited to 93 individuals with Braak-like stage 0–3, VeraBIND Tau demonstrated a significant association with entorhinal tau-PET signal ($\beta=1.69$, 95% CI [1.27-2.10], $p<0.001$), whereas plasma pTau217 concentration did not ($\beta=0.31$, 95% CI [-0.06-0.68], $p=0.10$).

VeraBIND Tau semi-quantitative measure is associated with cognitive, tau-PET and plasma measures

To assess the utility of the VeraBIND Tau score as a semi-quantitative measure, we correlated this novel plasma measure with the MMSE (Fig. 4.A), an episodic memory composite score (Fig. 4.B), the entorhinal and inferior temporal tau-PET signal (SUVR; Fig. 4.C-D), as well as the plasma concentration of pTau217 (Fig. 4.E), pTau181 (Fig. 4.F), and pTau231. All these associations were significant (all age-adjusted Spearman's $\rho>0.35$, all p -values <0.001). When restricting the analysis to the 79 CU participants, high VeraBIND Tau scores were still significantly associated with high entorhinal tau-PET signal ($r_s=0.52$, $p<0.0001$), inferior temporal tau-PET signal ($r_s=0.43$, $p<0.0001$), plasma pTau217 ($r_s=0.42$, $p=0.0001$) and pTau181 ($r_s=0.39$, $p=0.0005$) concentrations, indicating that these associations were not entirely driven by the CI participants. Consistent with the relatively low variability of cognitive performance on standard neuropsychological tests in CU individuals, VeraBIND Tau scores were not associated with the MMSE scores

($r_s=0.10$, $p=0.38$) and showed only marginal association with the episodic memory scores ($r_s=-0.20$, $p=0.08$). A marginal association was also found with plasma concentration of pTau231 in CU individuals (available in 70/79 individuals, $r_s=0.23$, $p=0.054$).

VeraBIND Tau annual rate of change is associated with disease severity proxy measures

The annual change in VeraBIND Tau Score correlated with cross-sectional entorhinal tau-PET SUVR (age-adjusted Spearman's $\rho=0.23$, $p=0.03$), Braak-like tau-PET stages ($r_s=0.29$, $p=0.006$), MMSE scores ($r_s=-0.38$, $p=0.0003$), episodic memory z-score ($r_s=-0.33$, $p=0.002$), plasma concentrations of pTau217 ($r_s=0.29$, $p=0.007$), and pTau181 ($r_s=0.30$, $p=0.005$), suggesting that VeraBIND Tau rate of change increases with disease progression. The association between the annual VeraBIND Tau change with pTau231 plasma concentration (available in 84/88 participants) was not significant ($r_s=0.08$, $p=0.48$), while the association with the inferior temporal tau-PET SUVR was marginal ($r_s=0.21$, $p=0.054$).

Five participants (5.7%) initially negative on VeraBIND Tau (VB-) converted to positivity (VB+) during follow-up (Fig. 5), while the other participants remained either positive ($n=32/88$, 36.4%) or negative ($n=51/88$, 57.9%). Progressors included three A-T- CU individuals who were VB- at the time of baseline tau-PET, one A+T+ CU individual with a Braak-like tau-PET stage 2 who had three closely spaced datapoints and was VB+ at the datapoint closest in time to baseline tau-PET, and one A+T+ CI participant with a Braak-like tau-PET stage 4 who was VB- two years before tau-PET but VB+ at the datapoint closest in time to baseline tau-PET.

DISCUSSION

In this study, we assessed VeraBIND Tau, a new plasma biomarker designed to reflect pathological pTau seeding activity, against [¹⁸F]MK6240 tau-PET imaging as standard of truth. Over a sample of 145 individuals, we found that the VeraBIND Tau assay outperformed the plasma levels of pTau217, pTau181, and pTau231 to predict tau-PET positivity, while, as in previous work^{14,53–57}, plasma pTau217 best predicted Aβ positivity. This high diagnostic performance was driven by the detection of individuals with low stages of brain tau deposition (Braak-like tau-PET stages 1-3). Consequently, VeraBIND Tau provided high positive predictive value (85.9%) in CU individuals, opening the possibility of screening older adults, which is not feasible with previously available plasma tests (e.g., pTau217 PPV=57.5% using a high threshold of 0.256pg/mL).

The development of plasma biomarkers reflecting tau aggregated pathology is of utmost importance, given its high prediction of cognitive decline^{5,6}. The National Institute on Aging and the Alzheimer's Association (NIA-AA) revised criteria for diagnosis and staging of AD¹ positioned emerging biofluid measures of pTau205^{58,59} and microtubule-binding region containing residue 243 (MTBR-tau243)⁶⁰ as Core 2 biomarkers, reflecting aggregated tau, besides tau-PET imaging. However, unlike tau-PET, none of these Core 2 fluid biomarkers are currently validated for clinical use. Both these fluid biomarkers have demonstrated high accuracy to predict tau-PET results when measured in the cerebrospinal fluid^{58–60}. In plasma, the endogenously cleaved MTBR-tau243 (eMTBR-tau243) showed the strongest correlation with tau-PET signal and cognition, compared to pTau205 and pTau217, in mixed samples of CI and CU individuals⁵³. In addition, the eMTBR-tau243 outperformed pTau205 and pTau217 to predict tau-PET status, especially at intermediate and advanced stages of NFT pathology (i.e., Braak-like tau-PET stages ≥3). Its accuracy to

predict abnormal tau status in low Braak-like tau-PET stages was higher than pTau205 but comparable to pTau217⁵³. High performance to identify individuals with advanced Braak stages was also evidenced for another ultrasensitive plasma assay targeting N-terminal containing tau fragments⁶¹. These results suggest that tracking early tau aggregation in blood remains challenging.

The major finding of this study was that the VeraBIND Tau assay showed high diagnostic performance to detect tau-PET positivity at both low (≤ 3 ; AUC=0.96) and high (≥ 4 ; AUC=0.98) Braak-like stages. Specifically, detection of individuals with low Braak-like stages was better with VeraBIND Tau (AUC=0.96, 88.2% sensitivity) than with pTau217 (AUC=0.74, 64.7% sensitivity using a low threshold=0.142pg/mL). In contrast, the ability to detect individuals with high Braak-like tau-PET stages was comparable for both assays (AUC=0.98). Furthermore, in individuals with Braak-like tau-PET stages 0-3, we found that only the VeraBIND Tau semi-quantitative measure predicted entorhinal tau-PET signal, whereas the plasma pTau217 did not. The low discriminative ability of plasma pTau217 to identify early tau-PET stages aligns with previous findings^{28,29,62}. This observation is also consistent with the lower accuracy of pTau217 to detect AD pathology in CU than in CI individuals^{24,25}, which is likely due to lower pathology burden in CU than in CI individuals and argues against any stand-alone application in CU individuals⁶³. In contrast, VeraBIND Tau demonstrated similar diagnostic accuracies in CI and CU individuals. While validation in independent cohorts is needed, these results suggest that the VeraBIND Tau assay is a highly promising, scalable tool to identify individuals with a high risk of clinical progression to AD, since the early tau deposition stages (i.e., the entorhinal stage). Improving the first-line screening workflow in CU individuals is key for informing clinical trials and future clinical practices focusing on prevention and brain health. Further research in larger CU cohorts will be needed to assess the isolated and/or

complementary value of the VeraBIND Tau assay to optimize triage strategies and reduce the need for costly confirmatory examinations.

Similarly, future work should investigate whether a two-test approach combining both assays may also constitute a valuable fluid-first workflow in a clinical setting. In symptomatic patients, our results suggest that the biological assessment may benefit from both plasma pTau217, as a Core 1 biomarker reflecting amyloid pathology, and VeraBIND Tau assays, as a Core 2 biomarker reflecting tau pathology. Concordant positive results on both plasma tests would be supportive of an AD etiology as the primary driver of cognitive impairment, while concordant negative results would exclude this possibility.

Moreover, this study highlighted that the VeraBIND Tau assay may have affinity for detecting non-AD tauopathy, as results were positive in 77.8% of A-T+ individuals (n=7/9), including five A-T+ CI patients who were suspected to suffer from primary age-related tauopathy (PART) or fronto-temporal lobar degeneration (FTLD), and in a single A-T- case clinically suspected to suffer from cortico-basal degeneration, a 4R tauopathy. The diagnostic performance of the VeraBIND Tau assay for non-AD tauopathies needs to be further investigated using radiotracers that are more sensitive to 4R tauopathies than [¹⁸F]MK6240⁵¹.

Further longitudinal work is required before considering the use of VeraBIND Tau for monitoring the effects of anti-tau therapies. The annual change in VeraBIND Tau Score correlated with cross-sectional disease severity proxy metrics, including the entorhinal tau-PET burden, Braak-like tau-PET stages, episodic memory scores, MMSE scores, as well as plasma concentrations of pTau217 and pTau181, suggesting that VeraBIND Tau rate of change increases with disease progression. Moreover, five participants (5.7%) converted to VeraBIND Tau positivity, whereas 83/88 (94.3%) maintained stable results (positive or negative) throughout follow-up, indicating good test-retest reproducibility. The five

individuals who converted to positivity included three A-T- CU individuals who were negative on VeraBIND Tau at the time of baseline tau-PET, and two A+T+ individuals (one CU, one CI) who were negative on a data-point preceding the one closest in time to tau-PET. Ongoing longitudinal follow-up will help further characterize the biological and cognitive trajectories of the A-T- CU individuals who converted on VeraBIND Tau over their follow-up. Together, our longitudinal results suggest that VeraBIND Tau may serve to monitor whether tau remains pathologically active or not, but its ability to parallel tau-PET signals over time needs further investigation.

Strengths of this study include head-to-head comparisons of VeraBIND Tau assay to several plasma biomarkers, a study cohort including well-characterized participants at the clinical and biological levels, and the inclusion of longitudinal plasma data. The VeraBIND Tau assay has the advantage of relying on isolation and enrichment techniques that are compatible with the immunoassay format, which is easier to integrate in clinical workflow compared to mass spectrometry. Limitations inherent to the current study include the lack of a validation cohort, and a relatively homogenous participant sample at the educational and racial levels. In addition, the CU individuals were enriched in *APOE* ϵ 4 carriers, leading to an overrepresentation of individuals at elevated risk for developing AD^{64,65} relative to the general population.

Despite these limitations, our study indicates that the VeraBIND Tau assay reliably captures early tau pathology regardless of amyloid status. Given its high accuracy for predicting tau-PET positivity, this assay can serve as a Core 2 plasma biomarker of underlying tau proteinopathy, as defined in the NIA-AA AD diagnostic framework¹. While further validation is needed, the VeraBIND Tau assay holds strong promise as a scalable and cost-effective alternative to tau-PET imaging for detecting active tau pathology in CU individuals and for diagnosing symptomatic patients with suspected 3R/4R tau pathology

(AD and PART). Moreover, our results support a potential utility of VeraBIND Tau for monitoring tau-related pathological activity in clinical trials.

Data Availability

Dr. Hanseeuw has full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Request for data for replication studies or meta-analyses can be sent to the following email address: bernard.hanseeuw@uclouvain.be.

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Competing interests

J. Soldo is coinventor of patents (pending) on the VeraBIND Assay and is Chief Scientific Officer & Co-Founder of Veravas, Inc., which holds equity in the assay. K. Shamsundar is R&D Manager of Veravas, Inc, and W. Pak is Head of Medical Affairs (Consulting) of Veravas, Inc.

K. Iqbal is coinventor of patents (pending) on VeraBIND Assay and is the Chief Scientific Officer of Phanes Biotech, Inc., which holds equity in the assay.

The other authors have nothing to disclose.

Author Contributions

Conceptualization: BH, KI; Methodology: BH, KS, WP, JS, KI; Investigation: LQ, JLB, EB, LC, YS, TG, LH, VM, RL, AI; Software: FBP; Formal analysis: BH, LQ; Visualization: BH, LQ; Writing – original draft: BH, LQ, JS, KI; Writing – review & editing: all authors; Supervision: BH, LD, PKC, KI; Funding acquisition: BH

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Tables and Figures

Table 1. Participants' characteristics

	All N=145 Mean (SD)	CU N=79 Mean (SD)	CI N=66 Mean (SD)	p-value
Age at tau-PET (years)	70.3 (8.4)	69.3 (7.8)	71.5 (9.0)	0.07
Male (%)	44.8%	39.2%	51.5%	0.19
Education (years)	16.3 (3.5)	16.9 (2.9)	15.5 (4.0)	0.02
APOE ε4 carriers (%)	53.8%	48.1%	60.1%	0.18
Aβ positivity (A+, %)	50.3%	26.6%	78.8%	<0.001
MMSE (/30)	26.8 (3.6)	28.9 (1.0)	24.3 (4.0)	<0.001
Episodic memory z-score	-1.3 (2.3)	0.2 (0.7)	-3.3 (2.0)	<0.001
Entorhinal tau burden (SUVr)	n=139 1.6 (0.9)	1.0 (0.4)	n=60 2.2 (1.0)	<0.001
Inferior temporal tau burden (SUVr)	n=141 1.5 (1.0)	1.0 (0.3)	n=62 2.1 (1.2)	<0.001
Plasma pTau181 (pg/mL)	n=142 33.8 (24.2)	n=77 24.7 (14.5)	n=65 44.6 (28.7)	<0.001
Plasma pTau231 (pg/mL)	n=118 6.4 (4.3)	n=70 5.2 (2.9)	n=48 8.1 (5.3)	<0.001
Plasma pTau217 (pg/mL)	0.37 (0.41)	0.19 (0.22)	0.58 (0.48)	<0.001
VeraBIND Tau Assay (RLU)	1.00 (0.23)	0.92 (0.14)	1.10 (0.27)	<0.001

Note. In case of missing data, the final sample size is reported below the Mean (SD). CU and CI groups were compared using t-tests for continuous normally distributed variables, Mann-Whitney tests for continuous non-normally distributed variables, χ^2 tests for categorical variables (i.e., sex, APOE ε4 carriership, and amyloid positivity). Aβ positivity (A+) was determined using lumbar puncture (CSF Aβ42 levels ≤ 544 pg/mL) or Aβ-PET with [¹⁸F]-Flutemetamol or Pittsburgh compound B (Centiloid ≥ 20).

Significant p-values (<0.05) are highlighted in bold.

CU = cognitively unimpaired; CI = cognitively impaired; SD = standard deviation; SUVr = Standard Uptake Value ratio; pg = picogram; mL = milliliter; RLU = Relative Luminescence Unit.

Table 2. Results of VeraBIND Tau Test by clinical and A/T status

	A-T-		A+T-		A+T+		A-T+	
CU (n=79)								
VeraBIND Tau result	53 –	1 +	8 –	0 +	0 –	13 +	2 –	2 +
% Positivity	1.9%		0%		100%		50%	
CI (n=66)								
VeraBIND Tau result	8 –	1 +	4 –	1 +	2 –	45 +	0 –	5 +
% Positivity	11.1%		20.0%		95.7%		100.0%	
Entire sample (n=145)								
VeraBIND Tau result	61 –	2 +	12 –	1 +	2 –	58 +	2 –	7 +
% Positivity	3.2%		7.7%		96.7%		77.8%	

Note. CU = cognitively unimpaired, CI = cognitively impaired. The – and + symbols denote the negative or positive test result. The A β (A) status was determined using lumbar puncture (positive for CSF A β 42 levels $\leq$ 544 pg/mL) or A β -PET (positive for Centiloid $\geq$ 20). The tau (T) status was defined using [^{18} F]MK6240 tau-PET (positive for Braak-like tau-PET stages $>$ 0). The A-T- individual with positive VeraBIND Tau score has a clinical diagnosis of apraxia of speech, a clinical phenotype suspected of cortico-basal degeneration.

Table 3. Diagnostic performance of VeraBIND Tau Test for predicting tau-PET positivity (Braak-like tau-PET stage>0), compared to plasma pTau217.

	VeraBIND Tau + for ≥ 1.0		Plasma pTau217 + for ≥ 0.142 pg/mL [†]		Plasma pTau217 + for ≥ 0.193 pg/mL [†]		Plasma pTau217 + for ≥ 0.256 pg/mL [†]	
	Value	95% CI	Value	95% CI	Value	95% CI	Value	95% CI
Entire sample (n=145)								
Sensitivity	94.2%	85.8 – 98.4	91.3%	82.0 – 96.7	85.5%	75.0 – 92.8	75.4%	63.5 – 85.0
Specificity	96.1%	88.9 – 99.2	71.1%	59.5 – 80.9	85.5%	75.6 – 92.6	92.1%	83.6 – 97.1
Accuracy	95.2%	90.3 – 98.0	80.7%	73.3 – 86.8	85.5%	78.7 – 90.8	84.1%	77.2 – 89.7
CU individuals (n=79)								
Sensitivity	88.2%	63.6 – 98.5	88.2%	63.6 – 98.5	76.5%	50.1 – 93.2	58.8%	32.9 – 81.6
Specificity	98.4%	91.3 – 100.0	80.7%	68.6 – 89.6	91.9%	82.2 – 97.3	95.2%	86.5 – 99.0
Accuracy	97.4%	91.0 – 99.7	81.4%	71.1 – 89.3	90.4%	81.7 – 95.9	91.5%	83.1 – 96.6
PPV [‡]	85.9%	46.3 – 97.7	33.6%	22.8 – 46.4	51.3%	30.4 – 71.8	57.5%	29.5 – 81.4
NPV [‡]	98.7%	95.3 – 99.6	98.4%	94.4 – 99.6	97.2%	93.7 – 98.8	91.5%	92.2 – 97.4
CI individuals (n=66)								
Sensitivity	96.2%	86.8 – 99.5	92.3%	81.5 – 97.9	88.5%	76.6 – 95.7	80.8%	67.5 – 90.4
Specificity	85.7%	57.2 – 98.2	28.6%	8.4 – 58.1	57.1%	28.9 – 82.3	78.6%	49.2 – 95.3
Accuracy	92.0%	82.6 – 97.2	66.8%	54.1 – 77.9	75.9%	63.8 – 85.6	79.9%	68.2 – 88.8
PPV [‡]	91.0%	73.7 – 97.3	66.0%	58.0 – 73.2	75.6%	62.7 – 85.1	85.0%	67.3 – 94.0
NPV [‡]	93.7%	79.0 – 98.3	71.2%	41.4 – 89.7	76.8%	57.8 – 88.8	73.2%	59.4 – 83.5

Note. CU = cognitively unimpaired, CI = cognitively impaired.

[†] The 0.142 pg/mL pTau217 threshold was found to provide 95% sensitivity for detecting brain amyloidosis in a large multicentric cohort of 411 individuals (partially overlapping with the current dataset), whereas the 0.193 pg/mL threshold optimally balanced sensitivity and specificity (~92%), and the 0.256 pg/mL threshold provided 95% specificity for excluding brain amyloidosis¹⁹.

[‡] Positive and negative predictive values (PPV, NPV) were calculated using the observed prevalence of tau-PET positivity in a large, recently published meta-analysis (i.e., 60% in CI individuals, 10% in CU individuals)⁶.

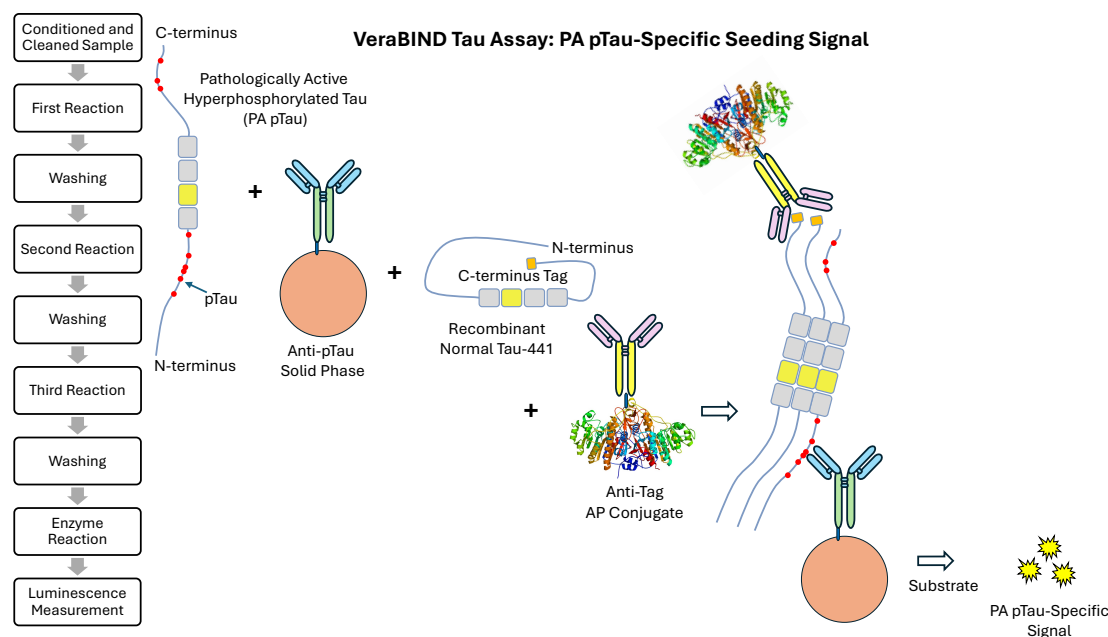


Fig. 1. VeraBIND Tau assay. The VeraBIND Tau assay uses a pool of different antibody-coated capture beads to capture hyperphosphorylated tau (pTau) in the plasma sample. The capture beads are washed to remove the plasma matrix, and buffer-exchanged/washed into a normal Tau binding buffer that mimic the pH, ionic and hydrophobic binding environment in the brain. The assay adds recombinant full-length normal Tau 1-441 with a recombinant tag at the C-terminus (recombinant normal tau) to determine if any of the pTau captured on the beads is pathologically active (PA pTau). If pTau captured from the plasma is pathologically active, it will bind the recombinant normal tau like it does in the brain with tau pathology. The final step washes the capture beads to remove unbound normal tau, adds an alkaline phosphatase conjugated anti-tag monoclonal antibody (AP Conjugate), washes the capture beads to remove unbound AP Conjugate, and adds Substrate to generate a relative luminescence signal (RLU) that is directly proportional to the amount of recombinant normal tau bound by PA pTau in the plasma sample.

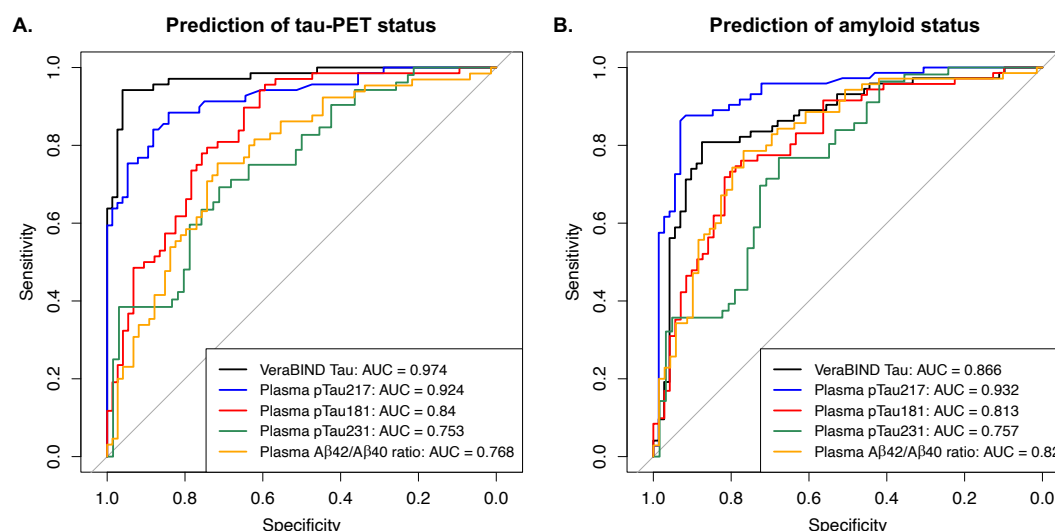


Fig. 2. Receiver Operating Characteristic (ROC) curves comparing the performance of VeraBIND Tau, plasma pTau217, pTau181 (missing data=3), pTau231 (missing data=27), and the Ab42/Ab40 ratio (missing data=6) for predicting: **A.** tau-PET positivity (Braak-like tau-PET stage>0), **B.** amyloid positivity (Centiloid $\geq$ 20 or CSF A β 42 $\leq$ 544 pg/mL).

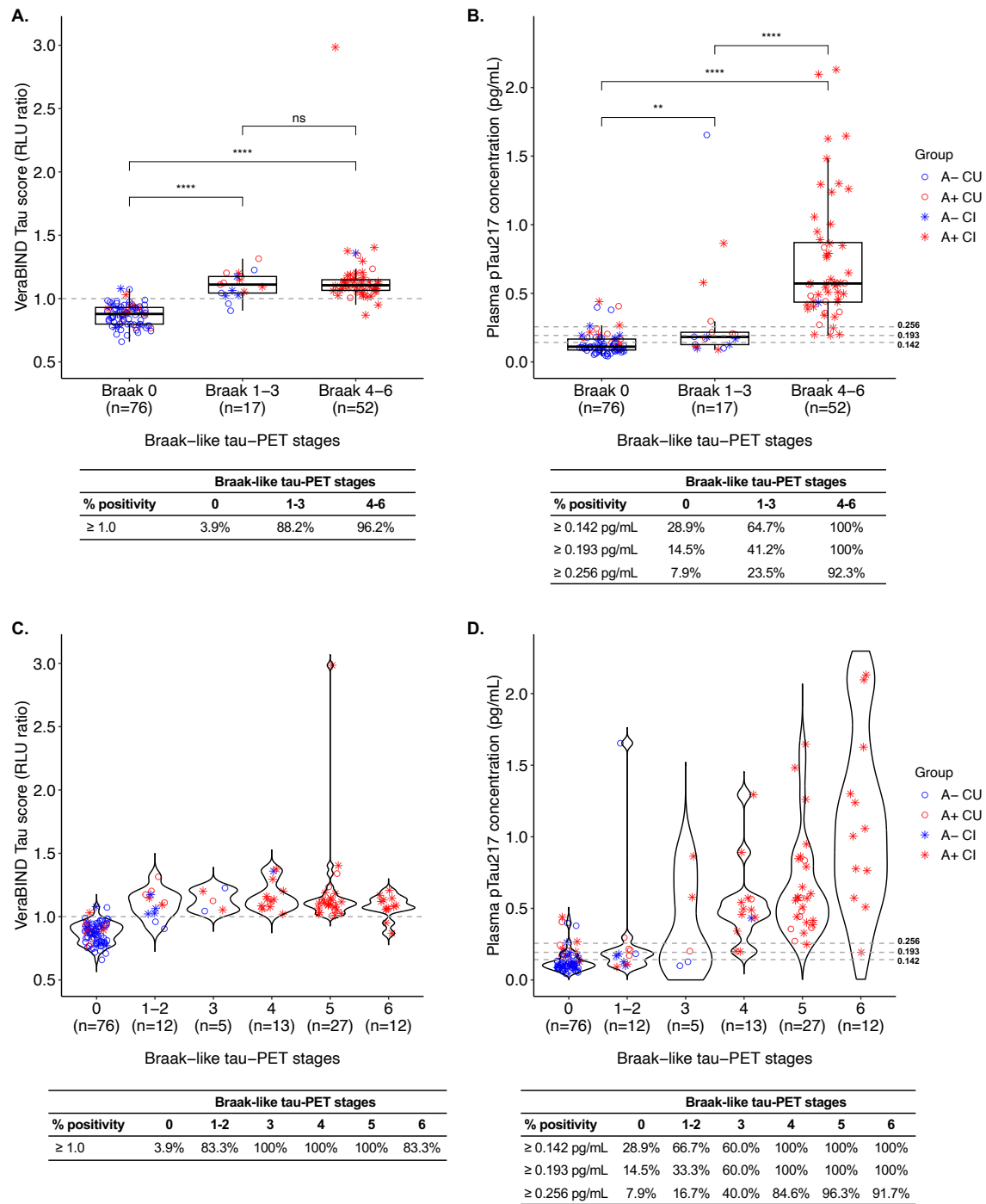


Fig. 3. Plasma levels and positivity rates for: **A-B.** VeraBIND Tau and different threshold plasma pTau217, respectively, in visually negative tau-PET individuals (Braak-like 0), low (Braak-like 1-3) and advanced (4-6) Braak-like tau-PET stage groups, **C.D.**

VeraBIND Tau and different threshold plasma pTau217, respectively, in fine-grained Braak-like tau-PET stage groups.

pg = picogram; mL = milliliter; A- = amyloid negative; A+ = amyloid positive; CU = cognitively unimpaired; CI = cognitively impaired.

ns $p \geq .05$; ** $p < .01$, **** $p < .0001$

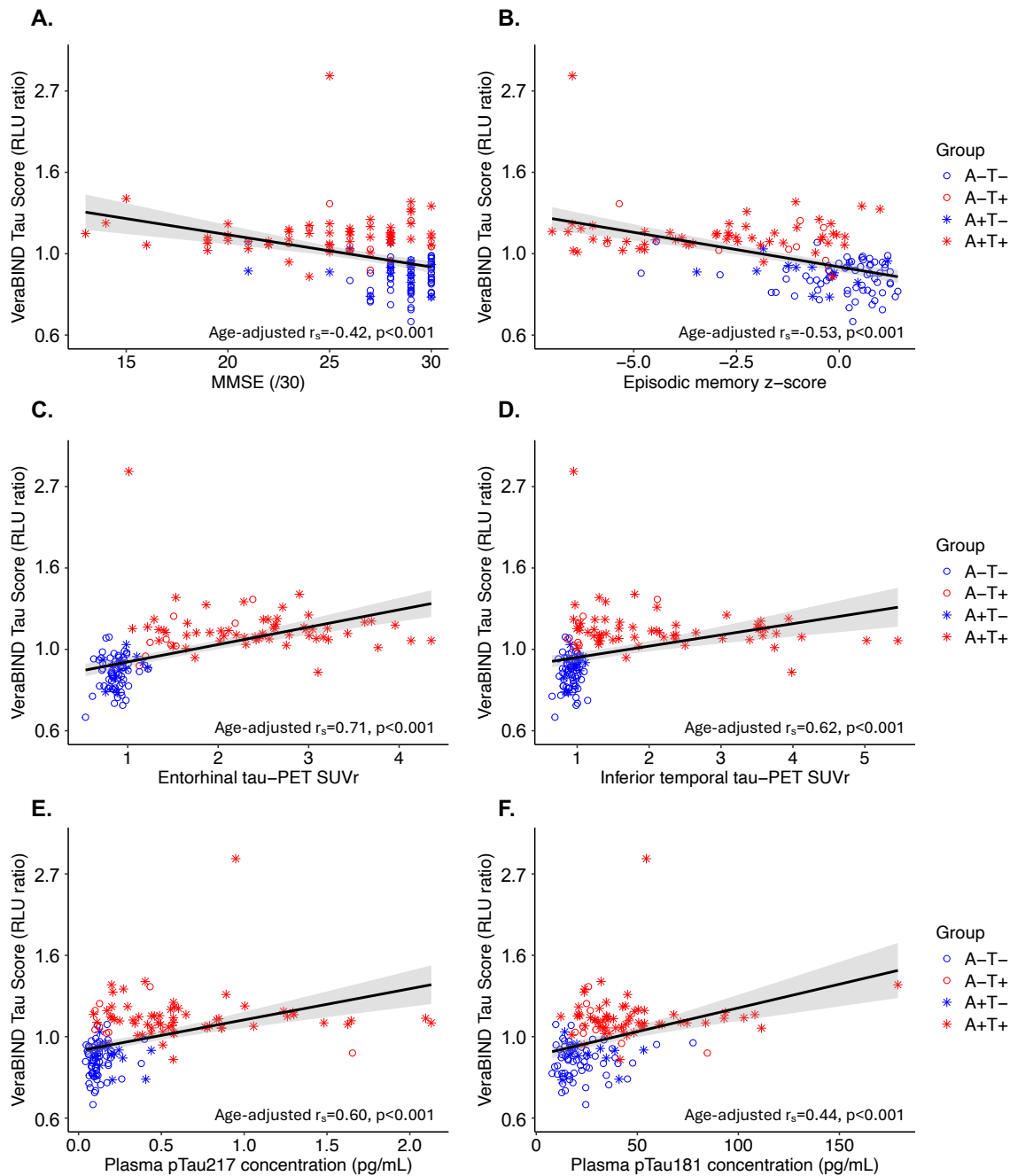


Fig. 4. Associations between VeraBIND Tau semi-quantitative measure and: **A.** Mini-Mental State Examination score (MMSE), **B.** Episodic memory composite score (z-score), **C.** Entorhinal tau burden, as measured using [^{18}F]MK6240 tau-PET Standard Uptake Value ratio (SUVR), **D.** Inferior temporal tau burden (SUVR), **E.** Plasma concentration of pTau217 (pg/mL), **F.** Plasma concentration of pTau181 (pg/mL).

VeraBIND Tau semi-quantitative scores were plotted on a logarithmic scale.

r_s = Spearman's rho.

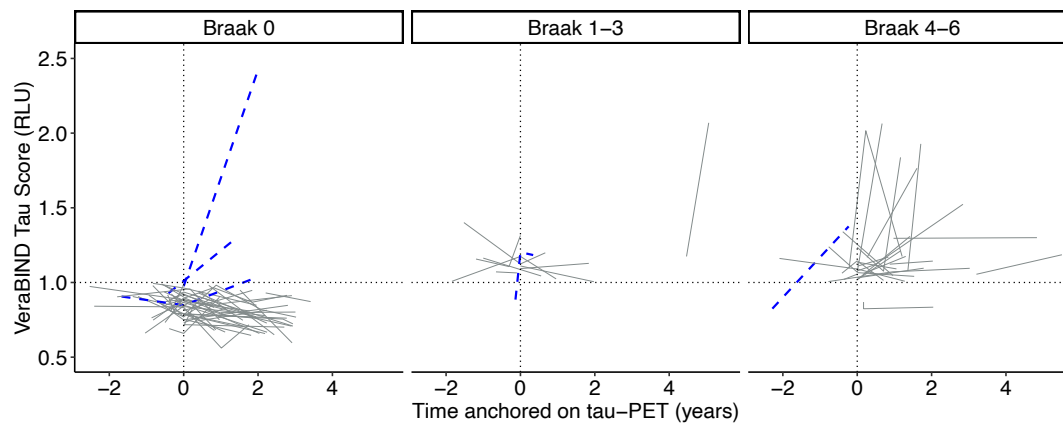


Fig. 5. Longitudinal VeraBIND Tau scores (relative radioluminescence ratio unit ; RLU) in Braak-like tau-PET stage groups. The blue dashed lines represent individuals who converted from a negative test result to a positive test result over their follow-up.