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Greater Specificity for Cerebrospinal Fluid P-tau231 over P-tau181 in the Differentiation of Healthy Controls from Alzheimer's Disease

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Abstract

Cerebrospinal fluid (CSF) measures of phosphorylated-tau (P-tau) 231 and P-tau181 are two biomarkers for the identification of tau pathology as related to Alzheimer's disease (AD). While both are pathologically validated, their relative diagnostic performances are not well known. This cross-sectional diagnostic study of 87 normal (NL) subjects and 28 AD subjects compared CSF P-tau231 with CSF P-tau181. Logistic regression modeling demonstrated that the P-tau231 was superior to the P-tau181 in the diagnostic classifications. At a fixed 85% sensitivity cutoff, the ROC analysis shows that P-tau231 has greater overall specificity than P-tau181. While both P-tau analytes demonstrated equivalent negative predictive accuracies, P-tau231 yielded significantly fewer false positives. Moreover, P-tau231, but not P-tau181, demonstrated sensitivity to the E4 genotype. A postmortem validation with 9 AD subjects confirmed the superiority of the CSF P-

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tau231 specificity. This study suggests that P-tau231 has the potential to improve the CSF tau biomarker diagnosis of AD.

Keywords

Alzheimer's disease; biomarkers; hyperphosphorylated tau; P-tau181; P-tau231

INTRODUCTION

In Alzheimer's disease (AD), hyperphosphorylated tau (P-tau) accumulates in the brain as neurofibrillary tangles and neuropil threads, which are associated with neuronal degeneration and cognitive deterioration [1]. Multiple hyperphosphorylation sites for tau have been identified and the most commonly used for CSF assays are tau phosphorylated at threonine 181 (P-tau181) and threonine 231 (P-tau231) (for review, see Hampel et al. [2]).

Prior pathological observations have shown that antibodies for both P-tau181 and P-tau231 stain neurofibrillary tangles in regions known to be vulnerable to tangle formation in AD; however, P-tau231 has advantages with respect to labeling vulnerable hippocampal formation tissues showing early tauopathy [3]. While one meta-analysis of clinical CSF studies showed that P-tau181 and P-tau231 measurements were comparably good biomarkers for the diagnosis of AD [4], a series of postmortem studies that examined both analytes reported that CSF P-tau231 was better associated with neocortical neurofibrillary pathology than CSF P-tau181 [5, 6]. As limited numbers of carefully screened aged normal volunteer subjects have participated in prior CSF analyte comparison studies, it has been challenging to assess the frequency of false positive P-tau measurements. In our study, we compared the diagnostic performance of P-tau181 and P-tau231 in 87 older normal control and 28 AD subjects of whom a subset received postmortem confirmation of AD. All subjects were derived from a single site using standardized clinical and postmortem criteria with the CSF samples studied in batch mode.

METHODS

Design

We conducted a cross sectional study of 115 subjects including 87 normal (NL) and 28 AD subjects. A postmortem validation study was available for 9 AD and one normal subject. We tested the null hypothesis that with a cut-off sensitivity of 85% (detection of AD), P-tau181 and P-tau231 were equivalent in the classification of the NL group (specificity).

Subjects and groups

The NL and AD subjects for this project were recruited from caregivers accompanying research patients and from voter registration lists of the area surrounding New York University. All subjects gave institutional review board (IRB) approved informed consent to participate in our National Institute of Health (NIH) supported CSF and imaging studies of aging and AD. The AD subjects enrolled were judged capable of understanding the consent forms as determined by an independent clinician. All patients and controls were evaluated

using standard NIH-National Alzheimer Coordinating Center (NACC) protocols that included: history, physical and neurological assessments, psychiatric screens, clinical labs, MRI imaging, and neuropsychological evaluations. The subjects ranged in age from 51 to 85 (mean age 66.0 ± 9.3) and had a minimum of 12 years education (see Table 1). After ruling out and excluding individuals with competing causes of dementia, the diagnosis of AD was based on progressive memory and cognitive complaints corroborated by an informant, and clinically observed cognitive impairment. The AD subjects had Global Deterioration Scale (GDS) scores of 4 or 5. All AD patients met DSM-IV [7] and NINCDS-ADRDA [8] criteria for dementia. The NL subjects were defined on the basis of consensus review of the above materials and achieved GDS scores of 1 or 2 [9] with Mini-Mental State Examination (MMSE) scores of 28 or higher [10]. The APOE genotypes were determined using published methods [11]. Postmortem diagnoses were conducted either using 1997 National Institute on Aging-Reagan Institute Consensus Recommendations for the Postmortem Diagnosis of Alzheimer's Disease [12] or according to 2012 National Institute on Aging guidelines for the Neuropathologic Assessment of Alzheimer's disease [13].

CSF sampling and analyses

CSF was handled according to the recommendations of Vanderstichle et al. [14]. After an overnight fast and a light breakfast before 9 am, the lumbar puncture procedure began at 12:00 p.m. using a 25-G needle guided by fluoroscopy. 15 mL of CSF was collected in three polypropylene tubes. Samples were kept on ice for up to 1 h until centrifuged for 10 min at 2,000 g at 4°C. 250 µL samples were aliquoted into 1 mL polypropylene tubes and stored at -80°C until thawed for the assays.

P-tau231

A sandwich ELISA (Applied NeuroSolutions) assay was used to detect tau phosphorylated at threonine 231 (P-tau231). In this assay, tau is captured with two backbone-directed antibodies, tau-1 and CP-27. The captured tau is then detected by CP9, which is specific for P-tau231 [15]. The quantitative limit of detection for this assay is 9 pg/ml and the coefficients of variation range from 6.0% to 10.3% (intra-assay) and 11.6% to 14.4% (inter-assay). The calibrator used for the assay was a synthetic peptide corresponding to amino acid numbers 186–236 of human tau, using the full-length tau [441 amino acids]. Calibration samples consisted of the tissue supernatant prepared from homogenized cortical gray matter from an AD brain. The peptide concentrations of the calibrators were used to construct a standard curve. The standard curve was constructed by 8 double down dilutions starting at a content of 312.50 pg/ml down to 1.25 pg/ml, plus a 0 point. Linearity was established by serial dilutions of CSF samples at high P-tau content and in separate tests by spiking negative CSF with a hot CSF sample. In all of the spike recovery experiments, we recovered 90% or better of the analyte. The lower limit of quantification (LLOQ) was determined as 9.8 pg/ml. Two concentrations of the calibrator samples consisting of 4 ng/well and 2 ng/well were each run in duplicate wells on every micro-titer plate. CSF samples were run in triplicate wells. The amount of peptide equivalents in the patient sample is calculated from the curve fit and is expressed in pg/ml of peptide. Values on or below the detection limit ($n = 46$, all controls) were conservatively coded as 9 pg/ml. This assay was conducted at Applied Neurosolutions, Chicago, Ill.

P-tau181

An Innogenetics sandwich ELISA assay with a synthetic phosphopeptide was used to detect phosphorylated threonine 181 (P-tau181). In this assay a phospho-dependent capture antibody, AT270 (P₁₇₆PAPKT_pP₁₃₂), and a human specific tau detection antibody, HT7 (P₁₅₉PGQK₁₆₃), are utilized. Prior work showed the detection limit for this assay is 15.6 pg/ml with less than 10% intra-assay and inter-assay coefficient variations [16]. The LLOQ was determined to be 25 pg/ml. None of the samples assayed were below the P-tau181 detection threshold. This assay was conducted at the Sahlgrenska Institute in Sweden.

Aβ₄₂

Amyloid-β (Aβ₄₂) levels were measured using ELISA (Innotest[®], Innogenetics, Ghent, Belgium). This assay was conducted at the Sahlgrenska Institute in Sweden.

Statistics

Group differences in demographic and descriptive measures were tested using *t*-tests for continuous variables and chi square analysis for categorical variables. The relationships of the demographic variables (age, gender, education, APOE genotype, and diagnostic group) with the CSF measures were examined by ANOVA. The log transformation was used to normalize the distributions of both P-tau181 and P-tau231.

Binary logistic regression models were used to determine the accuracy of the P-tau values in categorizing AD versus NL subjects after adjusting for age, APOE genotype, and education as confounds in the models. In addition, the results were examined in a subset of NL subjects (*n* = 28) who were randomly matched to the AD group for age. Following the recommendations of the consensus report of the working group on: “Molecular and Biochemical Markers of Alzheimer’s Disease” [17], in the ROC analyses the sensitivity level was set to 85%. Differences in biomarker specificity levels were compared using the McNemar test for the comparison of paired binomial proportions [18]. The positive and negative predictive values are reported for both P-tau assays. Results were considered statistically significant at *p* < 0.05 (2-sided tests). In addition, all the results were reexamined in the subset of data where the P-tau231 was above the detection limit of 9 pg/ml. Statistics were run using SPSS v.21 software (SPSS Inc., 2013).

RESULTS

With respect to the demographic measures, gender, APOE ε4 genotype, and race distributions did not differ between the groups; however, the AD group was older (*t* = 3.5, *p* < 0.01) and had less education (*t* = -2.3, *p* < 0.05). As expected, the AD group had significantly lower MMSE scores (*t* = -8.5, *p* < 0.001) and delayed recall performance (*t* = -13.8, *p* < 0.001) than the NL group (see Table 1).

ANOVA models were used to examine possible confounds on the two P-tau assays. In the model predicting P-tau181 levels, among the demographic measures only age (*F* = 4.0, *p* < 0.05) and diagnostic group (*F* = 20.3, *p* < 0.001) were significant predictors, but APOE ε4 genotype was not. In the prediction of P-tau231, elevated levels were associated with age (*F*

= 8.1, $p < 0.01$), diagnostic group ($F = 44.9$, $p < 0.001$), and APOE $\epsilon 4$ genotype ($F = 7.5$, $p < 0.01$). The 2-way interaction between APOE $\epsilon 4$ carriers and non-carriers and diagnostic group was not significant, indicating that the APOE $\epsilon 4$ genotype effect on P-tau231 was found in both diagnostic groups.

The overall correlation between P-tau181 and P-tau231 was significant ($r = 0.71$, $p < 0.001$), and this result was found separately in both controls ($r = 0.57$, $p < 0.001$) and in AD ($r = 0.84$, $p < 0.001$, see Fig. 1). Our findings show important similarities and differences between the analytes. Using binary logistic regression models to assess the diagnostic accuracy of the two assays, individually, both assays significantly improve the model after accounting for the significant confounds (P-tau181: χ^2 change = 22.6, $p < 0.001$; P-tau231: χ^2 change = 44.2, $p < 0.001$). There were no significant 2-way interactions between the confounds for either P-tau181 or P-tau231. Importantly, when combined in one model, P-tau231 significantly added to the predictive accuracy of the P-tau181 (χ^2 change = 24.4, $p < 0.001$). Conversely, P-tau181 did not add significantly to the P-tau231 model.

With the sensitivity fixed at 85%, P-tau231 yielded a specificity of 92%, whereas P-tau181 gave a specificity of 62% (Fig. 2). This difference is significant in the McNemar test ($p < 0.001$). Overall, P-tau181 incorrectly predicted 37 subjects (33 NL and 4 AD) whereas P-tau231 incorrectly predicted 11 (7 NL and 4 AD). Specifically, of the 37 incorrectly classified by P-tau181, P-tau231 correctly classified 30 (28 NL and 2 AD), whereas of the 11 incorrectly classified by P-tau231, P-tau181 correctly classified 4 (2 NL and 2 AD). Without considering the prevalence in this contrast between the two biomarkers, the negative predictive value (NPV) is high for both biomarkers (P-tau181 NPV = 93%, P-tau231 NPV = 95%), however, the positive predictive value (PPV) for P-tau181 is low at 42% compared to the 77% PPV for P-tau231 (Table 2).

The results demonstrating the P-tau231 specificity advantages were further confirmed in the 41 NL subjects with P-tau231 values > 9 pg/ml and the 28 AD. With the sensitivity threshold set at 85%, the specificity for P-tau231 was 83% and for P-tau181 was 42%. In the logistic models including the confounds, both biomarkers significantly added to the individual prediction models P-tau231: χ^2 change = 27.8, $p < 0.001$ and P-tau181: χ^2 change = 10.8, $p < 0.01$. As in the larger sample, P-tau231 significantly improved the combined model with the confounds and P-tau181 included (χ^2 change = 20.9, $p < 0.001$). P-tau181 did not contribute to the model with P-tau231 ($p > 0.05$).

In an effort to understand the significance of the P-tau231 levels below the detection threshold, we compared the 46 NL subjects below the 9 pg/mL threshold with 41 NL subjects above the threshold. The results show that subjects below the P-tau231 detection limit were younger ($t = -2.6$, $p < 0.05$), less likely to be APOE $\epsilon 4$ carriers (20% versus 39%, $\chi^2 = 4.0$, $p < 0.05$), had significantly lower P-tau 181 levels (38 ± 11 versus 59 ± 23 , $t = -5.9$, $p < 0.001$), and there were no CSF A β_{42} level differences observed (751 ± 203 versus 711 ± 248 , $p > 0.05$). All P-tau181 levels were above its 25 pg/ml detection threshold.

After age-matching the NL and AD groups (NL: $n = 28$, mean age = 71.0 ± 9.3 (52–85), AD: $n = 28$, mean age = 72.2 ± 10.2 (52–84)), there were no significant differences between

the groups in terms of any of the demographics. The diagnostic models were significant for both P-tau231 $\chi^2 = 36.1$, $p < 0.001$; and for P-tau181 $\chi^2 = 16.8$, $p < 0.001$. However, P-tau231 significantly improved the P-tau181 model (χ^2 change = 20.4 $p < 0.001$) but P-tau181 did not improve the P-tau231 model significantly. With the sensitivity set at 85%, P-tau231 had a specificity of 86% while for P-tau181 the specificity was 46%. As previously observed in the full model, the specificity level difference was significant (McNemar $p < 0.01$). In the matched sample, P-tau231 correctly identified 24/28 AD and 24/28 NL subjects whereas P-tau181 also correctly identified 24/28 AD but only 13/28 NL subjects.

As many diagnostic approaches rely on both an A β_{42} and a P-tau level, we examined the performance of the two analytes in models where A β_{42} is included. In the complete sample of 115 subjects, the addition of A β_{42} to a logistic regression model with prior entry of the confounds and P-tau181, significantly improved the model (χ^2 change = 18.0, $p < 0.001$); however, the addition of A β_{42} did not improve the logistic regression model with P-tau231 and the confounds. Additionally, we contrasted the diagnostic value of three tau and A β_{42} ratios. With the sensitivity constrained at 85%, there were no significant differences in the ratios for P-tau231/A β_{42} , P-tau181/A β_{42} , and total-tau/A β_{42} (i.e., specificity of 86%, 89%, and 97% respectively). As such, only by adding A β_{42} to the P-tau181 did the model achieve the same level of diagnostic accuracy as P-tau231 on its own.

From the study cohort, we conducted 9 AD and 1 NL postmortem exams that confirmed 100% of the *in vivo* diagnoses. The mean age for the 9 AD postmortem cases was 75.5 (sd = 9.2) and the group was 33% female. The average time between the spinal tap and the date of death was 4.4 years (sd = 3.2, range: 1–11 years). P-tau231 correctly classified 9/9 AD and P-tau181 8/9. Both assays correctly identified the NL subject. Comparing the 9 AD subjects to the 87 NL subjects with the sensitivity set at 85%, P-tau231 yielded 97% specificity and P-tau181 had a specificity of 69%. Based on the McNemar test, P-tau231 still demonstrated significantly higher sensitivity ($p < 0.001$).

DISCUSSION

The results of our study add to a body of knowledge showing that CSF levels of P-tau231 are superior to P-tau181 in separating AD subjects from normal control.

The present study, which includes the largest normal control group among head to head P-tau comparison studies, shows results that are both consistent with and that extend prior studies. With the sensitivity fixed at 85%, for P-tau181, we observed 62% specificity. A modest result similar in magnitude to those reported by Mattson (who reported an average specificity of approximately 50%) [19] and Mulder (who reported a specificity of 68%) [20].

Of importance, for P-tau231 we observed a specificity of 92% which is close to the 96% specificity reported by Hampel [21]. While both our study and Hampel et al used similar CSF P-tau231 assay procedures, our results are not consistent with those of Regeniter et al. [22] who used the Meso Scale Diagnostics (MSD) platform in a multiplex format to assay P-tau231. Regeniter et al. reported that only about 35% (55/156) of their 156 subjects' MSD P-tau231 samples were in a detectable range. They further reported that over half of their AD

population ($n = 44$) fell below the MSD assay's lower limit of quantification. We observed that 46/115 P-tau231 samples as compared with 0/115 P-tau181 samples were below the detection range. However, our samples below the detection range for P-tau231 were from the normal control group, they were younger, less likely to be $\epsilon 4$ carriers, and had lower P-tau181 levels. Examining this issue more specifically, we find the continued greater robustness of the P-tau231 assay after excluding subjects below the detection range, further highlighting the superior specificity of P-tau231 over the P-tau181. Thus, the data suggest that the absence of detectable CSF P-tau231 pathology carries a low probability of presymptomatic AD.

Moreover, our P-tau231 findings are also not consistent with those in the meta-analysis study reported by Mitchell [4]. Mitchell found no significant performance differences between P-tau181 and P-tau231 in the differentiation of AD from controls. Several reasons may account for the differences between our studies. We recruited a carefully characterized healthy control sample, whereas the meta-analysis drew on studies that examined controls from diverse sources. Importantly, the meta-analysis study gathered publications using controls derived primarily memory or neurological clinical settings. Two of the three P-tau231 studies used control subjects from neurologic or psychiatric clinics, one actually comparing AD to depressed non-demented subjects. Of the 15 P-tau181 studies included in the meta-analysis, two studies settings were unknown and 13 were from inpatient or outpatient clinics. Relative to the meta-analysis data, our study finds greater specificity (correct identification of the controls). Not a surprising finding when considering the increased likelihood of pathology in the controls from the meta-analysis.

Another important point of distinction was that the meta-analysis was designed to compare tau analytes across studies (i.e., from different subject samples not within subject), whereas our study examined both tau analytes in the same individuals. Additionally, we ran batch wise assays in contrast to many studies in the meta-analysis.

Our results show important performance differences between the analytes. We observed the negative predictive values were comparable across P-tau analytes ($>90\%$). However, the positive predictive values were nearly 45% lower for P-tau181 as compared with P-tau231. Specifically, the P-tau181 assay gave many more false positives than P-tau231 an effect reproduced after age-matching the NL and AD groups. Additionally, when the AD population was limited to postmortem confirmed cases, the superior specificity of P-tau231 was still seen. Overall, our data show that the CSF P-tau231 assay has superior value for recognizing subjects unaffected by tau pathology.

While it is well known that CSF $A\beta_{42}$ levels in both normal elderly and AD are lower in carriers of the $\epsilon 4$ genotype [23], a result also observed in our data (not shown), our results importantly show that CSF P-tau231 levels are elevated in APOE $\epsilon 4$ carriers, while P-tau181 levels were not different. Thus, our study replicates prior findings that P-tau231 was elevated with the APOE $\epsilon 4$ genotype [24]. Our study also replicates the findings of Engelborghs et al. [25] and Lautner et al. [23] who did not detect $\epsilon 4$ effects in association with P-tau181. Further, as we did not find a genotype by diagnosis interaction, our result suggests that P-tau231 levels in both normal elderly and AD are influenced by the ApoE

carrier status. We speculate that like the lower CSF A β ₄₂ level, an elevated P-tau231 in the presence of the $\epsilon 4$ allele is suggestive of an increased vulnerability to the preclinical pathology of AD. Clearly, this observation is in need of longitudinal and histological validation, which is not part of the present report.

Our analysis also explored the ratio between A β ₄₂ and P-tau181, P-tau231, and total tau. These analyses revealed that when any tau and the A β ₄₂ biomarker were combined, no significant difference in specificity were seen. These results support current efforts to optimize a biomarker diagnostic panel. However, the ability of P-tau181 to diagnose at similar specificity to P-tau231 when they are both combined with A β ₄₂ does not reconcile the apparent differences in their independent abilities, nor does it explain P-tau231's better correlation with AD neuropathology.

With respect to the pathological validation history of the P-tau assays, in a series of studies, Buerger et al. [5, 6] reported that CSF P-tau231 was more strongly associated with neocortical neurofibrillary pathology than P-tau181. Similarly, Augustinack et al. demonstrated that P-tau231 may be more sensitive to layer II entorhinal cortex tau pathology [3]. Layer II is considered to be the first limbic area in AD consistently targeted by neurofibrillary pathology [26, 27]. Thus, it appears from these pathology studies that P-tau231 has greater sensitivity for neurofibrillary tangles than P-tau181. Such histological findings are consistent with our clinical findings of greater P-tau231 than P-tau181 performance in the detection of normal. Overall, such studies warrant longitudinal preclinical investigation. While other reports exist for the utility of P-tau231 versus P-tau181 in the differential diagnosis of AD, this question was not part of the present report. Our findings may have implications for ordinal biomarker models in the AD pathological cascade. While the neuropathological data show that tauopathy precedes A β deposition in the staging of AD lesions [28], to the contrary CSF biomarker staging models suggest the primacy of amyloid beta over P-tau [29, 30]. While the CSF based staging models [30] acknowledge that earlier tau changes possibly lie beneath the detection threshold of the CSF assay, our study suggests this question could be examined with the use of the more specific P-tau231 assay. A better P-tau test could enable improved detection of early tau pathology making it of potential importance for preclinical detection, and further refine the interactions with other biomarkers of neurodegeneration [31].

The present study has several weaknesses: there were no longitudinal data and only 9 postmortem AD and one NL subject with which to test the diagnostic validity of the P-tau231 versus the P-tau181. Moreover, our clinical AD sample size was relatively small and did not include milder affected subjects such as mild cognitive impairment or those with subjective complaints. As such we did not evaluate the differential performance of the analytes in the early stage of AD.

In conclusion, our study shows that P-tau231 is superior to P-tau181 in the separation of AD from NL controls. The major performance difference between the two analytes is found in the greater accuracy for P-tau231 in the classification of cognitively normal subjects by virtue of fewer false positives. These data offer the suggestion that the P-tau231 assay is of potential interest in a prospective test for early AD diagnosis.

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REFERENCES

- [1]. Iqbal K, Alonso AC, Chen S, Chohan MO, El-Akkad E, Gong CX, Khatoon S, Li B, Liu F, Rahman A. Tau pathology in Alzheimer disease and other tauopathies. *Biochim Biophys Acta*. 2005; 1739:198–210. [PubMed: 15615638]
- [2]. Hampel H, Blennow K, Shaw LM, Hoessler YC, Zetterberg H, Trojanowski JQ. Total and phosphorylated tau protein as biological markers of Alzheimer's disease. *Exp Gerontol*. 2010; 45:30–40. [PubMed: 19853650]
- [3]. Augustinack JC, Schneider A, Mandelkow EM, Hyman BT. Specific tau phosphorylation sites correlate with severity of neuronal cytopathology in Alzheimer's disease. *Acta Neuropathol*. 2002; 103:26–35. [PubMed: 11837744]
- [4]. Mitchell AJ. CSF phosphorylated tau in the diagnosis and prognosis of mild cognitive impairment and Alzheimer's disease: A meta-analysis of 51 studies. *J Neurol Neurosurg Psychiatry*. 2009; 80:966–975. [PubMed: 19465413]
- [5]. Buerger K, Alafuzoff I, Ewers M, Pirttila T, Zinkowski R, Hampel H. No correlation between CSF tau protein phosphorylated at threonine 181 with neocortical neurofibrillary pathology in Alzheimer's disease. *Brain*. 2007; 130:e82. [PubMed: 17615094]
- [6]. Buerger K, Ewers M, Pirttila T, Zinkowski R, Alafuzoff I, Teipel SJ, DeBernardis J, Kerkman D, McCulloch C, Soininen H. CSF phosphorylated tau protein correlates with neocortical neurofibrillary pathology in Alzheimer's disease. *Brain*. 2006; 129:3035–3041. [PubMed: 17012293]
- [7]. American Psychiatric Association and American Psychiatric Association Task Force on DSM-IV. *Diagnostic and Statistical Manual of Mental Disorders: DSM-IV*. 1994.
- [8]. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease report of the NINCDS-ADRDA work group* under the auspices of department of health and human services task force on Alzheimer's disease. *Neurology*. 1984; 34:939–939. [PubMed: 6610841]
- [9]. Reisberg B, Ferris SH, de Leon MJ, Crook T. The Global Deterioration Scale for assessment of primary degenerative dementia. *Am J Psychiatry*. 1982; 139:1136–1139. [PubMed: 7114305]
- [10]. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state": A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res*. 1975; 12:189–198. [PubMed: 1202204]
- [11]. Main BF, Jones PJ, MacGillivray RT, Banfield DK. Apolipoprotein E genotyping using the polymerase chain reaction and allele-specific oligonucleotide primers. *J Lipid Res*. 1991; 32:183–187. [PubMed: 2010690]
- [12]. Hyman BT, Trojanowski JQ. Editorial on consensus recommendations for the postmortem diagnosis of Alzheimer disease from the National Institute on Aging and the Reagan Institute Working Group on diagnostic criteria for the neuropathological assessment of Alzheimer disease. *J Neuropathol Exp Neurol*. 1997; 56:1095–1097. [PubMed: 9329452]
- [13]. Hyman BT, Phelps CH, Beach TG, Bigio EH, Cairns NJ, Carrillo MC, Dickson DW, Duyckaerts C, Frosch MP, Masliah E. National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease. *Alzheimers Dement*. 2012; 8:1–13. [PubMed: 22265587]
- [14]. Vanderstichele H, Bibl M, Engelborghs S, Le Bastard N, Lewczuk P, Molinuevo JL, Parnetti L, Perret-Liaudet A, Shaw LM, Teunissen C. Standardization of preanalytical aspects of cerebrospinal fluid biomarker testing for Alzheimer's disease diagnosis: A consensus paper from the Alzheimer's Biomarkers Standardization Initiative. *Alzheimers Dement*. 2012; 8:65–73. [PubMed: 22047631]

- [15]. Kohnken R, Buerger K, Zinkowski R, Miller C, Kerkman D, DeBernardis J, Shen J, Moller H-J, Davies P, Hampel H. Detection of tau phosphorylated at threonine 231 in cerebrospinal fluid of Alzheimer's disease patients. *Neurosci Lett*. 2000; 287:187–190. [PubMed: 10863026]
- [16]. Vanmechelen E, Vanderstichele H, Davidsson P, Van Kerschaver E, Van Der Perre B, Sjoegren M, Andreasen N, Blennow K. Quantification of tau phosphorylated at threonine 181 in human cerebrospinal fluid: A sandwich ELISA with a synthetic phosphopeptide for standardization. *Neurosci Lett*. 2000; 285:49–52. [PubMed: 10788705]
- [17]. Radebaugh T, Khachaturian Z. Consensus report of the working group on: Molecular and biochemical markers of Alzheimer's disease. *Neurobiol Aging*. 1998; 19:109–116. [PubMed: 9558143]
- [18]. McNemar Q. Note on the sampling error of the difference between correlated proportions or percentages. *Psychometrika*. 1947; 12:153–157. [PubMed: 20254758]
- [19]. Mattsson N, Rosen E, Hansson O, Andreasen N, Parnetti L, Jonsson M, Herukka SK, van der Flier WM, Blankenstein MA, Ewers M. Age and diagnostic performance of Alzheimer disease CSF biomarkers. *Neurology*. 2012; 78:468–476. [PubMed: 22302554]
- [20]. Mulder C, Verwey NA, van der Flier WM, Bouwman FH, Kok A, van Elk EJ, Scheltens P, Blankenstein MA. Amyloid β (1-42), total tau, and phosphorylated tau as cerebrospinal fluid biomarkers for the diagnosis of Alzheimer disease. *Clin Chem*. 2010; 56:248–253. [PubMed: 19833838]
- [21]. Hampel H, Buerger K, Zinkowski R, Teipel SJ, Goernitz A, Andreasen N, Sjoegren M, DeBernardis J, Kerkman D, Ishiguro K. Measurement of phosphorylated tau epitopes in the differential diagnosis of Alzheimer disease: A comparative cerebrospinal fluid study. *Arch Gen Psychiatry*. 2004; 61:95–102. [PubMed: 14706948]
- [22]. Regeniter A, Kuhle J, Baumann T, Sollberger M, Herdener M, Kunze U, Camuso MC, Monsch AU. Biomarkers of dementia: Comparison of electrochemiluminescence results and reference ranges with conventional ELISA. *Methods*. 2012; 56:494–499. [PubMed: 22503775]
- [23]. Lautner R, Palmqvist S, Mattsson N, Andreasson U, Wallin A, Pallson E, Jakobsson J, Herukka SK, Owenius R, Olsson B. Apolipoprotein E genotype and the diagnostic accuracy of cerebrospinal fluid biomarkers for Alzheimer disease. *JAMA Psychiatry*. 2014; 71:1183–1191. [PubMed: 25162367]
- [24]. Buerger K, Teipel SJ, Zinkowski R, Sunderland T, Andreasen N, Blennow K, Ewers M, DeBernardis J, Shen Y, Kerkman D. Increased levels of CSF phosphorylated tau in apolipoprotein E4 carriers with mild cognitive impairment. *Neurosci Lett*. 2005; 391:48–50. [PubMed: 16165272]
- [25]. Engelborghs S, Sleegers K, Cras P, Brouwers N, Serneels S, De Leenheir E, Martin JJ, Vanmechelen E, Van Broeckhoven C, De Deyn PP. No association of CSF biomarkers with APOE ϵ 4, plaque and tangle burden in definite Alzheimer's disease. *Brain*. 2007; 130:2320–2326. [PubMed: 17586559]
- [26]. Braak H, Braak E. On areas of transition between entorhinal allocortex and temporal isocortex in the human brain. Normal morphology and lamina-specific pathology in Alzheimer's disease. *Acta Neuropathol*. 1985; 68:325–332. [PubMed: 4090943]
- [27]. Hyman BT, Van Hoesen GW, Kromer LJ, Damasio AR. Perforant pathway changes and the memory impairment of Alzheimer's disease. *Ann Neurol*. 1986; 20:472–481. [PubMed: 3789663]
- [28]. Braak H, Del Tredici K. Amyloid β may be released from non-junctional varicosities of axons generated from abnormal tau-containing brainstem nuclei in sporadic Alzheimer's disease: A hypothesis. *Acta Neuropathol*. 2013; 126:303–306. [PubMed: 23824268]
- [29]. Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: Progress and problems on the road to therapeutics. *Science*. 2002; 297:353–356. [PubMed: 12130773]
- [30]. Jack CR Jr, Knopman DS, Jagust WJ, Petersen RC, Weiner MW, Aisen PS, Shaw LM, Vemuri P, Wiste HJ, Weigand SD. Tracking pathophysiological processes in Alzheimer's disease: An updated hypothetical model of dynamic biomarkers. *Lancet Neurol*. 2013; 12:207–216. [PubMed: 23332364]

- [31]. Fortea J, Vilaplana E, Alcolea D, Carmona-Iragui M, Sanchez-Saudinos M, Sala I, Anton-Aguirre S, Gonzalez S, Medrano S, Pegueroles J. CSF β -amyloid and phosphotau biomarker interactions on brain structure in preclinical AD. *Ann Neurol*. 2014; 76:223–230. [PubMed: 24852682]

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Fig. 1.

Scatter plot of P-tau181 and P-tau231 for NL and AD. Figure 1 shows the correlation between P-tau231 and P-tau181 in both NL (green) and AD (black) subjects. It also shows, based on the ROC curves, each assays respective cutoff for diagnostic determination (dotted lines) with sensitivity fixed at 85%. The 10 subjects studied at postmortem are shown in yellow (NL) and red (AD).

Fig. 2. ROC curves for P-tau181 and P-tau231 in the diagnosis of NL ($n = 87$) and AD ($n = 28$). P-tau231 is represented by the dashed line and P-tau181 by the solid line. The 85% sensitivity is identified as previously recommended [17].

Table 1

Group demographics and descriptive measures

	NL	AD
<i>n</i>	87	28
Age ^a	64.0±8.1 (51–85)	72.2±10.2 (52–84) ^b
Education (in years) ^a	17.1±2.2 (12–22)	16.1±2.4 (12–20) ^b
% Female	59	64
% Caucasian	94	86
% ε4 carriers	29	46
MMSE ^a	29.4±0.8	23.0±4.0 ^b
Declarative Memory- z-score ^a	0.0±0.9	-2.7±0.8 ^b
P-tau231 pg/mL ^a	14.7±12.6	64.4±38.9 ^b
P-tau181 pg/mL ^a	47.6±20.3	94.8±55.2 ^b
Aβ ₄₂ pg/mL ^a	732 ± 225	434±284 ^b

^a values are mean±standard deviation (range).^b significant difference from NL group at $p < 0.05$.

Table 2

Specificity at 85% sensitivity, positive and negative predicted values

Analyte	85% Sensitivity Cut-off value (pg/ μ L)	Specificity	PPV	NPV
P-tau181	47.5	62%	42%	93%
P-tau231	25.3	92%	77%	95%