Letter to the Editor

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Diagnostic performances and cut-off verification of blood pTau 217 on the Lumipulse platform for amyloid deposition in Alzheimer's disease

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To the Editor,

Phosphorylated tau at threonine 217 (pTau 217) has been until recently considered a promising biomarker for predicting the risk of developing Alzheimer's disease (AD) [1], while compelling evidence has finally been collected to demonstrate its reliability as a diagnostic marker in primary and secondary care settings [2]. This paradigm shift has been already embodied by the Alzheimer's Association revised criteria for diagnosis and staging of AD [3] that have included plasma pTau 217 among both the core diagnostic and prognostic biomarkers, highlighting the preeminent need for a biological definition of the diagnosis rather than the syndromic presentation.

Blood-based biomarkers have been considered as the most significant diagnostic advance in the very last years [3], and are being implemented, along with the cerebrospinal fluid (CSF) biomarkers that are currently approved as *in vitro* diagnostic (IVD) tests for clinical use, i.e. Amyloid-β1-42 (AB 1–42), Amyloid-β1-40 (AB 1–40), pTau 181, in the diagnostic, staging and prognostic settings [4].

Largest cohorts have been tested for plasma pTau 217 with mass spectrometry (MS) assays [2], and more recently, an automated chemiluminescence method has also proved to be reliable and accurate in diagnosing AD [5] and predicting brain amyloid status [6], although a single cut-off that achieved ≥90 % in both sensitivity and specificity has not been found [5, 6], except for one study reporting 0.23 ng/L as discriminant cut-off for patients with both amyloid and tau pathology against no pathology according to CSF biomarkers [7].

Here we report the results of a preliminary study aiming to verify the diagnostic performances of the automated platform Lumipulse pTau 217 assay (Fujirebio Diagnostics, Japan), and particularly its association to amyloid pathology, and identify a clinical cut-off for AD diagnosis in a group of cognitively impaired subjects.

Twenty seven patients (median age 68 years, range 46–83) studied for suspected cognitive impairment were evaluated at the Regional Brain Aging Center and at the Neurology Clinic of University-Hospital of Padova, where a complete clinical workup involved also lumbar puncture for CSF AD biomarkers testing, amyloid positron emission tomography combined with brain MRI (PET/MRI) and blood drawing as standard of care. Patients were classified according to their amyloid PET status in A^+ and A^-; also, after a full clinical evaluation and according to their CSF results, patients were diagnosed as having AD (AD^+) or other neurodegenerative

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diseases (AD^-, i.e. cerebral amyloid angiopathy, frontotemporal dementia, corticobasal degeneration, Niemann-Pick disease).

pTau 217 was tested in K-2 ethylenediaminetetraacetic acid plasma or serum samples with a research use only (RUO) method on Lumipulse G1200 lot number 4080 having as measuring range 0.030–10.000 ng/L. Samples were frozen diagnostic leftovers that were aliquoted in polypropylene tubes; analyses were performed after thawing at room temperature for at least 30 min, vortexing for 10 s and centrifuging at 2,000 *g* for 5 min following manufacturer's specifications. Manufacturer's quality controls (QC) lot number D6C5034 (level 1 target mean 0.476 ± 0.095, level 2 target mean 3.777 ± 0.755) were tested in each analytical run, resulting within manufacturer's specifications with a CV <5 % for both levels, confirming the previous reported variability [6].

pTau 217 concentrations were summarized as range, mean ± standard deviation, median and interquartile range (IQR); mean values of pTau217 in A^+/A^- and AD^+/AD^- were reported and compared with one-way ANOVA and Bonferroni test; correlation between pTau217 and AB 1–42/1–40 ratio levels and with age was tested with Spearman's rank test. Diagnostic accuracy was calculated with receiver operating characteristic (ROC) curves analysis with confidence (CI) intervals 95 %; Youden's index cutoff was reported. Results of pTau217 were classified in negative/positive according to the best cutoff and we calculated sensitivity (Se%), specificity (Sp%), positive and negative predictive values (PPV and NPV) and the overall accuracy for the identification of PET-defined A^+ status. Analyses were performed on Stata SE v. 13.0.

In the whole cohort pTau 217 concentration ranged 0.03–1.353 ng/L, mean 0.398 ± 0.367, median 0.242 (IQR 0.08–0.664); mean concentrations were not different between males and females (p=0.45) and pTau 217 levels were not correlated to age (p=0.67). PTau 217 concentrations were significantly correlated to AB 1–42/1–40 ratio (Spearman's rho =–0.64, p=0.004). In A^+ patients (n. 14) mean concentration was 0.656 ± 0.338 ng/L, in A^- (n. 11) it was 0.108 ± 0.069 ng/L, they were significantly different (p<0.0001). When the final diagnosis was established, n. Fifteen patients were AD^+ and had a mean concentration of 0.633 ± 0.337 ng/L, significantly different from AD^- patients (n. 11) 0.107 ± 0.069 ng/L (p<0.0001).

ROC AUC for the identification of A^+/A^- status was 0.99 (CI 0.98-1) (Figure 1), Youden's best cutoff was 0.242 ng/L.

Diagnostic performances of pTau 217 at the 0.242 ng/L best cutoff are reported in Table 1: Se% was 92.9 % for A^+/A^- status and Sp% was 100 %, with a positive predictive value (PPV) also of 100 %.

In our study pTau 217 had higher AUC than those previously reported by Arranz with the same assay (0.99 vs. 0.95 for AD^+ and 0.94 for A^+) [5] and Figdore (0.93) [6], though this effect might be due to the lower number of subjects included in our study. Nevertheless, the cut-off identified by Arranz with the highest diagnostic accuracy to detect A^+ participant according to CSF biomarkers was 0.247 ng/L, very close to our finding [5], while Figdore identified 0.229 ng/L as best cut-off for prediction of amyloid PET positivity [6], also very close if considering a potential variability of 5 %.

A two-step cut-offs approach has been recently proposed [8] to optimize the diagnostic value and enhance sensitivity and specificity [6]; this approach could not be evaluated in this preliminary study, but future efforts from laboratory professionals should address this issue that has been raised by neurologists [9] particularly when evaluating this assay in different settings as primary and secondary care context [2, 9].

Blood pTau 217 has been recently highlighted as one of the most convincing AD blood biomarkers, our preliminary results confirm that it might be a promising minimally invasive tool to identify brain amyloid pathology and possibly accelerate the diagnosis of AD and/or reduce the load of more invasive, expensive and time-consuming procedures as lumbar puncture and PET. Nevertheless, the role of pTau 217 in the monitoring of response to disease-modifying drugs has not been yet clarified and therefore more comparison studies using PET as gold standard in this setting are to be deemed.

Moreover, beside the reported imprecision of CV <5 % the analytical performances of this assay are still to be verified, using different reagent lots and on higher number of analytical runs, as manufacturer has not yet provided any data on intra- or inter-assay imprecision; also, although no reference measurement procedures are currently defined [1], bias against MS method might be calculated. Likewise, pre-analytical conditions should be specifically evaluated, to exclude possible confounding factors e.g. hemolysis and storage temperature that have been previously found to be critical for blood pTau 181 on the same platform [10].

These encouraging results prompt to expand the studies on this biomarker with increased number of subjects, which is the main limitation of this preliminary study, and deeper comparison to CSF biomarkers and PET; blood pTau 217 might represent a turning point for the diagnosis of AD and the future management of upcoming disease-modifying drugs.

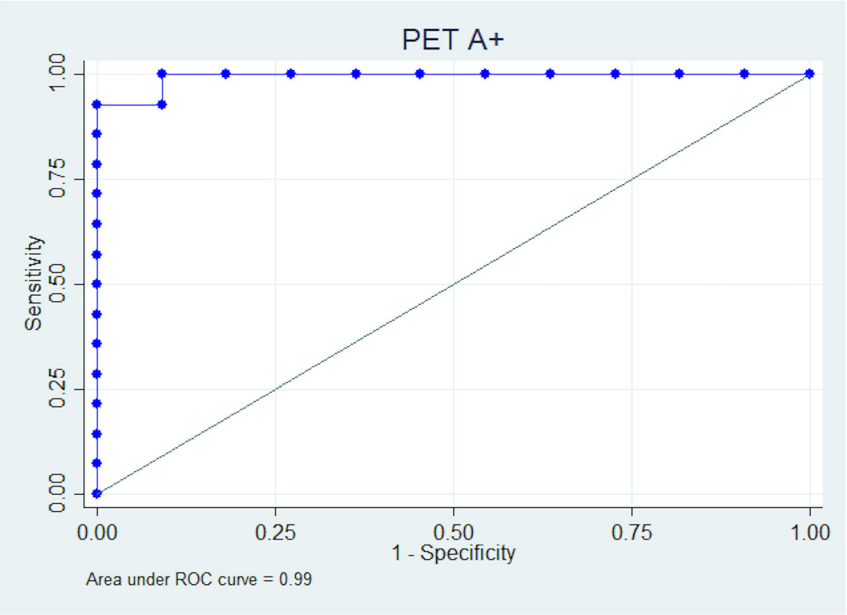

Figure 1: ROC curve for the identification of amyloid positive PET status (A^+) with blood pTau 217.

Table 1: Diagnostic performances of pTau 217 with cutoff 0.242 ng/L for the identification of A^+/A^- PET status; overall accuracy was calculated as (true positive+true negative)/total number of patients[a]100.

		A^+/A^- PET status
		95 % confidence intervals (CI)
Sensitivity	92.9 %	82.8–100 %
Specificity	100 %	100–100 %
Positive predictive value	100 %	100–100 %
Negative predictive value	91.7 %	80.8–100 %
Overall accuracy	96 %	

Research ethics: Study protocol CE:3950/AO/2016.

Informed consent: Informed consent was obtained from all individuals included in this study, or their legal guardians or wards.

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

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Data availability: The datasets generated during the current study are available from the corresponding author on reasonable request.

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