

## SHORT REPORT

# Elevation of plasma phosphorylated tau181 during neurological illnesses affecting consciousness and kidney dysfunction

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## Abstract

**Introduction:** Phosphorylated tau (p-tau)181 has become a promising blood-based Alzheimer's disease (AD) biomarker. We studied the agreement of plasma p-tau181 and cerebrospinal fluid (CSF) markers in patients with alteration of consciousness (AOC).

**Methods:** Plasma and CSF were simultaneously collected in participants presenting with AOC. Plasma p-tau181 was measured using the single-molecule array. CSF biomarkers were classified according to the amyloid/tau/neurodegeneration (AT[N]) framework.

**Results:** Among participants enrolled, the median (interquartile range) age was 57 (28.5–75) years and 5.8% had AD. Plasma p-tau181 yielded area under the curve of 0.85 and showed moderate correlation with CSF p-tau181 ( $Rho = 0.42, P < .001$ ). Using the historical cut-point, many non-AD participants had elevated plasma p-tau181 resulting in a specificity of 0.57. Plasma p-tau181 correlated with the glomerular filtration rate ( $Rho = -0.52, P < .001$ ). Among A– participants with elevated plasma p-tau181, 42% had kidney dysfunction.

**Discussion:** Plasma p-tau181 showed inadequate specificity in patients with AOC partially attributable to concomitant kidney dysfunction.

## KEYWORDS

alteration of consciousness, Alzheimer's disease, AT(N) framework, blood-based biomarker, phosphorylated tau

## 1 | BACKGROUND

Neuritic plaques comprising amyloid beta ( $A\beta$ ) and neurofibrillary tangles comprising phosphorylated tau (p-tau) are the defining pathologies in Alzheimer's disease (AD). They accumulate years before the onset of symptoms<sup>1</sup> are detectable in living patients through cerebrospinal fluid (CSF) and positron emission tomography (PET) biomark-

ers. Successful implementation of these biomarkers led to the proposal of a research framework for the diagnosis of AD. This framework introduced the AT(N) system, in which core biomarkers are classified into groups reflecting amyloid, tau, and neurodegeneration.<sup>2</sup>

Recently, sensitive methods for measuring biomarkers in blood have shown to be promising diagnostic tools for AD.<sup>3,4</sup> Nonetheless, before widespread implementation, more evidence is needed to address

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different aspects relating to clinical use.<sup>5,6</sup> Few studies had explored the influence of non-neurologic factors on plasma levels of neurofilament light chain (NfL) and recently for other biomarkers.<sup>7-10</sup> They consistently identified the association of chronic kidney disease and elevation of most biomarkers. However, studies so far largely excluded individuals with other significant neurological disorders. It is unknown how other brain diseases affect these biomarkers and their diagnostic performance. Obtaining such evidence can guide how we use these tools to diagnose AD accurately in more diverse settings.

In the present work, we measure plasma levels of p-tau181 in patients presenting with alteration of consciousness (AOC) from various causes and determine their agreement with the better-established CSF AD biomarkers.

## 2 | METHODS

### 2.1 | Participants

We measured plasma and CSF AD biomarkers from 69 consecutive participants who presented to King Chulalongkorn Memorial Hospital with AOC between December 2020 and 2021. These participants were enrolled in a separate study to evaluate NfL as a biomarker for classifying the etiology of AOC. Inclusion criteria for participants were patients over 15 years of age for whom which lumbar puncture was indicated for diagnostic purposes. Exclusion criteria were any history of central nervous system (CNS) disorders within 1 year or an established neurodegenerative disease (see supporting information).

### 2.2 | Standard protocol approvals

The institutional review boards (IRBs) of Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand approved the use of human subjects for this study (Med Chula IRB no. 0424/65). All methods in this study were in accordance with the Declaration of Helsinki. Informed consent was obtained from participants or their proxies, prior to the inclusion in the study.

### 2.3 | Biochemical procedures

CSF and ethylenediaminetetraacetic acid plasma were collected according to standard protocol.<sup>11</sup> CSF  $A\beta_{42}$ ,  $A\beta_{40}$ , and p-tau181 were measured with enzyme-linked immunosorbent assay (EUROIMMUN). We used the cut-offs of 0.129 for  $A\beta_{42}/A\beta_{40}$  ratio and 61.0 pg/mL for p-tau181 for classifying participants into AT(N) categories (see supporting information). P-tau 181 levels in plasma were measured using the Simoa SR-X instrument using the commercially available Simoa pTau-181 Advantage V2 Kit (Quanterix). Samples were analyzed in duplicate by technicians in a blinded fashion.

## RESEARCH IN CONTEXT

- 1. Systematic Review:** The authors reviewed the literature using PubMed focusing on plasma phosphorylated tau (p-tau), neurological diseases other than Alzheimer's disease (AD) or its differential diagnosis, and citations of review articles on plasma p-tau. Although many articles described plasma p-tau, none is informative about how its AD diagnostic performance is affected.
- 2. Interpretation:** Our findings showed that plasma p-tau181 can be highly elevated in many patients without AD but with a neurological illness affecting consciousness. Only some of them are attributable to kidney dysfunction or other non-neurological comorbidities.
- 3. Future Directions:** Effects of neurological disease can be validated in larger studies possibly along with other biomarkers to gain mechanistic insights. The results of these studies could help create the guideline for the use of plasma p-tau as a stand-alone biomarker in the real-world setting.

### 2.4 | Statistical analysis

Demographic characteristics and paraclinical data were summarized as median (interquartile range [IQR]) and number (percentage). Group comparisons were done using Mann-Whitney U test or chi-squared test as appropriate. Correlation between continuous variables were assessed by Spearman's rank coefficient. Diagnostic accuracy of plasma p-tau181 was assessed through receiver operating characteristic (ROC) analysis using CSF as the reference. Sensitivity, specificity, and overall agreement with CSF biomarkers were determined using the historical cut-off point of plasma p-tau181 (2.64 pg/mL). This cut-off was based on memory clinic patients who underwent  $A\beta$ -PET (see supporting information). Statistical significance was defined as a two-sided *P*-value of  $<.05$ . Analysis was performed using the base and the ROCit package of R.

## 3 | RESULTS

### 3.1 | Participant demographics, neurological diagnoses, and AT(N) profiles

Among 69 participants enrolled in the study, 37 (53.6%) were female. The median age was 57 years (IQR = 28.5–75, range = 15–93). Demographics and paraclinical data are summarized in Table 1. CSF biomarkers were consistent with AD (i.e., A+T+) in four participants (5.8%). Six additional participants had decreased  $A\beta_{42}/A\beta_{40}$  ratio with

**TABLE 1** Demographics, clinical characteristics, and biomarker profile

	AD (A+T+)	Alzheimer's continuum (A+)	Non-AD (A-T-)	All participants	P-value (A+ vs. A-)
N	4	10	59	69	
Age, years	80 (78.5–82)	70 (74.25–83.5)	54 (28.5–70)	57 (29–75)	.016
Female (%)	2 (50)	6 (60)	31 (53)	37 (54)	.925
Body mass index, kg/m <sup>2</sup>	20.64 (18.61–23.50)	22.68 (17.74–25.15)	21.30 (18.70–24.3)	21.63 (18.67–24.38)	.942
Comorbidities (%)					
Hypertension	2 (50)	3 (30)	21 (36)	24 (35)	1
Diabetes	1 (25)	3 (30)	14 (24)	17 (25)	.977
Dyslipidemia	3 (75)	5 (50)	22 (37)	27 (39)	.681
History of stroke	1 (25)	1 (10)	10 (17)	11 (16)	.930
History of myocardial infarction	0	0	4(7)	4 (6)	.907
Duration of neurological symptoms, days	53 (16–159)	10.5 (4.5–19.75)	7 (3–30)	7 (3–30)	.706
Glasgow coma score	14.5 (11.8–15)	14 (12.25–14.75)	14 (13–14.5)	14 (13–15)	.930
Glomerular filtration rate <sup>a</sup> , mL/min/1.73 m <sup>2</sup>	74.59 (55.17–78.24)	79.06 (67.86–91.43)	97.08 (73.84–116.6)	95.34 (71.77–111.20)	.131
Kidney dysfunction <sup>b</sup> (%)	1 (25)	2 (20)	14 (24)	16 (25)	1
Abnormal liver function test <sup>c</sup> (%)	1 (25)	2 (20)	13 (22)	15 (22)	1
CSF abnormality <sup>d</sup> (%)	1 (25)	7 (70)	31 (53)	38 (55)	.495
Pleocytosis <sup>e</sup> (%)	0	2 (20)	14 (24)	16 (23)	1
CSF leucocytes, cells/mL	2.5 (2–3.3)	3 (2–5.75)	3 (1–9.5)	3 (1–9)	.750
CSF protein, mg/dL	43.8 (40.6–56.5)	77.8 (46.5–68.3)	43.45 (28.30–66.55)	45.5 (28.6–75.9)	.108
CSF A $\beta_{42}$ /A $\beta_{40}$ ratio	0.066 (0.064–0.071)	0.089 (0.066–0.118)	0.193 (0.165–0.209)	0.181 (0.157–207)	<.001
CSF p-tau181, pg/mL	95.25 (86.55–106.98)	47.65 (24.50–86.55)	21 (15.20–28.55)	23.4 (15.2–32.1)	.012
Plasma p-tau181, pg/mL	13.18 (5.95–23.39)	5.11 (3.00–13.00)	2.25 (1.47–70)	2.38 (1.47–4.41)	.040

Abbreviations: AD, Alzheimer's disease; A $\beta$ , amyloid beta; CSF, cerebrospinal fluid; p-tau181, phosphorylated tau on threonine 181.

Note: Unless specified, values are presented as median (interquartile range).

<sup>a</sup>Estimated using the 2009 Chronic Kidney Disease Epidemiology Collaboration creatinine equation.

<sup>b</sup>Defined here as glomerular filtration rate < 60.0 mL/min/1.73 m<sup>2</sup> or underlying kidney disease that met the criteria of chronic kidney disease regardless of glomerular filtration rate.

<sup>c</sup>Defined here as transaminase level above 2 $\times$  upper normal limit or hyperbilirubinemia.

<sup>d</sup>Abnormal CSF means having pleocytosis or elevated protein.

<sup>e</sup>Pleocytosis means CSF corrected leukocytes counts < 10 cells/mL.

normal CSF p-tau181 (i.e., A+T-) while none had abnormal CSF p-tau181 with normal A $\beta_{42}$ /A $\beta_{40}$  ratio (i.e., A-T+).

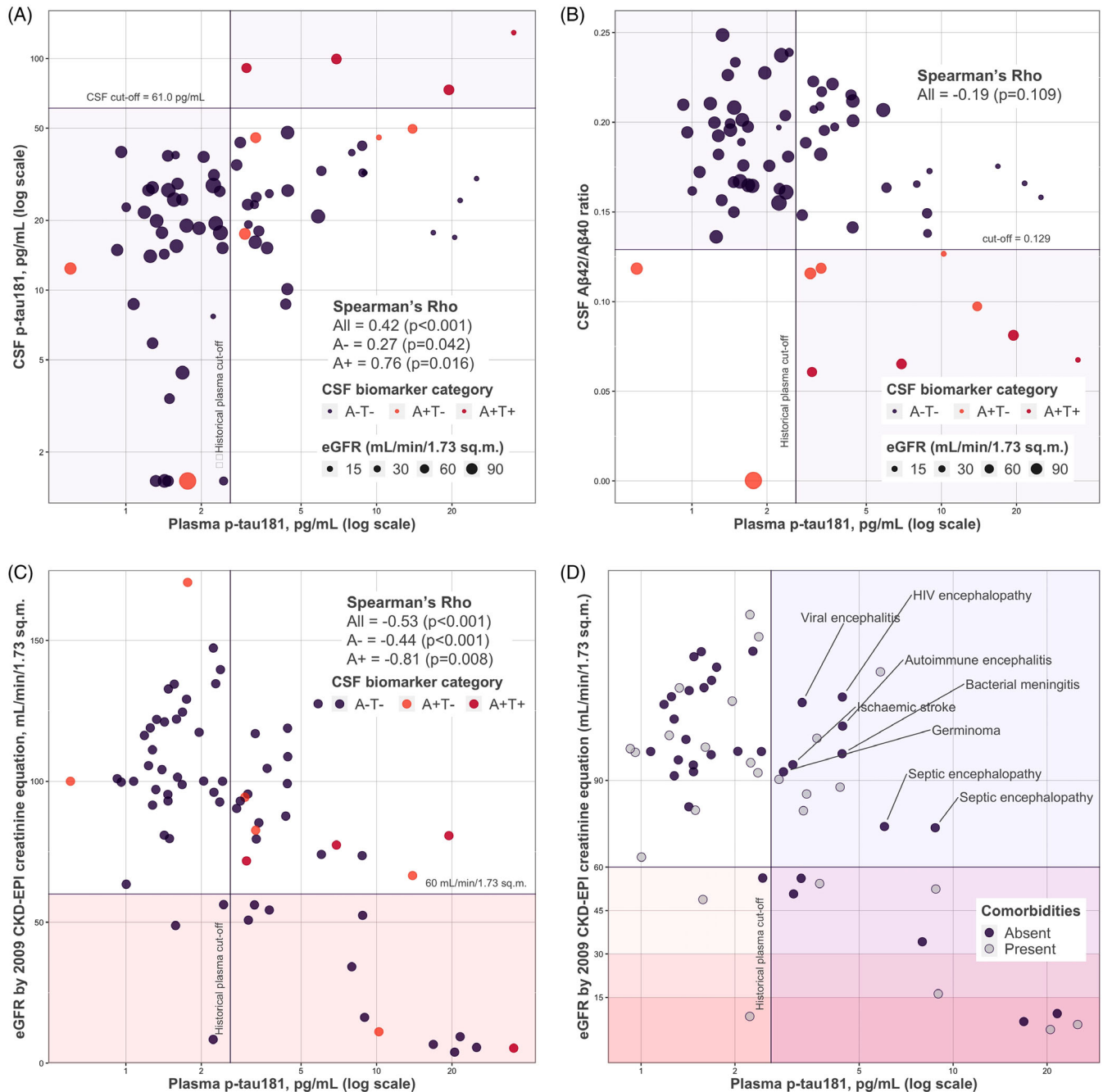
$P = .012$ ) but not plasma p-tau181 (Rho = -0.19,  $P = .109$ ; Figure 1).

### 3.2 | Correlation of plasma p-tau181 with CSF biomarkers

Plasma levels of p-tau181 showed moderate correlation with that of CSF (Rho = 0.42,  $P < .001$ ). For A+ participants ( $N = 10$ ), strong correlation was observed (Rho = 0.76,  $P = .016$ ). CSF A $\beta_{42}$ /A $\beta_{40}$  ratio also showed weak correlation with CSF p-tau181 (Rho = -0.30,

### 3.3 | Diagnostic performance of plasma p-tau181

ROC analysis of plasma p-tau181 during neurological illness for diagnosing AD ( $N = 4$ ) revealed an area under the curve (AUC) of 0.85 (95% confidence interval [CI] 0.62–1.00). The AUC for identifying participants with A+ status ( $N = 10$ ) was 0.71 (95% CI 0.51–0.90; Figure S1 in supporting information). The sensitivity, specificity, and overall



**FIGURE 1** Correlation between log-transformed plasma p-tau181 and CSF p-tau181 (A) and CSF A $\beta_{42}$ /A $\beta_{40}$  ratio (B) along with the value of each participant. The purple lines represent the biomarker cut-offs. Shaded areas show the agreement between CSF and plasma biomarkers. C, Correlation between eGFR and plasma p-tau181. The shaded areas encompass participants with significant kidney dysfunction (eGFR less than 60 mL/min/1.73 m $^2$ ). D, Plasma p-tau181 and eGFR of the A- participants. Empty circles represent participants with other comorbidities known to increase p-tau181 (history of stroke or myocardial infarction) or abnormal liver function test (transaminase level above 2 $\times$  upper normal limit or hyperbilirubinaemia). The solid vertical line represents the historical p-tau181 cut-off whereas the horizontal line represents the eGFR of 60 mL/min/1.73 m $^2$ . Areas shaded in blue represent false positive participants whereas the areas shaded in red signify kidney dysfunction. The non-overlapping blue areas at the top right show potentially “divergent” false positive cases not attributable to kidney dysfunction. The neurological diagnoses of those cases that also lack other comorbidities are displayed here but more details can be found in Table S2. A $\beta$ , amyloid beta; CSF, cerebrospinal fluid; eGFR, estimated glomerular filtration rate; p-tau181, phosphorylated tau on threonine 181

agreement were 1.0 (95% CI 0.40–1.0), 0.57 (95% CI 0.44–0.69), and 0.59 (95% CI 0.47–0.71), respectively.

### 3.4 | The effect of comorbidities

Some participants were critically ill. Plasma p-tau181 showed clear correlation with the estimated glomerular filtration rate (eGFR) using the 2009 Chronic Kidney Disease Epidemiology Collaboration creatinine equation ( $Rho = -0.52$ ,  $P < .001$ ; Figure 1C) and differed significantly between participants with eGFR below and above 60 mL/min/1.73 m<sup>2</sup> (median 8.33 vs. 2.04,  $P < .01$ ). Among A– participants, kidney dysfunction could explain only 10/24 false positive cases (42%). Regarding acute liver dysfunction, plasma p-tau181 did not differ between those with ( $N = 17$ ) and without abnormal liver function tests (median 2.78 vs. 2.38 pg/mL,  $P = .902$ ). Only two participants had cirrhosis. Correlation between plasma p-tau181 and liver parameters are shown in Table S1 in supporting information.

## 4 | DISCUSSION

In the present study, we found that plasma p-tau181 is elevated beyond the usual threshold in 24 out of 59 participants without AD biomarker (i.e., A–T– profile) resulting in a poor overall specificity of 0.57 for AD (i.e., A+T+ profile) in patients with AOC. We hypothesized that the integrity of the blood-brain barrier (BBB) may have been compromised by certain intercurrent neurological illnesses allowing excessive leakage into the circulation. We also found an unequivocal negative correlation between plasma p-tau181 and kidney function, which is consistent with previous reports.<sup>9,10</sup> Indeed, plasma p-tau can reach several fold of the cut-off values in non-AD patients with severe kidney dysfunction. Nonetheless, impaired renal clearance cannot explain all false positive cases. Many “divergent” false positive cases that lack confounding comorbidities previously reported were identified. Interestingly, most of them have diagnoses that are known to disrupt the BBB, supporting our hypothesis (Figure 1D and Table S2 in supporting information). Future studies can evaluate these divergent cases in a larger population through markers of BBB integrity such as the albumin CSF/serum concentration quotient.<sup>12</sup> Alternatively, quotient of CNS-derived proteins other than p-tau may also be used.

This non-AD-specific elevation of p-tau181 in the plasma could not be explained by false negative CSF biomarkers from using an inappropriate cut-off. Even if the threshold for CSF p-tau181 was lowered to 43.8 pg/mL (see supporting information for further discussion), there will be only four more T+ participants (one of which is A–) and the specificity remained low (0.60). Consequently, plasma p-tau181 should be used with caution in the setting of confounding neurological diseases or kidney impairment. P-tau at other phosphorylation sites have different trafficking dynamics between CNS and the periphery.<sup>13</sup> While plasma p-tau217 seemed to be slightly less affected by chronic kidney disease,<sup>8,10</sup> it remains to be seen if other p-tau species are similarly affected by CNS disorders.

To our knowledge, this is the first study to identify interfering neurological illnesses as potential confounders when using plasma p-tau to diagnose AD. While our data may raise more questions than answers, we believe that further research can address the mechanistic view that is crucial for CNS biomarkers. Moreover, it is one of the few studies that describe plasma AD biomarkers in populations of Asian ancestry,<sup>14,15</sup> and the first in Thailand. Racial disparity in AD research remains problematic and will obstruct generalization to the underrepresented population.

There are few limitations to our study. We rely on an assumption that CSF biomarkers are unaffected by the intercurrent illness. While this could be true in most circumstances,<sup>16–20</sup> we remained cautious. We found that CSF biomarkers identified AD participants that were of appropriate age (all > 73 years old) and followed AD continuum (no A–T+), which supported the validity of this assumption. This consistency was not observed for plasma p-tau181. Our small sample size confirmed only four AD participants, which limited the extrapolation of sensitivity. Recent studies also found that cirrhosis, history of stroke, and myocardial infarction is associated with increased plasma p-tau181.<sup>9,10</sup> Such associations cannot be evaluated in our small study. Finally, due to the design, we cannot obtain the baseline cognitive performance in this population.

To summarize, we described plasma p-tau181 and CSF biomarkers in individuals with AOC. We had shown that plasma p-tau181 can be erroneously elevated in this setting, despite accounting for kidney dysfunction. This knowledge can guide further research and would be helpful when plasma biomarker become widely used clinically.

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### CONFLICTS OF INTEREST

P.T. received traveling support from The Alzheimer's Association and The Thai Red Cross Society. The authors declare that there are no conflicts of interest. Author disclosures are available in the supporting information.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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