

SHORT REPORT

Impact of preanalytical delay on the performance of plasma A β 42, A β 42/40, p-tau217, and p-tau217/A β 42 in detecting brain amyloidosis in the ALZAN cohort

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Abstract

BACKGROUND: Alzheimer disease (AD), the leading cause of dementia, has increased interest in the development of blood-based biomarkers for early diagnosis and monitoring. The plasma amyloid beta (A β)42/A β 40 ratio and phosphorylated tau (p-tau) isoforms closely align with cerebrospinal fluid and positron emission tomography markers. The recent approval of the plasma p-tau217/A β 42 ratio marks a key step toward non-invasive diagnostics. However, known A β 42's preanalytical and analytical challenges raise concerns about the reliability of this ratio in routine clinical practice.

METHODS: Using the ALZAN prospective cohort of cognitively impaired individuals, we examined how delays between blood collection and laboratory processing affected the performance of plasma biomarkers in detecting.

RESULTS: The preanalytical delay has a significant impact for A β 40 and A β 42. However, the performance of p-tau217 and p-tau217/A β 42 ratio were similar, thereby supporting the use of the ratio as a robust and efficient marker for AD diagnosis.

DISCUSSION: Our findings reinforce the high diagnostic accuracy of the p-tau217/A β 42 ratio in AD, regardless of preanalytical delays.

KEYWORDS

Alzheimer's disease, biomarkers, plasma, preanalytical

Highlights

- Blood-based biomarkers are playing an increasingly important role in the routine clinical management of AD. A significant advancement in this field was marked by the FDA's recent approval of the Lumipulse immunoassay, which measures the plasma

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p-tau217/A β 42 ratio for AD diagnosis. However, this development has sparked notable concern among researchers.

- One major issue is that the p-tau217/A β 42 ratio has not been extensively investigated. Only recently have studies begun to show that it offers slightly better diagnostic performance than p-tau217 alone (<https://doi.org/10.1101/2024.12.07.24318640>). In addition, the known instability of A β 42 under different preanalytical conditions could potentially compromise the reliability of the ratio in routine clinical settings.
- Using data from the ALZAN cohort, where we previously confirmed the enhanced diagnostic value of the p-tau217/A β 42 ratio, we specifically examined how pre-analytical factors, specifically delays in blood sample processing, might affect its performance. Our results highlight the limited effect of this preanalytical parameter on clinical performance of the ratio, thereby supporting its robustness and practical value for routine use in AD diagnostic and care.

1 | INTRODUCTION

Amyloid beta peptides (A β 42, A β 40) and tau (total and phosphorylated [p-tau]) are central to Alzheimer's disease (AD) pathology, with A β 42 forming extracellular plaques and hyperphosphorylated tau leading to the formation of intracellular neurofibrillary tangles. Traditionally, detecting these pathological features in vivo has required invasive and costly methods like cerebrospinal fluid (CSF) analysis and positron emission tomography (PET) imaging.¹⁻³ However, these techniques are associated with high costs, limited availability, and reduced accessibility for patients. Recent advancements have focused on detecting A β peptides and p-tau in blood plasma as a non-invasive alternative.⁴ Numerous studies have demonstrated that plasma biomarkers, including the A β 42/A β 40 ratio and p-tau isoforms such as p-tau181, show dynamic changes across the AD continuum.⁵⁻⁹ These biomarkers are prospectively associated with cognitive decline and correlate strongly with cerebral amyloid burden as assessed by amyloid PET.^{10,11}

Recent advances have underscored the utility of the p-tau217 isoform as a highly specific biomarker for distinguishing AD from other neurodegenerative disorders,¹² as well as for tracking AD-related pathophysiological changes across the disease continuum.^{9,13} We recently demonstrated that the plasma p-tau217/A β 42 ratio provided higher diagnostic accuracy for identifying A β positivity compared with individual biomarkers, while also reducing the intermediate, uncertain zone in a two-cut-offs approach.¹⁴ These findings underscore the clinical utility of plasma biomarker ratios for the early detection of AD and support their broader adoption in both clinical practice and research frameworks. Importantly, the U.S. Food and Drug Administration (FDA) approved the G1200 Lumipulse p-tau217/A β 42 plasma ratio test for the early diagnosis of AD, marking a significant milestone toward a non-invasive and accessible alternative to traditional methods such as PET imaging and CSF analysis. This assay demonstrated high diagnostic accuracy, with an area under the receiver operating characteristic (ROC) curve (AUC) of 0.94, aligning closely with PET imaging results.¹⁵

However, A β 42 is known for its high tendency to rapidly aggregate into oligomers and fibrils, leading to challenges in accurately and routinely measuring its concentration, especially in plasma samples.^{16,17} This instability can result in fluctuating or underestimated A β 42 levels, raising concerns about the reliability and reproducibility of the p-tau217/A β 42 plasma ratio for AD diagnosis. Therefore, continued validation in diverse cohorts and clinical settings is critical to establish standardized protocols and to ensure the robustness of these biomarkers for routine clinical application.

Drawing on the real-life ALZAN cohort of individuals with cognitive impairment, we assessed the influence of preanalytical delays, from blood collection to laboratory processing, on the diagnostic accuracy of p-tau217 and the p-tau217/A β 42 ratio for AD diagnosis.

2 | METHODS

2.1 | Study cohorts

This study included participants from the ALZAN multicenter prospective cohort (ClinicalTrials.gov Identifier No. NCT05427448). The ALZAN cohort is a clinical study designed to evaluate the diagnostic performance of blood biomarkers for AD. Participants with cognitive complaints are recruited from memory clinics and neurology departments at the Montpellier, Nîmes, and Perpignan hospitals in France. Each participant had a lumbar puncture with CSF analysis as part of their routine care. Additional information on the cohort was published earlier.¹⁴

2.2 | Biological measurement in ALZAN

CSF was collected using standardized protocols for collection, centrifugation, and storage.¹⁸ CSF A β 1-42 and A β 1-40 (referred to here as

RESEARCH IN CONTEXT

1. **Systematic review:** Blood-based biomarkers are essential tools for the diagnosis of AD, enabling earlier detection and tracking of disease progression. The FDA's recent approval of the p-tau217/A β 42 assay marks a significant advancement in the development of non-invasive diagnostic methods for AD. Maintaining consistent preanalytical conditions is especially important for the reliable measurement of this ratio, given the known instability of A β 42 during sample handling. In the present study involving participants from the ALZAN multicenter prospective cohort, we assessed the impact of the delay between blood collection and laboratory arrival on the diagnostic performance of blood biomarkers for AD, specifically p-tau217 and the p-tau217/A β 42 ratio.
2. **Interpretation:** Our findings validate the diagnostic accuracy of the plasma p-tau217/A β 42 ratio and highlight the limited influence of preanalytical delay on its reliability.
3. **Future directions:** Building on previous studies and the recent FDA approval of the plasma p-tau217/A β 42 ratio for AD diagnosis, our results further support the ratio's strong performance in detecting A β + status, even when accounting for delays in sample processing. This reinforces the potential for wider adoption of routine blood tests in clinical practice, enabling earlier diagnosis while potentially reducing reliance on more invasive or costly procedures like lumbar punctures and PET imaging.

A β 42 and A β 40) were measured using commercially available assays (Fujirebio Europe, Ghent, Belgium) on the Lumipulse G1200 analyzer, according to the manufacturer's protocols. Participants were categorized as amyloid-positive (A β +) or amyloid-negative (A β -) based on their CSF A β 42/A β 40 ratio, using a threshold set at 7%, which corresponds well to the optimal cut-off identified for detecting amyloid PET status¹⁹ as well as AD.²⁰

Blood samples were collected in EDTA-K2 tubes on the same day as the lumbar puncture and sent to the local clinical laboratory for processing (2000 g $\times$ 10 min, room temperature [RT]). The delay between collection and processing in the laboratory (referred to as "blood delay") was recorded. No significant differences in storage duration were observed between samples with a preanalytical delay of less than 4 h and those with a delay exceeding 4 h. Following the preanalytical delay, all samples were handled in an identical manner: They were centrifuged, aliquoted, and subsequently stored at -80°C in low-binding Eppendorf LoBind microtubes (Eppendorf, ref 022431064, Hamburg, Germany) until testing. Estimated glomerular filtration rate

(eGFR) based on creatinine, age, and sex was calculated using the apolipoprotein E gene chronic kidney disease (CKD) Epidemiology Collaboration (CKD-EPI) equation, revised in 2021 without inclusion of race.²¹

For biomarker measurement and in order to avoid bias, plasma aliquots were thawed at $+4^{\circ}\text{C}$, gently homogenized, and centrifuged for 5 min at 2000 g before analysis in the morning. One aliquot was used for p-tau217 measurement using commercial kits on the Lumipulse G1200 as reported.²² Another aliquot was used for A β 42, A β 40 using the Elecsys research use only (RUO) assays on the e402 Cobas analyzer as described.^{14,23}

2.3 | Statistical analyses

Categorical variables were analyzed as percentages (%), continuous variables as mean (standard deviation) or medians (25th to 75th percentiles) if they did not follow a normal distribution as assessed by the Shapiro–Wilk test. The performance of individual and ratio biomarkers for A β + detection was assessed using a non-parametric bootstrap resampling to estimate the AUC and its 95% confidence interval (obtained as the 2.5th and 97.5th percentiles). Subsequently, pairwise comparisons between AUCs were conducted using the DeLong method.²⁴ To assess the relationship between plasma biomarkers and preanalytical delay while accounting for potential confounders, we employed a two-step approach. First, we computed partial Spearman correlations between each biomarker and the continuous delay variable, adjusting for covariates such as age and AD diagnosis using ordinary least-squares regression to obtain residuals. Spearman correlations were then calculated on these residuals to quantify rank-based associations independent of confounding. In addition, to ensure robustness against non-normality and the influence of outliers, we performed quantile regression analyses at the median ($\tau = 0.5$), again using preanalytical delay as a continuous predictor. This allowed us to estimate the conditional effect of delay on the median biomarker level while controlling for covariates. We thus define the "influence" of preanalytical delay as the degree to which the time elapsed between blood collection and sample processing is associated with measurable variation in plasma biomarker concentrations, independent of biological or clinical confounders.

To examine the relationship between biomarker concentrations and sample collection delay, we first assessed whether other variables should be included as covariates in the analysis. We therefore used Spearman's method to assess the association between blood collection delay and (1) age (correlation coefficient [cc] = -0.238 , $p < 0.001$), (2) sex (cc = -0.041 , $p = 0.405$), (3) body mass index (cc = -0.025 , $p = 0.626$), (4) eGFR (cc = 0.064 , $p = 0.190$), and (5) AD diagnosis (cc = -0.209 , $p < 0.001$). Accordingly, age and AD diagnosis were included as relevant covariates and were further used to statistically adjust the relationship between biomarker concentrations and preanalytical delay.

TABLE 1 Distribution of preanalytical delays among ALZAN participants.

Blood delay (H:m)	≤04:00	04:01 to 08:00	08:01 to 24:00	>24:00
N	214	79	83	38
Percentage of cohort	51.7%	19.1%	20.0%	9.2%
Median delay (IQR 25th to 75th)	01:35 (00:51 to 02:20)	05:30 (04:50 to 06:20)	20:20 (20:00 to 21:30)	29:25 (26:00 to 68:00)
Median collection time (IQR 25th to 75th)	10:10 (09:35 to 10:56)	09:00 (08:30 to 10:00)	14:10 (14:10 to 15:10)	13:00 (10:30 to 14:10)

Abbreviation: IQR, interquartile range.

3 | RESULTS

3.1 | Participants and biomarker profile

Of the 433 ALZAN participants initially enrolled, 414 were included in this analysis, based on the availability of sufficient samples to measure all biomarkers, as well as on the availability of the preanalytical delay. The mean age at baseline was 71.1 (SD 10.5) years, 46.6% were male, and 43.0% were apolipoprotein E gene (APOE) ε4 carriers, as determined by the Elecsys immunoassay.²³ In terms of kidney function, the mean eGFR was 84.7 (16.1) ml/min/1.73 m². Based on the CSF Aβ42/40 ratio,²⁰ 57.0% of the cohort was classified as Aβ+. Aβ+ participants were significantly older (74.2 [7.1] vs 67.0 [12.6] years, $p < 0.0001$), were more APOE ε4 carriers (59.3% vs 21.3%) and with lower Mini-Mental State Examination scores (21.9 [4.6] vs 24.6 [4.0], $p < 0.0001$). For plasma biomarkers, in the Aβ+ group, Aβ42 and Aβ42/40 values were significantly lower (32.3 [7.2] vs 38.7 [8.5] pg/mL and 0.107 [0.017] vs 0.134 [0.031], $p < 0.0001$, respectively), and p-tau217 and p-tau217/Aβ42 values were significantly higher (0.591 [0.426] vs 0.162 [0.238] pg/mL and 1.925 [1.478] vs 0.434 [0.643], $p < 0.0001$, respectively). Complete information on the ALZAN demographic, clinical, and biomarker readings are available in a previous publication.¹⁴

3.2 | Impact of blood delay on biomarker values

Blood sample processing time (preanalytical delay) was distributed in the cohort as follows: 51.7% of patients had samples processed within <4 h, 19.1% within 4 to 8 h, 20.0% within 8 to 24 h, and 9.2% after >24 h (Table 1).

As illustrated in Figure 1A–E, only Aβ40 and Aβ42 levels were associated with preanalytical delay; no other variables showed a significant association.

3.2.1 | Global performance of biomarkers in cohort and subcohorts stratified by preanalytical delay

The ability of the plasma biomarkers to detect Aβ+ status was assessed using ROC curve and AUC computation. Values of AUC for Aβ42, Aβ42/40, p-tau217, and p-tau217/Aβ42 were 0.747 (0.700 to 0.796), 0.848 (0.807 to 0.885), 0.910 (0.880 to 0.937), and 0.929 (0.900 to

0.953), respectively. As assessed by the Delong test, p-tau217/Aβ42 AUC outperformed all others including that of p-tau217 ($z = 4.358$, $p < 0.0001$). The optimal cut-offs determined by the Youden index were 35.6 pg/mL for Aβ42, 0.1162 for Aβ42/40, 0.225 pg/mL for p-tau217, and 0.546 for the p-tau217/Aβ42 ratio. The Youden index at these cut-offs in the general cohort, as well as in the cohort stratified by the preanalytical delay, is illustrated in Figure 1F. The drop in diagnostic performance observed in the subgroup with a preanalytical delay of over 4 h was 0.097 for Aβ42, 0.030 for Aβ42/40, 0.008 for p-tau217, and 0.006 for the p-tau217/Aβ42 ratio. Only the drop of Aβ42 is significant. An additional illustration of the impact of preanalytical time was investigated by computing and plotting the AUCs in the subcohort (Figure 1G–J). Only Aβ42 showed a significant difference between AUCs for samples processed before and after 4 h (0.831 [0.772 to 0.884] vs 0.669 [0.595 to 0.743], DeLong $p = 0.0006$).

3.2.2 | Impact of blood delay in a two-cut-offs “rule-in/rule-out” approach

We applied a two-cut-off approach²⁵ to stratify our cohort by Aβ+ risk, thereby creating a rule-in/rule-out framework similar to methods used in cardiovascular research.²⁶ This approach corresponds to the recently proposed strategy of using two cut-offs when applying blood biomarkers for AD. The rationale is that a low biomarker value can be used to exclude the presence of amyloidosis (with a negative predictive value of 90% or higher, meaning that if an individual in our cohort is below this value, it is Aβ– with at least a 90% probability). The second cut-off is high, and if an individual is above this value, it has a probability above 90% of being Aβ+. The performance of this approach is reflected notably in the proportion of individuals who fall between these two cut-offs, that is, in the intermediate (or “gray”) zone. Following the recommendations of the Global CEO Initiative on AD,²⁷ we selected cut-offs corresponding to 90% specificity or sensitivity for Aβ+, for the different biomarkers (Table 2).

As previously reported,¹⁴ the intermediate gray zone, which reflects the discriminative power of the biomarker, is already low in this cohort for p-tau217 at 15.4%, and it was further reduced to 8.5% when using the p-tau217/Aβ42 ratio. The rule-in/out approach is ultimately used to identify Aβ+ and Aβ– individuals, with negative and positive predictive values (NPV, PPV) that depend on the prevalence of the condition. In our cohort, predictive values for plasma Aβ42 and p-tau217 were affected by preanalytical delay, with differences between samples

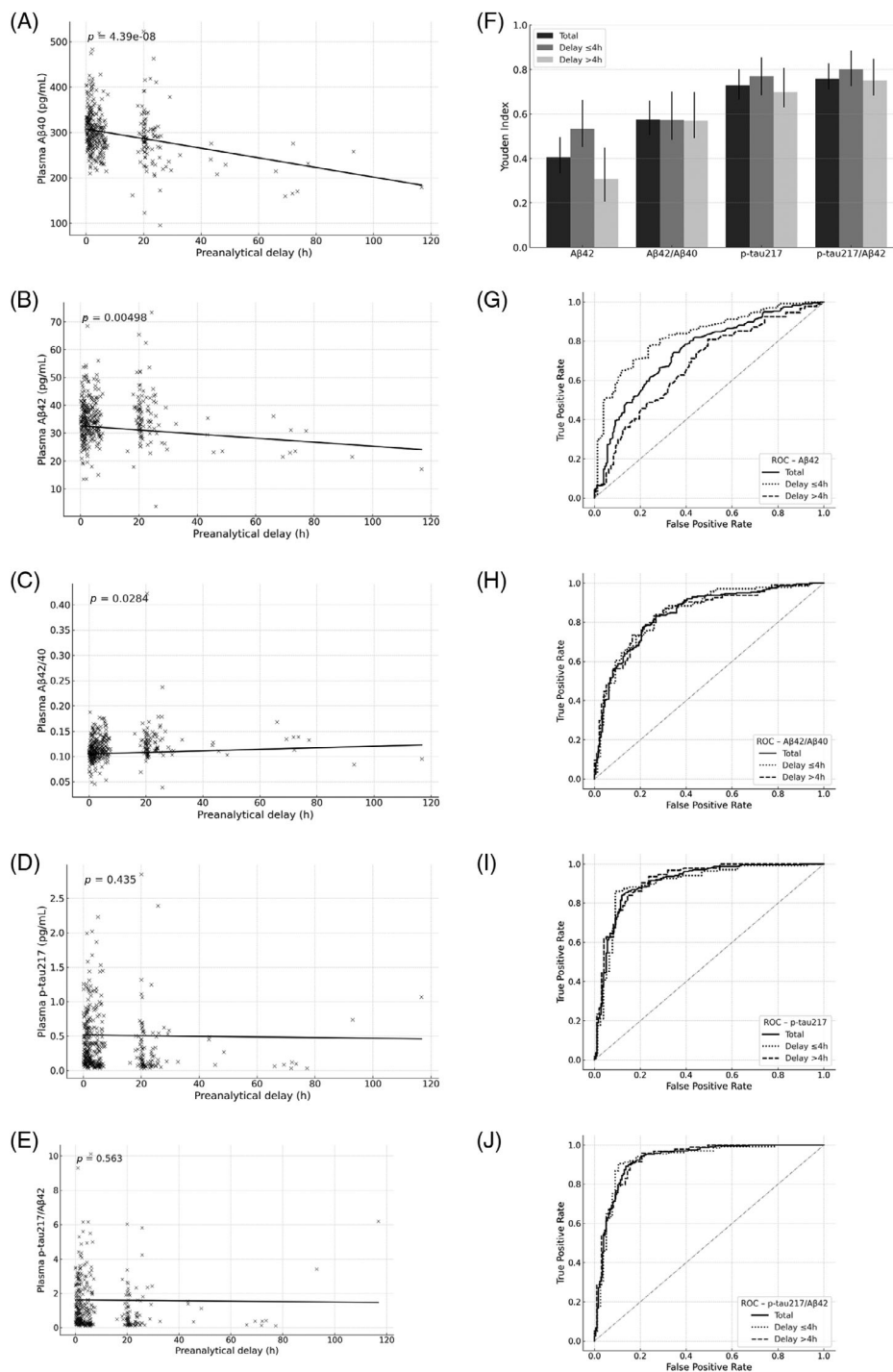


FIGURE 1 Impact of preanalytical delay on biomarkers. (A–E) Quantile regression showing relationship between Aβ40, Aβ42, Aβ42/Aβ40, p-tau217, and p-tau217/Aβ42 and outcome across different quantiles of the distribution. *P* values (adjusted Pearson correlation) are indicated on the graphs. (F) Youden index with 95% confidence intervals for each biomarker, stratified by preanalytical delay and using cut-off of the total cohort. Values for Aβ42, Aβ42/Aβ40, p-tau217, and p-tau217/Aβ42 in the total cohort and in subgroups with delay below and above 4 h were as follows: Aβ42: 0.405 (0.335 to 0.501), 0.000 (0.000 to 0.035), and 0.010 (0.000 to 0.063), respectively; Aβ42/Aβ40: 0.575 (0.509 to 0.658), 0.000 (0.000 to 0.024), and 0.000 (0.000 to 0.024), respectively; p-tau217: 0.729 (0.659 to 0.794), 0.770 (0.681 to 0.853), and 0.699 (0.630 to 0.809), respectively; p-tau217/Aβ42: 0.758 (0.704 to 0.824), 0.801 (0.716 to 0.883), and 0.750 (0.682 to 0.840), respectively. (G–J) Receiver operating characteristic (ROC) curves for total cohort and in cohort stratified by preanalytical delay. AUCs for Aβ42, Aβ42/Aβ40, p-tau217, and p-tau217/Aβ42 in the total cohort and in subgroups with delay below and above 4 h were as follows: Aβ42: 0.747 (0.700 to 0.796), 0.831 (0.772 to 0.884), and 0.669 (0.595 to 0.743), respectively; Aβ42/Aβ40: 0.848 (0.807 to 0.885), 0.847 (0.788 to 0.899), and 0.839 (0.778 to 0.891), respectively; p-tau217: 0.910 (0.880 to 0.937), 0.900 (0.849 to 0.944), and 0.918 (0.873 to 0.955), respectively; p-tau217/Aβ42: 0.929 (0.898 to 0.953), 0.924 (0.878 to 0.964), and 0.930 (0.893 to 0.961), respectively. Aβ, amyloid beta; AUC, area under the ROC curve; p-tau, phosphorylated tau; ROC: receiver operating characteristic.

TABLE 2 Stratification of cohort using two cut-off (rule in/out) risk stratification for brain amyloidosis.

Plasma biomarker	Cut-off (sensitivity ≥ 90%)	Sensitivity	Specificity	Cut-off (specificity ≥ 90%)	Sensitivity	Specificity	Rule in (high risk)	Rule out (low risk)	Gray zone
Aβ42	41.1	90.0% (85.4 to 93.1)	30.7% (24.3 to 37.8)	30.4	39.9% (33.5 to 45.8)	90.3% (85.1 to 93.9)	26.0%	18.7%	55.3%
Aβ42/Aβ40	0.127	90.8% (86.4 to 93.8)	60.8% (53.4 to 67.7)	0.109	57.6% (50.8 to 63.3)	90.3% (85.1 to 93.9)	36.6%	31.7%	31.7%
p-tau217	0.160	90.0% (85.4 to 93.1)	76.1% (69.3 to 81.8)	0.298	72.7% (66.3 to 77.6)	90.3% (85.1 to 93.9)	47.0%	37.6%	15.4%
p-tau217/Aβ42	0.509	90.3% (85.9 to 93.5)	84.1% (78.0 to 88.8)	0.844	75.6% (69.4 to 80.3)	90.3% (85.1 to 93.9)	50.1%	41.4%	8.5%

Note: Optimal cut-off prioritizing 90% specificity or sensitivity. Corresponding sensitivity and specificity, along with their 95% confidence intervals using the Wilson score method. The proportion of the cohort with high risk (rule in) was defined using the 90% specificity cut-off, the high-risk cohort (rule in) with the 90% sensitivity cut-off. The proportion between these two cut-offs represents the intermediate "gray zone."

Abbreviations: Aβ, amyloid beta; p-tau, phosphorylated tau.

TABLE 3 Predictive values according to sample preanalytical delay.

Plasma biomarker	PPV total	PPV delay ≤ 4 h	PPV delay > 4 h	NPV total	NPV delay ≤ 4 h	NPV delay > 4 h
Aβ42	84.7% (76.8 to 90.2)	71.7% (57.5 to 82.7)	95.1% (86.5 to 98.3)	69.3% (59.0 to 78.0)	68.9% (54.3 to 80.5)	72.5% (57.2 to 83.9)
Aβ42/Aβ40	89.0% (83.0 to 93.0)	90.0% (78.6 to 95.7)	89.1% (81.5 to 93.8)	80.9% (73.5 to 86.6)	82.4% (72.9 to 89.0)	81.2% (68.1 to 89.8)
p-tau217	91.0% (86.1 to 94.3)	87.5% (78.5 to 93.1)	93.4% (87.0 to 96.8)	85.4% (79.0 to 90.0)	87.5% (79.0 to 92.9)	83.6% (72.9 to 90.6)
p-tau217/Aβ42	91.4% (86.6 to 94.5)	88.9% (80.2 to 94.0)	93.7% (87.6 to 96.9)	86.5% (80.6 to 90.9)	89.1% (81.1 to 94.0)	85.5% (75.9 to 91.7)

Note: Positive predictive value (PPV) and negative predictive value (NPV) in the total study cohort and in subgroups defined by preanalytical delay (≤4 h vs >4 h). Cut-offs defined in the total cohort were used. PPV derived from specificity cut-off and NPV from sensitivity cut-off. PVs were reported with 95% confidence intervals calculated using the Wilson method.

Abbreviations: Aβ, amyloid beta; NPV, negative predictive value; PPV, positive predictive value; p-tau, phosphorylated tau.

processed within <4 h and those processed after >4 h, compared with values in the overall cohort (Table 3). In contrast, PPV and NPV for plasma Aβ42/Aβ40 and the p-tau217/Aβ42 ratio remained consistent with those of the general cohort, regardless of whether the delay was below or above 4 h. Similarly, NPV remained stable across delay categories for all biomarkers.

4 | DISCUSSION

The integration of blood-based assays into clinical practice holds promise for enhancing early diagnosis, monitoring therapeutic responses, and facilitating patient stratification for clinical trials. In a significant milestone for AD diagnosis and care, the FDA recently approved the first blood test for AD (Fujirebio's Lumipulse G1200 p-tau217/Aβ42 immunoassay), which is intended to support the diagnosis of AD in cognitively impaired individuals aged 55 and older. While this clearly represents a major advancement in the diagnosis and management of patients, it also raises certain concerns, particularly due to the low stability of Aβ42 peptides,¹⁷ which are sensitive to pre-

analytical variability. This could potentially challenge the routine use of the p-tau217/Aβ42 ratio, as such variability may lead to significant overestimation of the ratio and, consequently, an overestimation of the underlying cerebral amyloidosis reflected by this plasma biomarker. However, it should be noted that plasma biomarkers demonstrated overall acceptable stability, remaining stable for up to 24 h at 4°C when stored as whole blood or EDTA plasma.²⁸

In the present work, we took advantage of the ALZAN cohort, which offers a unique opportunity to assess, in real-life clinical conditions, whether preanalytical factors, particularly blood collection delay, impact the performance of plasma biomarkers. Specifically, we addressed the question of whether this effect could be more pronounced for the p-tau217/Aβ42 ratio compared to p-tau217 alone.

Importantly, although our general preanalytical recommendation is to maintain a preanalytical time of less than 4 h, 50% of the samples in our cohort exceeded this threshold, with 25% processed the following day. This delay was primarily due to blood being collected in the afternoon, reflecting real-life conditions in the clinical centers involved. When comparing samples processed before and after 4 h, we observed a difference in the mean age between the two groups, which may be

related to the fact that older patients, who often remain in the hospital overnight, typically have their blood drawn in the morning. The pre-analytical delay has a significant impact for A β 40 and A β 42. However, the performance of p-tau217 and p-tau217/A β 42 ratio were similar, thereby supporting the use of the ratio as a robust and efficient marker for AD diagnosis.

Looking at the diagnostic performance, we first confirm in our ALZAN subcohort that p-tau217/A β 42 AUC for A β 42+ detection outperforms all other biomarkers AUCs. We further observed that the preanalytical delay significantly affected the AUCs (and corresponding Youden indices) only when A β 42 was analyzed independently. Calculating the A β 40/A β 42 ratio or using p-tau217 in combination with A β 42 yields diagnostic performance that remains robust despite pre-analytical delays. This finding was further supported by applying a rule-in/rule-out approach using two cut-offs, where the p-tau217/A β 42 ratio outperformed the other biomarkers and was only marginally affected by preanalytical delays.

The main limitation of our study is that it was conducted within a single cohort, and confirmation of the results would benefit from validation in other cohorts. We wish to highlight the significance of these results, especially given the recent FDA approval of the blood-based p-tau217/A β 42 ratio assay for AD diagnosis. This ratio is expected to build confidence for its use in routine clinical practice. However, further evaluation of other preanalytical variability factors is necessary to guarantee consistent implementation across different centers and ensure reliable and comparable results. While the p-tau217/A β 42 ratio demonstrates preserved performance under varying preanalytical delays, its reliability may be compromised in the presence of major deviations, such as extended RT storage, hemolysis, or very long processing delays, which warrant particular attention in clinical settings. Another limitation of this study is that, due to supplier availability, we had to use different platforms to measure the biomarkers p-tau217 and A β 42. Consequently, future studies should confirm these results using identical platforms to ensure greater robustness.

In conclusion, this work represents an important step toward establishing standard operating procedures for the assessment of the p-tau217/A β 42 ratio. Validation in diverse cohorts and clinical settings will be crucial to harmonize preanalytical protocols and to guarantee consistent biomarker performance for routine AD diagnosis.

AUTHOR CONTRIBUTIONS

Sylvain Lehmann, Constance Delaby, Germain Busto, and Karim Bennys accessed and verified the underlying data. They take responsibility for the integrity of the data and the accuracy of the data analysis. Concept and design: Sylvain Lehmann, Audrey Gabelle, Karim Bennys. Acquisition of biological data: Marie Duchiron, Mehdi Morchikh. Analysis or interpretation of data: All authors. Drafting of the manuscript: Sylvain Lehmann. Critical revision of the manuscript for important intellectual content: All authors. Statistical analysis: Sylvain Lehmann, Germain Busto. Funding acquisition: Sylvain Lehmann. All authors had full access to the data and contributed to revision and editing of the manuscript. They read and approved the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

Consultant or advisory role: S. Lehmann, Advisory Board for Roche Diagnostics, Biogen, Lilly, and Fujirabio. A. Gabelle, Advisory Board for Biogen, Lilly, and Esai. No other conflicts of interest. Author disclosures are available in the [Supporting Information](#).

DATA AVAILABILITY STATEMENT

Data and informed consent forms are available upon request (CHU Montpellier). Requests will be considered by each study investigator, based on the information provided by the requester, regarding the study and analysis plan. If the use is appropriate, a data sharing agreement will be put in place before distributing a fully de-identified version of the dataset, including the data dictionary used for analysis with individual participant data.

CONSENT STATEMENT

All participants gave written informed consent to be part of the study. The ALZAN study was approved by the "Comité de Protection des Personnes Nord Ouest IV" under # 2022-A00565-38. Clinical trial registration: NCT05427448.

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SUPPORTING INFORMATION

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