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Diagnostic performance of plasma $pTau_{217}/A\beta_{42}$ ratio and a three-zone threshold model for Alzheimer's disease

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ABSTRACT

Early and accurate diagnosis of Alzheimer's disease (AD) typically relies on invasive or expensive methods like cerebrospinal fluid (CSF) biomarkers and amyloid PET imaging. Blood-based biomarkers, particularly plasma phosphorylated tau ($pTau_{181}$, $pTau_{217}$) and amyloid-beta ratios ($A\beta_{42/40}$), offer a more accessible diagnostic alternative. This study assessed the diagnostic accuracy of plasma biomarkers and developed a three-zone classification model to reduce reliance on invasive confirmatory tests. We retrospectively evaluated 109 participants referred to a tertiary memory clinic. Participants underwent cognitive assessments, brain MRI, CSF biomarker analyses ($pTau_{181}$, $A\beta_{42/40}$), and plasma biomarker measurements ($pTau_{181}$, $pTau_{217}$, $A\beta_{42/40}$, $pTau_{217}/A\beta_{42}$ ratio). Diagnostic performance was evaluated using ROC analyses, and thresholds achieving $\geq$ 95 % sensitivity and specificity were used to define low, intermediate and high-risk zones. Plasma biomarkers correlated significantly with CSF biomarkers. For identifying AD pathology (A+/T + vs. others), plasma $pTau_{217}$ and the $pTau_{217}/A\beta_{42}$ ratio demonstrated the highest accuracy (both AUC=0.95), outperforming plasma $pTau_{181}$ (AUC=0.88) and $A\beta_{42/40}$ ratio (AUC=0.73). At optimal thresholds, plasma $pTau_{217}$ showed 87.5 % sensitivity and 93.4 % specificity, whereas the $pTau_{217}/A\beta_{42}$ ratio showed higher sensitivity (95.8 %) but lower specificity (85.2 %). Using the three-zone model, plasma $pTau_{217}$ enabled definitive classification in 80.7 % of patients, increasing to 84.4 % with the $pTau_{217}/A\beta_{42}$ ratio. Among patients with mild cognitive impairment, plasma $pTau_{217}$ achieved excellent accuracy (AUC=0.98). Plasma $pTau_{217}$, alone or combined with $A\beta_{42}$, provides highly accurate and scalable identification of AD pathology, substantially reducing the need for invasive diagnostic procedures.

1. Background

Alzheimer's disease (AD) is the most common cause of dementia worldwide and its prevalence is expected to triple by 2050 due to population aging (Scheltens et al., 2021). Early and accurate diagnosis is crucial for timely therapeutic interventions, appropriate patient management and enrollment in clinical trials. Current diagnostic criteria rely on the amyloid/tau/neurodegeneration (ATN) framework proposed by the National Institute on Aging-Alzheimer's Association (NIA-AA), which is typically implemented using cerebrospinal fluid (CSF) biomarkers and positron emission tomography (PET) imaging (Hansson, 2021; Jack et al., 2018). Although highly accurate, these approaches are invasive, costly and not universally accessible in routine clinical practice.

Recent advances in blood-based biomarkers have substantially improved the accessibility of AD diagnostics (Hampel et al., 2018;

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Teunissen et al., 2022). Among these, plasma phosphorylated tau isoforms (particularly $pTau_{181}$ and $pTau_{217}$) and amyloid-β (Aβ) ratios ($Aβ_{42}/_{40}$) show strong concordance with CSF and PET markers of AD pathology and high diagnostic accuracy (Benussi et al., 2020; Karikari et al., 2020). Plasma $pTau_{217}$, in particular, often outperforms other blood-based markers and approaches the performance of CSF and PET (Ashton et al., 2024; Mattsson-Carlgren et al., 2022; Palmqvist et al., 2020). These advancements have supported recent regulatory and guideline changes, including FDA clearance of a commercial plasma assay for AD and the incorporation of blood-based biomarkers into the latest AA diagnostic criteria (FDA, 2025; Jack et al., 2024).

For clinical implementation, however, defining operational diagnostic thresholds is critical. Binary cutoffs that classify patients simply as biomarker positive or negative may inadequately capture borderline or uncertain cases and can still lead to frequent use of confirmatory CSF or PET (Hansson et al., 2023). Several groups have therefore proposed a three-zone classification (negative, positive and an intermediate "grey zone") to stratify diagnostic confidence and triage the need for additional testing (Benussi et al., 2025b; Brum et al., 2023; Hansson et al., 2022; Hazan et al., 2025). While this approach is promising, optimal three-zone thresholds for plasma $pTau_{217}$ remain under evaluation, and data from real-world memory clinic populations are still limited.

The aim of the present study was to assess the diagnostic accuracy of plasma biomarkers ($pTau_{181}$, $pTau_{217}$, the $Aβ_{42}/_{40}$ ratio and the $pTau_{217}/Aβ_{42}$ ratio) for AD and to develop a two-step diagnostic algorithm based on the most accurate marker, defining low-, intermediate- and high-risk categories to reduce the need for confirmatory CSF analysis and/or amyloid PET.

2. Methods

2.1. Study design and participants

We conducted a retrospective observational study of consecutive patients referred for cognitive assessment to the Memory Clinic of the University Hospital of Trieste, Italy, between March 2022 and December 2024. All patients underwent a standardized diagnostic work-up including clinical and neurological examination, Montreal cognitive assessment (MoCA), clinical dementia rating (CDR, global score and sum of boxes), structural brain MRI, routine blood tests and CSF analysis as part of routine care.

CSF tTau, $pTau_{181}$, $Aβ_{42}$ and $Aβ_{40}$ were measured using validated assays, and patients were classified according to the 2024 Alzheimer's Association research framework (Jack et al., 2024) using manufacturer cutoffs for CSF $Aβ_{42}/_{40}$ (A) and $pTau_{181}$ (T1), as previously reported (Benussi et al., 2022b). Plasma and CSF samples were collected within a maximum interval of 7 days. Additional procedural details are reported in the Supplementary Methods.

2.2. Blood based biomarkers measurements

Blood was collected in K2-EDTA tubes, processed according to standardized pre-analytical procedures and stored at −80°C. Plasma $pTau_{181}$, $pTau_{217}$, $Aβ_{42}$ and $Aβ_{40}$ were quantified on the fully automated LUMIPULSE G platform (Fujirebio), and $Aβ_{42}/_{40}$ and $pTau_{217}/Aβ_{42}$ ratios were calculated. Full pre-analytical and assay procedures are provided in the Supplementary Methods.

2.3. Statistical analysis

Demographic, clinical and biomarker characteristics were summarized using descriptive statistics. Continuous variables were compared across CSF A/T profiles and plasma risk strata using generalized linear models (Gaussian family, identity link) with group as predictor and, where appropriate, age and sex as covariates; FDR-corrected pairwise comparisons were used for *post hoc* tests. Categorical variables were compared using Fisher's exact tests. Correlations between CSF and plasma biomarkers and between biomarkers and clinical measures were assessed using Spearman's rho. Further details on model assumptions, handling of skewness and effect size estimation are given in the Supplementary Methods.

Diagnostic performance of plasma biomarkers for CSF-defined A and T status and for clinical AD vs non-AD was evaluated using receiver operating characteristic (ROC) curve analyses, with AUCs (95 % CI) compared using DeLong's test (DeLong et al., 1988). Moreover, logistic regression models including the biomarker with age/sex adjustment were fitted and the corresponding AUCs compared. For the three-zone classification, we used ROC coordinates to derive "rule-out" and "rule-in" thresholds on the most accurate biomarker ($pTau_{217}$ or $pTau_{217}/Aβ_{42}$ ratio) based on pre-specified sensitivity and specificity targets, defining low-, intermediate- and high-risk categories. All tests were two-sided with $p < 0.05$; FDR correction (Benjamini and Hochberg, 1995), was applied where appropriate. Analyses were performed using IBM SPSS Statistics 29.0 and GraphPad Prism 10.0.

3. Results

3.1. Demographic and clinical features

We enrolled a total of 109 patients. Among the CSF A/T profiles, 44 were categorized as A-/T- (40.4 %), 4 as A-/T + (3.7 %), 13 as A+ /T- (11.9 %), and 48 as A+ /T + (44.0 %). As expected, the A-/T + profile was rare in this memory clinic CSF cohort, consistent with the low prevalence (approximately 3–5 %) reported in larger ATN studies, and results involving this subgroup should therefore be interpreted with caution. Demographic and clinical features of different A/T groups are summarized in Table 1. The patient groups exhibited similar distributions in terms of age, age at symptom onset, sex, education, MoCA and CDR scores. The prevalence of vascular, metabolic and psychiatric comorbidities was similar across A/T groups and across $pTau_{217}/Aβ_{42}$ risk strata, with no statistically significant between-group differences.

3.2. CSF and plasma biomarker correlations

In the whole sample, we observed a significant negative correlation between age and CSF $Aβ_{42/40}$ ratio ($r = -0.19$, $p = 0.049$), plasma $Aβ_{42/40}$ ratio ($r = -0.23$, $p = 0.014$), and a positive correlation with CSF $pTau_{181}$ ($r = 0.23$, $p = 0.017$). MoCA scores significantly correlated with CSF $Aβ_{42/40}$ ratio ($r = 0.22$, $p = 0.025$), plasma $pTau_{181}$ ($r = -0.27$, $p = 0.005$) and plasma $pTau_{217}$ ($r = -0.38$, $p < 0.001$).

We observed strong correlations between CSF and plasma biomarkers, in particular CSF $pTau_{181}$ correlated with plasma $pTau_{181}$ ($r = 0.55$, $p < 0.001$), plasma $pTau_{217}$ ($r = 0.74$, $p < 0.001$), and plasma $Aβ_{42/40}$ ratio ($r = -0.41$, $p < 0.001$). The CSF $Aβ_{42}/_{40}$ ratio correlated similarly with plasma $pTau_{181}$ ($r = -0.57$, $p < 0.001$), plasma $pTau_{217}$ ($r = -0.77$, $p < 0.001$), and plasma $Aβ_{42/40}$ ratio ($r = 0.47$, $p < 0.001$) (see Figure 1).

3.3. Plasma biomarkers across different A/T groups

Given the association between several CSF and blood-based biomarkers and age, plasma biomarkers were analyzed using GLMs with A/T group as predictor and age and sex as covariates. Unadjusted models were also run as a sensitivity analysis and showed the same pattern of significance, therefore only the adjusted models are reported below. In the adjusted models, A/T group had a highly significant effect on plasma $pTau_{181}$, $pTau_{217}$, Amyloid-$β_{42}$, the Amyloid-$β_{42/40}$ ratio and the $pTau_{217}$/Amyloid-$β_{42}$ ratio (all $p < 0.001$), whereas Amyloid-$β_{40}$ did not differ significantly across A/T profiles ($p = 0.36$) (Table 1). Patients in the A+ /T + group showed substantially higher concentrations of both $pTau_{181}$ and $pTau_{217}$ compared with all other groups, together with markedly increased $pTau_{217}$/Amyloid-$β_{42}$ ratios (all *post hoc*

Table 1
Demographic and clinical features of participants according to A/T status.

	A-/T- (n = 44)	A-/T + (n = 4)	A+ /T- (n = 13)	A+ /T + (n = 48)
Age, yrs	68.8 (62.7–72.7)	72.0 (67.8–75.4)	72.8 (69.4–77.6)	70.8 (66.2–75.7)
Sex, males (%)	24 (54.5 %)	1 (25.0 %)	6 (46.2 %)	28 (58.3 %)
Age at onset, yrs	64.0 (57.3–69.8)	66.1 (60.0–72.1)	68.0 (64.4–75.5)	66.0 (60.3–73.0)
Education	13.0 (8.3–17.0)	10.5 (8.0–13.5)	9.8 (6–12.5)	12.5 (8.0–16.5)
MoCA	20.0 (17.9–24.0)	17.5 (14.9–25.2)	19.0 (14.0–22.5)	18.5 (13.3–22.0)
Global CDR	0.5 (0.5–0.88)	0.8 (0.5–1.0)	0.5 (0.5–1.0)	0.5 (0.5–1.0)
CDR Sum of boxes	1.3 (0.5–3.0)	3.3 (1.3–7.1)	2.0 (1.0–4.3)	2.0 (1.1–5.0)
CSF biomarkers				
$pTau_{181}$ (pg/mL)	35.30 (28.45–47.60)†§	66.90 (61.73–102.23)*	47.70 (37.30–52.70)	105.70 (80.60–142.90)*
$A\beta_{42/40}$ (pg/mL)	0.101 (0.091–0.105)‡§	0.091 (0.075–0.111)‡§	0.059 (0.054–0.064)*†§	0.043 (0.038–0.053)*†‡
Plasma biomarkers	0.905 (0.655–1.390)§	0.990 (0.865–1.123)§	1.090 (0.840–1.535)§	1.920 (1.425–2.648)*†‡
$pTau_{181}$ (pg/mL)	0.905 (0.655–1.390)§	0.990 (0.865–1.123)§	1.090 (0.840–1.535)§	1.920 (1.425–2.648)*†‡
$pTau_{217}$ (pg/mL)	0.113 (0.082–0.181)‡§	0.117 (0.137–0.248)§	0.207 (0.140–0.381)*§	0.643 (0.366–1.215)*†‡
$A\beta_{42/40}$ (pg/mL)	0.097 (0.082–0.112)§	0.082 (0.077–0.104)	0.081 (0.068–0.098)	0.071 (0.065–0.09)*
$pTau_{217}/A\beta_{42}$ (pg/mL)	0.004 (0.003–0.006)‡§	0.008 (0.006–0.008)§	0.009 (0.006–0.015)*§	0.030 (0.017–0.058)*†‡

Data are median (IQR) or n (%). n = number of participants; yrs = years; MoCA = Montreal Cognitive assessment; CDR = clinical dementia rating; CSF = cerebrospinal fluid.
*$p < 0.05$ vs A-/T-
†$p < 0.05$ vs A-/T +
‡$p < 0.05$ vs A+ /T-
§$p < 0.05$ vs A+ /T + .
Continuous demographic, clinical and biomarker variables were compared across A/T groups using generalized linear models (Gaussian family, identity link) with A/T group as the main predictor; for CSF and plasma biomarker analyses, models were additionally adjusted for age and sex. Pairwise comparisons between A/T groups were derived from these models and corrected for multiple testing using the false discovery rate (FDR). Categorical variables were compared using Fisher's exact test.

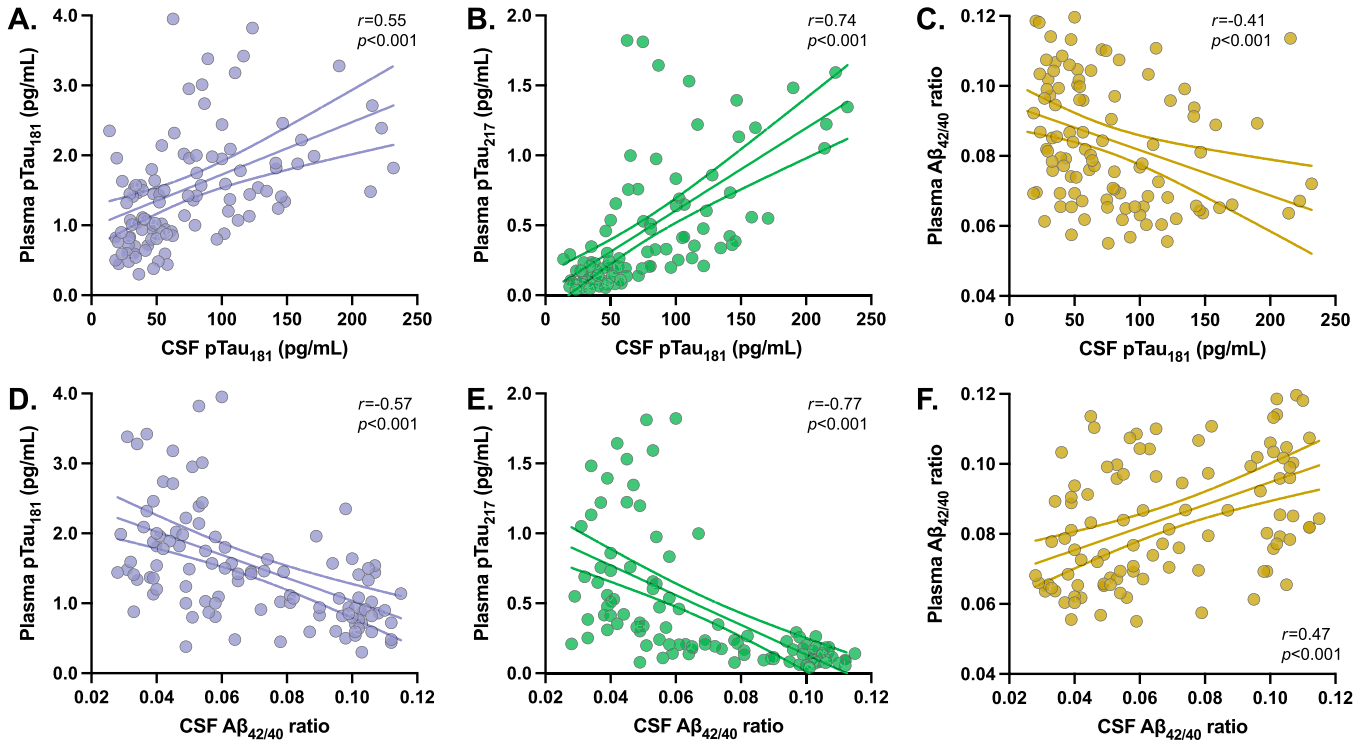

Fig. 1. Correlations between CSF and plasma biomarkers. Correlations between CSF $pTau_{181}$ and A) plasma $pTau_{181}$, B) plasma $pTau_{217}$, and C) plasma $A\beta_{42/40}$ ratio. Correlations between CSF $A\beta_{42/40}$ ratio and D) plasma $pTau_{181,}$ E) plasma $pTau_{217}$ and F) plasma $A\beta_{42/40}$ ratio.

comparisons after correction for multiple testing significant for contrasts involving A+/T + vs other groups). Conversely, the Amyloid-$\beta_{42/40}$ ratio and Amyloid-β_{42} levels were significantly lower in A+ / T + participants compared with A-/T-, whereas differences among the A-/T + , A+ /T- and A-/T- groups were smaller and often non-significant (see Figure 2). The magnitude of these effects was large to very large for contrasts involving A+ /T + versus A-/T-, with Cohen's *d* of 1.68 for $pTau_{217}$, 1.43 for $pTau_{181}$ and 1.58 for the $pTau_{217}$/Amyloid-β_{42} ratio, and −0.83 and −0.86 for Amyloid-β_{42} and the Amyloid-$\beta_{42/40}$ ratio, respectively.

3.4. Diagnostic accuracy of blood-based biomarkers

We performed ROC curve analysis to identify the biomarker with the highest accuracy in distinguishing A+ from A-, and A+ /T + from the other groups.

For A+ vs A-, the biomarker with the highest AUC was the $pTau_{217}/A\beta_{42}$ ratio (AUC 0.94, 95 % CI 0.89–0.99, $p < 0.001$), followed by

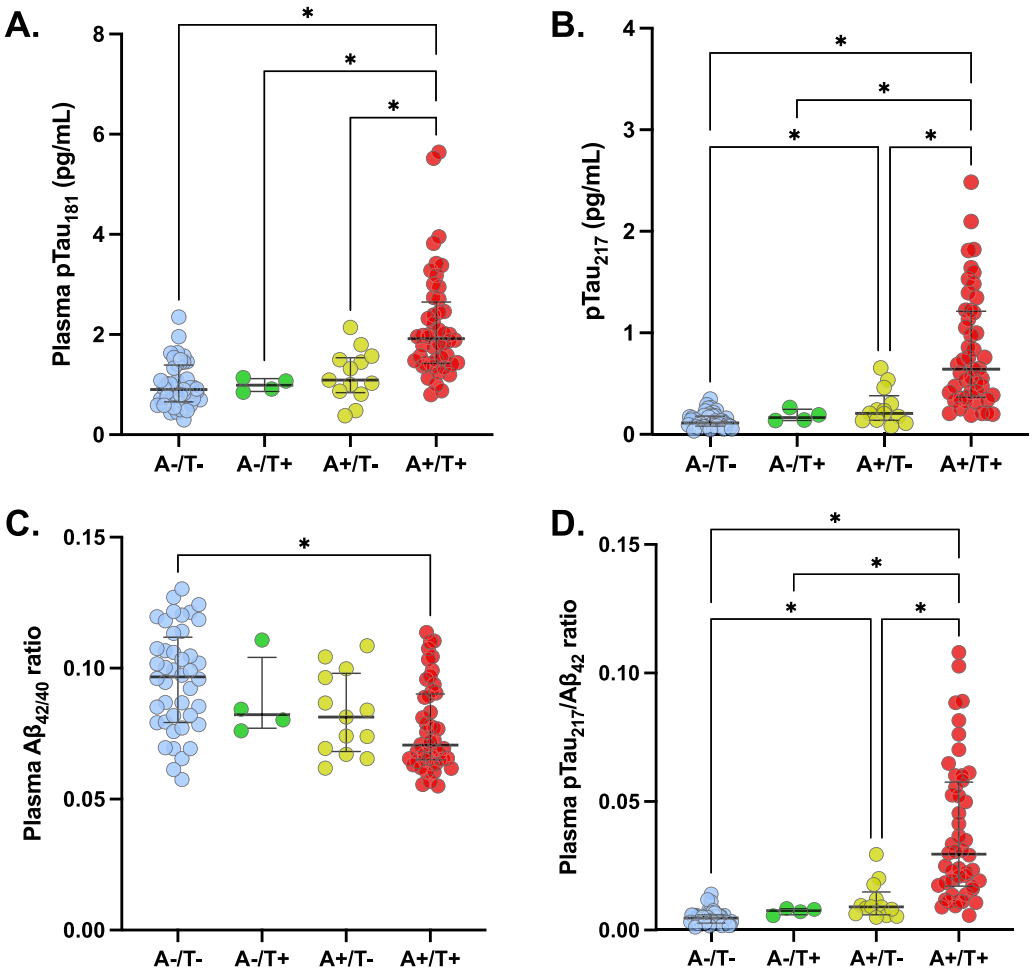

Fig. 2. Plasma biomarkers according to A/T status. A) plasma $pTau_{181}$, B) plasma $pTau_{217}$, C) plasma $A\beta_{42/40}$ ratio and D) plasma $pTau_{217}/A\beta_{42}$ ratio. Thick horizontal lines represent median values while thin horizontal lines represent the interquartile range. Between group differences were tested using generalized linear models (Gaussian family, identity link) with A/T group as predictor and age and sex as covariates, followed by FDR corrected pairwise comparisons. *$p < 0.05$ for *post hoc* comparisons between A/T groups. Given the very small number of A-/T + cases (n = 4), estimates for this subgroup are less precise and should be interpreted with caution.

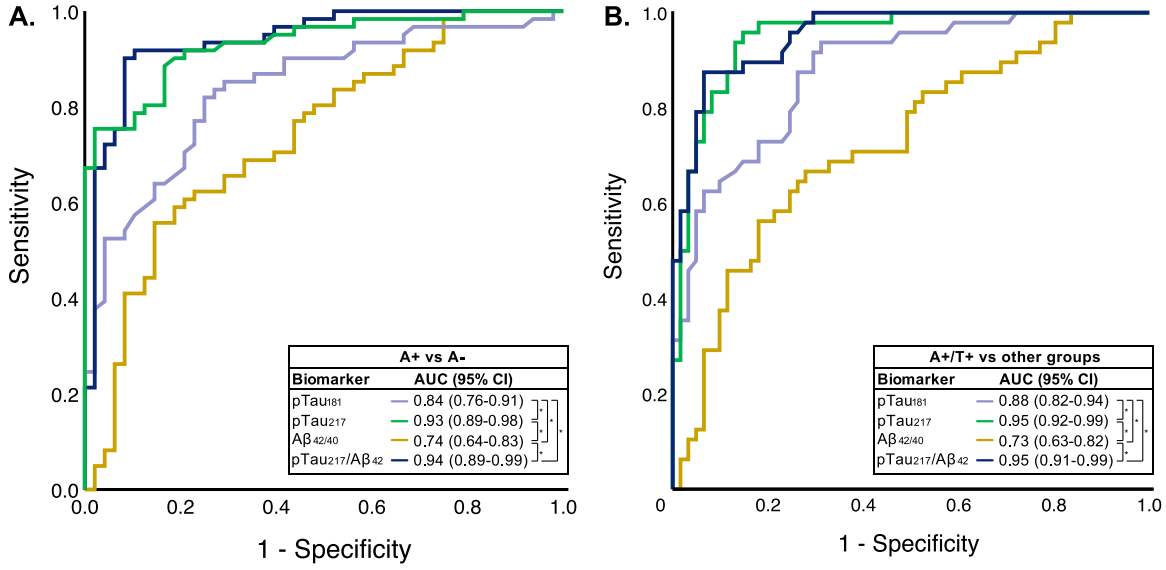

Fig. 3. ROC curve analysis. ROC curves for differentiating A) A+ vs A- and B) A+ /T + vs other A/T groups, with AUCs and 95 % CI. Comparisons between AUCs were performed using DeLong statistics: *$p < 0.05$. ROC = receiver operating characteristic; AUC = area under the curve; 95 % CI = 95 % confidence interval.

$pTau_{217}$ (AUC 0.93, 95 % CI 0.89–0.98), $pTau_{181}$ (AUC 0.84, 95 % CI 0.76–0.91, $p < 0.001$) and the $A\beta_{42/40}$ ratio (AUC 0.74, 95 % CI 0.64–0.83, $p < 0.001$) (see Figure 3A). According to DeLong's test, there was a significant AUC difference between the $pTau_{217}/A\beta_{42}$ ratio and both $pTau_{181}$ ($p = 0.009$) and the $A\beta_{42/40}$ ratio ($p < 0.001$), but not with the $pTau_{217}$ alone ($p = 0.739$). According to Youden's index, the $pTau_{217}/A\beta_{42}$ ratio (at a value of 0.0085) showed a sensitivity of 90.2 % and a specificity of 91.7 %, while $pTau_{217}$ alone (at a value of 0.296 pg/mL) showed a sensitivity of 75.4 % and a specificity of 97.9 %

For A+ /T + vs other groups (A-/T-, A-/T + , A+/T-), the biomarker with the highest AUC was $pTau_{217}$ (AUC 0.95, 95 % CI 0.92–0.99, $p < 0.001$), followed by the $pTau_{217}/A\beta_{42}$ ratio (AUC 0.95, 95 % CI 0.91–0.99, $p < 0.001$), $pTau_{181}$ (AUC 0.88, 95 % CI 0.82–0.94, $p < 0.001$) and the $A\beta_{42/40}$ ratio (AUC 0.73, 95 % CI 0.63–0.82, $p < 0.001$) (see Figure 3B). There was a significant AUC difference between $pTau_{217}$ and both $pTau_{181}$ ($p = 0.002$) and the $A\beta_{42/40}$ ratio ($p < 0.001$), but not with the $pTau_{217}/A\beta_{42}$ ratio ($p = 0.739$). According to Youden's index, the $pTau_{217}/A\beta_{42}$ ratio (at a value of 0.0092) showed a sensitivity of 95.8 % and a specificity of 85.2 %, while $pTau_{217}$ alone (at a value of 0.306 pg/mL) showed a sensitivity of 87.5 % and a specificity of 93.4 %.

When we selected patients with a global CDR score of 0.5 (*i.e.*, patients with MCI) (A+/T + , n = 31 vs other groups, n = 41), we observed very similar results. In particular, the biomarker with the highest AUC was $pTau_{217}$ (AUC 0.98, 95 % CI 0.96–1.00, $p < 0.001$), followed by the $pTau_{217}/A\beta_{42}$ ratio (AUC 0.96, 95 % CI 0.92–1.00, $p < 0.001$), $pTau_{181}$ (AUC 0.91, 95 % CI 0.82–0.94, $p < 0.001$) and the $A\beta_{42/40}$ ratio (AUC 0.73, 95 % CI 0.63–0.82, $p < 0.001$).

In additional ROC analyses, we evaluated whether including age and/or sex alongside the plasma biomarkers improved discrimination for A+ /T + status. Across all biomarkers, AUC values for models including age, sex, or both were very similar to those of the corresponding biomarker-only models, and DeLong's tests did not show any significant increase in accuracy.

To approximate real-world diagnostic performance, we also evaluated how plasma biomarkers discriminated between clinically diagnosed AD and non-AD disorders. Using the final consensus clinical diagnosis as the outcome, plasma $pTau_{217}$ and the $pTau_{217}/A\beta_{42}$ ratio again showed the highest accuracy in ROC analyses (AUC 0.94 and 0.95, respectively), followed by $pTau_{181}$ (AUC 0.87) and the $A\beta_{42/40}$ ratio (AUC 0.71).

3.5. Three-zone classification strategy

We applied a three-zone threshold model to each biomarker, using A+ /T + status as the reference standard. For each plasma biomarker, we defined a lower cutoff that achieved ≥ 95 % sensitivity to identify individuals at low-risk (*i.e.*, low probability of having an A+/T + status) and an upper cutoff that achieved ≥ 95 % specificity to identify individuals at high-risk (*i.e.*, high probability of A+/T + status). Values falling between these cutoffs defined an intermediate "grey zone", for which additional testing would be recommended.

For plasma $pTau_{217}$, we identified a lower cutoff of 0.206 pg/mL and an upper cutoff of 0.353 pg/mL. Based on these thresholds, 40 participants (36.7 %) were classified as high-risk, 48 participants (44.0 %) as low-risk, and 21 participants (19.3 %) fell within the grey zone. This approach enabled a definitive classification in 88 out of 109 participants (80.7 %) without the need for additional invasive testing (see Figure 4A).

For the $pTau_{217}/A\beta_{42}$ ratio, a lower cutoff of 0.009 and an upper cutoff of 0.019 were identified. Using these thresholds, 55 participants (50.5 %) were classified as high-risk, 37 participants (33.9 %) as low-risk, and 17 participants (15.6 %) fell within the grey zone. When comparing these three groups, intermediate-zone patients showed an age and degree of cognitive impairment intermediate between low- and high-risk individuals (see Supplementary Table S1), with high-risk patients displaying overall lower MoCA scores and higher CDR-SB than the other groups. This approach allowed 92 out of 109 participants (84.4 %) to be definitively classified without further testing (see Figure 4B), representing an improvement of 6 individuals (5.5 %) compared to $pTau_{217}$ alone.

When applying more stringent cutoffs that achieved ≥ 97.5 % sensitivity and specificity, the number of participants in the grey zone increased to 34 for both $pTau_{217}$ and the $pTau_{217}/A\beta_{42}$ ratio. Nevertheless, this strategy still enabled the avoidance of CSF or amyloid PET testing in 68.9 % of participants for each biomarker.

To facilitate comparison with the FDA cleared Lumipulse G $pTau_{217}$/Amyloid-β_{42} ratio, we also applied the manufacturer recommended cutoffs in our cohort (ratio ≤0.00370 negative, 0.00371–0.00737 indeterminate, ≥0.00738 positive). Under this categorisation, 47 of 48 A+T + participants were classified as positive and 1 as indeterminate, with none classified as negative. Among participants in other A/T groups (n = 61), 19 were classified as negative, 25 as indeterminate and 17 as positive. When considering only participants classified as negative

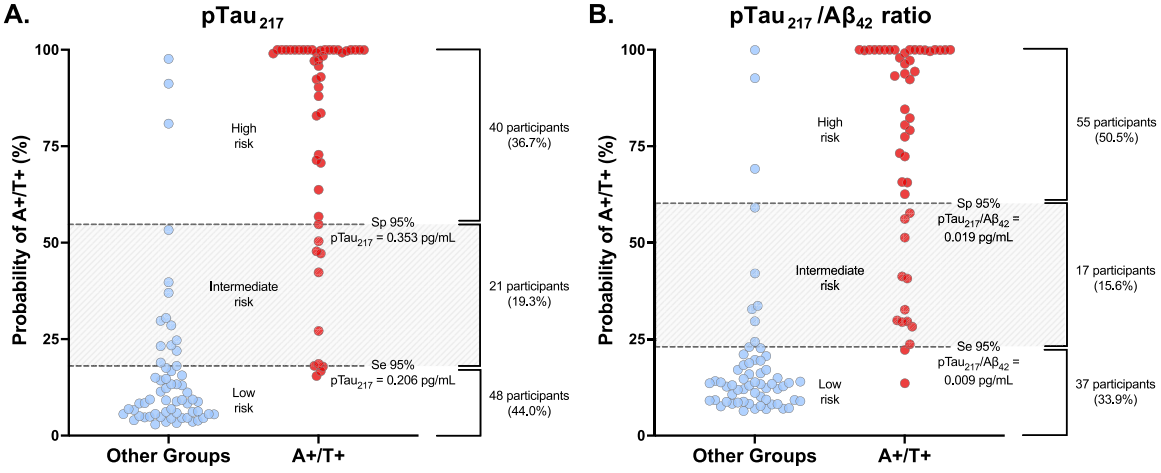

Fig. 4. Risk stratification with a plasma $pTau_{217}$ and $pTau_{217}/A\beta42$ ratio-based models. Distribution of predicted probabilities of A+ /T + obtained from logistic regression models including A) $pTau_{217}$ and B) $pTau_{217}$/Amyloid-β_{42} ratio as predictors. The $pTau_{217}$ and $pTau_{217}$/Amyloid-β_{42} ratio values corresponding to the evaluated risk thresholds are indicated by vertical dashed lines and were derived from the ROC curves, corresponding to the biomarker values achieving 95 % sensitivity (low risk threshold) and 95 % specificity (high risk threshold) for A+ /T + status. The lower dashed line demonstrates where the 95 % sensitivity low risk threshold falls on the probability distribution, while the upper dashed line corresponds to the 95 % specificity high risk threshold.

or positive by the FDA cutoffs (excluding indeterminate; n = 83), classification was concordant with A+T + status in 66 of 83 cases (79.5 %) (see Supplementary Figure).

4. Discussion

This study demonstrates the clinical feasibility and diagnostic value of plasma biomarkers, particularly plasma $pTau_{217}$ and the $pTau_{217}/A\beta_{42}$ ratio, in accurately identifying AD pathology. By adopting a three-zone classification model (low-risk, intermediate-risk or "grey zone," and high-risk), we were able to effectively stratify patients based on their likelihood of AD biomarker positivity, substantially reducing reliance on invasive or expensive confirmatory diagnostic procedures such as CSF sampling and amyloid PET imaging. These results align closely with recent updates to Alzheimer's Association guidelines, endorsing the use of blood-based biomarkers as robust first-line diagnostic tools (Hansson et al., 2022; Palmqvist et al., 2025b), and are consistent with several recent multicenter and memory-clinic studies using the fully automated Lumipulse platform that have reported very high diagnostic accuracy for plasma $pTau_{217}$ and the $pTau_{217}/A\beta_{42}$ ratio (AUCs>0.95) (Arranz et al., 2024; Cecchetti et al., 2024; Martínez-Dubarbie et al., 2025; Wang et al., 2025). Because the study was embedded in the routine workflow of a tertiary memory clinic, these findings support the real-world applicability of plasma-based triage to guide when CSF or PET confirmation is needed.

In line with prior studies, our results confirm plasma $pTau_{217}$ as a highly accurate and reliable marker for AD diagnosis, achieving diagnostic accuracy comparable to CSF and PET biomarkers, demonstrating its remarkable sensitivity and specificity in detecting cerebral amyloid and tau pathology, and strongly suggesting that it could effectively replace or at least complement invasive testing in clinical practice (Arranz et al., 2025; Bali et al., 2025; Groot et al., 2022; Lehmann et al., 2025, 2024; Palmqvist et al., 2020; Rudolph et al., 2025). These findings have culminated in a recent milestone, as the U.S. Food and Drug Administration (FDA) approved the Lumipulse G plasma $pTau_{217}/A\beta_{42}$ ratio assay in May 2025, underscoring the biomarker's maturity and readiness for widespread clinical application.

The main novelty of this work is that it evaluates Lumipulse plasma $pTau_{217}$ and the $pTau_{217}$/Amyloid-β_{42} ratio in an unselected, real-world memory clinic where CSF is the predominant confirmatory test, and derives a three-zone risk classification calibrated to CSF-defined A+ / T + status to support practical triage of which patients may safely avoid, or should be prioritized for, further CSF or PET investigations.

Interestingly, our study highlighted the incremental diagnostic value of incorporating the $pTau_{217}/A\beta_{42}$ ratio compared to $pTau_{217}$ alone. Although both biomarkers exhibited similarly high diagnostic accuracy, the ratio modestly improved classification by reducing the number of patients assigned to the intermediate "grey zone," thus providing greater diagnostic certainty. A recent large-scale study similarly reported only marginal gains in overall diagnostic accuracy when using the $pTau_{217}/A\beta_{42}$ ratio versus $pTau_{217}$ alone, aiding in reducing intermediate test results (Palmqvist et al., 2025a). Therefore, while the ratio could help minimize diagnostic uncertainty in ambiguous cases, the clinical benefit should be weighed against practical considerations.

From a practical perspective, it is also important to consider our ROC derived thresholds in relation to the manufacturer recommended cut-offs, which in our cohort yielded a larger low risk group, a slightly smaller grey zone and a more restricted high-risk group. This pattern likely reflects the different reference standards and optimization goals: the Lumipulse thresholds were derived against amyloid PET or combined PET/CSF amyloid positivity, whereas our cutoffs are anchored to CSF defined A+ /T + status in a real world memory clinic population and should therefore be viewed as context specific, CSF based operational cutoffs that complement rather than replace the manufacturer recommendations and require external validation in independent cohorts. Our results also suggest that threshold performance may vary with case mix and pretest probability, which can differ across clinics and populations. We therefore encourage multicenter studies that validate and, when necessary, recalibrate thresholds in diverse settings, including community and tertiary memory clinics.

In contrast, the plasma $A\beta_{42/40}$ ratio displayed relatively poor diagnostic accuracy, consistent with numerous previous reports in the literature (Doecke et al., 2025; Giudici et al., 2020; Janelidze et al., 2021; Pyun et al., 2024; Quaresima et al., 2024; Verberk et al., 2018). Multiple biological and analytical factors likely contribute to this limited performance. A significant factor is the weak and inconsistent correlation between plasma and CSF $A\beta_{42}$ levels (Verberk et al., 2018), with only a minor fraction of plasma $A\beta_{42}$ originating from central nervous system sources (Toombs and Zetterberg, 2020). Peripheral tissues, particularly platelets, are major contributors to circulating $A\beta_{42}$, significantly diluting brain-derived signals (Padovani et al., 2001; Sun et al., 2018). Additionally, plasma $A\beta_{42}$ levels demonstrate stronger correlations with brain pathology only when blood-brain barrier permeability is compromised, particularly in *ApoE* ε4 carriers (Bellaver et al., 2023; Libri et al., 2024). Consequently, in early or MCI cases, plasma $A\beta_{42}$ provides little discriminatory value and are highly susceptible to even minor pre-analytical variations, and modest coefficients of variation can dramatically diminish diagnostic accuracy (Benedet et al., 2022; Karikari et al., 2022). Taken together, these biological and technical challenges likely explain why plasma $A\beta_{42/40}$ has consistently underperformed as a diagnostic biomarker for AD in clinical studies.

The diagnostic utility of plasma biomarkers observed in our cohort was equally strong when we restricted analyses to patients with MCI (CDR=0.5). This finding has crucial clinical implications, as disease-modifying therapies and clinical trials increasingly target the earliest symptomatic stages of AD. Plasma biomarkers, therefore, represent valuable tools not only for accurate diagnosis but also for early patient stratification, potentially maximizing therapeutic effectiveness. Given recent promising results from phase 3 trials of anti-amyloid agents, such as lecanemab and donanemab, the ability to identify suitable patients at the earliest disease stages using minimally invasive blood tests is especially timely and relevant (Sims et al., 2023; van Dyck et al., 2023).

Despite these promising findings, several limitations must be considered. *ApoE* genotype was not systematically available and could therefore not be included in the multivariable models; although adjustment for age and sex did not change the pattern of between-group differences in plasma biomarkers, residual confounding by *ApoE* status cannot be entirely excluded. Nonetheless, prior work indicates that *ApoE* explains only a modest proportion of variance in plasma $pTau_{217}$ and provides limited incremental value over high-performing $pTau_{217}$-based models (Pandey et al., 2025). Our study was conducted in a single specialized Italian memory clinic, with a moderate sample size and a cohort consisting almost exclusively of White individuals, which limits generalizability to other settings and more diverse populations. In addition, the number of participants in some AT strata, particularly the A-/T + group, was small, reflecting the rarity of this profile in CSF-based ATN cohorts and resulting in less precise estimates; our main inferences therefore rely on contrasts between the more prevalent A+ /T + and A-/T- groups, and findings for A-/T + should be viewed as exploratory.

Finally, referral patterns to a tertiary clinic and the cross-sectional design may influence biomarker performance and do not capture longitudinal trajectories, despite growing evidence that changes over time in plasma biomarkers, including $pTau_{217}$, predict amyloid-dependent progression, cognitive decline and progression to dementia (Ashton et al., 2022; Contador and Suárez-Calvet, 2024; Kivisäkk et al., 2022). This underscores the need for external validation in larger, multi-center and more ethnically diverse cohorts with longitudinal follow-up.

Another important consideration is that A and T status was defined using CSF rather than amyloid or tau PET. Although CSF and PET show good overall agreement for amyloid-β pathology when ratio-based CSF measures and validated cutoffs are used, they are not fully interchangeable and discrepancies may occur, particularly around decision

thresholds and in early disease stages (Guillén et al., 2025; Knopman et al., 2025). The plasma cutoffs reported here are therefore anchored to a CSF-defined A+ /T + status and should be interpreted in that context, with validation in independent cohorts, ideally including amyloid PET. At the same time, using CSF as the reference standard reflects routine practice in many European memory clinics, including in Italy (Benussi et al., 2025a; Frisoni et al., 2024), where CSF analysis is far more common than amyloid PET, which may enhance the ecological validity and immediate applicability of our proposed thresholds in real-life clinical settings.

An area for future exploration is the reduction of diagnostic uncertainty in intermediate-risk cases, which constituted approximately 15–20 % of participants even after applying the biomarker ratio. Incorporating additional blood biomarkers reflecting neurodegeneration or neuroinflammation, such as neurofilament light (NfL) or glial fibrillary acidic protein (GFAP), could further refine diagnostic precision (Benussi et al., 2022; Chatterjee et al., 2023; González-Escalante et al., 2025; Grande et al., 2025; Palmqvist et al., 2023; Rabl et al., 2024; Therriault et al., 2024).

5. Conclusions

In conclusion, this study strongly supports plasma $pTau_{217}$ and the $pTau_{217}/A\beta_{42}$ ratio as clinically valuable blood-based biomarkers for accurately diagnosing Alzheimer's disease. The implementation of a three-zone diagnostic model significantly improves clinical feasibility by reducing reliance on invasive confirmatory procedures. Plasma $pTau_{217}$ alone demonstrates robust diagnostic accuracy and clinical practicality, while the $pTau_{217}/A\beta_{42}$ ratio offers incremental benefits primarily by reducing diagnostic uncertainty in borderline cases. Ultimately, our findings reinforce the promising potential of blood-based biomarkers to transform AD diagnostics, providing scalable, cost-effective, and patient-friendly solutions for early diagnosis and optimal patient management.

CRediT authorship contribution statement

Alina Menichelli: Writing – review & editing, Investigation, Data curation. **Francesco Capacchione:** Writing – review & editing, Investigation, Data curation. **Francesca Sirianni:** Writing – review & editing, Supervision, Methodology, Investigation, Formal analysis, Data curation. **Alberto Perego:** Writing – review & editing, Resources, Funding acquisition. **Marco Michelutti:** Writing – original draft, Methodology, Investigation, Formal analysis. **Paolo Manganotti:** Writing – review & editing, Supervision, Project administration, Investigation. **Alberto Benussi:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation. **Tatiana Cattaruzza:** Writing – review & editing, Supervision, Investigation, Formal analysis, Data curation, Conceptualization. **Barbara Toffoletto:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Tiziana Maria Isabella Lombardo:** Writing – original draft, Methodology, Formal analysis, Data curation. **Valentina Cenacchi:** Writing – review & editing, Investigation, Data curation. **Federica Palacino:** Writing – review & editing, Methodology, Investigation, Data curation. **Luca Pelusi:** Writing – review & editing, Investigation, Data curation.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the University of Trieste. All participants or their legal representatives provided written informed consent to participate in the study, in accordance with the Declaration of Helsinki.

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Declaration of Competing Interest

None.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.neurobiolaging.2025.12.005.

Data Availability

The datasets analyzed during the current study are not publicly available due to the inclusion of sensitive clinical information but are available from the corresponding author on reasonable request.

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