Fluids and Barriers of the CNS

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Exploring the ability of plasma pTau217, pTau181 and beta-amyloid in mirroring cerebrospinal fluid biomarker profile of Mild Cognitive Impairment by the fully automated Lumipulse® platform

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Abstract

Background The approval of new disease-modifying therapies by the U.S. Food and Drug Administration and the European Medicine Agency makes it necessary to optimize non-invasive and cost-effective tools for the identification of subjects at-risk of developing Alzheimer's Disease (AD). Plasma biomarkers are excellent candidates. However, their ability to reflect the cerebrospinal fluid (CSF) profile - that remains to date the gold standard for the biochemical diagnosis of AD - needs to be confirmed and validated before their implementation in clinical practice. The aims of this study are to analyse the correlation between CSF and plasma Aβ40, Aβ42, Aβ42/Aβ40 and pTau181, and to assess the diagnostic performance of plasma biomarkers in a cohort of subjects affected by Mild Cognitive Impairment (MCI).

Methods The study was performed on 306 subjects affected by MCI, enrolled in the context of the Italian Interceptor Project. Aβ40, Aβ42 and pTau181 were analysed in plasma and CSF, and pTau217 was measured in plasma. The fully automated chemiluminescence enzyme immunoassay and the Lumipulse® G600II (Fujirebio) instrument were used for all measurements. We analysed the correlations between CSF and plasma biomarkers and the differences of plasma biomarker concentrations after grouping MCI cases according to AT classification of CSF AD biomarker profiles.

Results We found statistically significant positive correlations between CSF and plasma Aβ42, Aβ42/Aβ40 ratio and pTau181. All the biomarkers, except Aβ40, showed differences in A+ vs. A-, A+T+ vs. A-T- and A+T- vs. A-T- patients. Moreover, Aβ42 and Aβ42/Aβ40 plasma levels were lower in A+T- compared to A-T- and A-T+ groups, and pTau181

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and pTau217 plasma levels were higher in A+T+ compared to A+T-. Aβ42/Aβ40 and pTau217 showed a robust performance in distinguishing A+ from A- (AUC = 0.857 and 0.862, respectively) and A+T+ from A-T- (AUC = 0.866 and 0.911) subjects.

Conclusions Our results suggest that plasma biomarkers, and especially Aβ42/Aβ40 ratio and pTau217, are promising candidates for the early detection of AD pathology.

Keywords Alzheimer's disease, Mild Cognitive Impairment, CSF, Plasma, Biomarkers, Diagnosis, Aβ, pTau, Lumipulse®

Background

Alzheimer's Disease (AD) is the most common cause of dementia, accounting for 50–75% of all cases. More than 30 million people are estimated to be affected by AD worldwide, and this number is predicted to increase as the life expectancy raises [1].

In recent years, the U.S. Food and Drug Administation (FDA) approved three monoclonal antibodies as disease-modifying treatments for AD [2, 3], and few weeks ago also the European Medicine Agency (EMA) accepted one of them (Lecanemab) as a therapeutic strategy for AD. These drugs have shown statistically significant cognitive benefits in phase III trials. While the clinical relevance of these effects is debated, the possibility that this therapeutic approach is useful in the first stages of disease underlines the importance of an accurate early diagnosis [4].

According to the revised diagnostic guidelines by the National Institute on Aging and Alzheimer's Association (NIA-AA), AD is defined by the neuropathological changes – presence of extracellular amyloid plaques composed by Aβ peptides, and intraneuronal aggregates made up of hyperphosphorylated tau (pTau) – underlying the clinical symptoms [5]. Recently, a huge effort was made to demonstrate that cerebrospinal fluid (CSF) biomarkers are able to mirror these neuropathological changes [6–9] and can be used for the diagnosis of AD in living subjects [5, 10]. In particular, low levels of Aβ42, or Aβ42/Aβ40 ratio, are suggestive of the brain Aβ pathology, and high pTau levels are indicative of pathologic changes correlated with pTau accumulation in brain tissue. Since the neurodegenerative process begins years before the onset of symptoms, the use of CSF biomarkers is essential for identifying the disease at the very early stages, when the chances of preventing neuronal loss by disease-modifying treatments are greater [11].

The lumbar puncture required for CSF collection is an invasive procedure and gives limitations due to low compliance to repeat the test in order to monitor the progression of the disease or the effects of therapeutic treatments [12]. Hence the need to develop ultrasensitive, cost-effective methods to measure Aβ42/Aβ40 ratio and pTau in easily accessible biofluids like blood [13–15]. Recent studies showed the correlation between CSF and plasma Aβ and tau phosphorylated at Threonine 181 (pTau181) by using different methods [16–20]. However, the use of plasma biomarkers in clinical practice is still limited by the large variability among centres, mainly due to differences in the platforms and assays used [21], that have still not allowed the definition of standardized cut-off values. The implementation of the fully automated platform Lumipulse® (Fujirebio) with chemiluminescent enzyme immunoassays (CLEIA) specific for plasma Aβ40, Aβ42, pTau181 and pTau217 is a potential breakthrough for the development of high throughput and high reproducible tests for the diagnosis of AD [22–27]. The Lumipulse® platform is largely used in clinical practice for the measurement of AD biomarker levels in CSF. It provides high throughput, wide availability, and high reproducibility even for plasma biomarkers [25]. These features can facilitate the implementation of plasma biomarker analysis as a promising diagnostic tool for AD in clinical practice.

Here, we aimed to demonstrate the correlation between CSF and plasma biomarkers and assess the diagnostic performance of plasma Aβ42, Aβ40/Aβ42, pTau181 and pTau217 in a large cohort of subjects with Mild Cognitive Impairment (MCI) (Interceptor cohort) using the Lumipulse® G assays. The Italian Interceptor Project was launched and funded by the Italian Ministry of Health and Drug Agency in 2018 with the aim to create and validate an instrument for the early recognition of subjects with MCI with very high-risk of developing dementia and accurate distinction from those with middle-low risk. In order to reach this aim, this project recruited in the Italian territory (20 different recruiting centres) 499 MCI subjects on the basis of harmonized neuropsychological tests [28] and collected at baseline 6 different 'biomarkers' including - besides neuropsychological tests - volumetric MRI, PET-FDG, EEG for connectivity analysis, CSF and blood including ApoE genotyping. Biomarkers were collected after harmonization meetings for procedure standardization; moreover, their final evaluation was carried out in centralized 'expert centres' highly specialized for each biomarker [29]. At the end of the 3-year follow-up period (neuropsychological test battery every six months, progression to dementia having been validated by experts in two successive trials) the Interceptor database comprised 356 subjects with MCI.

Methods

Study participants

Plasma samples collected from 306 out of the 356 patients included in the Interceptor cohort were used in this study for the measurement of Aβ40, Aβ42, pTau181 and pTau217. 160 of the 306 subjects included in the study were females and 146 were males, and the mean age was 72 years (Standard Deviation, SD = 7). The patients were divided into Amyloid positive (A+) and Amyloid negative (A-) based on Amyloid CSF status (A+ = CSF Aβ42/Aβ40 < 0.069, $n = 174$; A- = Aβ42/Aβ40 > 0.069, $n = 132$). In a second analysis, the subjects were divided in Amyloid positive and Tau negative (A+T-), Amyloid positive and Tau positive (A+T+), Amyloid negative and Tau negative (A-T-), Amyloid negative and Tau positive (A-T+) based on CSF Amyloid and Tau levels (A+T- = CSF Aβ42/Aβ40 < 0.069 and pTau181 < 56.5 pg/ml, $n = 30$; A+T+ = CSF Aβ42/Aβ40 < 0.069 and pTau181 > 56.5 pg/ml, $n = 144$; A-T- = CSF Aβ42/Aβ40 > 0.069 and pTau181 < 56.5 pg/ml, $n = 110$; A-T+ = CSF Aβ42/Aβ40 > 0.069 and pTau181 > 56.5 pg/ml, $n = 22$). A description of the cohort, the distribution according to AT classification and a descriptive analysis of the CSF and plasma biomarkers are reported in Table 1.

Table 1 Sample description

	***n* = 306**
Characteristics	
Females, n (%)	160 (52%)
Age, mean ± SD	72 ± 7
CSF biomarkers	
Aβ42, mean ± SD, pg/ml	739 ± 391
Aβ40, mean ± SD, pg/ml	11,193 ± 3,934
Aβ42/Aβ40, mean ± SD	0.069 ± 0.029
Total tau, mean ± SD, pg/ml	474 ± 321
pTau 181, mean ± SD, pg/ml	73.0 ± 55.3
Plasma biomarkers	
Aβ42, mean ± SD, pg/ml	21.59 ± 5.34
Aβ40, mean ± SD, pg/ml	260.18 ± 59.55
Aβ42/Aβ40, mean ± SD	0.083 ± 0.011
pTau 181, mean ± SD, pg/ml	2.40 ± 1.51
pTau 217, mean ± SD, pg/ml	0.400 ± 0.366
A classification, n(%)	
A+	174 (57%)
Females, n (%)	103 (59%)
Age, mean ± SD	73 ± 6
MMSE, score ± SD	27 ± 2
A-	132 (43%)
Females, n (%)	57 (43%)
Age, mean ± SD	71 ± 8
MMSE, score ± SD	27 ± 2
AT classification, n(%)	
A+T-	30 (10%)
Females, n (%)	17 (57%)
Age, mean ± SD	72 ± 6
MMSE, score ± SD	27 ± 2
A+T+	144 (47%)
Females, n (%)	86 (60%)
Age, mean ± SD	73 ± 6
MMSE, score ± SD	27 ± 2
A-T-	110 (36%)
Females, n (%)	43 (39%)
Age, mean ± SD	70 ± 8
MMSE, score ± SD	28 ± 2
A-T+	22 (7%)
Females, n (%)	14 (64%)
Age, mean ± SD	73 ± 7
MMSE, score ± SD	26 ± 2

Abbreviations: SD, Standard Deviation; Aβ, amyloid beta; pTau181, tau protein phosphorylated at residue 181; pTau217, tau protein phosphorylated at residue 217; A-, amyloid negative; A+, amyloid positive; T-, tau negative, T+, tau positive; MMSE, Mini Mental State Examination

CSF and plasma collection

CSF samples were collected in the morning by lumbar puncture (L4-L5) and centrifuged at 2,000 xg for 10 min within 1 h from collection. The samples were aliquoted in 500 µl polypropylene tubes (Sarstedt) and stored at -80 °C.

Whole blood was collected in EDTA tubes after an overnight fast. 15 ml of Ficoll-Paque (Cytiva) were added in a 50 ml Arnika tube and centrifuged at 1,400 xg for 5 min to force the Ficoll under the filter. The undiluted blood was transferred to the Arnika tube and centrifuged at 1,400 xg for 15 min. After centrifugation, the plasma was collected, aliquoted in 500 µl polypropylene tubes (Sarstedt) and stored at -80 °C.

CSF and plasma samples were shipped in dry ice to Fondazione IRCCS – Istituto Neurologico Carlo Besta, where the biomarkers analyses were performed.

Biomarkers analysis

CSF Aβ40, Aβ42, Total Tau and pTau181 were measured by the fully automated chemiluminescence enzyme immunoassay (CLEIA) and the Lumipulse® G600II instrument (Fujirebio) with the kits Lumipulse® G β-Amyloid 1–40 (lot number 4072), Lumipulse® G β-Amyloid 1–42 (lot number 4121), Lumipulse® G Total Tau (lot number 4063) and Lumipulse® G pTau181 (lot number 4074) (Fujirebio).

Plasma Aβ40, Aβ42, pTau181 and pTau217 were measured by the fully automated chemiluminescence enzyme immunoassay (CLEIA) and the Lumipulse® G600II instrument (Fujirebio) with the kits Lumipulse® G β-Amyloid 1–40 Plasma (lot numbers 3105 and 4056), Lumipulse® G β-Amyloid 1–42 Plasma (lot numbers 3105 and 4056), Lumipulse® G pTau181 Plasma (lot numbers 4025 and 4066) and Lumipulse® G pTau217 Plasma (lot number 4097) (Fujirebio). The Quality Controls (QC) were provided by the manufacturer and were measaured

at the beginning of each analytic session; their interrun variation was lower than 6%.

CSF samples were thawed for 30 min at room temperature and shaked by vortex for 10 s before the analysis. Plasma samples were thawed for 30 min at room temperature, shaked by vortex for 10 s and centrifuged at 2,000 xg for 5 min before the analysis.

Statistical analysis

A normality assumption of continuous variables was evaluated with a Kolmogorov Smirnov test. The Student t test or the Mann Whitney test were used to compare plasma biomarkers in A+ and A- subjects. The Kruskal-Wallis test followed by Steel-Dwass-Critchlow-Fligner test was used to make all possible pairwise comparisons across the four groups A+T+, A+T-, A-T+ and A-T-. The correlations between CSF and plasma Aβ42/Aβ40 ratio, pTau181, Aβ42 and Aβ40 levels were visualized using scatter plots and analysed with Pearson's correlation coefficient. The diagnostic performance of plasma Aβ42, Aβ42/Aβ40, pTau181 and pTau217 in distinguishing A+T+ from A-T- patients was analysed by means of a Receiver Operating Characteristic (ROC) curve analysis using Wilcoxon-Mann-Witney AUC estimator. To compare the ROC curves the Area Under the Curve (AUC) was taken into account. The thresholds were determined by maximizing the Youden index, and sensitivity and specificity were calculated for each biomarker. To evaluate the combined diagnostic performance of the plasma Aß42/Aß40 ratio and pTau217, a bivariate ROC analysis was performed. A logistic regression model was fitted with both biomarkers as predictors for the A or AT status. The predicted probabilities from this model were then used to generate the ROC curve. The AUC for each model was compared using the DeLong test to determine statistical significance, to assess the added value of combining the two biomarkers compared to using each biomarker alone. The statistical analysis was performed by Excel Analyse-it ® v. 6.15.4 (Leeds, UK) and the pROC package in R for ROC analysis. p-values < 0.05 were considered significant (*<0.05; **<0.01; ***<0.001; ****<0.0001).

Results

Plasma Aβ42, Aβ42/Aβ40 and pTau181 correlation with CSF biomarkers

Analysis of plasma and CSF biomarkers showed a weak or moderate, but significant, positive correlation for Aβ42 ($r = 0.18$, $p = 0.0007$) (Fig. 1a), Aβ42/Aβ40 ($r = 0.62$, $p < 0.0001$) (Fig. 1c) and pTau181 ($r = 0.29$, $p < 0.0001$) (Fig. 1d). Conversely, no correlation was observed between plasma and CSF Aβ40 levels (Fig. 1b).

Plasma biomarkers in amyloid and AT classification

The patients were divided into A+ ($n = 174$) and A- ($n = 132$) based on Amyloid CSF status. Plasma Aβ42/Aβ40 ratio and Aβ42, Aβ40, pTau181 and pTau217 levels were then analysed in the two groups. Aβ42 was significantly lower in A+ compared to A- patients ($p < 0.0001$) (Fig. 2a), while Aβ40 levels did not show any difference between the two groups (Fig. 2b). Aβ42/Aβ40 ratio was significantly lower in A+ compared to A- patients ($p < 0.0001$) (Fig. 2c). Conversely, pTau181 and pTau217 levels were significantly higher in A+ compared to A- patients ($p < 0.0001$) (Fig. 2d and e).

In a second analysis, the subjects were divided in A+T- ($n = 30$), A+T+ ($n = 144$), A-T- ($n = 110$) and A-T+ ($n = 22$) based on CSF Amyloid and Tau levels. Plasma Aβ42 was significantly decreased in A+T+ ($p = 0.0001$) and A+T- ($p = 0.0125$) compared to A-T-, and in A+T+ ($p = 0.0004$) and A+ T- ($p = 0.0034$) compared to A-T+ (Fig. 3a). Aβ42/Aβ40 was significantly decreased in A+T+ and A+T- compared to A-T- ($p < 0.0001$), and in A-T+ compared to A+T+ ($p < 0.0001$) and A+T- ($p = 0.0006$) (Fig. 3c). Plasma pTau181 was significantly increased in A+T+ compared to A-T- ($p < 0.0001$), A-T+ ($p = 0.0002$) and A+T- ($p = 0.0082$) (Fig. 3d). pTau217 was significantly increased in A+T+ compared to A+T-, A-T- and A-T+ ($p < 0.0001$), and in A-T- compared to A+T- ($p = 0.0013$) (Fig. 3e). No differences were observed in Aβ40 levels among groups (Fig. 3b).

Plasma pTau181/Aβ42 and pTau217/Aβ42 in amyloid and AT classification

We compared pTau181/Aβ42 and pTau217/Aβ42 between A+ and A- subjects, and among A+T-, A+T+, A-T- and A-T+ patients. pTau181/Aβ42 and pTau217/Aβ42 were both significantly higher in A+ compared to A- subjects ($p < 0.0001$) (Fig. 4a and b). pTau181/Aβ42 was significantly increased in A+T+ compared to A-T-, A-T+ ($p < 0.0001$), and A+T- ($p = 0.0259$) (Fig. 4c). pTau217/Aβ42 was significantly increased in A+T+ compared to A-T-, A-T+ ($p < 0.0001$) and A+T- ($p = 0.0002$), and in A+T- compared to A-T- ($p = 0.0002$) (Fig. 4d).

Diagnostic performance of plasma biomarkers

To assess the diagnostic performance of plasma Aβ42, pTau181, pTau217, Aβ42/Aβ40, pTau181/Aβ42 and pTau217/Aβ42 in distinguishing A+ from A- and A+T+ from A-T- patients belonging to the Interceptor cohort, a ROC curve analysis was performed. When comparing A+ and A- patients, the AUC curves were: 0.678 (95% CI 0.616–0.740) for Aβ42, 0.857 (95% CI 0.813–0.901) for Aβ42/Aβ40, 0.733 (95% CI 0.672–0.794) for pTau181, 0.862 (95% CI 0.820–0.904) for pTau217, 0.766 (95% CI 0.707–0.825) for pTau181/Aβ42 and 0.873 (95% CI 0.833–0.913) for pTau217/Aβ42 (Fig. 5a). The optimal

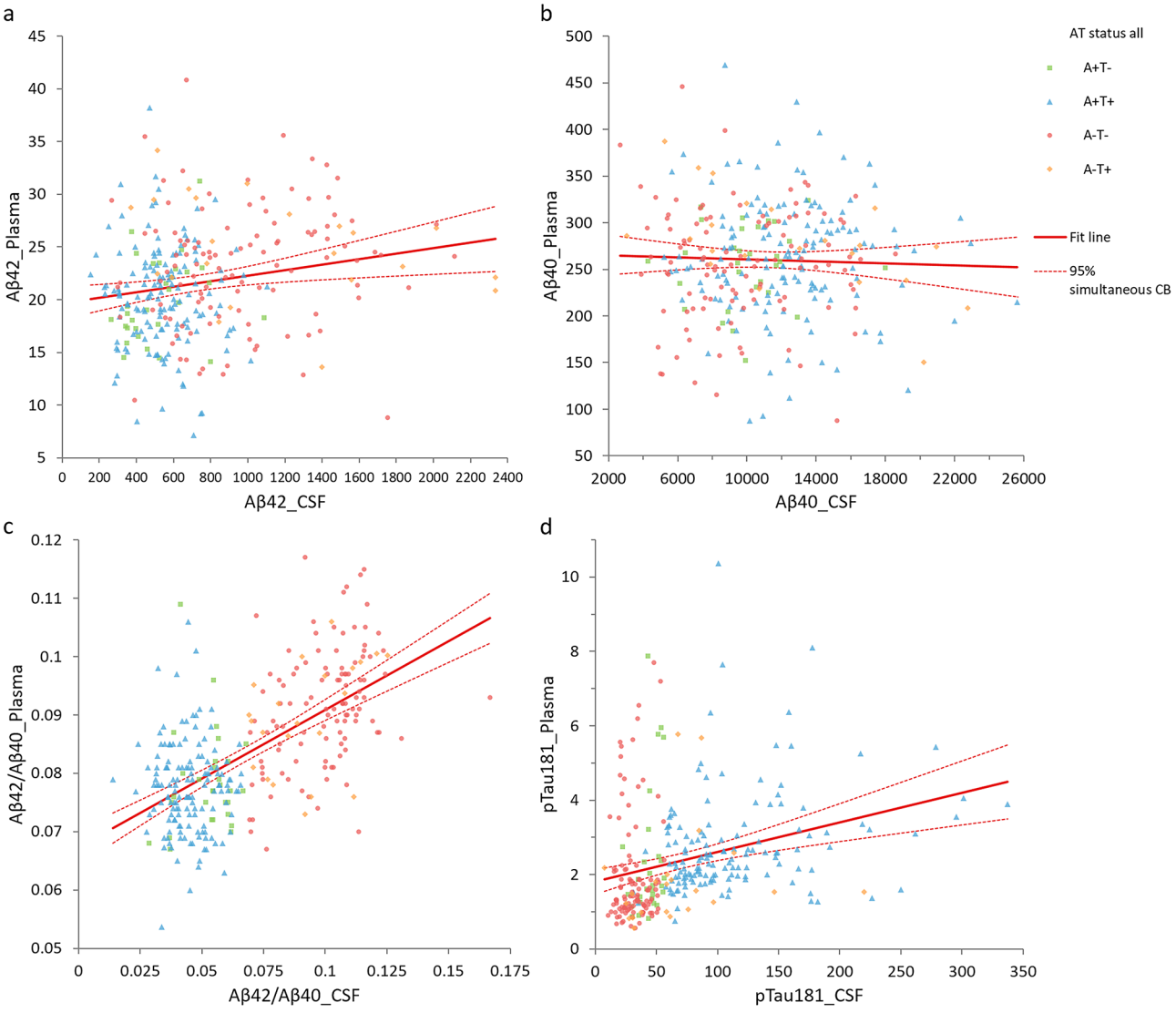

Fig. 1 Biomarker correlation between plasma and CSF. The graphs represent (**a**) Aβ42 concentrations, (**b**) Aβ40 concentrations, (**c**) Aβ42/Aβ40 ratios, (**d**) pTau181 concentrations in CSF and plasma. The values are expressed as pg/ml, except for Aβ42/Aβ40 ratio. The symbols represent the CSF and plasma concentration of a single sample, with the colours corresponding to CSF AT status (green A+T-, blue A+T+, red A-T-, yellow A-T+). *Abbreviations*: Aβ, amyloid beta; pTau181, tau protein phosphorylated at residue 181; CSF, cerebrospinal fluid

thresholds were 23.19 pg/ml (sensitivity 77.0%, specificity 54.5%) for Aβ42, 0.086 (sensitivity 87.9%, specificity 73.5%) for Aβ42/Aβ40, 1.87 pg/ml (sensitivity 72.4%, specificity 72.0%) for pTau181, 0.194 pg/ml (sensitivity 89.1%, specificity 72.0%) for pTau217, 0.087 (sensitivity 75.9%, specificity 74.2%) for pTau181/Aβ42 and 0.011 (sensitivity 85.6%, specificity 78.8%) for pTau217/Aβ42 (Table 2). When comparing A+T+ and A-T- subjects, the AUC curve was 0.656 (95% CI 0.586–0.725) for Aβ42, 0.866 (95% CI 0.819–0.913) for Aβ42/Aβ40, 0.758 (95% CI 0.691–0.824) for pTau181, 0.911 (95% CI 0.875–0.947) for pTau217, 0.781 (95% CI 0.715–0.846) for pTau181/Aβ42 and 0.912 (95% CI 0.875–0.949) for pTau217/Aβ42 (Fig. 5b). The optimal thresholds were 23.19 pg/ml (sensitivity 76.4%, specificity 53.6%) for Aβ42, 0.086 (sensitivity 88.9%, specificity 73.6%) for Aβ42/Aβ40, 1.88 pg/ml (sensitivity 77.8%, specificity 72.7%) for pTau181, 0.280 pg/ml (sensitivity 84.7%, specificity 86.4%) for pTau217, 0.087 (sensitivity 81.3%, specificity 74.5%) for pTau181/Aβ42 and 0.011 (sensitivity 91.7%, specificity 82.7%) for pTau217/Aβ42 (Table 2).

The combination of the two best performing plasma biomarkers, Aβ42/Aβ40 and pTau217, resulted in a further improvement of their ability to distinguish A+ from A- (AUC 0.907, 95% CI: 0.872–0.942, Fig. 5c). The DeLong test indicated that the AUC obtained by combining Aβ42/Aβ40 and pTau217 was significantly higher than the AUC of the Aß42/Aß40 ratio ($p = 0.00024$)

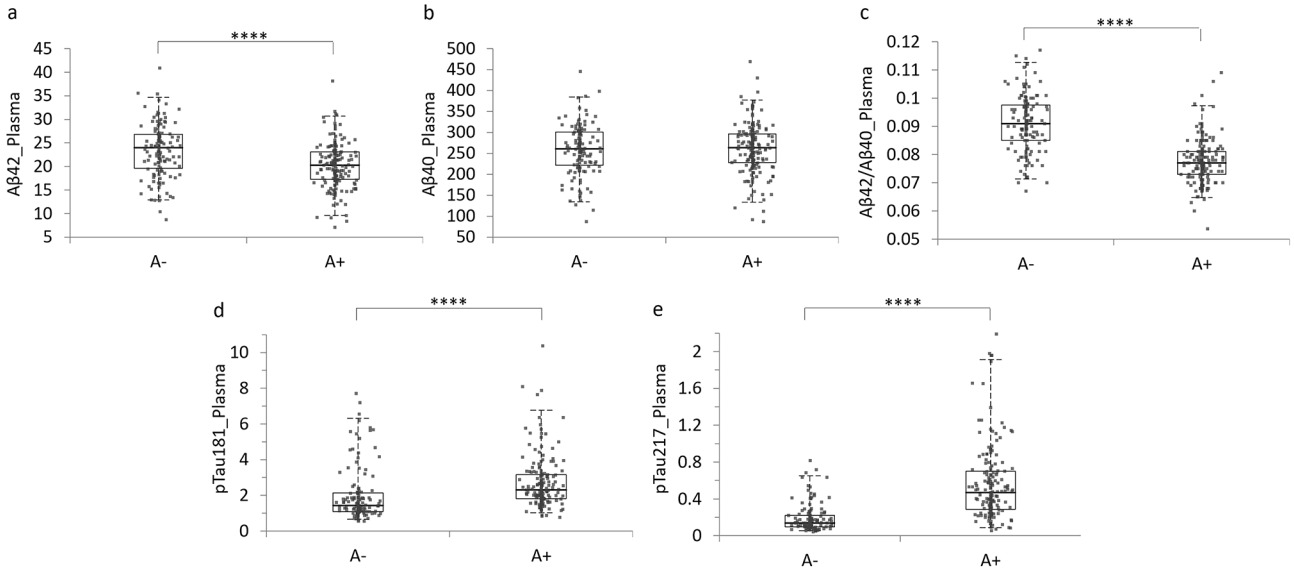

Fig. 2 Comparison of plasma biomarkers in A+ and A- groups. The graphs represent the box and whiskers plots of (**a**) Aβ42 concentrations, (**b**) Aβ40 concentrations, (**c**) Aβ42/Aβ40 ratios, (**d**) pTau181 concentrations and (**e**) pTau217 concentrations. The values are expressed as pg/ml, except for Aβ42/Aβ40 ratio. Each point corresponds to an individual value. Significant differences were assessed using the Student t test or the Mann Whitney test and are indicated by asterisks: ****$p < 0.0001$. A+, $n = 174$; A-, $n = 132$. *Abbreviations*: Aβ, amyloid beta; pTau181, tau protein phosphorylated at residue 181; pTau217, tau protein phosphorylated at residue 217; A-, amyloid negative; A+, amyloid positive

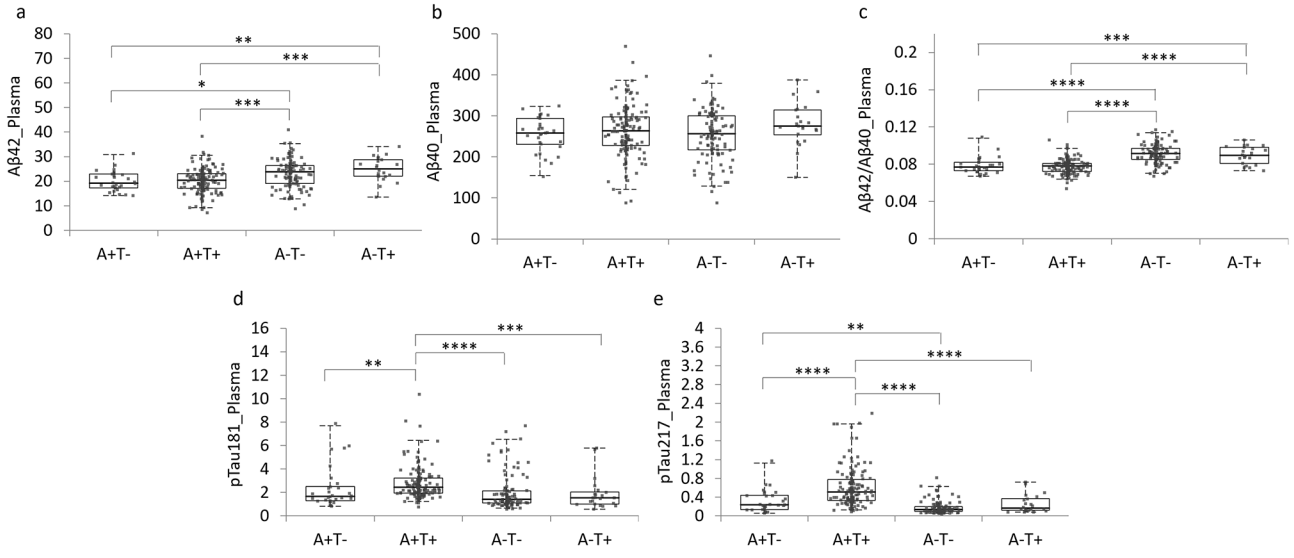

Fig. 3 Comparison of plasma biomarkers according to CSF status. The graphs represent the box and whiskers plots of (**a**) Aβ42 concentrations, (**b**) Aβ40 concentrations, (**c**) Aβ42/Aβ40 ratios, (**d**) pTau181 concentrations and (**e**) pTau217 concentrations. The values are expressed as pg/ml, except for Aβ42/Aβ40 ratio. Each point corresponds to an individual value. Significant differences were assessed using the Kruskal-Wallis test followed by Steel-Dwass-Critchlow-Fligner test and are indicated by asterisks: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. A+T-, $n = 30$; A+T+, $n = 144$; A-T-, $n = 110$; A-T+, $n = 22$. *Abbreviations*: Aβ, amyloid beta; pTau181, tau protein phosphorylated at residue 181; pTau217, tau protein phosphorylated at residue 217; A-, amyloid negative; A+, amyloid positive; T-, tau negative, T+, tau positive

and pTau217 ($p = 0.0012$) alone. There was no significant difference between the AUCs of the individual biomarkers ($p = 0.83$). The combination of Aβ42/Aβ40 and pTau217 showed an excellent performance also in distinguishing A+T+ from A-T- groups (AUC 0.939, 95% CI 0.907–0.970, Fig. 5d). Even in this case, the DeLong test indicated that the AUC obtained by combining Aβ42/Aβ40 and pTau217 was significantly higher than the AUC of the Aß42/Aß40 ratio ($p < 0.0001$) and pTau217 ($p = 0.022$) alone. There was no significant difference between the AUCs of the individual biomarkers ($p = 0.071$). These results suggest that the combined use of plasma Aß42/Aß40 ratio and pTau217 improves the diagnostic accuracy for distinguishing both A+T+ from

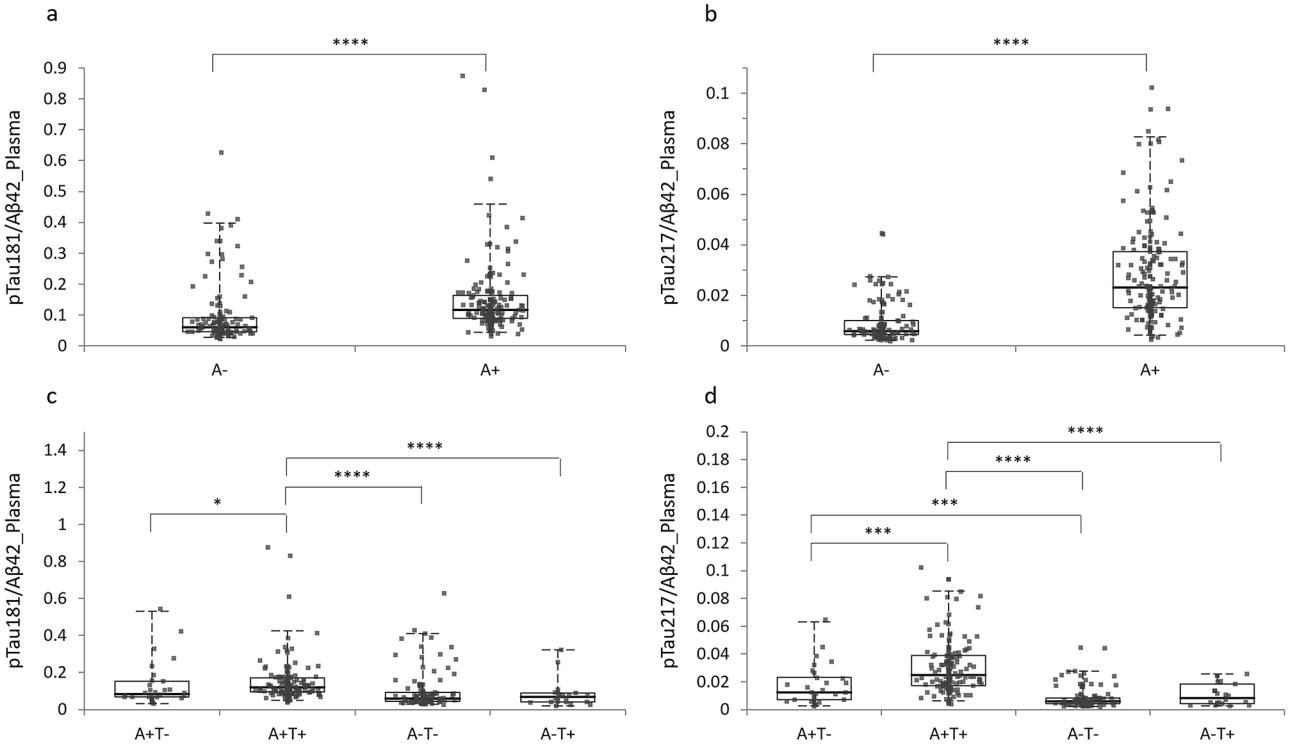

Fig. 4 Comparison of plasma biomarkers according to CSF status. The graphs represent the box and whiskers plots of (**a**) pTau181/Aβ42 and (**b**) pTau217/Aβ42 concentrations in A+ and A- subjects, and (**c**) pTau181/Aβ42 and (**d**) pTau217/Aβ42 concentrations in A+T-, A+T+, A-T- and A-T+ subjects. Each point corresponds to an individual value, expressed as pg/ml. Significant differences were assessed using the Student t test or the Mann Whitney test for the comparison between two groups, and the Kruskal-Wallis test followed by Steel-Dwass-Critchlow-Fligner test for the comparisons among four groups, and are indicated by asterisks: *$p < 0.05$, ***$p < 0.001$, ****$p < 0.0001$. A+, $n = 174$; A-, $n = 132$; A+T-, $n = 30$; A+T+, $n = 144$; A-T-, $n = 110$; A-T+, $n = 22$. *Abbreviations*: Aβ, amyloid beta; pTau181, tau protein phosphorylated at residue 181; pTau217, tau protein phosphorylated at residue 217; A-, amyloid negative; A+, amyloid positive; T-, tau negative, T+, tau positive

A-T- patients and A+ from A- subjects compared to using each biomarker individually.

Discussion

Growing evidence coming from the most recent scientific literature suggests that AD plasma biomarkers may provide an unprecedented non-invasive approach to the molecular diagnosis of the disease [21, 30, 31] and that automated platforms - by reducing the intrinsic variability of pre-analytical procedures - may assure consistent reliability and quality of the biomarker analysis that may lead to the development of efficient diagnostic tools in clinical practice [23, 32–34]

In this study we investigated the ability of plasma biomarkers to mirror CSF profile and the diagnostic performance of plasma Aβ40, Aβ42, Aβ42/Aβ40, pTau181, pTau217, pTau181/Aβ42 and pTau217/Aβ42 in an Italian cohort composed of MCI subjects (Interceptor cohort), using the chemiluminescence-based Lumipulse® G assays. Our results showed a significant positive correlation between CSF and plasma for Aβ42, pTau181 and, in particular, for Aβ42/Aβ40 ratio, while plasma Aβ40 levels did not correlate with CSF. The best results in reflecting CSF profile were achieved by pTau217 and Aβ42/Aβ40, followed by pTau181, suggesting that pTau217 is a promising plasma biomarker for intercepting AD patients at the very early clinical stages of the disease. These data are consistent with previously published results, showing that pTau217 is the most accurate plasma biomarker for distinguishing A+ and A- subjects in a cohort of cognitively unimpaired individuals, MCI individuals and dementia patients [35] (AUC = 0.94, 95% CI 0.92–0.97). In our study the AUC of pTau217 was lower (0.862, 95% CI 0.820–0.904), probably due to the composition of our population (MCI subjects) and the multicentre nature of our research. Furthermore, we demonstrated that pTau217 diagnostic performance is increased when also tau pathology is taken into account, showing a robust AUC (0.911, 95% CI 0.875–0.947) for the comparison between A+T+ and A-T-.

Plasma pTau181 is usually described as a powerful biomarker [22, 24–26]. Martínez-Dubarbie et al. and Bellomo et al. demonstrated that Aβ42/Aβ40 ratio showed the best performance when A+ and A- groups were compared, while pTau181 was an effective biomarker in differentiating A+T+ from A-T- subjects. Our results

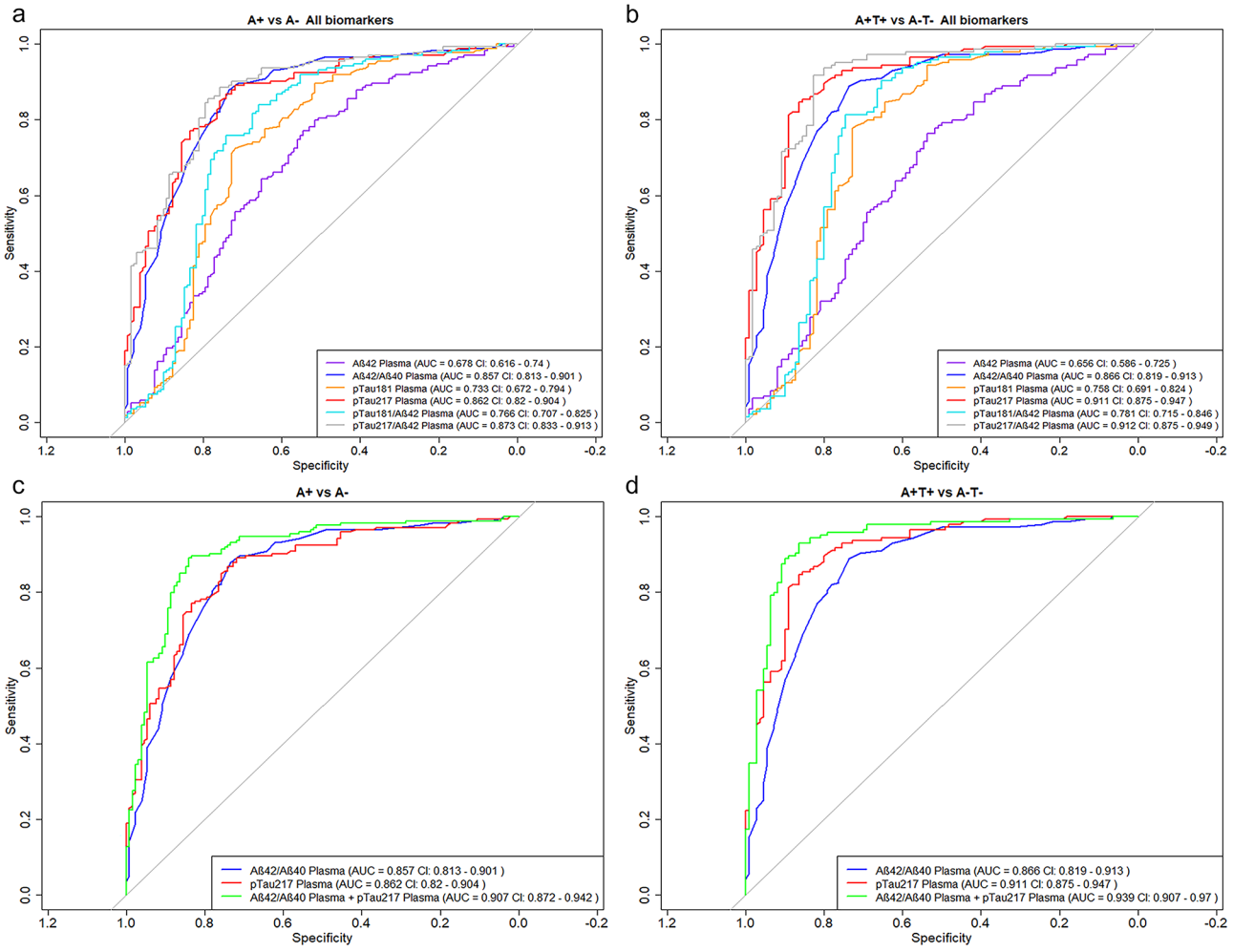

Fig. 5 Plasma biomarker diagnostic performance. ROC curves describing the ability of plasma biomarkers in distinguishing (**a**, **c**) A+ from A-, and (**b**, **d**) A+T+ from A-T- subjects. The ROC curves are described by the AUC, indicated in parenthesis for each biomarker. *Abbreviations*: Aβ, amyloid beta; pTau181, tau protein phosphorylated at residue 181; pTau217, tau protein phosphorylated at residue 217; ROC, Receiver Operating Characteristic, AUC, Area Under the Curve

are consistent with these findings when we refer to the classification based on CSF amyloid status: we obtained AUC values for the Aβ42/Aβ40 ratio (0.857) and pTau181 (0.733) that are very close to those described by Martínez-Dubarbie (0.9 and 0.73) and Bellomo (0.864 and 0.859), respectively. However, in our cohort Aβ42/Aβ40 ratio showed better results than pTau181 also in the comparison of A+T+ versus A-T- groups. In fact, we found an AUC value of 0.866 for Aβ42/Aβ40 (0.89 and 0.858 described by Martínez-Dubarbie and Bellomo, respectively) and 0.758 for pTau181 (0.86 and 0.912 in Martínez-Dubarbie and Bellomo). Since Aβ42/Aβ40 and pTau217 are the first biomarkers to be altered along the course of the disease while pTau181 is reported by several studies to be increased later along AD [19, 26, 36] progression, it is reasonable to assume that the lower performance of pTau181 in our study is due to the composition of the Interceptor cohort that includes only MCI cases. Our interpretation is also supported by studies that assume that plasma pTau181 reaches abnormal levels 6.5 and 5.7 years after CSF and PET measurement of amyloid-beta, respectively, following a similar dynamic as pTau181 in CSF [37]

An interesting observation is that the diagnostic performance of Aβ42/Aβ40 ratio in our cohort resembles the one found in most previously published papers [22, 24, 35], highlighting the robustness of this plasma biomarker. Noteworthy, the combination of Aβ42/Aβ40 and pTau217 in our study led to an increase of the diagnostic performance compared to individual biomarkers, confirming data [38–40] from scientific literature.

Plasma pTau181 is more efficient in differentiating A+T+ from A-T- than A+T- from A-T-. This is a confirmation that plasma biomarkers mirror CSF biomarkers. In comparison with pTau181, pTau217 shows a better performance in distinguishing A+T- from A-T-, probably

Table 2 ROC curve analysis describing the diagnostic performance of plasma biomarkers

A+ vs. A-					
	AUC	**95% CI**	**Threshold (pg/ml)**	**Sensitivity (%)**	**Specificity (%)**
Aβ42	0.678	0.616–0.740	23.190	77.0	54.5
Aβ42/Aβ40	0.857	0.813–0.901	0.086	87.9	73.5
pTau181	0.733	0.672–0.794	1.870	72.4	72.0
pTau217	0.862	0.820–0.904	0.194	89.1	72.0
pTau181/Aβ42	0.766	0.707–0.825	0.087	75.9	74.2
pTau217/Aβ42	0.873	0.833–0.913	0.011	85.6	78.8
A+T+ vs. A-T-					
	AUC	**95% CI**	**Threshold (pg/ml)**	**Sensitivity (%)**	**Specificity (%)**
Aβ42	0.656	0.586–0.725	23.190	76.4	53.6
Aβ42/Aβ40	0.866	0.819–0.913	0.086	88.9	73.6
pTau181	0.758	0.691–0.824	1.880	77.8	72.7
pTau217	0.911	0.875–0.947	0.280	84.7	86.4
pTau181/Aβ42	0.781	0.715–0.846	0.087	81.3	74.5
pTau217/Aβ42	0.912	0.875–0.949	0.011	91.7	82.7

Abbreviations: AUC, Area Under the Curve; CI, Confidence Interval; Aβ, amyloid beta; pTau181, tau protein phosphorylated at residue 181; pTau217, tau protein phosphorylated at residue 217; A-, amyloid negative; A+, amyloid positive; T-, tau negative; T+, tau positive

due to the fact that its levels change earlier than pTau181 along the course of the disease, as suggested by previous reports [41].

Emerging evidence suggest that plasma concentrations of Aβ42, pTau181 and pTau217 are affected by confounding factors, especially due to kidney diseases [35, 42, 43]. The use of amyloid ratio is very useful to overcome this issue, since Aβ40 and Aβ42 are similarly affected by glomerular filtration rate, and this finding further strengthens the relevance of Aβ42/Aβ40 to predict AD CSF profile. The effects of confounding factors on pTau levels are less clear, since some authors did not find significant effects [42, 44], while others suggested that pTau levels are increased in patients affected by kidney diseases [22].

Of note, in agreement with previously published studies [22], we did not find any statistically significant difference of the analysed biomarkers between A-T- and A-T+ groups, reinforcing the hypothesis that A-T+ profile is not relevant from a clinical point of view and should be considered normal or not associated with AD pathology. It has been suggested that the A-T+ profile is not an expression of a neurodegenerative condition but is associated with physiological features correlated with CSF turnover [45]. However, based on our data, we cannot rule out that A-T+ group is composed by subjects with a mixed AD pathology. This and other hypotheses on the interpretation of biomarkers profile in A-T+ group need further studies in larger cohorts.

In the present study we propose a single cut-off value for each biomarker - obtained by maximizing the Youden index - resembling the strategy currently used for the interpretation of CSF biomarker levels. However, we can hypothesize to use a two-cutoff model, especially for pTau217, as proposed by Brum [46], for the screening of "at risk" AD population.

Our study on plasma biomarkers is based on the correlation with CSF profile. Further studies matching plasma profile and molecular PET findings will be useful to confirm the consistency of our results.

A strength of our work is that the analysis of CSF and plasma biomarkers was performed on a large cohort of patients in the frame of a multicentre study. The work, however, has the following limitations: (i) the lack of enough information on non-neurological comorbidities, so it was not possible to study their effects on plasma biomarkers in our population; (ii) the absence of independent validation of our results in other cohorts.

Conclusions

Overall, our results suggest the utility of plasma biomarkers as non-invasive and cost-effective tool to support AD diagnosis. Among them, Aβ42/Aβ40 and pTau217 show the best diagnostic performances and for this reason they seem the most promising candidates for pre-screening analyses on 'at risk' AD population and even for the replacement of CSF biomarkers in the detection of AD pathology, especially in MCI-stage subjects.

It would be of great interest to verify whether plasma biomarkers can also be useful to monitor disease progression and responsiveness to therapies [34, 47]. Taking especially into account that the most promising pharmacological treatments against AD need to be initiated at very early stages of the disease, particularly relevant is to investigate on the performance of blood biomarkers in predicting the conversion from MCI to AD; this performance will be assessed in a follow-up study on the Interceptor cohort, in the near future. The follow-up study could also provide further insights on the ability of plasma biomarkers in predicting disease trajectories of patients across the dementia continuum.

Abbreviations

AD	Alzheimer's Disease
FDA	Food and Drug Administration
MCI	Mild Cognitive Impairment
NIA-AA	National Institute on Aging and Alzheimer's Association
Aβ	Amyloid β
pTau	Hyperphosphorylated tau
CSF	Cerebro Spinal Fluid
MMSE	Mini Mental State Examination
CLEIA	Chemi Luminescent Enzyme Immuno Assay
QC	Quality Control
MRI	Magnetic Resonance Imaging
PET-FDG	Positron Emission Tomography-fluoro Deoxy Glucose
EEG	Electro Encephalo Graphy
APOE	Apolipoprotein E
SD	Standard Deviation
IQR	Inter Quartile Range
EDTA	Ethylene Diamine Tetraacetic Acid
ROC	Receiver Operating Characteristic
AUC	Area Under the Curve
CI	Confidence Interval
KD	Kidney Disease

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Author contributions
G.D.F., P.T. and F.T. designed and supervised the study. M.C., C.B., E.M., F.A.C. managed biological samples and performed CSF and plasma analyses. E.S. performed the statistical analysis. M.C. and G.D.F. interpreted the data and drafted the manuscript. A.P. provided technical assistance for CSF and plasma Aβ measurement. P.M.R, C.M., N.V., A.R., D.P., P.S., F.T., M.Co., S.C. and N.C. coordinated the Interceptor Project. All the authors read and approved the manuscript.

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Data availability
Row data are available upon reasonable request. The requests should be addressed to the corresponding author.

Declarations

Ethics approval and consent to participate
The study was conducted in compliance with the Declaration of Helsinki for the protection of human participants and was approved by the Ethics Committee of Fondazione Policlinico Universitario Agostino Gemelli (approval code 2251). Additionally, approval was obtained from all local ethics committees of the participating clinical centres. Written informed consent was obtained from participants.

Consent for publication
N/A.

Competing interests
A.P. is an employee of Fujirebio.

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