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Concordance between cerebrospinal fluid biomarkers with Alzheimer's Disease pathology between three independent assay platforms.

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Running title: Assay-independent CSF biomarker and PET Aβ-amyloid concordance.

Abstract and Keywords

Background: To enhance the accuracy of clinical diagnosis for Alzheimer's disease (AD), pre-mortem biomarkers have become increasingly important for diagnosis and for participant recruitment in disease-specific treatment trials. Cerebrospinal fluid (CSF) biomarkers provide a low-cost alternative to positron emission tomography (PET) imaging for *in vivo* quantification of different AD pathological hallmarks in the brains of affected subjects; however, consensus around the best platform, most informative biomarker and correlations across different methodologies are controversial.

Objective: Assessing levels of Aβ-amyloid and tau species determined using three different versions of immunoassays, the current study explored the ability of CSF biomarkers to predict PET Aβ-amyloid (32 Aβ-amyloid- and 45 Aβ-amyloid+), as well as concordance between CSF biomarker levels and PET Aβ-amyloid imaging.

Methods: Prediction and concordance analyses were performed using a sub-cohort of 77 individuals (48 healthy controls, 15 with mild cognitive impairment and 14 with AD) from the Australian Imaging Biomarker and Lifestyle study of aging.

Results: Across all three platforms, the T-tau/Aβ42 ratio biomarker had modestly higher correlation with SUVR/BeCKeT (ρ=0.69-0.8) as compared with Aβ42 alone (ρ=0.66-0.75). Differences in CSF biomarker levels between the PET Aβ-amyloid– and Aβ-amyloid+ groups were strongest for the Aβ42/Aβ40 and T-tau/Aβ42 ratios ($p<0.0001$), however comparison of predictive models for PET Aβ-amyloid showed no difference between Aβ42 alone and the T-tau/Aβ42 ratio.

Conclusion: This study confirms strong concordance between CSF biomarkers and PET Aβ-amyloid status is independent of immunoassay platform, supporting their utility as

biomarkers in clinical practice for the diagnosis of AD and for participant enrichment in clinical trials.

Key words: CSF, Biomarker, Amyloid, PET, concordance

Abbreviations: HC: healthy control, MCI: mild cognitive impairment, AD: Alzheimer's disease, CSF: cerebrospinal fluid, PET: positron emission tomography, BeCKeT: Before the Centiloid Kernel Transformation, Aβ: Aβ-amyloid, Aβ40, Aβ42.

Introduction

Alzheimer's disease (AD) is a growing healthcare and economic burden that requires a suitable intervention strategy to prevent a potential economic healthcare crisis [1]. Despite promising *in vitro* and animal model disease interventions, most human therapeutic trials targeting the Aβ-amyloid pathway have failed to achieve definite efficacy (as reviewed in [2]) suggesting that these interventions might be administered too late in patients with established clinical disease, highlighting the urgent need for accurate screening tools for the earliest symptoms and detection at the preclinical stage of AD [3].

Indeed, PET imaging studies for neocortical Aβ-amyloid have shown that there is a lengthy preclinical stage before development of mild cognitive impairment (MCI) and AD wherein the underlying progressive pathology precedes the symptomatic onset of AD by one or two decades [4]. Analysis of cerebrospinal fluid (CSF) biomarkers can also detect changes decades before the onset of clinical symptoms in both sporadic [5] and in dominantly inherited AD [6]. In contrast to PET imaging, biochemical testing of the CSF for Aβ-amyloid and tau biomarkers can detect simultaneously the abnormalities predictive of the presence of plaques and neurofibrillary tangles, respectively. While concordance between PET and CSF biomarkers has been positive [7], there is still discordance between the two modalities [8] due to either threshold variance or measurement at different stages of the disease biomarker cascade [9]. Despite early CNS changes, there still is no consensus on an optimal blood-based biomarker or biomarker signature panel for AD (as reviewed in [10]). Until this is achieved, CSF analysis is the most expedient, inexpensive and widely available method to screen for preclinical or prodromal AD.

CSF biomarkers aimed at identification of AD pathology in the brain of affected subjects have been evaluated using traditional enzyme-linked immunosorbent assay (ELISA) platforms with a major focus on Aβ1-42 amyloid (Aβ42), total tau protein (T-tau) and phosphorylated-tau protein 181p (P-tau181p, referred to here as P-tau) [11]. Markers have also been assessed on a multiplex bead capture version of the assay (xMAP technology) [12]. Most of these first generation CSF biomarker assays have some limitations with respect to their analytical performance (for example: assay imprecision, [13]) and matrix interference ([14], [15]), thereby hampering their world-wide integration into routine clinical testing. No reference methods are available yet for these analytes [16].

The clinical utility of the different platforms assaying CSF biomarkers to predict the presence of neocortical Aβ-amyloid pathology has been compared, with variable results across platforms and studies suggesting there are innate differences in the affinity and specificity of the antibodies [17]. Reports on the concordance between CSF biomarkers and neocortical Aβ-amyloid have been quite strong, with accuracy up to 92% for the T-tau/Aβ42 ratio [7], [18],[19]. Variability in concordance with neocortical Aβ-amyloid however may be due to many different factors in both the CSF assay and the PET tracer, with error rates and threshold differences between assays being quite variable between study site and antibody [20, 21].

For diagnostic purposes, disparate absolute concentrations of analytes across assays are arguably less concerning if the individual assay can reliably detect preclinical or prodromal AD, wherein a decrease in Aβ42 and increase in T-tau and P-tau can be easily discerned by a change in the Aβ42/T-tau ratio [22, 23]. Although research has shown that these assays

appear capable of discerning changes in CSF biomarker levels between MCI and AD [24], little is known about how these biomarkers track with preclinical disease. As mentioned above, there appears to be a pre-symptomatic phase in which the early changes in CSF biomarker concentration might be more subtle, and so there is still a need for highly standardised, robust and sensitive methods to detect these earliest changes.

This study compared the performance of the ADx-EUROIMMUN assays (developed by ADx NeuroSciences, and commercialized by EUROIMMUN AG) to two of the most routinely employed CSF biomarker assays that are purported to have clinical utility for detecting neuropathological changes reflected in the CSF. We hypothesized that the EUROIMMUN assay, providing good dilutional linearity (equating to no matrix interference) compared to the first generation of CSF biomarker assays, would improve the quantification accuracy reflecting changes associated with AD neuropathology both prior to and after the onset of clinical disease.

Materials and Methods

Participants

A sub-cohort of 77 individuals from the Australian Imaging Biomarker and Lifestyle study of ageing (AIBL) [25], comprising 48 healthy controls (HC), 15 MCI and 14 AD participants who had undergone both lumbar puncture (LP) and PET Aβ-amyloid imaging (32 PET Aβ- and 45 PET Aβ+) were included in the current study. Ethical approval was provided by the institutional ethics committees of Austin Health, St Vincent's Health, Hollywood Private Hospital and Edith Cowan University. Individuals gave written informed consent before participating in the study.

Lumbar puncture and CSF processing

Detailed protocols regarding the CSF collection have been previously published ([18, 26]) and align with the Alzheimer's Biomarkers Standardization Initiative [27]. CSF was collected in the morning from fasted participants. Aseptic technique was adhered to at all times, with the participants sitting upright. CSF (8mL) was collected by gravity after LP using a Temena (Polymedic®, EU) spinal needle micro-tip (22/27G x 103mm) (CAT 21922-27) into 15mL polypropylene tubes (Greiner Bio-One188271), and placed onto wet ice immediately. Samples were kept between 2-8^{o}C during transport to the laboratory and processed within 1 hour. The CSF was centrifuged (2,000 x g, 4^{o}C, for 10 minutes) and supernatant transferred to a fresh polypropylene tube (Greiner Bio-One188271, 15mL) and gently inverted. Aliquots (500 µL) were snap-frozen in 1mL screw-cap 2D barcoded polypropylene Nunc Cryotubes (NUN374088) for long-term storage until use. Samples were transferred to liquid nitrogen vapour tanks for long term storage until use, and thawed once immediately before analysis. Besides the information below, further details on the analysis platforms are shown in Supplementary Table 1.

xMAP biomarker assay

All samples were measured in duplicate using the INNO-BIA AlzBio3 xMAP assay (Fujirebio Europe N.V.) (multiplex assay including Aβ42, T-tau, and P-tau, subsequently referred to as AlzBio3) according to the manufacturer's instructions. Briefly, all reagents, calibrators, controls, and samples were brought to room temperature (18-30ºC) and pulse-vortexed immediately before the start of the assay. The filter plate was washed once using

225 µL/well of 1x wash buffer and vacuum aspirated immediately before use. One hundred µL of bead suspension (3,000 beads/analyte) was added to each well, followed by vacuum aspiration of the plate. Twenty-five µL of the conjugate working solution (mixture of two biotinylated detector antibodies) was added to each well. Seventy-five µL of calibrators (ready-to-use formulation), controls, and samples were added to the plate in duplicate. Sample diluent was included as blank in the assay format. The filter plate was sealed, covered in aluminium foil, incubated overnight (at least 14 hours) on an orbital plate shaker at room temperature. The filter plate was then aspirated and washed 3 times with 225 µL of 1x wash buffer. One hundred µL of diluted detection reagent (phycoerythrin-labelled streptavidin) was then added to each well and the plate re-covered in foil for 1 hour on an orbital plate shaker. The filter plate was aspirated and washed 3 times with 225 µL of 1x wash buffer. One hundred µL of reading solution was added to each well and the plate was finally incubated on an orbital plate shaker, for 5 minutes covered in foil, at room temperature. The data was fitted to calibration curves constructed with the median fluorescence values for each replicate of the standards. The sample concentrations were then quantified using a Bio-Rad Bioplex 200 instrument (Bio-Rad Laboratories, USA) using 5PL logistic regression. The analyses were performed with one production batch of the kit. Data from duplicate sample measurements that had a percentage coefficient of variance (%CV) above 20% (as recommended by the manufacturer) (N=9) were discarded and fresh samples were re-analysed. Run-validation was undertaken with an internal control CSF "pool". The mean intra-assay %CV (based on the internal CSF control) for Aβ42 was 6.9%, for T-tau 11.2%, and for P-tau 8.1%, while the inter-assay %CV (based on the internal CSF control pool) for Aβ42 was 12.5%, for T-tau 11.3%, and for P-tau 9.9%.

Innogenetics (INNOTEST) enzyme linked immunosorbent assay.

All samples were analysed in duplicate using INNOTEST ELISA immune-affinity capture and detection: Aβ42, T-tau and P-tau (Innogenetics, now from Fujirebio, Ghent, Belgium, from herein referred to as INNOTEST) according to manufacturer's procedures and published standard methods [18]. The analyses were performed across four batches of kits. Run-validation was undertaken with an internal control CSF "pool". Based on the internal CSF control, the mean intra-assay percentage %CV for Aβ42 was 7.4%, for T-tau 4.0%, and for P-tau 1.6%, and the inter-assay %CV for Aβ42 was 15.9%, for T-tau 8.4%, and for P-tau 6.7%. This data was generated during contemporaneous parallel studies.

EUROIMMUNE-ADx NeuroSciences HV kit enzyme linked immunosorbent assay

The EUROIMMUNE-ADx ELISA assays (referred to subsequently as EUROIMMUN) for CSF Aβ42, Aβ1-40 (Aβ40), and T-tau were performed according to the manufacturer's instructions. Briefly, the calibrator series and run validation kit controls were reconstituted for each assay. The loading volume for Aβ42 was 15 µL of calibrators, controls and undiluted CSF. For Aβ40 the loading volume was 15 µL of calibrators, controls and 1:21 pre-diluted CSF. For T-tau the loading volume was 25 µL of calibrators, controls and undiluted CSF. For each assay, 100 µL of biotinylated detector antibody and specified volume of the calibrators, controls and CSF were added in duplicates to respective coated wells. The plates were incubated for 180 minutes at room temperature (23ºC). The wells were subsequently washed five times using 1x wash buffer, 100 µL of enzyme conjugate (streptavidin-peroxidase) was added into each of the respective microplate wells. The plates were incubated for 30 minutes at room temperature. After the final wash, 100 µL of substrate was added and incubated for 30 minutes at room temperature. Finally, 100 µL of stop solution was added and colour intensity was measured at a wavelength of 450 nm in a plate

reader. The test procedure and components were harmonized for the three analytes. Run-validation control was undertaken with pooled CSF. The analyses were performed using one batch of the kit. The mean intra-assay percentage coefficient of variation %CV for Aβ40 was 8.2%, for Aβ42 5.8%, and for T-tau 4.5%, while the inter-assay %CV for Aβ40 was 9.9%, for Aβ42 6.8%, and for T-tau 6.4%.

PET imaging

To determine brain Aβ-amyloid levels, participants underwent PET imaging conducted using one of three different tracers; either ^{11}C-Pittsburgh Compound-B (PiB; N=28), ^{18}F-flutemetamol (FLUTE; N=32) or ^{18}F-florbetapir (FBP; N=17). The PET methodology for each tracer has been previously described, [28-30]. For semi-quantitative analysis, a volume of interest template was applied to the summed and spatially normalized PET images in order to obtain a standardized uptake value (SUV). The images were then scaled to the SUV of each tracer's recommended reference region to generate a tissue ratio termed SUV ratio (SUVR). A global measure of Aβ-amyloid burden was computed using the mean SUVR in the frontal, superior parietal, lateral temporal, lateral occipital, and anterior and posterior cingulate regions. For PiB, the SUVs were normalized to the cerebellar cortex and, as advocated by the respective pharmaceutical companies, the whole cerebellum was used as the reference region for FBP [31] whilst for FLUTE the reference region was the pons [32]. In order to use the results of all PET tracers as a single continuous variable, FLUTE and FBP results were transformed into PiB-like SUVR termed Before the Centiloid Kernel Transformation (BeCKeT) [33]. The SUVR/BeCKeT was dichotomised as high (Aβ+) or low (Aβ-) Aβ-amyloid burden with an SUVR ≥1.4 used as the threshold [34].

PET Aβ-amyloid-CSF biomarker concordance

CSF biomarker thresholding to define high (CSF+) or low (CSF-) CSF biomarker levels were primarily established for Aβ42 alone and for the T-tau/Aβ42 ratio; that is, comparative analyses focused on those biomarkers that were available for assessment by all three platforms at the time of analyses. Participants were scored as either CSF+ or CSF- according to the standardised platform thresholds (where available, [18], [35]). Thresholds for the EUROIMMUN platform Aβ42 and T-tau (not previously published) were defined by taking the average of the group specific multivariate normal Expectation Maximisation (mnEM) mean values (mnEM calculated using the EM algorithm with one CSF biomarker with SUVR/BeCKeT; mu ±SD calculated as: [mu1 – 1SD + mu2 +1SD/2], Supplementary Figure 1). For EUROIMMUN, we used a threshold of <649 pg/mL for Aβ42 and >0.618 (401 pg/mL/649 pg/mL) for the T-tau/Aβ42 ratio (via multinomial expectation maximisation (mnEM) modelling). For INNOTEST, we used a threshold of <544 pg/mL for Aβ42 and >0.748 (407 pg/mL/544 pg/mL as per [18]) for the T-tau/Aβ42 ratio. For AlzBio3, we used a threshold of <416 pg/mL for Aβ42 and >0.184 (76.7pg/mL/416pg/mL as per [35]) for the T-tau/Aβ42 ratio. Thresholds for the T-tau/Aβ42 ratio were derived by dividing the platform specific threshold for T-tau by the platform specific threshold for Aβ42. Concordance was investigated via assessment of proportions of true negatives (TN) and true positives (TP) for the binary CSF and PET biomarkers.

Statistical analysis

Demographic characteristics were compared between neocortical Aβ-amyloid burden status and clinical classification (HC, MCI and AD) using Analysis of Variance and Independent Samples T-test (Age), Chi-Square test (*APOE ε4*, gender), and Kruskal Wallis and Wilcoxon test of Ranks (MMSE, CDR score). Comparison of CSF biomarkers between PET Aβ-

amyloid status and clinical classification, adjusted for age, gender and APOE ε4 status was performed using proportional odds logistic regression (three group comparisons) and generalized linear models (all pairwise comparisons). Receiver Operating Characteristic (ROC) analyses were used to define non-cross validated predictive statistics including AUC (95%CI), p-value, threshold chosen via ROC (Youden, closest top left), sensitivity, specificity, PPV, NPV and accuracy. Comparison of ROC models was performed using DeLong's method for model comparison. Spearman's Rho (ρ) was used to compute correlations between BeCKeT SUVR and CSF biomarker levels. Concordance using binary biomarkers post thresholding was conducted using Cohen's Kappa (κ) method for agreement. Biomarker data were log transformed and scaled prior to analyses to ensure that data fit a normal distribution. In the text, where the p-values are less than 0.0001, values are presented as $p < 0.0001$, while full values are shown in the tables. P-values (Aβ-amyloid and clinical classification) were considered significant after correcting for multiple comparisons using the Bonferroni adjustment for seven biomarkers (α=0.05/7, 0.007). All statistical analyses were performed using the R statistical environment (R version 3.2.3).

Results

Demographic comparisons

Cohort demographics for the PET and clinical groups are shown in Tables 1A and 1B, respectively. Within the HC group there were 27 Aβ- and 21 Aβ+ PET scans, while in the MCI group there were 4 Aβ- and 11 Aβ+ scans, and in the AD group there was 1 Aβ- and 13 Aβ+ scans. There were no significant differences in age, gender or APOE ε4 status between both clinical classification or PET groups (binary variable cut at SUVR 1.4, $p > 0.05$). There were significant differences in MMSE ($p<0.001$) and CDR ($p<0.001$) between clinical classifications; however, the difference was somewhat reduced for the PET sub-group

(MMSE (p=0.12) and CDR (p=0.004)) due to a high proportion of Aβ+ HC participants (21/48).

Correlation of biomarkers between platforms

We performed linear correlation analyses between platforms to assess the relationships for Aβ42, T-tau, and the ratio of T-tau/Aβ42. Relationships were plotted stratified by clinical classification (Figure 1). Strongest correlation for Aβ42 was seen between the EUROIMMUN and INNOTEST platforms (R=0.94, Figure 1A), while the weakest correlation was between the INNOTEST and AlzBio3 platforms (R=0.67, Figure 1C). Overall the strongest correlations across Aβ42, T-tau, and the ratio of T-tau/Aβ42 were observed for EUROIMMUN and INNOTEST (R=0.94 [Figure 1A], R=0.91 [Figure 1D] and R=0.89 [Figure 1G] respectively). Interestingly, the T-tau/Aβ42 ratio correlation for the INNOTEST and AlzBio3 platforms was strong (R=0.94, Figure 1I).

Biomarker mean differences per platform

Table 2 and Supplementary Table 3 present mean CSF biomarker levels and associated p-values between both PET Aβ-amyloid status groups and clinical classifications respectively. Assessing the ratios for T-tau/Aβ42 and Aβ42/Aβ40 against PET Aβ-amyloid status (Figures 2C and 3A, Table 2), it was apparent that the combination of biomarkers performed better in separating Aβ-amyloid PET- from Aβ-amyloid PET+ status compared to each biomarker alone. In particular, the Aβ42/Aβ40 ratio performed the best (p = 0.000007), however this ratio was assessed only in the EUROIMMUN platform; comparisons could therefore not be made between platforms.

Receiver operating characteristic analyses per platform

To evaluate the performance of each biomarker in predicting PET Aβ-amyloid status, we assessed the predictive performance (not cross validated) using the complete cohort. All biomarkers from each platform were tested, including the Aβ42/Aβ40 ratio and T-tau/Aβ42 ratios. ROC derived threshold values along with associated predictive values per platform and biomarker are shown in Table 3. Of the individual markers, Aβ42 was comparable to the ratio biomarkers, albeit values were lower (not significantly) to that from the T-tau/Aβ42 ratio. Comparing the ROC models for Aβ42 alone and the T-tau/Aβ42 ratio for each platform using DeLong's method, there were no significant differences in model performance across the three platforms (AlzBio3: p = 0.85, EUROIMMUN: p = 0.30, INNOTEST: p = 0.62). Further comparisons of ROC models for the individual (Aβ42) and ratio (T-tau/Aβ42) can be found in Supplementary Table 3.

PET Aβ-amyloid-CSF biomarker concordance per platform

Assessing the total concordance via binary biomarker comparisons from all three platforms, the true negative (TN) rate was high for most comparisons (CSF- /PET-, ranging from 24-31/32), while the true positive (TP) rate was more variable (CSF+/PET+, ranging from 31-44/45) across both the individual Aβ42 analyte results and the T-tau/Aβ42 ratio. In general, the T-tau/Aβ42 ratio performed better than the individual Aβ42 biomarker with regard to delineating between true negatives and true positives. The highest performance was seen for the EUROIMMUN T-tau/Aβ42 ratio (30/32 TN, 44/45 TP (Supplementary Table 4). Concordance using Cohen's Kappa coefficient between CSF and PET measures was

strongest for the individual Aβ42 biomarker using the EUROIMMUN platform (κ = 0.763, *p* <0.0001), followed by INNOTEST and AlzBio3 (κ = 0. 615, *p* <0.0001 and (κ = 0.581, *p* = 0.105 respectively). Concordance values were higher for the T-tau/Aβ42 ratio, with the EUROIMMUN platform having the best concordance (κ = 0.919, *p* <0.0001), followed by AlzBio3 and INNOTEST (κ = 0. 689, *p* <0.0001 and κ = 0.621, <0.0001, respectively).

Assessing the correlations between CSF Aβ42 and the T-tau/Aβ42 ratio against SUVR/BeCKeT (Figure 4), both the INNOTEST and EUROIMMUN platforms showed strongest correlations between the T-tau/Aβ42 ratio and SUVR/BeCKeT (INNOTEST: ρ = 0.79, EUROIMMUN: ρ = 0.80), while the correlation between the individual INNOTEST CSF Aβ42 and SUVR/BeCKeT was strongest of the three platforms assessed (ρ = 0.75). The LOESS lines plotted for each comparison depict the approximate correlation between CSF biomarker and PET SUVR, and shows the extent of the correlation decreases substantially for SUVR values greater than ~1.7.

Discussion

The use of CSF biomarkers as a means to interrogate the pathological status of the brain has been the subject of much attention in the recent literature. In the current study, we aimed to assess the CSF biomarkers Aβ42, Aβ40, T-tau and P-tau and their associated ratios within clinical classification groups (HC, MCI, AD) and especially PET Aβ-amyloid status groups across three separate immunoassays, developed on two different technology platforms (ELISA, xMAP). Based on a sub-cohort from the AIBL study, each of the biomarkers from the three platforms performed particularly well at separating both clinical classification and

PET Aβ-amyloid status, with the Aβ42/Aβ40 (limited to EUROIMMUNE) ratio and T-tau/Aβ42 ratio the strongest markers to predict PET Aβ-amyloid status. Whilst the predictive values were higher for the ratio biomarkers in comparison to Aβ42 alone, comparison of ROC models for all three platforms showed the differences were not significant. Across the three platforms, agreement was quite strong for both individual biomarkers and ratios, with the EUROIMMUN and INNOTEST platforms having very high correlation for Aβ42 (R=0.94), while the INNOTEST and AlzBio3 platforms had the weakest correlation for Aβ42 (R=0.67).

Of the individual biomarkers tested, CSF Aβ42 in each platform performed similarly to distinguish Aβ- from Aβ+ participants with large differences in biomarker levels between groups for all platforms. Approximating an internal normalisation step for each platform, biomarker ratios had similar performance at separating PET Aβ-amyloid status compared with individual biomarkers. Contrary to previous studies [36-38] where the CSF Aβ42/Aβ40 ratio performed better than Aβ42 alone to predict both Aβ-Amyloid pathology and clinical AD, the current study using the EUROIMMUN platform did not see any appreciable difference in prediction accuracies between the CSF Aβ42/Aβ40 and CSF Aβ42 biomarker alone. After adjusting for age, gender and APOE ε4 status, the INNOTEST and AlzBio3 T-tau/Aβ42 ratio performed similarly (p=0.00005 for both), with the p-value marginally lower than the same comparison for the EUROIMMUN platform (p=0.0001). Although not the primary focus for our study, for clinical classification, the T-tau/Aβ42 ratio was the strongest to separate HC from AD participants for all three platforms. Using standard ROC analyses to find predictive values, highest negative and positive predictive values for an individual biomarker to predict PET Aβ-amyloid status was found from Aβ42 from the EUROIMMUN platform (NPV: 0.97, PPV: 0.92), while the best predictive values for a ratio were found from

the T-tau/Aβ42 ratio also from the EUROIMMUN platform (NPV: 0.97, PPV: 0.96). Given the prevalence of amyloid positivity in the test sample was higher than what would be expected in the general population over the age 60 years, it is possible that the PPV may be over-estimated and the NPV may be under-estimated; however this does not detract from the statistical comparison of predictive accuracy between platforms.

Concordance between dichotomised CSF biomarkers and PET Aβ-amyloid status was quite strong across all platforms. This concordance was apparent after plotting the quantitative biomarker data along with a ±5% "grey zone" of uncertainty around each threshold, with the EUROIMMUN platform 98% accurate for PET Aβ+ and 94% accurate for PET Aβ-. Such concordance statistics are highly variable via small changes in quantitative biomarker thresholds. We note here that although the threshold derived yielded good results for the EUROIMMUN platform, future studies may benefit from a comparison of prediction values arising from minor changes to the optimal threshold. Correlation between SUVR/BeCKeT and CSF biomarkers was also relatively strong, with Spearman's Rho values ranging between 0.66 and 0.8.

Collectively, our results corroborate what has been recently reported, including in other cohorts interrogated with a number of biomarker platforms. Leuzy et al., [7] demonstrated strong correlation for Aβ42 between the INNOTEST, Mesoscale Scale Discovery (MSD) and mass spectrometry based reference measurement procedure (MS-RMP) platforms with strong agreement with PiB PET. Similar to our study, the authors also showed increased concordance to PET Aβ-amyloid when using ratios as compared to individual biomarkers. In addition, Leuzy et al., [39] and Wang et al., [40] also demonstrated highest concordance rates

with PiB PET using the T-tau/Aβ42 ratio. In a large study assessing ratios for the diagnosis of AD, Janelidze et al., [19] showed that the Aβ42/Aβ40 and Aβ42/Aβ38 ratios were significantly better than Aβ42 alone at predicting PET Aβ-amyloid status using two different technologies (EUROIMMUN and MSD). Further, in a study of 38 community-recruited cognitively intact older adults, Adamczuk et al., [41] showed that decisions for determining pre-clinical AD should be based on an Aβ ratio rather than Aβ42 alone, supporting our results for the ratio over the individual Aβ42 even in pre-symptomatic persons harbouring Aβ-amyloid pathology. In the current study the Aβ42/Aβ40 ratio was only able to be assessed in the EUROIMMUN platform, and the P-tau/Aβ42 ratio in the INNOTEST and AlzBio3 platforms. Whilst the Aβ42/Aβ40 ratio continues to show promise to predict PET Aβ-amyloid status, further work needs to be done to investigate the utility of the biomarker.

Several studies have compared and reported the analytical, as well as the clinical performance, of the first generation of CSF biomarker assays. Apart from a few exceptions, an identical clinical utility could not be identified, independent of the context of use or the technologies included in the study. Few reports have directly compared platforms to determine if there is a relative consensus on clinical utility. In one study (N=103), both techniques were able to identify individuals with neocortical Aβ-amyloid pathology but showed differences in absolute biomarker values suggesting that there are assay specific cut-offs that need to be established [42]. In another study (N=140 including 17 participants with Parkinson's disease), good concordance was reported between two platforms; however, when applying a conversion model for the xMAP assay, the ELISA phosphorylated-tau was more correlated to xMAP T-tau than xMAP P-tau [17]. The authors suggested that this discrepancy was a result of inherent differences in the platform technology methods and in particular, differences in the affinity and specificity of the antibodies in each platform. Reijn

and colleagues [43] also compared two platforms in mild-to-moderate AD (N=69) and vascular dementia (N=26). Results showed that the high difference in concentration inherent in the xMAP data made it impossible for a single correction factor to be applied to transform the xMAP values into a relative ELISA result. The authors concluded that although the xMAP assay is better suited to high throughput sample interrogation and has utility in differentiating AD subjects from controls and those with vascular dementia, absolute and comparative biomarker values could not be simply extrapolated. More recently another comparison (N=58), resulted in a similar conclusion, again suggesting that the two platforms were correlated, but that the ELISA yielded higher absolute values for each marker [44]. Therefore, the definition of new reference range cut-offs are required before the xMAP assay can be reliably employed in widespread clinical application or population screening.

Clinical decision making derived from a combination of cognitive performance testing and biomarker assessment appears optimal for achieving the most accurate diagnosis of cognitive impairment due to AD. Further research is necessary however, to ascertain the degree of correlation and precision of CSF biomarker assays to A) better understand the pathological process, and B) provide confidence to clinical decisions made by utilising the biomarkers to inform clinical diagnosis. Similar to the recently proposed A/T/N biomarker classification scheme derived by Jack et al., [45] for the diagnosis of clinical disease, we believe that the most well informed decision on underlying AD pathology (estimated via PET imaging (surrogate gold standard) in the absence of post-mortem histopathology (true gold standard)[46]) will arise from the combination of Aβ42 in a ratio with another key CSF biomarker, whether it is the Aβ42/Aβ40 ratio, the T-tau/Aβ42 ratio or the P-tau/Aβ42 ratio remains to be determined.

In the current study, we primarily aimed to assess the concordance of the main AD related CSF biomarkers both across three different molecular platforms and with PET Aβ-amyloid status. Although for the most part, the biomarkers were comparable, we acknowledge that there may be a decrease in concordance due to data from the INNOTEST platform arising from assessing multiple batches of kits, while data from the AlzBio3 and EUROIMMUN platforms are both obtained from single batch kits. While there have been many assessments confirming the concordance between INNOTEST and AlzBio3 kits previously ([47], [43], [42], [17],[44]), results from one recent study ([48]) concur with the results from this study, with stronger association between EUROIMMUN and INNOTEST compared with EUROIMMUN and AlzBio3. Even though it is tempting to rank the performance of one assay over another, the data shows very high agreement between both EUROIMMUN and INNOTEST for Aβ42 (R=0.94), and between INNOTEST and AlzBio3 for the T-tau/Aβ42 ratio (R=0.94). Further comparisons, especially using neat CSF in a larger cohort are warranted.

Similar to the experience of a recent study [48], our results highlight the challenges in achieving harmonisation across different assays measuring Aβ-amyloid using routine diagnostic sources and underscores the utility of employing clinically accessible candidate reference materials across analytical platforms. The assays included in the current paper differ with respect to presence of matrix interference (INNOTEST, INNO-BIA) or no matrix interference (EUROIMMUN assays). The latter used a smaller amount of CSF per well during sample measurement, as such reducing the interference in the assay of proteins, known to be bound with the analyte. In addition, it has been shown that the correlation of assays with mass spectrometric reference methods is better when there is no matrix

interference, which was documented for several assay formats [49]. Once universal reference materials become available, it will be possible to harmonize concentrations when samples are analyzed using different technology platforms although this can only be done very efficiently if the same protein isoform is measured, the assays do not suffer from matrix interference and there is a good correlation (constant over the whole concentration range) with the mass spectrometric method.

While concordance and correlation results from this research have been quite positive, we acknowledge certain limitations that may have impacted the results. Some of these include a relatively low number of samples per study group and the use of different amyloid PET imaging tracers; however, the latter limitation is to some degree circumvented by the BeCKeT calculation and the separate (not simultaneous) analysis of CSF on the different technology platforms. Furthermore previous work has shown strong correlations between cortical uptake across different tracers [50, 51], providing confidence in the analysis of data from different PET sources. While we report herein the accuracy from the three assays to predict Aβ-amyloid pathology, it is important to remember that our predictive accuracies arise from approximate biomarker thresholds applied to samples from participants across three separate clinical classifications. Assessment of predictive accuracy using only a single clinical group such as a healthy control population [18] has been shown previously to be more accurate with results directly transferable into the clinic. Furthermore, it is possible that differences in protein concentrations between platforms is due to the assay design. These inter-assay variances include calibrators which are not provided in a CSF-like matrix [52], varying affinity and specificity of the antibodies, especially those linked to the specificity at the carboxy-terminus (Aβ1-40, Aβ1-42) or a possible interference within the assay by other Aβ isoforms, or lastly varying due to the degree of matrix interference, present in both AlzBio3 and INNOTEST but not EUROIMMUNE assays. While we did not intentionally

select for any specific APOE allele for our analyses, we note an under-representation of APOE ε4 alleles in the MCI group, which may have affected the overall biomarker means.

In summary, we have shown that three currently available CSF biomarker platforms perform well to separate low from high brain Aβ-amyloid burden, correlating highly with clinical status. Results from this study further support the need for further investigation of CSF biomarker ratios incorporating Aβ42 for providing the strongest accuracy for predicting AD pathology.

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Conflict of interest/Disclosure Statement

There are no conflicts of interest.

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Tables

Table 1a: PET cohort demographic details

	Aβ PET-	Aβ PET+	*p*–value
N	32	45	
Age (years)	73.5 (7.9)	73.3 (4.9)	0.91
Sex (F%)	53	44	0.45
APOE ε4 %	22	27	0.63
MMSE (median IQR)	29 (2)	27 (4)	0.12
CDR (median IQR)	0 (0.4)	0.5 (0.5)	0.004

Table 1b: Clinical classification cohort demographic details

	HC	MCI	AD	*p*–value
N	48	15	14	
Age (years)	72.9 (5.3)	75.8 (8.6)	72.5 (5.4)	0.24
Sex (F%)	51	53	41	0.74
APOE ε4 %	21	7	41	0.07
MMSE (median IQR)	29 (2)	27 (3)	22 (2)	<0.0001
CDR (median IQR)	0 (0)	0.5 (0)	1 (0.5)	<0.0001

Table 1: Study demographic comparisons between PET Aβ-amyloid status groups (1A) and clinical classification groups (1B). N; number, HC, Healthy Control, MCI; Mild Cognitive Impairment, AD; Alzheimer's disease, *APOE ε4*; Apolipoprotein epsilon 4 allele. MMSE; mini–mental state examination, CDR; Clinical Dementia Rating, IQR; inter-quartile range.

Table 2 Biomarker concentration [pg/mL] by PET Aβ-amyloid status and assay platform

		Aβ PET-	Aβ PET+	Unadjusted	Adjusted
Biomarker	N	32	45		
Aβ40	EUROIMMUN	7053.07 (3655.99)	5153.23 (1986.5)	0.0114	0.0115
Aβ42	EUROIMMUN	1015.57 (345.9)	434.02 (182.37)	0.00001	0.00001
	INNOTEST	829.71 (246.37)	452.74 (108.56)	0.00004	0.00005
	AlzBio3	463.33 (66.31)	330.88 (82.18)	0.00001	0.00002
T-tau	EUROIMMUN	364.97 (121.38)	525.81 (191.24)	0.00107	0.00124
	INNOTEST	315.88 (135.72)	511.52 (249.36)	0.00072	0.00081
	AlzBio3	77.83 (40.57)	125.6 (54.6)	0.00070	0.00085
P-tau					
	INNOTEST	58 (20.34)	76.76 (23.98)	0.00179	0.00163
	AlzBio3	28.65 (8.5)	50.68 (20.48)	0.00006	0.00008
Aβ42/Aβ40	EUROIMMUN	0.16 (0.04)	0.09 (0.02)	0.000005	0.000007
T-tau/Aβ42	EUROIMMUN	0.38 (0.15)	1.37 (0.62)	0.00003	0.00013
	INNOTEST	0.39 (0.16)	1.18 (0.59)	0.00007	0.00005
	AlzBio3	0.17 (0.09)	0.4 (0.2)	0.00004	0.00005
P-tau/Aβ42					
	INNOTEST	0.07 (0.02)	0.18 (0.06)	0.00006	0.00017
	AlzBio3	0.06 (0.02)	0.17 (0.09)	0.00024	0.00043

Table 2: Biomarker concentration [pg/mL] by PET Aβ-amyloid status and assay platform: Biomarker mean and standard deviation (SD) values, including unadjusted and adjusted (age, gender *APOE* ε4 allele status) p-values from GLM for PET Aβ-amyloid status. An Aβ40 test was not available for the INNOTEST and AlzBio3 platforms, while the P-tau test was not available for the EUROIMMUN platform at the time of performing assays.

Table 3: CSF biomarker ROC results for predicting PET Aβ status.

		AUC 95%CI	p-value	Threshold	Sensitivity	Specificity	PPV	NPV	Accuracy
Aβ40	EUROIMMUN	0.67 (0.61 - 0.73)	0.01	5062.30	0.60	0.69	0.77	0.54	0.64
Aβ42	EUROIMMUN	0.95 (0.92 - 0.97)	<0.0001	775.12	0.98	0.88	0.92	0.97	0.94
	INNOTEST	0.94 (0.91 - 0.97)	<0.0001	576.91	0.87	0.84	0.86	0.89	0.87
	AlzBio3	0.89 (0.85 - 0.93)	<0.0001	393.10	0.76	0.88	0.89	0.74	0.81
T-tau	EUROIMMUN	0.76 (0.71 - 0.81)	0.00010	391.28	0.80	0.72	0.80	0.72	0.77
	INNOTEST	0.77 (0.71 - 0.82)	0.00008	332.30	0.80	0.66	0.77	0.70	0.74
	AlzBio3	0.78 (0.73 - 0.83)	0.00003	90.63	0.76	0.75	0.79	0.71	0.75
P-tau									
	INNOTEST	0.72 (0.66 - 0.77)	0.0012	54.77	0.84	0.56	0.73	0.72	0.73
	AlzBio3	0.86 (0.82 - 0.9)	<0.0001	32.76	0.78	0.84	0.94	0.71	0.81
Aβ42/Aβ40	EUROIMMUN	0.94 (0.91 - 0.97)	<0.0001	0.11	0.93	0.91	0.93	0.91	0.92
T-tau/Aβ42	EUROIMMUN	0.98 (0.96 - 0.99)	<0.0001	0.61	0.98	0.94	0.96	0.97	0.96
	INNOTEST	0.95 (0.93 - 0.98)	<0.0001	0.62	0.84	0.94	0.95	0.81	0.88
	AlzBio3	0.90 (0.87 - 0.94)	<0.0001	0.22	0.88	0.81	0.84	0.89	0.86
P-tau/Aβ42									
	INNOTEST	0.97 (0.95 - 0.99)	<0.0001	0.103	0.93	0.94	0.98	0.89	0.94
	AlzBio3	0.92 (0.89 - 0.95)	<0.0001	0.081	0.83	0.91	0.92	0.81	0.86

Table 3: CSF biomarker ROC results for PET prediction: Predictive statistics calculated using ROC derived thresholds. An Aβ40 test was not available for the INNOTEST and AlzBio3 platforms, while the P-tau test was not available for the EUROIMMUN platform at the time of performing assays.

Figures
Figure 1

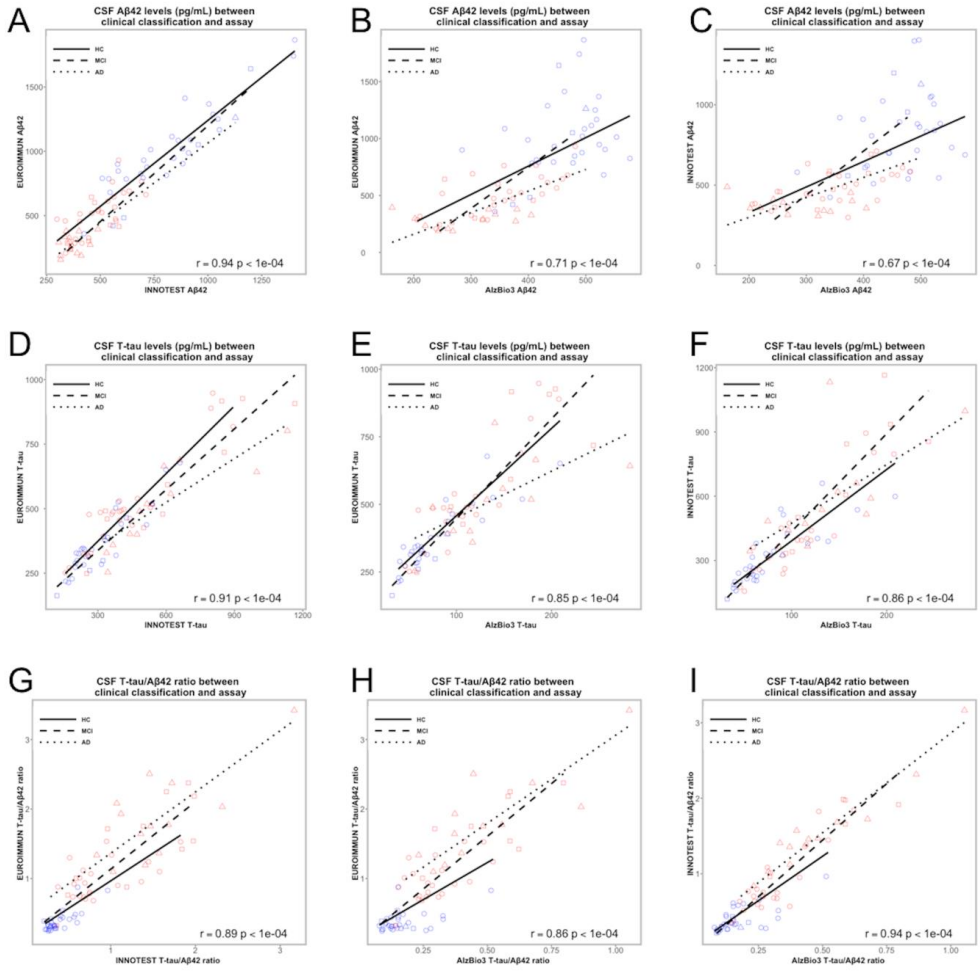


Figure 2

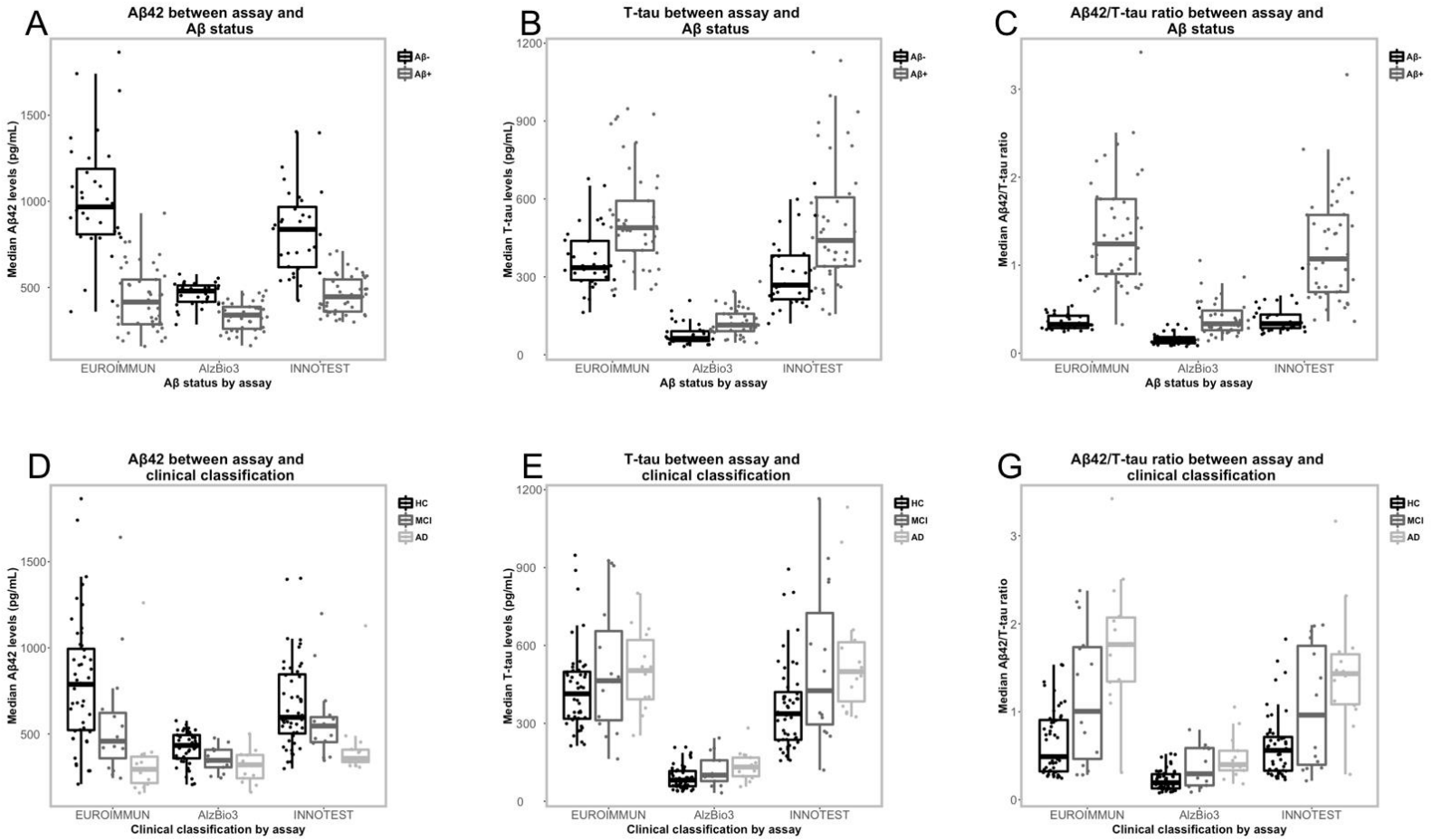

A
Aβ42 between assay and Aβ status
Median Aβ42 levels (pg/mL)
Aβ status by assay
B
T-tau between assay and Aβ status
Median T-tau levels (pg/mL)
Aβ status by assay
C
Aβ42/T-tau ratio between assay and Aβ status
Median Aβ42/T-tau ratio
Aβ status by assay
Aβ-
Aβ+
D
Aβ42 between assay and clinical classification
Median Aβ42 levels (pg/mL)
Clinical classification by assay
E
T-tau between assay and clinical classification
Median T-tau levels (pg/mL)
Clinical classification by assay
G
Aβ42/T-tau ratio between assay and clinical classification
Median Aβ42/T-tau ratio
Clinical classification by assay
HC
MCI
AD
EUROIMMUN
AlzBio3
INNOTEST

Figure 3

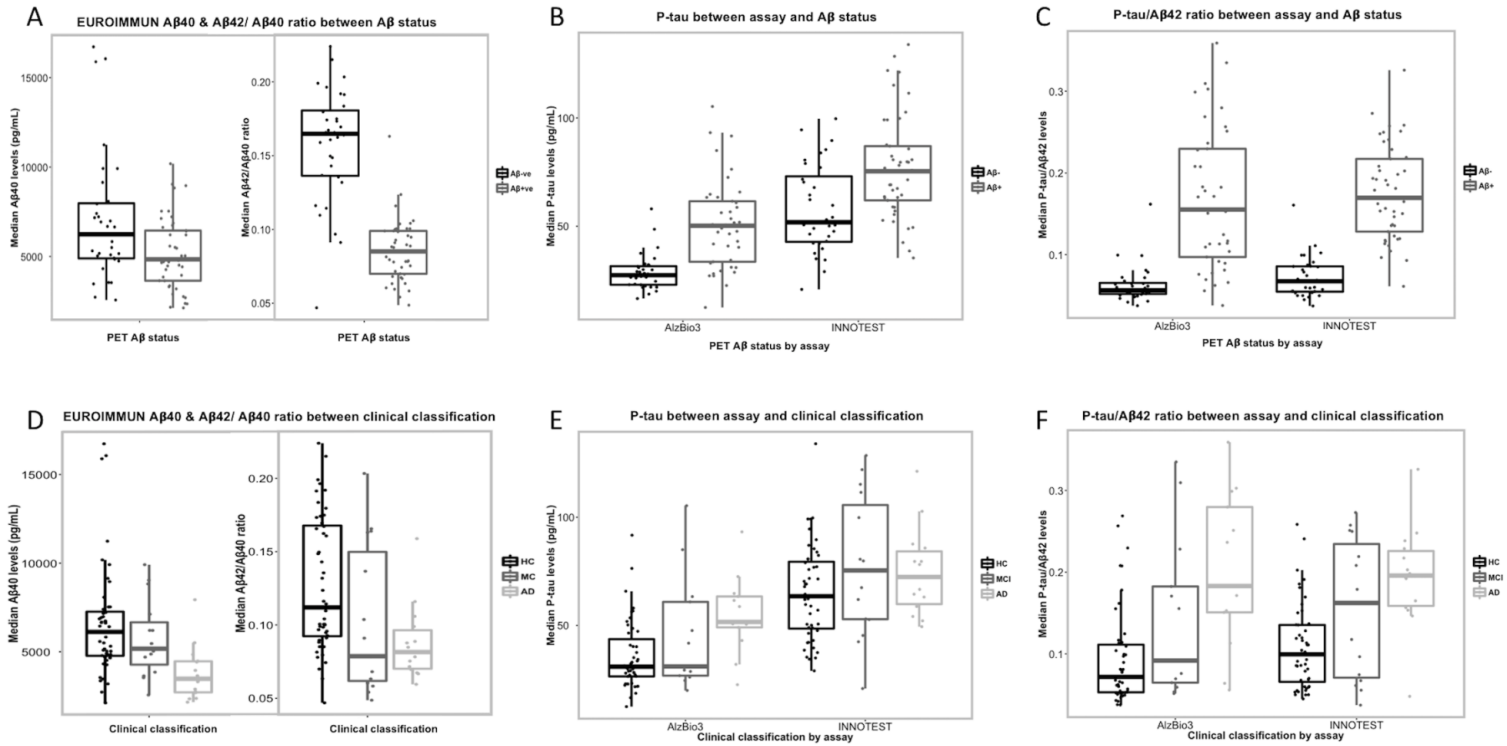

A
EUROIMMUN Aβ40 & Aβ42/ Aβ40 ratio between Aβ status
Median Aβ40 levels (pg/mL)
Median Aβ42/Aβ40 ratio
PET Aβ status
PET Aβ status
Aβ-ve
Aβ+ve
B
P-tau between assay and Aβ status
Median P-tau levels (pg/mL)
AlzBio3
INNOTEST
PET Aβ status by assay
Aβ-
Aβ+
C
P-tau/Aβ42 ratio between assay and Aβ status
Median P-tau/Aβ42 levels
AlzBio3
INNOTEST
PET Aβ status by assay
Aβ-
Aβ+
D
EUROIMMUN Aβ40 & Aβ42/ Aβ40 ratio between clinical classification
Median Aβ40 levels (pg/mL)
Median Aβ42/Aβ40 ratio
Clinical classification
Clinical classification
HC
MC
AD
E
P-tau between assay and clinical classification
Median P-tau levels (pg/mL)
AlzBio3
INNOTEST
Clinical classification by assay
HC
MCI
AD
F
P-tau/Aβ42 ratio between assay and clinical classification
Median P-tau/Aβ42 levels
AlzBio3
INNOTEST
Clinical classification by assay
HC
MCI
AD

Figure 4

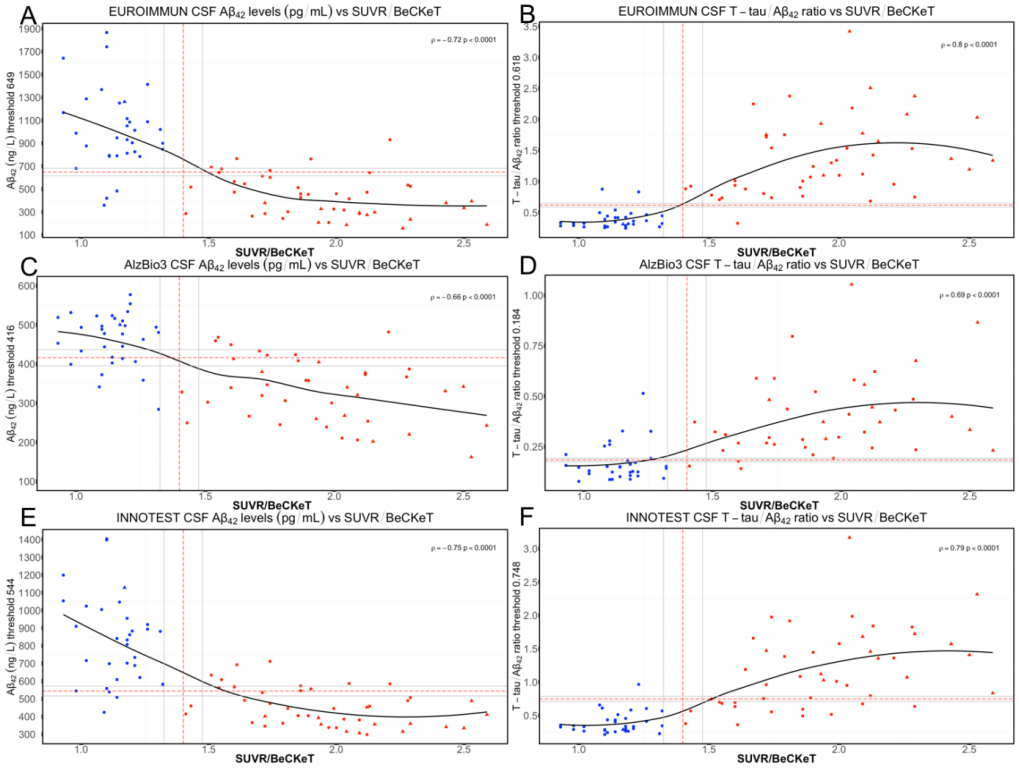

A
EUROIMMUN CSF Aβ42 levels (pg/mL) vs SUVR/BeCKeT
ρ = −0.72 p < 0.0001
Aβ42 (ng/L) threshold 649
SUVR/BeCKeT
B
EUROIMMUN CSF T−tau/Aβ42 ratio vs SUVR/BeCKeT
ρ = 0.8 p < 0.0001
T−tau/Aβ42 ratio threshold 0.618
SUVR/BeCKeT
C
AlzBio3 CSF Aβ42 levels (pg/mL) vs SUVR/BeCKeT
ρ = −0.66 p < 0.0001
Aβ42 (ng/L) threshold 416
SUVR/BeCKeT
D
AlzBio3 CSF T−tau/Aβ42 ratio vs SUVR/BeCKeT
ρ = 0.69 p < 0.0001
T−tau/Aβ42 ratio threshold 0.184
SUVR/BeCKeT
E
INNOTEST CSF Aβ42 levels (pg/mL) vs SUVR/BeCKeT
ρ = −0.75 p < 0.0001
Aβ42 (ng/L) threshold 544
SUVR/BeCKeT
F
INNOTEST CSF T−tau/Aβ42 ratio vs SUVR/BeCKeT
ρ = 0.79 p < 0.0001
T−tau/Aβ42 ratio threshold 0.748
SUVR/BeCKeT

Figure Legends

Figure 1: Correlation of CSF biomarkers by clinical classification across assay platforms. Coloured points represent PET Aβ-amyloid status: PET Aβ- = blue, PET Aβ+ = red. Circles represent HC, squares represent those participants with MCI, triangles represent those participants with AD. 1A: EUROIMMUN vs INNOTEST CSF Aβ42 correlation, 1B: EUROIMMUN vs AlzBio3 CSF Aβ42 correlation, 1C: INNOTEST vs AlzBio3 CSF Aβ42 correlation, 1D: EUROIMMUN vs INNOTEST CSF T-tau correlation, 1E: EUROIMMUN vs AlzBio3 CSF T-tau correlation, 1F: INNOTEST vs AlzBio3 CSF T-tau correlation, 1G: EUROIMMUN vs INNOTEST CSF T-tau/Aβ42 ratio correlation, 1H: EUROIMMUN vs AlzBio3 CSF T-tau/Aβ42 ratio correlation, 1I: INNOTEST vs AlzBio3 CSF T-tau/Aβ42 ratio correlation.

Figure 2: Assay comparisons for those biomarkers measured by all three platforms by classification and PET Aβ-amyloid status. 2A: CSF Aβ42 levels between PET Aβ-amyloid status and assay, 2B: CSF T-tau levels between PET Aβ-amyloid status and assay, 2C: CSF T-tau/Aβ42 ratio between PET Aβ-amyloid status and assay, 2D: CSF Aβ42 levels between clinical classification and assay, 2E: CSF T-tau levels between clinical classification and assay, 2F: CSF T-tau/Aβ42 ratio between clinical classification and assay.

Figure 3: Assay comparisons for those biomarkers not measured by all three platforms by classification and PET Aβ-amyloid status. 3A: EUROIMMUN CSF Aβ40 & Aβ42/Aβ40 levels between PET Aβ-amyloid status, 3B: CSF P-tau levels between PET Aβ-amyloid status and assay, 3C: CSF P-tau/Aβ42 ratio between PET Aβ-amyloid status and assay, 3D:

EUROIMMUN CSF Aβ40 & Aβ42/Aβ40 levels between clinical classification, 3E: CSF P-tau levels between clinical classification and assay, 3F: CSF P-tau/Aβ42 ratio between clinical classification and assay.

Figure 4: CSF biomarker – BeCKeT SUVR correlation. 4A: EUROIMMUN CSF Aβ42 levels vs BeCKeT SUVR, 4B: EUROIMMUN CSF T-tau/Aβ42 ratio vs BeCKeT SUVR, 4C: AlzBio3 CSF Aβ42 levels vs BeCKeT SUVR, 4D: AlzBio3 CSF T-tau/Aβ42 ratio vs BeCKeT SUVR, 4E: INNOTEST CSF Aβ42 levels vs BeCKeT SUVR, 4F: INNOTEST CSF T-tau/Aβ42 ratio vs BeCKeT SUVR. Lines represent linear correlation between CSF biomarker and BeCKeT SUVR. Shown is the Spearman's Rho correlation, asociated p-value, and estimated loess slope.

Supplementary material

Supplementary Table 1

Analyte	Aβ42		
Technology	ELISA	ELISA	xMAP
Vendor	Euroimmun	Fujirebio	Fujirebio
Critical raw materials			
Capture antibody	21F12	21F12	4D7A3
Detector antibody	3D6	3D6	3D6
Calibrator	rec. protein	synthetic peptide	synthetic peptide
Sample incubation	Simultaneous with detector antibody		
Total volume (µL)	115	100	100
CSF (µL)	15	25	75
Detector antibody (µL)	100	75	25
% CSF in the well	13.0	25	75
Sample dilution before test	no	no	no
Incubation time (hrs)	3	1	O/N

Supplementary Table 1: CSF Aβ42 assay details per platform.

Supplementary Table 2 Biomarker concentration [pg/mL] by clinical classification and assay platform

					HC vs MCI vs AD		HC vs AD		HC vs MCI		MCI vs AD	
		HC	MCI	AD	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted
Biomarker	N	48	15	14								
Aβ40	EUROIMMUN	6628.08 (3163.38)	5742.86 (2169.66)	3807.33 (1575.5)	0.000000002	0.1886	0.002	0.002	0.318	0.184	0.028	0.062
Aβ42	EUROIMMUN	803.34 (364.34)	569.97 (365.11)	351.35 (273.1)	0.00009	0.00005	0.001	0.002	0.042	0.0105	0.128	0.048
	INNOTEST	672.89 (255.61)	577.66 (229.67)	425.73 (208.98)	0.0020	0.0015	0.006	0.012	0.205	0.0594	0.119	0.064
	AlzBio3	420.8 (93.28)	354.16 (74.9)	308.53 (94.29)	0.0002	0.0002	0.002	0.002	0.028	0.0166	0.183	0.087
T-tau	EUROIMMUN	430.16 (162.1)	511.97 (252.05)	500.95 (156.6)	0.004	0.0024	0.007	0.008	0.024	0.0258	0.706	0.604
	INNOTEST	364.35 (173.33)	521.18 (304.04)	558.6 (240.74)	0.004	0.0039	0.010	0.013	0.060	0.0558	0.520	0.968
	AlzBio3	90.89 (45.14)	121.98 (64.45)	137.14 (58.59)	0.126	0.107	0.159	0.164	0.149	0.1418	0.884	0.914
P-tau												
	INNOTEST	64.81 (21.78)	77.1 (32.42)	74.51 (20.44)	0.087	0.070	0.148	0.128	0.103	0.1486	0.792	0.904
	AlzBio3	35.97 (15.94)	45.4 (26.28)	54.9 (17.8)	0.004	0.002	0.003	0.002	0.123	0.1109	0.284	0.153

Comment [Office1]: One line

Comment [Office2]: One line

Aβ42/Aβ40	EUROIMMUN	0.13 (0.05)	0.1 (0.05)	0.09 (0.03)	0.004	0.004	0.011	0.012	0.082	0.056	0.332	0.244
T-tau/Aβ42	EUROIMMUN	0.66 (0.37)	1.18 (0.76)	1.77 (0.74)	0.000001	0.000001	0.0003	0.0007	0.003	0.0021	0.055	0.094
	INNOTEST	0.61 (0.37)	1.05 (0.69)	1.46 (0.68)	0.00001	0.00001	0.0002	0.0003	0.006	0.004	0.121	0.200
	AlzBio3	0.23 (0.12)	0.38 (0.23)	0.48 (0.25)	0.00009	0.00007	0.001	0.0013	0.007	0.0053	0.279	0.406
P-tau/Aβ42												
	INNOTEST	0.11 (0.05)	0.15 (0.09)	0.19 (0.06)	0.00008	0.00008	0.0004	0.0005	0.020	0.014	0.166	0.267
	AlzBio3	0.09 (0.06)	0.14 (0.1)	0.2 (0.1)	0.00013	0.00008	0.0004	0.0004	0.042	0.038	0.133	0.046

Supplementary Table 2: Biomarker concentration [pg/mL] by clinical classification and assay platform: Biomarker mean and standard deviation (SD) values, including unadjusted and adjusted (age, gender *APOE* ε4 allele status) p-values from GLM for clinical classification.

Supplementary Table 3: ROC model comparisons between platform for Aβ42 biomarker and the T-tau/Aβ42 ratio

Biomarker	Model	AUC	D	p-value
Aβ42	INNOTEST	0.936		
	AlzBio3	0.893	-1.674	0.094
	INNOTEST	0.936		
	EUROIMMUNE	0.946	-0.431	0.666
	AlzBio3	0.893		
	EUROIMMUNE	0.946	-1.233	0.218
T-tau/Aβ42	INNOTEST	0.953		
	AlzBio3	0.903	2.313	0.021
	INNOTEST	0.953		
	EUROIMMUNE	0.978	1.548	0.122
	AlzBio3	0.903		
	EUROIMMUNE	0.978	1.837	0.066

Supplementary Table 3: Platform ROC model comparisons using DeLongs test. Values shown include ROC AUC, DeLongs statistic D and related p-value.

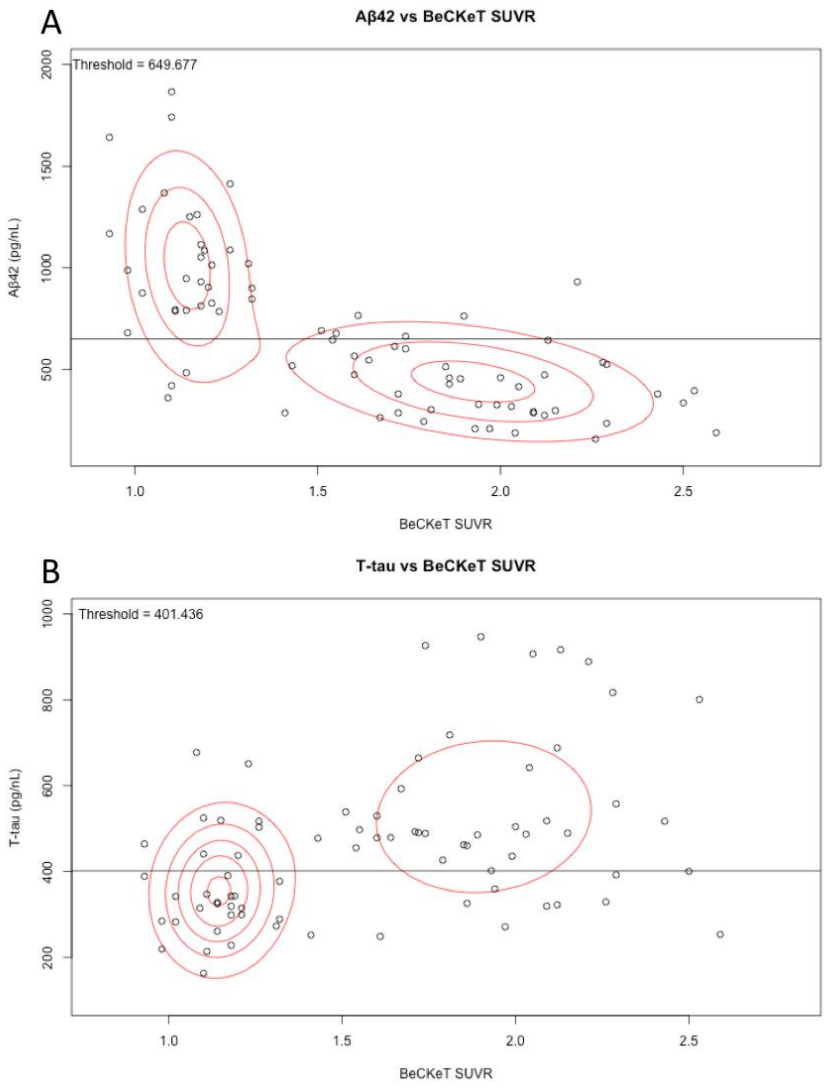
Supplementary Table 4: Concordance of CSF biomarkers per platform with PET Aβ-amyloid status by clinical classification

Platform	Biomarker	CSF- PET Aβ- (TN) HC/MCI/AD	CSF- PET Aβ+ (FN) HC/MCI/AD	CSF+ PET Aβ- (FP) HC/MCI/AD	CSF+ PET Aβ+ (TP) HC/MCI/AD
N PET		32	45	32	45
N Classification**		27/4/1	21/11/13	27/4/1	21/11/13
EUROIMMUN	Aβ1–42	26/2/1/ (29)	5/1/0/ (6)	1/2/0/ (3)	16/10/13/ (39)
	T-tau/Aβ42 ratio	25/4/1/ (30)	0/1/0/ (1)	2/0/0/ (0)	21/10/13/ (44)
INNOTEST	Aβ42	24/4/1/ (29)	8/4/0/ (12)	3/0/0/ (3)	13/7/13/ (43)
	T-tau/Aβ42 ratio	26/4/1 (31)	11/3/0/ (14)	1/0/0/ (1)	10/8/13/ (31)
AlzBio3*	Aβ42	21/2/1/ (24)	7/0/0/ (7)	4/2/0 (6)	13/9/12 (34)
	T-tau/Aβ42 ratio	21/3/1/ (25)	2/1/0/ (3)	6/1/0/ (7)	18/8/12/ (38)

* There were 4 missing values from this platform, ** (HC/MCI/AD)

Supplementary Table 4: Concordance of CSF biomarkers per platform with PET Aβ-amyloid status by clinical classification. Values shown are frequencies of true negatives (TN), false negatives (FN), true positives (TP), false positives (FP) for HC, MCI, AD and totals (in brackets).

Supplementary Figure 1:



Supplementary Figure 1: EUROIMMUN CSF biomarker threshold detection: Scatterplot of multivariate normal expectation maximisation (mnEM) group and threshold estimation using CSF biomarkers Aβ42 (S1A) and T-tau (S1B). Clusters of contour lines represent individual groups. Calculated threshold represented via horizontal line.