

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

James D. Doecke<sup>a,b</sup>, Alan Rembach<sup>c,\*\*</sup>, Victor L. Villemagne<sup>c,d</sup>, Shiji Varghese<sup>c</sup>, Stephanie Rainey-Smith<sup>e</sup>, Shannon Sarros<sup>c</sup>, Lisbeth A. Evered<sup>f</sup>, Christopher J. Fowler<sup>g</sup>, Kelly K. Pertile<sup>c</sup>, Rebecca L. Rumble<sup>c</sup>, Brett Tronson<sup>c</sup>, Kevin Taddei<sup>e</sup>, Simon M. Laws<sup>c</sup>, S. Lance Macaulay<sup>a</sup>, Ashley I Bush<sup>c</sup>, Kathryn A Ellis<sup>g</sup>, Ralph Martins<sup>c</sup>, David Ames<sup>g</sup>, Brendan Silbert<sup>f</sup>, Vanderstichele H<sup>h</sup>, Colin L. Masters<sup>c</sup>, David G. Darby<sup>c</sup>, Qiao-Xin Li<sup>c</sup>, Steven Collins<sup>c,i</sup>, and the AIBL Research Group<sup>j</sup>.

<sup>a</sup> CSIRO Health and Biosecurity/Australian e-Health Research Centre, Brisbane, QLD, 4029, Australia.

<sup>b</sup> Cooperative Research Centre for Mental Health, Parkville, Victoria, Australia.

<sup>c</sup> The Florey Institute of Neuroscience and Mental Health, The University of Melbourne, Victoria, 3010, Australia.

<sup>d</sup> Department of Nuclear Medicine and Centre for PET, Austin Health, Heidelberg, VIC, 3084, Australia

<sup>e</sup> Sir James McCusker Alzheimer's Disease Research Unit (Hollywood Private Hospital), Perth, WA, 6009, Australia.

<sup>f</sup> Centre for Anaesthesia and Cognitive Function, Department of Anaesthesia and Perioperative Pain Medicine, St Vincent's Hospital, Melbourne.

<sup>g</sup> Academic Unit for Psychiatry of Old Age, The University of Melbourne

<sup>h</sup> ADx NeuroSciences, EUROIMMUN, Gent, Belgium

<sup>i</sup> Department of Medicine (RMH), The University of Melbourne, Parkville, 3010, Australia.

<sup>j</sup> aibl.csiro.au

\*\* Unexpectedly deceased 20 Nov 2014

Corresponding author: Steven Collins, Department of Medicine (RHM) & The Florey  
Institute of Neuroscience and Mental Health, Parkville, VIC 3052, Ph: +61 3 9035 3000,  
eMail: s.collins@unimelb.edu.au

Running title: Assay-independent CSF biomarker and PET A $\beta$ -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 A $\beta$ -amyloid and tau species determined using three different versions of immunoassays, the current study explored the ability of CSF biomarkers to predict PET A $\beta$ -amyloid (32 A $\beta$ -amyloid- and 45 A $\beta$ -amyloid+), as well as concordance between CSF biomarker levels and PET A $\beta$ -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/A $\beta$ 42 ratio biomarker had modestly higher correlation with SUVR/BeCKeT ( $\rho=0.69-0.8$ ) as compared with A $\beta$ 42 alone ( $\rho=0.66-0.75$ ). Differences in CSF biomarker levels between the PET A $\beta$ -amyloid- and A $\beta$ -amyloid+ groups were strongest for the A $\beta$ 42/A $\beta$ 40 and T-tau/A $\beta$ 42 ratios ( $p<0.0001$ ), however comparison of predictive models for PET A $\beta$ -amyloid showed no difference between A $\beta$ 42 alone and the T-tau/A $\beta$ 42 ratio.

**Conclusion:** This study confirms strong concordance between CSF biomarkers and PET A $\beta$ -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, A $\beta$ : A $\beta$ -amyloid, A $\beta$ 40, A $\beta$ 42.

## 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 A $\beta$ -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 A $\beta$ -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 A $\beta$ -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 A $\beta$ 1-42 amyloid (A $\beta$ 42), 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 A $\beta$ -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 A $\beta$ -amyloid have been quite strong, with accuracy up to 92% for the T-tau/A $\beta$ 42 ratio [7], [18],[19]. Variability in concordance with neocortical A $\beta$ -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 A $\beta$ 42 and increase in T-tau and P-tau can be easily discerned by a change in the A $\beta$ 42/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 A $\beta$ -amyloid imaging (32 PET A $\beta$ - and 45 PET A $\beta$ +) 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<sup>®</sup>, 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°C during transport to the laboratory and processed within 1 hour. The CSF was centrifuged (2,000 x g, 4°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 (18-30°C) 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: A $\beta$ 42, 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 A $\beta$ 42 was 7.4%, for T-tau 4.0%, and for P-tau 1.6%, and the inter-assay %CV for A $\beta$ 42 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 A $\beta$ 42, A $\beta$ 1-40 (A $\beta$ 40), 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 A $\beta$ 42 was 15  $\mu$ L of calibrators, controls and undiluted CSF. For A $\beta$ 40 the loading volume was 15  $\mu$ L of calibrators, controls and 1:21 pre-diluted CSF. For T-tau the loading volume was 25  $\mu$ L of calibrators, controls and undiluted CSF. For each assay, 100  $\mu$ L 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 (23°C). The wells were subsequently washed five times using 1x wash buffer, 100  $\mu$ L 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  $\mu$ L of substrate was added and incubated for 30 minutes at room temperature. Finally, 100  $\mu$ L 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 A $\beta$ 40 was 8.2%, for A $\beta$ 42 5.8%, and for T-tau 4.5%, while the inter-assay %CV for A $\beta$ 40 was 9.9%, for A $\beta$ 42 6.8%, and for T-tau 6.4%.

### ***PET imaging***

To determine brain A $\beta$ -amyloid levels, participants underwent PET imaging conducted using one of three different tracers; either  $^{11}\text{C}$ -Pittsburgh Compound-B (PiB; N=28),  $^{18}\text{F}$ -flutemetamol (FLUTE; N=32) or  $^{18}\text{F}$ -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 A $\beta$ -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 (A $\beta$ +) or low (A $\beta$ -) A $\beta$ -amyloid burden with an SUVR  $\geq 1.4$  used as the threshold [34].

### ***PET A $\beta$ -amyloid-CSF biomarker concordance***

CSF biomarker thresholding to define high (CSF+) or low (CSF-) CSF biomarker levels were primarily established for A $\beta$ 42 alone and for the T-tau/A $\beta$ 42 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 A $\beta$ 42 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;  $\mu \pm SD$  calculated as:  $[\mu_1 - 1SD + \mu_2 + 1SD]/2$ , Supplementary Figure 1). For EUROIMMUN, we used a threshold of <649 pg/mL for A $\beta$ 42 and >0.618 (401 pg/mL/649 pg/mL) for the T-tau/A $\beta$ 42 ratio (via multinomial expectation maximisation (mnEM) modelling). For INNOTEST, we used a threshold of <544 pg/mL for A $\beta$ 42 and >0.748 (407 pg/mL/544 pg/mL as per [18]) for the T-tau/A $\beta$ 42 ratio. For AlzBio3, we used a threshold of <416 pg/mL for A $\beta$ 42 and >0.184 (76.7pg/mL/416pg/mL as per [35]) for the T-tau/A $\beta$ 42 ratio. Thresholds for the T-tau/A $\beta$ 42 ratio were derived by dividing the platform specific threshold for T-tau by the platform specific threshold for A $\beta$ 42. 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 A $\beta$ -amyloid burden status and clinical classification (HC, MCI and AD) using Analysis of Variance and Independent Samples T-test (Age), Chi-Square test (*APOE*  $\epsilon 4$ , gender), and Kruskal Wallis and Wilcoxon test of Ranks (MMSE, CDR score). Comparison of CSF biomarkers between PET A $\beta$ -

amyloid status and clinical classification, adjusted for age, gender and APOE  $\epsilon$ 4 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 ( $\rho$ ) was used to compute correlations between BeCKeT SUVR and CSF biomarker levels. Concordance using binary biomarkers post thresholding was conducted using Cohen's Kappa ( $\kappa$ ) 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 ( $A\beta$ -amyloid and clinical classification) were considered significant after correcting for multiple comparisons using the Bonferroni adjustment for seven biomarkers ( $\alpha=0.05/7$ , 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  $A\beta$ - and 21  $A\beta$ + PET scans, while in the MCI group there were 4  $A\beta$ - and 11  $A\beta$ + scans, and in the AD group there was 1  $A\beta$ - and 13  $A\beta$ + scans. There were no significant differences in age, gender or APOE  $\epsilon$ 4 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 A $\beta$ + HC participants (21/48).

### ***Correlation of biomarkers between platforms***

We performed linear correlation analyses between platforms to assess the relationships for A $\beta$ 42, T-tau, and the ratio of T-tau/A $\beta$ 42. Relationships were plotted stratified by clinical classification (Figure 1). Strongest correlation for A $\beta$ 42 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 A $\beta$ 42, T-tau, and the ratio of T-tau/A $\beta$ 42 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/A $\beta$ 42 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 A $\beta$ -amyloid status groups and clinical classifications respectively. Assessing the ratios for T-tau/A $\beta$ 42 and A $\beta$ 42/A $\beta$ 40 against PET A $\beta$ -amyloid status (Figures 2C and 3A, Table 2), it was apparent that the combination of biomarkers performed better in separating A $\beta$ -amyloid PET- from A $\beta$ -amyloid PET+ status compared to each biomarker alone. In particular, the A $\beta$ 42/A $\beta$ 40 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 A $\beta$ -amyloid status, we assessed the predictive performance (not cross validated) using the complete cohort. All biomarkers from each platform were tested, including the A $\beta$ 42/A $\beta$ 40 ratio and T-tau/A $\beta$ 42 ratios. ROC derived threshold values along with associated predictive values per platform and biomarker are shown in Table 3. Of the individual markers, A $\beta$ 42 was comparable to the ratio biomarkers, albeit values were lower (not significantly) to that from the T-tau/A $\beta$ 42 ratio. Comparing the ROC models for A $\beta$ 42 alone and the T-tau/A $\beta$ 42 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 (A $\beta$ 42) and ratio (T-tau/A $\beta$ 42) can be found in Supplementary Table 3.

### ***PET A $\beta$ -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 A $\beta$ 42 analyte results and the T-tau/A $\beta$ 42 ratio. In general, the T-tau/A $\beta$ 42 ratio performed better than the individual A $\beta$ 42 biomarker with regard to delineating between true negatives and true positives. The highest performance was seen for the EUROIMMUN T-tau/A $\beta$ 42 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 A $\beta$ 42 biomarker using the EUROIMMUN platform ( $\kappa = 0.763$ ,  $p < 0.0001$ ), followed by INNOTEST and AlzBio3 ( $\kappa = 0.615$ ,  $p < 0.0001$  and ( $\kappa = 0.581$ ,  $p = 0.105$  respectively). Concordance values were higher for the T-tau/A $\beta$ 42 ratio, with the EUROIMMUN platform having the best concordance ( $\kappa = 0.919$ ,  $p < 0.0001$ ), followed by AlzBio3 and INNOTEST ( $\kappa = 0.689$ ,  $p < 0.0001$  and  $\kappa = 0.621$ ,  $p < 0.0001$ , respectively).

Assessing the correlations between CSF A $\beta$ 42 and the T-tau/A $\beta$ 42 ratio against SUVR/BeCKeT (Figure 4), both the INNOTEST and EUROIMMUN platforms showed strongest correlations between the T-tau/A $\beta$ 42 ratio and SUVR/BeCKeT (INNOTEST:  $\rho = 0.79$ , EUROIMMUN:  $\rho = 0.80$ ), while the correlation between the individual INNOTEST CSF A $\beta$ 42 and SUVR/BeCKeT was strongest of the three platforms assessed ( $\rho = 0.75$ ). 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 A $\beta$ 42, A $\beta$ 40, T-tau and P-tau and their associated ratios within clinical classification groups (HC, MCI, AD) and especially PET A $\beta$ -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 A $\beta$ -amyloid status, with the A $\beta$ 42/A $\beta$ 40 (limited to EUROIMMUNE) ratio and T-tau/A $\beta$ 42 ratio the strongest markers to predict PET A $\beta$ -amyloid status. Whilst the predictive values were higher for the ratio biomarkers in comparison to A $\beta$ 42 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 A $\beta$ 42 (R=0.94), while the INNOTEST and AlzBio3 platforms had the weakest correlation for A $\beta$ 42 (R=0.67).

Of the individual biomarkers tested, CSF A $\beta$ 42 in each platform performed similarly to distinguish A $\beta$ - from A $\beta$ + 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 A $\beta$ -amyloid status compared with individual biomarkers. Contrary to previous studies [36-38] where the CSF A $\beta$ 42/A $\beta$ 40 ratio performed better than A $\beta$ 42 alone to predict both A $\beta$ -Amyloid pathology and clinical AD, the current study using the EUROIMMUN platform did not see any appreciable difference in prediction accuracies between the CSF A $\beta$ 42/A $\beta$ 40 and CSF A $\beta$ 42 biomarker alone. After adjusting for age, gender and APOE  $\epsilon$ 4 status, the INNOTEST and AlzBio3 T-tau/A $\beta$ 42 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/A $\beta$ 42 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 A $\beta$ -amyloid status was found from A $\beta$ 42 from the EUROIMMUN platform (NPV: 0.97, PPV: 0.92), while the best predictive values for a ratio were found from

the T-tau/A $\beta$ 42 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 A $\beta$ -amyloid status was quite strong across all platforms. This concordance was apparent after plotting the quantitative biomarker data along with a  $\pm 5\%$  “grey zone” of uncertainty around each threshold, with the EUROIMMUN platform 98% accurate for PET A $\beta$ + and 94% accurate for PET A $\beta$ -. 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 A $\beta$ 42 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 A $\beta$ -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/A $\beta$ 42 ratio. In a large study assessing ratios for the diagnosis of AD, Janelidze et al., [19] showed that the A $\beta$ 42/A $\beta$ 40 and A $\beta$ 42/A $\beta$ 38 ratios were significantly better than A $\beta$ 42 alone at predicting PET A $\beta$ -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 A $\beta$  ratio rather than A $\beta$ 42 alone, supporting our results for the ratio over the individual A $\beta$ 42 even in pre-symptomatic persons harbouring A $\beta$ -amyloid pathology. In the current study the A $\beta$ 42/A $\beta$ 40 ratio was only able to be assessed in the EUROIMMUN platform, and the P-tau/A $\beta$ 42 ratio in the INNOTEST and AlzBio3 platforms. Whilst the A $\beta$ 42/A $\beta$ 40 ratio continues to show promise to predict PET A $\beta$ -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 A $\beta$ -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 A $\beta$ 42 in a ratio with another key CSF biomarker, whether it is the A $\beta$ 42/A $\beta$ 40 ratio, the T-tau/A $\beta$ 42 ratio or the P-tau/A $\beta$ 42 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 A $\beta$ -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 A $\beta$ 42 (R=0.94), and between INNOTEST and AlzBio3 for the T-tau/A $\beta$ 42 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 A $\beta$ -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 A $\beta$ -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 (A $\beta$ 1-40, A $\beta$ 1-42) or a possible interference within the assay by other A $\beta$  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  $\epsilon$ 4 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 A $\beta$ -amyloid burden, correlating highly with clinical status. Results from this study further support the need for further investigation of CSF biomarker ratios incorporating A $\beta$ 42 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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## Reference List

- [1] Takizawa C, Thompson PL, van Walsem A, Faure C, Maier WC (2015) Epidemiological and economic burden of Alzheimer's disease: a systematic literature review of data across Europe and the United States of America. *J Alzheimers Dis* **43**, 1271-1284.
- [2] Cummings J, Aisen PS, DuBois B, Frolich L, Jack CR, Jr., Jones RW, Morris JC, Raskin J, Dowsett SA, Scheltens P (2016) Drug development in Alzheimer's disease: the path to 2025. *Alzheimers Res Ther* **8**, 39.
- [3] Karran E, Hardy J (2014) A critique of the drug discovery and phase 3 clinical programs targeting the amyloid hypothesis for Alzheimer disease. *Ann Neurol* **76**, 185-205.
- [4] Villemagne VL, Burnham S, Bourgeat P, Brown B, Ellis KA, Salvado O, Szeoke C, Macaulay SL, Martins R, Maruff P, Ames D, Rowe CC, Masters CL, Australian Imaging B, Lifestyle Research G (2013) Amyloid beta deposition, neurodegeneration, and cognitive decline in sporadic Alzheimer's disease: a prospective cohort study. *Lancet Neurol* **12**, 357-367.
- [5] Toledo JB, Xie SX, Trojanowski JQ, Shaw LM (2013) Longitudinal change in CSF Tau and Aβeta biomarkers for up to 48 months in ADNI. *Acta Neuropathol* **126**, 659-670.
- [6] Bateman RJ, Xiong C, Benzinger TL, Fagan AM, Goate A, Fox NC, Marcus DS, Cairns NJ, Xie X, Blazey TM, Holtzman DM, Santacruz A, Buckles V, Oliver A, Moulder K, Aisen PS, Ghetti B, Klunk WE, McDade E, Martins RN, Masters CL, Mayeux R, Ringman JM, Rossor MN, Schofield PR, Sperling RA, Salloway S, Morris JC, Dominantly Inherited Alzheimer N (2012) Clinical and biomarker changes in dominantly inherited Alzheimer's disease. *N Engl J Med* **367**, 795-804.
- [7] Leuzy A, Chiotis K, Hasselbalch SG, Rinne JO, de Mendonca A, Otto M, Lleó A, Castelo-Branco M, Santana I, Johansson J, Anderl-Straub S, von Arnim CA, Beer A, Blesa R, Fortea J, Herukka SK, Portelius E, Pannee J, Zetterberg H, Blennow K, Nordberg A (2016) Pittsburgh compound B imaging and cerebrospinal fluid amyloid-beta in a multicentre European memory clinic study. *Brain* **139**, 2540-2553.
- [8] Vos SJ, Gordon BA, Su Y, Visser PJ, Holtzman DM, Morris JC, Fagan AM, Benzinger TL (2016) NIA-AA staging of preclinical Alzheimer disease: discordance and concordance of CSF and imaging biomarkers. *Neurobiol Aging* **44**, 1-8.
- [9] Iturria-Medina Y, Sotero RC, Toussaint PJ, Mateos-Perez JM, Evans AC, Alzheimer's Disease Neuroimaging I (2016) Early role of vascular dysregulation on late-onset Alzheimer's disease based on multifactorial data-driven analysis. *Nat Commun* **7**, 11934.
- [10] O'Bryant SE, Gupta V, Henriksen K, Edwards M, Jeromin A, Lista S, Bazenet C, Soares H, Lovestone S, Hampel H, Montine T, Blennow K, Foroud T, Carrillo M, Graff-Radford N, Laske C, Breteler M, Shaw L, Trojanowski JQ, Schupf N, Rissman RA, Fagan AM, Oberoi P, Umek R, Weiner MW, Grammas P, Posner H, Martins R, Star B, groups Bw (2015) Guidelines for the standardization of preanalytic variables for blood-based biomarker studies in Alzheimer's disease research. *Alzheimers Dement* **11**, 549-560.
- [11] Blennow K, Hampel H (2003) CSF markers for incipient Alzheimer's disease. *Lancet Neurol* **2**, 605-613.
- [12] Olsson A, Vanderstichele H, Andreasen N, De Meyer G, Wallin A, Holmberg B, Rosengren L, Vanmechelen E, Blennow K (2005) Simultaneous measurement of beta-amyloid(1-42), total tau, and phosphorylated tau (Thr181) in cerebrospinal fluid by the xMAP technology. *Clin Chem* **51**, 336-345.
- [13] Mattsson N, Andreasson U, Persson S, Carrillo MC, Collins S, Chalbot S, Cutler N, Dufour-Rainfray D, Fagan AM, Heegaard NH, Robin Hsiung GY, Hyman B, Iqbal K, Kaeser SA, Lachno DR, Lleó A, Lewczuk P, Molinuevo JL, Parchi P, Regeniter A, Rissman RA, Rosenmann H, Sancesario G, Schroder J, Shaw LM, Teunissen CE, Trojanowski JQ, Vanderstichele H, Vandijck M, Verbeek MM, Zetterberg H, Blennow K, Alzheimer's Association QCPWG (2013) CSF biomarker variability in the Alzheimer's Association quality control program. *Alzheimers Dement* **9**, 251-261.

- [14] Bjerke M, Portelius E, Minthon L, Wallin A, Anckarsater H, Anckarsater R, Andreassen N, Zetterberg H, Andreasson U, Blennow K (2010) Confounding factors influencing amyloid Beta concentration in cerebrospinal fluid. *Int J Alzheimers Dis* **2010**.
- [15] Cullen VC, Fredenburg RA, Evans C, Conliffe PR, Solomon ME (2012) Development and advanced validation of an optimized method for the quantitation of Abeta42 in human cerebrospinal fluid. *AAPS J* **14**, 510-518.
- [16] Kuhlmann J, Andreasson U, Pannee J, Bjerke M, Portelius E, Leinenbach A, Bittner T, Korecka M, Jenkins RG, Vanderstichele H, Stoops E, Lewczuk P, Shaw LM, Zegers I, Schimmel H, Zetterberg H, Blennow K, proteins IWGoSoC (2016) CSF Abeta1-42 - an excellent but complicated Alzheimer's biomarker - a route to standardisation. *Clin Chim Acta*.
- [17] Wang LS, Leung YY, Chang SK, Leight S, Knapik-Czajka M, Baek Y, Shaw LM, Lee VM, Trojanowski JQ, Clark CM (2012) Comparison of xMAP and ELISA assays for detecting cerebrospinal fluid biomarkers of Alzheimer's disease. *J Alzheimers Dis* **31**, 439-445.
- [18] Li QX, Villemagne VL, Doecke JD, Rembach A, Sarros S, Varghese S, McGlade A, Loughton KM, Pertile KK, Fowler CJ, Rumble RL, Trounson BO, Taddei K, Rainey-Smith SR, Laws SM, Robertson JS, Evered LA, Silbert B, Ellis KA, Rowe CC, Macaulay SL, Darby D, Martins RN, Ames D, Masters CL, Collins S, Group AR (2015) Alzheimer's Disease Normative Cerebrospinal Fluid Biomarkers Validated in PET Amyloid-beta Characterized Subjects from the Australian Imaging, Biomarkers and Lifestyle (AIBL) study. *J Alzheimers Dis* **48**, 175-187.
- [19] Janelidze S, Zetterberg H, Mattsson N, Palmqvist S, Vanderstichele H, Lindberg O, van Westen D, Stomrud E, Minthon L, Blennow K, Swedish Bio Fsg, Hansson O (2016) CSF Abeta42/Abeta40 and Abeta42/Abeta38 ratios: better diagnostic markers of Alzheimer disease. *Ann Clin Transl Neurol* **3**, 154-165.
- [20] Mattsson N, Insel PS, Palmqvist S, Stomrud E, van Westen D, Minthon L, Zetterberg H, Blennow K, Hansson O (2016) Increased amyloidogenic APP processing in APOE varepsilon4-negative individuals with cerebral beta-amyloidosis. *Nat Commun* **7**, 10918.
- [21] Palmqvist S, Mattsson N, Hansson O (2016) Cerebrospinal fluid analysis detects cerebral amyloid-beta accumulation earlier than positron emission tomography. *Brain* **139**, 1226-1236.
- [22] Harari O, Cruchaga C, Kauwe JS, Ainscough BJ, Bales K, Pickering EH, Bertelsen S, Fagan AM, Holtzman DM, Morris JC, Goate AM, Alzheimer's Disease Neuroimaging I (2014) Phosphorylated tau-Abeta42 ratio as a continuous trait for biomarker discovery for early-stage Alzheimer's disease in multiplex immunoassay panels of cerebrospinal fluid. *Biol Psychiatry* **75**, 723-731.
- [23] Molinuevo JL, Blennow K, Dubois B, Engelborghs S, Lewczuk P, Perret-Liaudet A, Teunissen CE, Parnetti L (2014) The clinical use of cerebrospinal fluid biomarker testing for Alzheimer's disease diagnosis: A consensus paper from the Alzheimer's Biomarkers Standardization Initiative. *Alzheimers Dement*.
- [24] Jongbloed W, Kester MI, van der Flier WM, Veerhuis R, Scheltens P, Blankenstein MA, Teunissen CE (2013) Discriminatory and predictive capabilities of enzyme-linked immunosorbent assay and multiplex platforms in a longitudinal Alzheimer's disease study. *Alzheimers Dement* **9**, 276-283.
- [25] Ellis KA, Bush AI, Darby D, De Fazio D, Foster J, Hudson P, Lautenschlager NT, Lenzo N, Martins RN, Maruff P, Masters C, Milner A, Pike K, Rowe C, Savage G, Szoek C, Taddei K, Villemagne V, Woodward M, Ames D, Group AR (2009) The Australian Imaging, Biomarkers and Lifestyle (AIBL) study of aging: methodology and baseline characteristics of 1112 individuals recruited for a longitudinal study of Alzheimer's disease. *Int Psychogeriatr* **21**, 672-687.
- [26] Rembach A, Evered LA, Li QX, Nash T, Vidaurre L, Fowler CJ, Pertile KK, Rumble RL, Trounson BO, Maher S, Mooney F, Farrow M, Taddei K, Rainey-Smith S, Laws SM, Macaulay SL, Wilson W, Darby DG, Martins RN, Ames D, Collins S, Silbert B, Masters CL, Doecke JD (2015)

- Alzheimer's disease cerebrospinal fluid biomarkers are not influenced by gravity drip or aspiration extraction methodology. *Alzheimers Res Ther* **7**, 71.
- [27] Vanderstichele H, Bibl M, Engelborghs S, Le Bastard N, Lewczuk P, Molinuevo JL, Parnetti L, Perret-Liaudet A, Shaw LM, Teunissen C, Wouters D, Blennow K (2012) Standardization of preanalytical aspects of cerebrospinal fluid biomarker testing for Alzheimer's disease diagnosis: a consensus paper from the Alzheimer's Biomarkers Standardization Initiative. *Alzheimers Dement* **8**, 65-73.
  - [28] Rowe CC, Ellis KA, Rimajova M, Bourgeat P, Pike KE, Jones G, Frapp J, Tochon-Danguy H, Morandau L, O'Keefe G, Price R, Raniga P, Robins P, Acosta O, Lenzo N, Szeke C, Salvado O, Head R, Martins R, Masters CL, Ames D, Villemagne VL (2010) Amyloid imaging results from the Australian Imaging, Biomarkers and Lifestyle (AIBL) study of aging. *Neurobiology of Aging* **31**, 1275-1283.
  - [29] Wong DF, Rosenberg PB, Zhou Y, Kumar A, Raymond V, Ravert HT, Dannals RF, Nandi A, Brašić JR, Ye W (2010) In vivo imaging of amyloid deposition in Alzheimer disease using the radioligand 18F-AV-45 (florbetapir F 18). *Journal of Nuclear Medicine* **51**, 913-920.
  - [30] Vandenberghe R, Van Laere K, Ivanoiu A, Salmon E, Bastin C, Triau E, Hasselbalch S, Law I, Andersen A, Korner A (2010) 18F-flutemetamol amyloid imaging in Alzheimer disease and mild cognitive impairment: A phase 2 trial. *Annals of neurology* **68**, 319-329.
  - [31] Clark CM, Schneider JA, Mintun MM, Bedell BJ, Beach TG, Sadowsky CH, Doraiswamy P, Gamez J, Sabbagh MN, Fleisher AS (2010) Phase III trial results for the amyloid PET imaging agent florbetapir F 18 (18F-AV-45): imaging to histopathologic correlations in an end-of-life human subject study. *Alzheimer's & Dementia: The Journal of the Alzheimer's Association* **6**.
  - [32] Thurfjell L, Lundqvist R, Buckley C, Smith A, Sherwin P (2013) in *Society of Nuclear Medicine Annual Meeting Abstracts Soc Nuclear Med*, p. 302.
  - [33] Villemagne VL, Dore V, Yates P, Brown B, Mulligan R, Bourgeat P, Veljanoski R, Rainey-Smith S, Ong K, Rembach A, Williams R, Burnham SC, Laws SM, Salvado O, Taddei K, Macaulay SL, Martins RN, Ames D, Masters CL, Rowe CC (2014) *En Attendant Centiloid. Advances in Research* **2**, 723-729.
  - [34] Chetelat G, Villemagne VL, Villain N, Jones G, Ellis KA, Ames D, Martins RN, Masters CL, Rowe CC, Group AR (2012) Accelerated cortical atrophy in cognitively normal elderly with high beta-amyloid deposition. *Neurology* **78**, 477-484.
  - [35] Forlenza OV, Radanovic M, Talib LL, Aprahamian I, Diniz BS, Zetterberg H, Gattaz WF (2015) Cerebrospinal fluid biomarkers in Alzheimer's disease: Diagnostic accuracy and prediction of dementia. *Alzheimers Dement (Amst)* **1**, 455-463.
  - [36] Hansson O, Zetterberg H, Buchhave P, Andreasson U, Londos E, Minthon L, Blennow K (2007) Prediction of Alzheimer's disease using the CSF Abeta42/Abeta40 ratio in patients with mild cognitive impairment. *Dement Geriatr Cogn Disord* **23**, 316-320.
  - [37] Sauvee M, DidierLaurent G, Latarche C, Escanye MC, Olivier JL, Malaplate-Armand C (2014) Additional use of Abeta(4)(2)/Abeta(4)(0) ratio with cerebrospinal fluid biomarkers P-tau and Abeta(4)(2) increases the level of evidence of Alzheimer's disease pathophysiological process in routine practice. *J Alzheimers Dis* **41**, 377-386.
  - [38] Lewczuk P, Lelental N, Spitzer P, Maler JM, Kornhuber J (2015) Amyloid-beta 42/40 cerebrospinal fluid concentration ratio in the diagnostics of Alzheimer's disease: validation of two novel assays. *J Alzheimers Dis* **43**, 183-191.
  - [39] Leuzy A, Carter SF, Chiotis K, Almkvist O, Wall A, Nordberg A (2015) Concordance and Diagnostic Accuracy of [11C]PIB PET and Cerebrospinal Fluid Biomarkers in a Sample of Patients with Mild Cognitive Impairment and Alzheimer's Disease. *J Alzheimers Dis* **45**, 1077-1088.
  - [40] Wang MJ, Yi S, Han JY, Park SY, Jang JW, Chun IK, Giau VV, Bagyinszky E, Lim KT, Kang SM, An SS, Park YH, Youn YC, Kim S (2016) Analysis of Cerebrospinal Fluid and [11C]PIB PET Biomarkers for Alzheimer's Disease with Updated Protocols. *J Alzheimers Dis* **52**, 1403-1413.

- [41] Adamczuk K, Schaeverbeke J, Vanderstichele HM, Lilja J, Nelissen N, Van Laere K, Dupont P, Hilven K, Poesen K, Vandenberghe R (2015) Diagnostic value of cerebrospinal fluid Abeta ratios in preclinical Alzheimer's disease. *Alzheimers Res Ther* **7**, 75.
- [42] Fagan AM, Shaw LM, Xiong C, Vanderstichele H, Mintun MA, Trojanowski JQ, Coart E, Morris JC, Holtzman DM (2011) Comparison of analytical platforms for cerebrospinal fluid measures of beta-amyloid 1-42, total tau, and p-tau181 for identifying Alzheimer disease amyloid plaque pathology. *Arch Neurol* **68**, 1137-1144.
- [43] Reijn TS, Rikkert MO, van Geel WJ, de Jong D, Verbeek MM (2007) Diagnostic accuracy of ELISA and xMAP technology for analysis of amyloid beta(42) and tau proteins. *Clin Chem* **53**, 859-865.
- [44] Monge-Argiles JA, Munoz-Ruiz C, Sanchez-Paya J, Gasparini Berenguer R, Blanco Canto ME, Leiva-Santana C (2014) Comparison of two analytical platforms for CSF biomarkers of Alzheimer's disease. *Biomed Res Int* **2014**, 765130.
- [45] Jack CR, Jr., Bennett DA, Blennow K, Carrillo MC, Feldman HH, Frisoni GB, Hampel H, Jagust WJ, Johnson KA, Knopman DS, Petersen RC, Scheltens P, Sperling RA, Dubois B (2016) A/T/N: An unbiased descriptive classification scheme for Alzheimer disease biomarkers. *Neurology* **87**, 539-547.
- [46] Palmqvist S, Zetterberg H, Blennow K, Vestberg S, Andreasson U, Brooks DJ, Owenius R, Hagerstrom D, Wollmer P, Minthon L, Hansson O (2014) Accuracy of brain amyloid detection in clinical practice using cerebrospinal fluid beta-amyloid 42: a cross-validation study against amyloid positron emission tomography. *JAMA Neurol* **71**, 1282-1289.
- [47] Elshal MF, McCoy JP (2006) Multiplex bead array assays: performance evaluation and comparison of sensitivity to ELISA. *Methods* **38**, 317-323.
- [48] Bjerke M, Andreasson U, Kuhlmann J, Portelius E, Pannee J, Lewczuk P, Umek RM, Vanmechelen E, Vanderstichele H, Stoops E, Lewis J, Vandijck M, Kostanjevecki V, Jeromin A, Salamone SJ, Schmidt O, Matzen A, Madin K, Eichenlaub U, Bittner T, Shaw LM, Zegers I, Zetterberg H, Blennow K (2016) Assessing the commutability of reference material formats for the harmonization of amyloid-beta measurements. *Clin Chem Lab Med* **54**, 1177-1191.
- [49] Kuhlmann J, Andreasson U, Pannee J, Bjerke M, Portelius E, Leinenbach A, Bittner T, Korecka M, Jenkins RG, Vanderstichele H, Stoops E, Lewczuk P, Shaw LM, Zegers I, Schimmel H, Zetterberg H, Blennow K, proteins IWGoSoC (2017) CSF Abeta1-42 - an excellent but complicated Alzheimer's biomarker - a route to standardisation. *Clin Chim Acta* **467**, 27-33.
- [50] Landau SM, Thomas BA, Thurfjell L, Schmidt M, Margolin R, Mintun M, Pontecorvo M, Baker SL, Jagust WJ, Alzheimer's Disease Neuroimaging I (2014) Amyloid PET imaging in Alzheimer's disease: a comparison of three radiotracers. *Eur J Nucl Med Mol Imaging* **41**, 1398-1407.
- [51] Yousefi BH, von Reutern B, Scherubl D, Manook A, Schwaiger M, Grimmer T, Henriksen G, Forster S, Drzezga A, Wester HJ (2015) FIBT versus florbetaben and PiB: a preclinical comparison study with amyloid-PET in transgenic mice. *EJNMMI Res* **5**, 20.
- [52] Vanderstichele HM, Shaw L, Vandijck M, Jeromin A, Zetterberg H, Blennow K, Teunissen C, Engelborghs S (2013) Alzheimer disease biomarker testing in cerebrospinal fluid: a method to harmonize assay platforms in the absence of an absolute reference standard. *Clin Chem* **59**, 710-712.

## Tables

Table 1a: PET cohort demographic details

	A $\beta$ PET-	A $\beta$ PET+	<i>p</i> -value
N	32	45	
Age (years)	73.5 (7.9)	73.3 (4.9)	0.91
Sex (F%)	53	44	0.45
<i>APOE</i> $\epsilon 4$ %	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	<i>p</i> -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
<i>APOE</i> $\epsilon 4$ %	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 A $\beta$ -amyloid status groups (1A) and clinical classification groups (1B). N; number, HC, Healthy Control, MCI; Mild Cognitive Impairment, AD; Alzheimer's disease, *APOE*  $\epsilon 4$ ; 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 A $\beta$ -amyloid status and assay platform

		A $\beta$ PET-	A $\beta$ PET+	Unadjusted	Adjusted
Biomarker	N	32	45		
A $\beta$ 40	EUROIMMUN	7053.07 (3655.99)	5153.23 (1986.5)	0.0114	0.0115
A $\beta$ 42	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
A $\beta$ 42/A $\beta$ 40	EUROIMMUN	0.16 (0.04)	0.09 (0.02)	0.000005	0.000007
T-tau/A $\beta$ 42	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/A $\beta$ 42	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 A $\beta$ -amyloid status and assay platform: Biomarker mean and standard deviation (SD) values, including unadjusted and adjusted (age, gender *APOE*  $\epsilon$ 4 allele status) p-values from GLM for PET A $\beta$ -amyloid status. An A $\beta$ 40 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 A $\beta$  status.

		AUC 95%CI	p-value	Threshold	Sensitivity	Specificity	PPV	NPV	Accuracy
A $\beta$ 40	EUROIMMUN	0.67 (0.61 - 0.73)	0.01	5062.30	0.60	0.69	0.77	0.54	0.64
A $\beta$ 42	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
A $\beta$ 42/A $\beta$ 40	EUROIMMUN	0.94 (0.91 - 0.97)	<0.0001	0.11	0.93	0.91	0.93	0.91	0.92
T-tau/A $\beta$ 42	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/A $\beta$ 42	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 A $\beta$ 40 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**

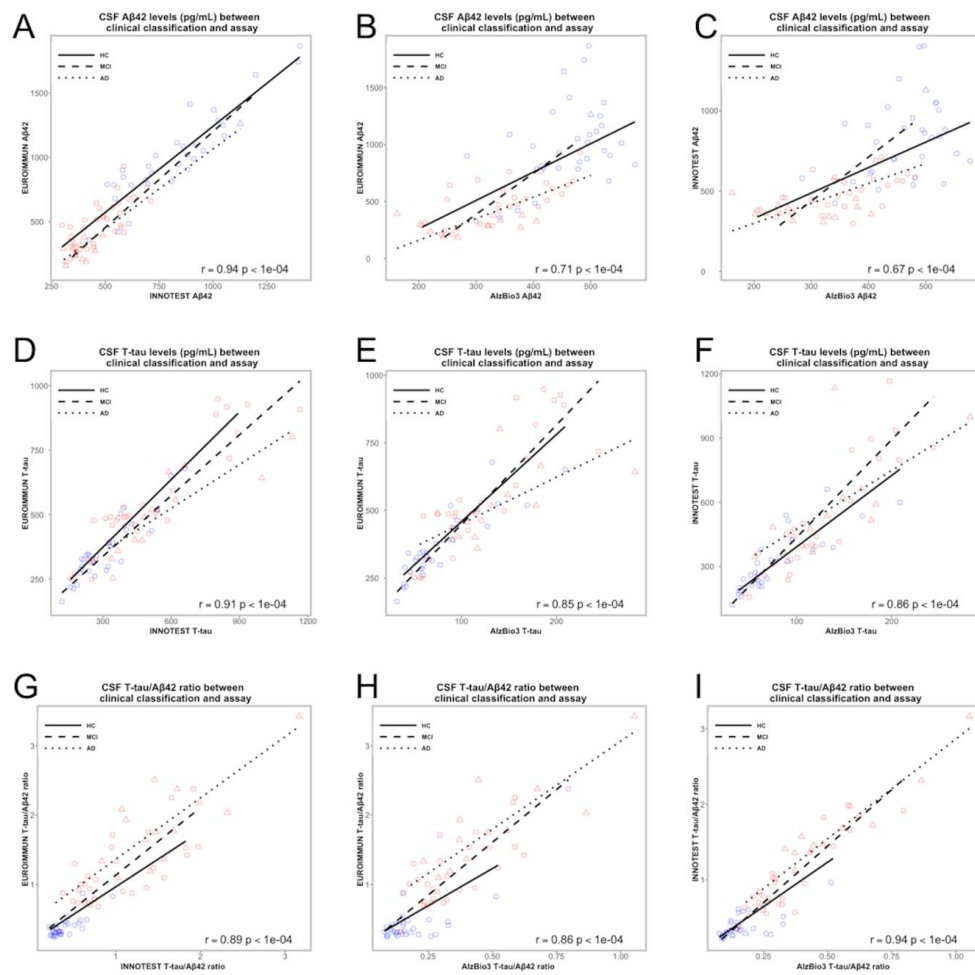


Figure 2

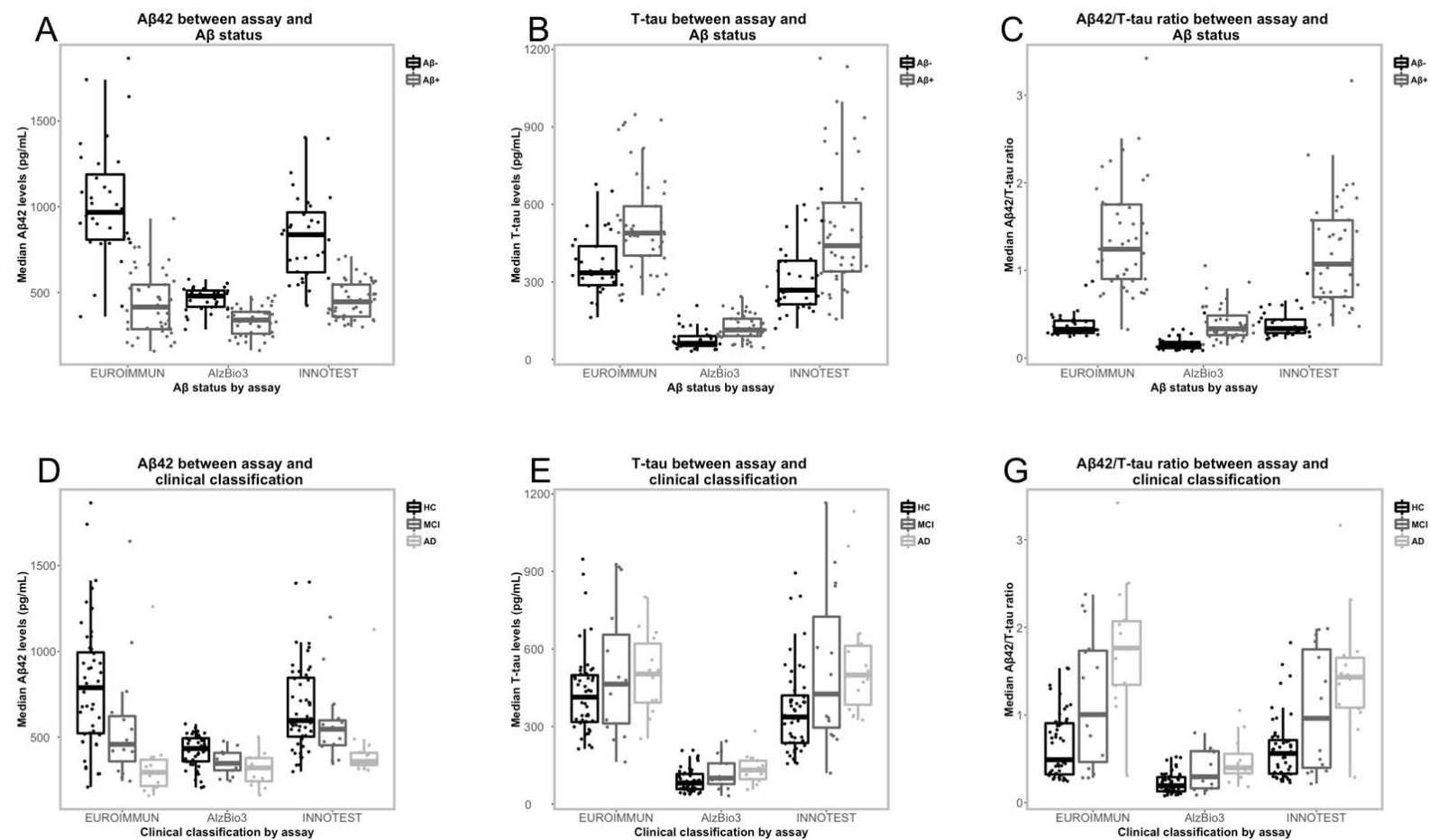


Figure 3

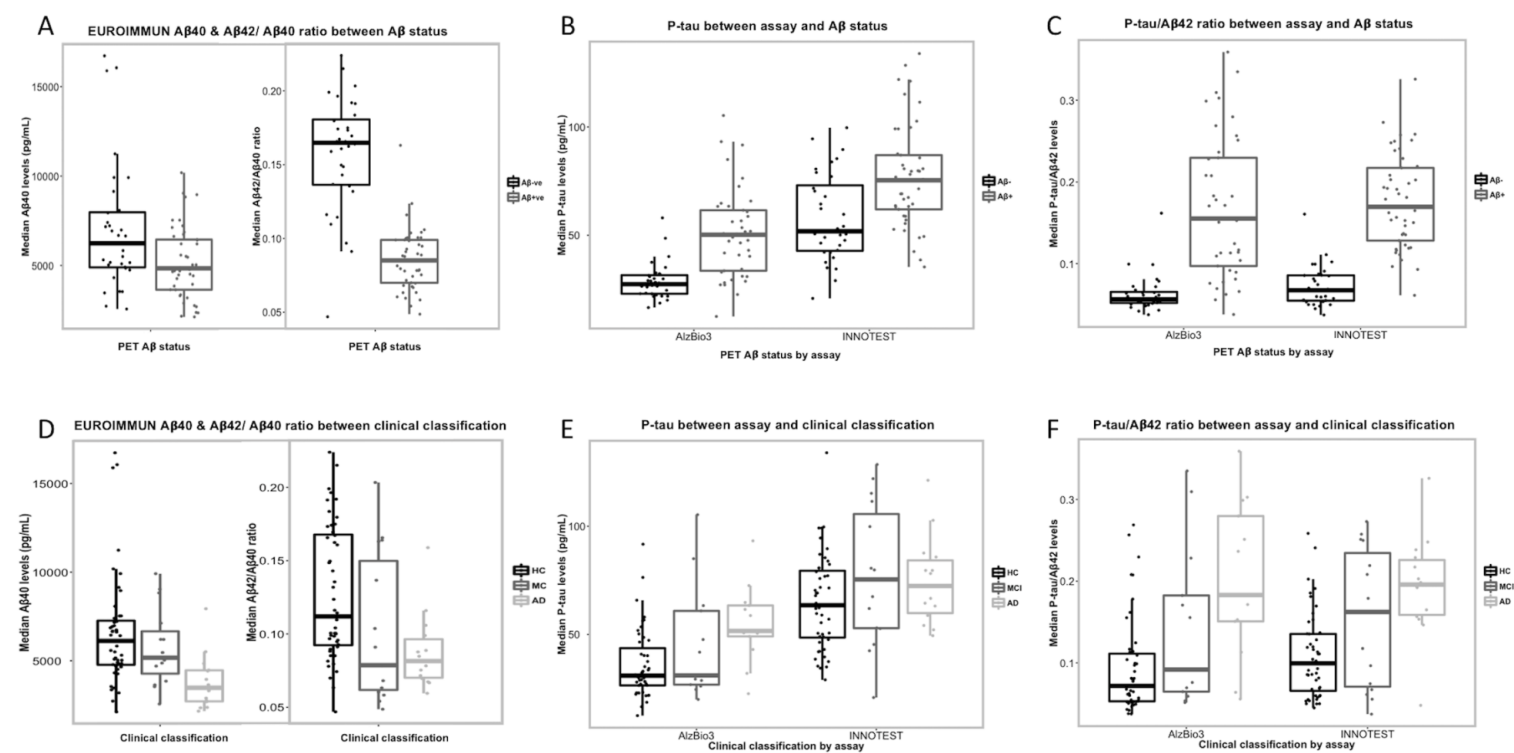
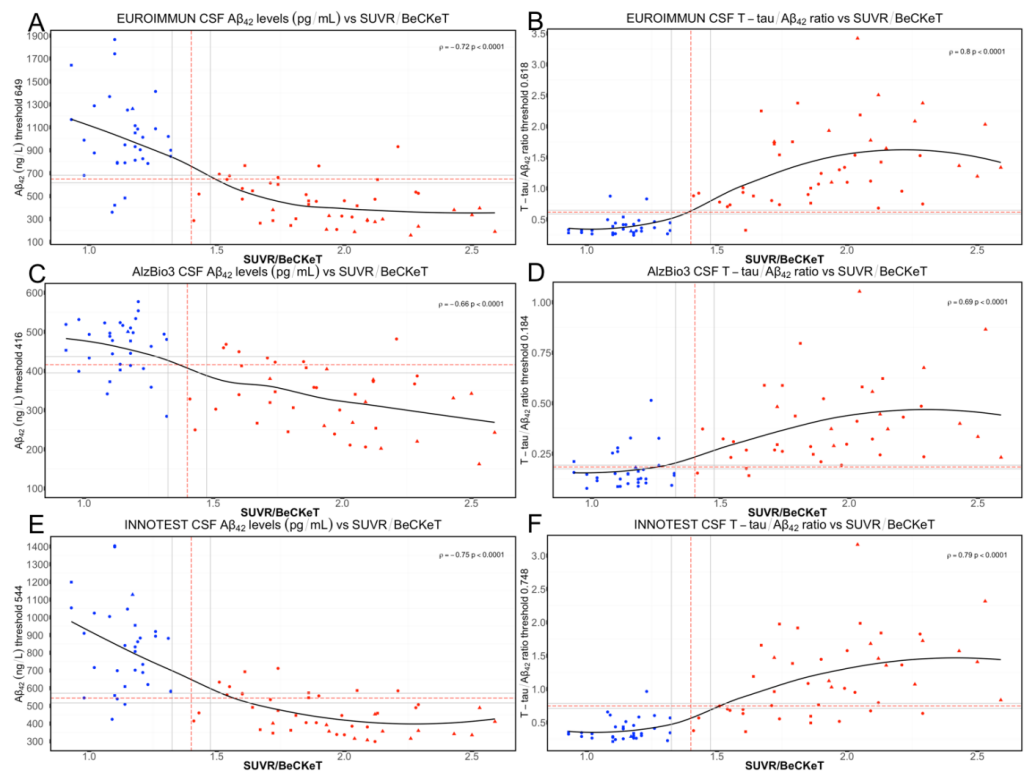


Figure 4



## Figure Legends

Figure 1: Correlation of CSF biomarkers by clinical classification across assay platforms. Coloured points represent PET A $\beta$ -amyloid status: PET A $\beta$ - = blue, PET A $\beta$ + = red. Circles represent HC, squares represent those participants with MCI, triangles represent those participants with AD. 1A: EUROIMMUN vs INNOTEST CSF A $\beta$ 42 correlation, 1B: EUROIMMUN vs AlzBio3 CSF A $\beta$ 42 correlation, 1C: INNOTEST vs AlzBio3 CSF A $\beta$ 42 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/A $\beta$ 42 ratio correlation, 1H: EUROIMMUN vs AlzBio3 CSF T-tau/A $\beta$ 42 ratio correlation, 1I: INNOTEST vs AlzBio3 CSF T-tau/A $\beta$ 42 ratio correlation.

Figure 2: Assay comparisons for those biomarkers measured by all three platforms by classification and PET A $\beta$ -amyloid status. 2A: CSF A $\beta$ 42 levels between PET A $\beta$ -amyloid status and assay, 2B: CSF T-tau levels between PET A $\beta$ -amyloid status and assay, 2C: CSF T-tau/A $\beta$ 42 ratio between PET A $\beta$ -amyloid status and assay, 2D: CSF A $\beta$ 42 levels between clinical classification and assay, 2E: CSF T-tau levels between clinical classification and assay, 2F: CSF T-tau/A $\beta$ 42 ratio between clinical classification and assay.

Figure 3: Assay comparisons for those biomarkers not measured by all three platforms by classification and PET A $\beta$ -amyloid status. 3A: EUROIMMUN CSF A $\beta$ 40 & A $\beta$ 42/A $\beta$ 40 levels between PET A $\beta$ -amyloid status, 3B: CSF P-tau levels between PET A $\beta$ -amyloid status and assay, 3C: CSF P-tau/A $\beta$ 42 ratio between PET A $\beta$ -amyloid status and assay, 3D:

EUROIMMUN CSF A $\beta$ 40 & A $\beta$ 42/A $\beta$ 40 levels between clinical classification, 3E: CSF P-tau levels between clinical classification and assay, 3F: CSF P-tau/A $\beta$ 42 ratio between clinical classification and assay.

Figure 4: CSF biomarker – BeCKeT SUVR correlation. 4A: EUROIMMUN CSF A $\beta$ 42 levels vs BeCKeT SUVR, 4B: EUROIMMUN CSF T-tau/A $\beta$ 42 ratio vs BeCKeT SUVR, 4C: AlzBio3 CSF A $\beta$ 42 levels vs BeCKeT SUVR, 4D: AlzBio3 CSF T-tau/A $\beta$ 42 ratio vs BeCKeT SUVR, 4E: INNOTEST CSF A $\beta$ 42 levels vs BeCKeT SUVR, 4F: INNOTEST CSF T-tau/A $\beta$ 42 ratio vs BeCKeT SUVR. Lines represent linear correlation between CSF biomarker and BeCKeT SUVR. Shown is the Spearman's Rho correlation, associated p-value, and estimated loess slope.

## Supplementary material

**Supplementary Table 1**

Analyte	A $\beta$ 42		
Technology	ELISA	ELISA	xMAP
Vendor	Euroimmun	Fujirebio	Fujirebio
<b>Critical raw materials</b>			
Capture antibody	21F12	21F12	4D7A3
Detector antibody	3D6	3D6	3D6
Calibrator	rec. protein	synthetic peptide	synthetic peptide
<b>Sample incubation</b>	Simultaneous with detector antibody		
Total volume ( $\mu$ L)	115	100	100
CSF ( $\mu$ L)	15	25	75
Detector antibody ( $\mu$ L)	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 A $\beta$ 42 assay details per platform.

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

Biomarker		HC	MCI	AD	HC vs MCI vs AD		HC vs AD		HC vs MCI		MCI vs AD	
					Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted
	N	48	15	14								
Aβ40	EUROI	6628.08	5742.86	3807.33	0.000000							
	MMUN	(3163.38)	(2169.66)	(1575.5)	002	0.1886	0.002	0.002	0.318	0.184	0.028	0.062
Aβ42	EUROI	803.34	569.97	351.35								
	MMUN	(364.34)	(365.11)	(273.1)	0.00009	0.00005	0.001	0.002	0.042	0.0105	0.128	0.048
	INNOT	672.89	577.66	425.73								
	EST	(255.61)	(229.67)	(208.98)	0.0020	0.0015	0.006	0.012	0.205	0.0594	0.119	0.064
	AlzBio 3	420.8	354.16	308.53								
		(93.28)	(74.9)	(94.29)	0.0002	0.0002	0.002	0.002	0.028	0.0166	0.183	0.087
T-tau	EUROI	430.16	511.97	500.95								
	MMUN	(162.1)	(252.05)	(156.6)	0.004	0.0024	0.007	0.008	0.024	0.0258	0.706	0.604
	INNOT	364.35	521.18	558.6								
	EST	(173.33)	(304.04)	(240.74)	0.004	0.0039	0.010	0.013	0.060	0.0558	0.520	0.968
	AlzBio 3	90.89	121.98	137.14								
		(45.14)	(64.45)	(58.59)	0.126	0.107	0.159	0.164	0.149	0.1418	0.884	0.914
P-tau												
	INNOT	64.81	77.1	74.51								
	EST	(21.78)	(32.42)	(20.44)	0.087	0.070	0.148	0.128	0.103	0.1486	0.792	0.904
	AlzBio 3	35.97	45.4	54.9								
		(15.94)	(26.28)	(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	EUROI MMUN	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/Aβ 42	EUROI MMUN INNOT EST AlzBio 3	0.66 (0.37) 0.61 (0.37) 0.23 (0.12)	1.18 (0.76) 1.05 (0.69) 0.38 (0.23)	1.77 (0.74) 1.46 (0.68) 0.48 (0.25)	0.000001 0.000001 0.000001 0.000001 0.000009	0.000001 0.000001 0.000001 0.000001 0.000007	0.00003 0.00003 0.00002 0.00003 0.001	0.00007 0.00003 0.00003 0.00003 0.0013	0.0003 0.0003 0.0006 0.0006 0.007	0.0021 0.0004 0.0004 0.0004 0.0053	0.055 0.121 0.121 0.121 0.279	0.094 0.200 0.200 0.200 0.406
P- tau/Aβ 42	INNOT EST AlzBio 3	0.11 (0.05) 0.09 (0.06)	0.15 (0.09) 0.14 (0.1)	0.19 (0.06) 0.2 (0.1)	0.000008 0.000008 0.000013	0.000008 0.000008 0.000008	0.00004 0.00004 0.00004	0.00005 0.00005 0.00004	0.020 0.020 0.042	0.014 0.014 0.038	0.166 0.166 0.133	0.267 0.267 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 A $\beta$ 42 biomarker and the T-tau/A $\beta$ 42 ratio**

Biomarker	Model	AUC	D	p-value
A $\beta$ 42	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/A $\beta$ 42	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 DeLong's test. Values shown include ROC AUC, DeLong's statistic D and related p-value.

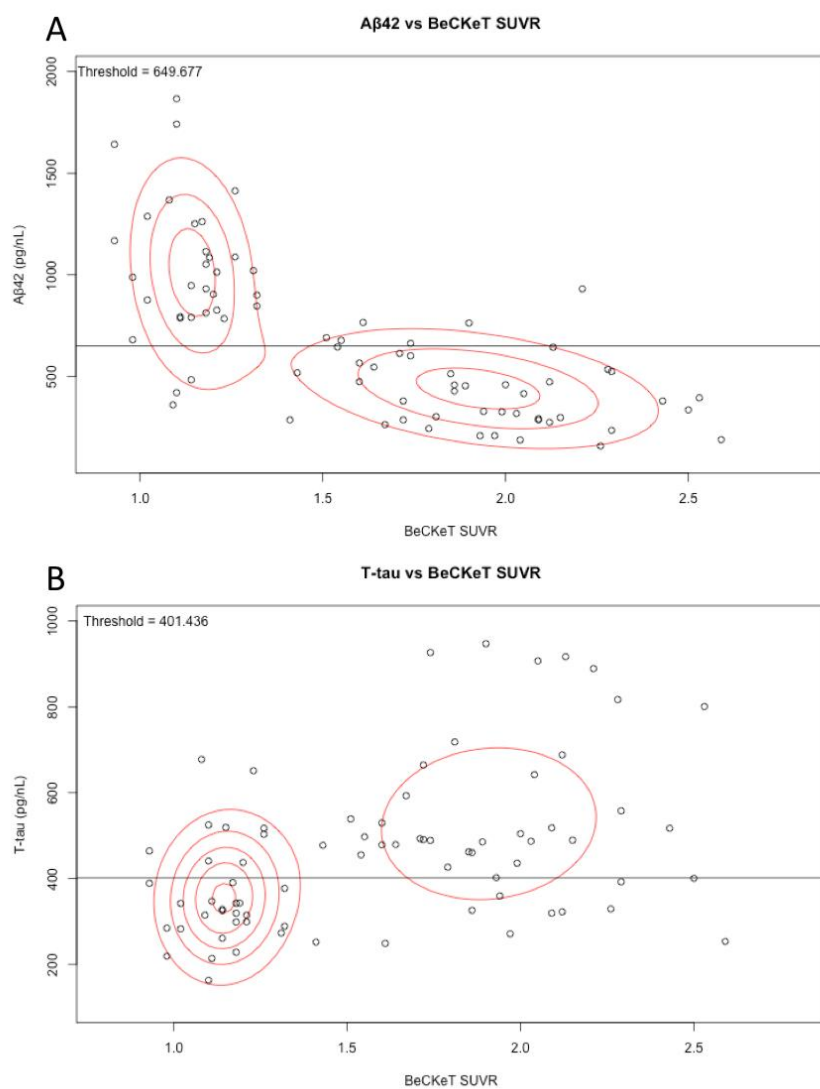
**Supplementary Table 4: Concordance of CSF biomarkers per platform with PET A $\beta$ -amyloid status by clinical classification**

Platform	Biomarker	CSF- PET A $\beta$ - (TN) HC/MCI/AD	CSF- PET A $\beta$ + (FN) HC/MCI/AD	CSF+ PET A $\beta$ - (FP) HC/MCI/AD	CSF+ PET A $\beta$ + (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	A $\beta$ 1–42	26/2/1/ (29)	5/1/0/ (6)	1/2/0/ (3)	16/10/13/ (39)
	T-tau/A $\beta$ 42 ratio	25/4/1/ (30)	0/1/0/ (1)	2/0/0/ (0)	21/10/13/ (44)
INNOTEST	A $\beta$ 42	24/4/1/ (29)	8/4/0/ (12)	3/0/0/ (3)	13/7/13/ (43)
	T-tau/A $\beta$ 42 ratio	26/4/1 (31)	11/3/0/ (14)	1/0/0/ (1)	10/8/13/ (31)
AlzBio3*	A $\beta$ 42	21/2/1/ (24)	7/0/0/ (7)	4/2/0 (6)	13/9/12 (34)
	T-tau/A $\beta$ 42 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 A $\beta$ -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.