

Pittsburgh compound B imaging and cerebrospinal fluid amyloid- β in a multicentre European memory clinic study

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The aim of this study was to assess the agreement between data on cerebral amyloidosis, derived using Pittsburgh compound B positron emission tomography and (i) multi-laboratory INNOTEST enzyme linked immunosorbent assay derived cerebrospinal fluid concentrations of amyloid- β_{42} ; (ii) centrally measured cerebrospinal fluid amyloid- β_{42} using a Meso Scale Discovery enzyme linked immunosorbent assay; and (iii) cerebrospinal fluid amyloid- β_{42} centrally measured using an antibody-independent mass spectrometry-based reference method. Moreover, we examined the hypothesis that discordance between amyloid biomarker measurements may be due to interindividual differences in total amyloid- β production, by using the ratio of amyloid- β_{42} to amyloid- β_{40} . Our study population consisted of 243 subjects from seven centres belonging to the Biomarkers for Alzheimer's and Parkinson's Disease Initiative, and included subjects with normal cognition and patients with mild cognitive impairment, Alzheimer's disease dementia, frontotemporal dementia, and vascular dementia. All had Pittsburgh compound B positron emission tomography data, cerebrospinal fluid INNOTEST amyloid- β_{42} values, and cerebrospinal fluid samples available for reanalysis. Cerebrospinal fluid samples were reanalysed (amyloid- β_{42} and amyloid- β_{40}) using Meso Scale Discovery electrochemiluminescence enzyme linked immunosorbent assay technology, and a novel, antibody-independent, mass spectrometry reference method. Pittsburgh compound B standardized uptake value ratio results were scaled using the Centiloid method. Concordance between Meso Scale Discovery/mass spectrometry reference measurement procedure findings and Pittsburgh compound B was high in subjects with mild cognitive impairment and Alzheimer's disease, while more variable results were observed for cognitively normal and non-Alzheimer's disease groups. Agreement between Pittsburgh compound B classification and Meso Scale Discovery/mass spectrometry reference measurement procedure findings was further improved when using amyloid- $\beta_{42/40}$. Agreement between Pittsburgh compound B visual ratings and Centiloids was near complete. Despite improved agreement between Pittsburgh compound B and centrally analysed cerebrospinal fluid, a minority of subjects showed discordant findings. While future studies are needed, our results suggest that amyloid biomarker results may not be interchangeable in some individuals.

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Abbreviations: AD-100 = amyloid- β -positive Alzheimer's disease subjects used as part of Centiloid standardization; FTD = frontotemporal dementia; MCI = mild cognitive impairment; MSD = Meso Scale Discovery ELISA; MS-RMP = mass spectrometry-based candidate reference measurement procedure; PiB = Pittsburgh compound B; SUV_r = standardized uptake value ratio; VaD = vascular dementia; YC-0 = amyloid- β -negative young controls used as part of Centiloid standardization

Introduction

Current thinking ascribes the pathogenesis of Alzheimer's disease to the aggregation of amyloid- β in the brain. Specifically, the accumulation of various species of aggregated amyloid- β is believed to set in motion tau pathology and neurodegeneration, leading to cognitive impairment and, ultimately, dementia (Jack *et al.*, 2013). Brain amyloid- β accumulation can be identified *in vivo* using CSF levels of the 42-amino acid form of amyloid- β (amyloid- β ₄₂), and PET with fibrillar amyloid- β specific radiotracers, such as the carbon-11 labelled thioflavin-T derivative, Pittsburgh compound B (PiB) (Blennow *et al.*, 2015).

While brain retention of amyloid tracers such as PiB is elevated in Alzheimer's disease, CSF levels of amyloid- β ₄₂ are decreased, an observation hypothesized to reflect the sequestration of brain soluble amyloid- β into insoluble plaques, with a resultant reduction in the amount of amyloid- β ₄₂ available for clearance into the CSF (Kawarabayashi *et al.*, 2001; DeMattos *et al.*, 2002). This inverse relationship has been confirmed by many groups across cognitively normal, mild cognitive impairment (MCI) and Alzheimer's disease subjects, leading to the view that these biomarkers are interchangeable in defining 'amyloid-positivity' (Fagan, 2015). Though this inverse relationship has generally been observed between both measures, a subset of cases shows discordant results, with either abnormal CSF amyloid- β ₄₂ but normal amyloid PET, or normal CSF amyloid- β ₄₂ but abnormal amyloid PET (Forsberg *et al.*, 2008; Jagust *et al.*, 2009; Degerman Gunnarsson *et al.*, 2010; Landau *et al.*, 2013; Palmqvist *et al.*, 2014; Zwan *et al.*, 2014; Mattsson *et al.*, 2015). While discordance in most cases is due to

abnormal CSF amyloid- β ₄₂ in subjects with normal amyloid PET, isolated PET positivity has been reported in both MCI and Alzheimer's disease (Koivunen *et al.*, 2008; Forsberg *et al.*, 2010; Landau *et al.*, 2013; Zwan *et al.*, 2014; Leuzy *et al.*, 2015; Mattsson *et al.*, 2015; Palmqvist *et al.*, 2016).

With the exception of a recent European multicentre study (Zwan *et al.*, 2016), however, large-scale studies addressing agreement between amyloid PET and CSF amyloid- β ₄₂ have to date been conducted using cohorts evaluated according to standardized clinical and biomarker assessment protocols (Landau *et al.*, 2013; Mattsson *et al.*, 2015; Palmqvist *et al.*, 2016). In addition, there are no studies comparing the agreement between amyloid PET and CSF amyloid- β ₄₂ concentrations obtained by the novel antibody-free mass spectrometry (MS)-based reference measurement procedure (RMP) (Leinenbach *et al.*, 2014). Moreover, there have as yet been no studies implementing the Centiloid method, a recently proposed standardization approach that aims to facilitate cross-centre comparison/combination of amyloid PET outcome data using a scaling procedure (Klunk *et al.*, 2015). The aim of the present study was thus to assess agreement between CSF amyloid- β ₄₂ and PiB PET in a mixed memory clinic sample drawn from different academic European research centres, with patients assessed according to local clinical routines and imaged using differing acquisition protocols. Given the established between-centre variability in enzyme-linked immunosorbent assay (ELISA)-derived CSF amyloid- β ₄₂ values (Wiltfang *et al.*, 2007; Bjerke *et al.*, 2010; Andreasson *et al.*, 2012; Mattsson *et al.*, 2013), we likewise aimed to determine whether concordance rates would

be affected by centralized reanalysis of CSF using Meso Scale Discovery ELISA technology (MSD) and an MS-based candidate RMP (Leinenbach *et al.*, 2014). Finally, we sought to examine the hypothesis that discordance between amyloid biomarker results may be due to interindividual differences in total amyloid- β production (Wiltfang *et al.*, 2007; Lewczuk *et al.*, 2015), by adjusting MSD and MS-RMP determined amyloid- β_{42} levels, for those of amyloid- β_{40} .

Materials and methods

Study population

Our study population consisted of 243 subjects for whom PiB PET data and CSF data and samples were available, collected across seven European academic centres belonging to the Biomarkers for Alzheimer's and Parkinson's Disease (BIOMARKAPD) initiative. Participating sites included the Hospital de Sant Pau, Barcelona, Spain ($n = 24$); Coimbra University Hospital, Coimbra, Portugal ($n = 22$); Danish Dementia Research Centre, Copenhagen, Denmark ($n = 31$); University of Lisbon, Lisbon, Portugal ($n = 23$); Karolinska Institutet, Stockholm, Sweden ($n = 32$); Turku University Hospital, Turku, Finland ($n = 87$); and the Ulm University Hospital, Ulm, Germany ($n = 24$). Patients had been referred for cognitive complaints and assessed according to standard local clinical routines. All diagnoses were made in a multidisciplinary team setting using a consensus-based approach.

Patients with Alzheimer's disease ($n = 122$) fulfilled the 1984 National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria for probable Alzheimer's disease dementia (McKhann *et al.*, 2011), with MCI ($n = 81$) diagnosed according to the Petersen criteria (Petersen *et al.*, 1999). Other diagnoses were made according to the Neary criteria for frontotemporal dementia (FTD; $n = 20$), including behavioural, semantic, and progressive non-fluent variants (Neary *et al.*, 1998), and the National Institute of Neurological Disorders and Stroke-Association Internationale pour la Recherche et l'Enseignement en Neurosciences (NINDS-AIREN) criteria for vascular dementia (VaD; $n = 7$) (Román *et al.*, 1993).

In addition, 13 with normal cognition were recruited from relatives and carers of patients. Inclusion criteria were: the absence of memory or other cognitive complaints; independence in basic and instrumental activities of daily living; and no active neurological or psychiatric disease. All subjects provided written informed consent to participate in the investigation, which was conducted according to the Declaration of Helsinki and subsequent revisions. Ethical approval was obtained from local regional ethics committees.

Apolipoprotein E genotype

Apolipoprotein E (*APOE*) genotyping was performed in a subset of subjects ($n = 106$; four cognitively normal, 44 MCI, 52 Alzheimer's disease, six FTD) via polymerase chain reaction (PCR) of genomic DNA extracted from EDTA-anticoagulated

blood. Subjects were classified as $\epsilon 4$ allele carriers or non-carriers.

Local CSF amyloid- β_{42} and tau measurements

All centres used a similar protocol for CSF collection and processing. CSF samples were collected by lumbar puncture, between 8 am and 3 pm. A total of 10 ml was drawn, and stored in polypropylene tubes. After removal of the first 0.5 ml, samples were centrifuged at 1500g (3000–4000 rpm) for 10 min at +4°C. Samples were then stored at –80°C in 1 ml portions pending biochemical analysis, without being thawed or refrozen. Amyloid- β_{42} , total tau, and phosphorylated tau were measured using commercially available sandwich ELISAs (INNOTEST, Fujirebio-Europe), according to kit inserts.

Centralized CSF reanalysis for amyloid- β

All CSF samples were those remaining from the clinical routine at participating centres. Samples were stored on dry ice and sent via express mail to the Clinical Neurochemistry Laboratory, Gothenburg University, Mölndal, Sweden. Given the low volume of CSF available for reanalysis per centre (~500 μ l), MSD was selected over the INNOTEST platform, given its lower sample volume requirement. MSD electrochemiluminescence analyses (amyloid- β_{42} and amyloid- β_{40}) were performed according to the manufacturer's protocol. For calibration and sample preparation for MS-RMP, native (unlabelled) and ^{15}N uniformly labelled amyloid- β_{40} and amyloid- β_{42} and ^{13}C uniformly labelled amyloid- β_{42} (rPeptide) were dissolved in 20% acetonitrile and 1% ammonium hydroxide (NH_4OH) to a concentration of 50 $\mu\text{g}/\text{ml}$. Aliquots were stored at –80°C. Artificial CSF was prepared as described elsewhere (Dillen *et al.*, 2011). Calibration samples for amyloid- β_{42} were prepared in human CSF as previously described (Leinenbach *et al.*, 2014). For amyloid- β_{40} , artificial CSF was spiked to a final concentration of 1.5, 5, 10, 20, 30 and 40 ng/ml native amyloid- β_{40} and a constant concentration of ^{15}N -amyloid- β_{40} at 1600 pg/ml as internal standard. Unknown samples (180 μ l) were spiked with 20 μ l internal standard to a final concentration of 1600 pg/ml ^{13}C -amyloid- β_{42} and ^{15}N -amyloid- β_{40} . Solid phase extraction (SPE), liquid chromatography, MS analysis and data processing were conducted as previously described (Leinenbach *et al.*, 2014).

Determination of CSF amyloid- β cut-offs

Local amyloid- β_{42} values (pooled) were classified as positive (abnormal) or negative (normal) using an optimized cut-off of 557 pg/ml, established in a recent study using a large cohort of BIOMARKAPD subjects (Zwan *et al.*, 2016). Unbiased cut-offs for MSD- and MS-RMP-derived amyloid- β_{42} and amyloid- $\beta_{42/40}$ ratios were determined by mixture modelling (Benaglia *et al.*, 2009), implemented in R (v.3.2.3, R Foundation for Statistical Computing, Vienna, Austria, 2015).

Pittsburgh compound B-PET imaging

PiB was synthesized using a previously described method at the individual centres according to good manufacturing practice requirements (Mathis *et al.*, 2003; Klunk *et al.*, 2004). PiB-PET acquisition protocols varied across sites, with late summation images created according to local clinical practice: Barcelona, four 5-min frames, 50- to 70-min post-injection (50- to 70-min summation); Coimbra, Lisbon, Stockholm, 60-min dynamic acquisition (40- to 60-min summation); Copenhagen, 30-min dynamic acquisition, 40-min post-injection (40- to 70-min summation); Turku, 30-min dynamic acquisition 60 min post-injection (60- to 90-min summation), and Ulm, four 5-min frames, 40- to 60-min post-injection (40- to 60-min summation). PiB summation images were rated locally by nuclear medicine physicians as either positive (binding in more than one cortical region; abnormal) or negative (predominantly white matter binding; normal), blinded to CSF results.

Pittsburgh compound B-PET image analysis

PiB summation images (40–60, 40–70, 50–70, and 60–90 min) were first non-linearly spatially normalized to a population-based PiB template (Nordberg *et al.*, 2013), using the normalize function in SPM8 (Functional Imaging Laboratory, Wellcome Department of Imaging Neuroscience, UCL, London, UK). Spatially normalized images were then resampled using a 23-region grey matter atlas, created in parallel to the PiB template (Nordberg *et al.*, 2013). Standardized uptake value ratio (SUVr) images were then calculated by normalizing PiB uptake within frontal, temporal, parietal, occipital, parahippocampal, anterior and posterior cingulate regions to mean cerebellar grey matter uptake.

Owing to between-centre variability in PiB scanning windows, SUVr data were standardized using a recent method that allows for linear scaling of amyloid PET outcome data to a 100-point scale (Klunk *et al.*, 2015), the units of which have been termed ‘Centiloids’. After downloading de-identified PiB and MRI data from the Global Alzheimer’s Association Information Network website (GAAIN; <http://www.gaain.org>) for 34 amyloid-negative young controls (YC-0) and 45 amyloid-positive Alzheimer’s disease subjects (AD-100), we confirmed the validity of our analysis pipeline via replication of the Level 1 analysis (linear correlation between downloaded/recalculated PiB Centiloid values: slope of 0.99, intercept of 1.03, and R^2 of 0.99; Fig. 1) (Klunk *et al.*, 2015). As a result of having scanning intervals up to 90 min, we were restricted to the subset of subjects with PET data over this time interval (0–90 min; 16 YC-0 and 40 AD-100) in the Level 2 calibration of our non-standard approach. Following exclusion of scans where there had been difficulty with subject positioning in the scan field, the final sample used to derive slope and intercept parameters for calculation of ‘PiB calculated’ SUVr values ($^{PiB-Calc}SUVr$), were 11 for YC-0 and 35 for AD-100. After comparing SUVr values obtained using both standard (50–70 min, global cortical target region and whole cerebellum reference volumes of interest) and non-standard approaches via linear regression ($^{PiB-UNIT}_{IND}$: R^2 between 0.96 and 0.97), $^{PiB-Calc}SUVr$ data for each of the four time windows

was converted into Centiloid values ($^{PiB}Centiloid_{std}$ YC-0: SD between 6.62 and 25.4, relative variance between 0.26 and 5.17; $^{PiB}Centiloid_{std}$ AD-100: SD between 21.31 and 22.95, relative variance between 1 and 1.08), using the mean SUVr of the 34 YC-0 and 45 AD-100 subjects.

To set a threshold for PiB positivity using Centiloids, we adopted a composite cortical SUVr cut-off of 1.41, representing the upper 95% confidence limit from a previously characterized population of normally distributed healthy controls for whom PiB data have been acquired 40–60 min after injection (Nordberg *et al.*, 2013). Using 40–60-min slope and intercept parameters, this cut-off was converted to Centiloid units, giving a cut-off of 34. This value was then used to subdivide subjects into PiB+ (>34) and PiB- (≤ 34).

Statistical analysis

Statistical analyses were performed with R, v.3.2.3. Between-group comparisons were done using Kruskal-Wallis ANOVA for continuous values and chi-square (sex, education) or Fisher’s exact tests (*APOE*) for categorical values. *Post hoc* Mann Whitney U-tests were performed where appropriate. These analyses were corrected for multiple comparisons using false discovery rate (FDR). The ratio of amyloid- β_{42} to amyloid- β_{40} (MSD and MS-RMP) was calculated according to a previously published formula: $[(\text{amyloid-}\beta_{42}) / (\text{amyloid-}\beta_{40}) \times 10]$ (Hansson *et al.*, 2007). Linear regression analyses were performed to assess the relationship between values from the different CSF analytical platforms, as well as between published Centiloid values and those calculated at our site.

Concordance between PiB PET (visual, Centiloid) and CSF (amyloid- β_{42} , amyloid- $\beta_{42/40}$) was defined as the proportion of subjects positive or negative for both biomarkers (i.e. concordant positive, PiB+/CSF+, or concordant negative, PiB-/CSF-). Discordance between PiB and CSF was defined as the proportion of individuals with only one abnormal biomarker (i.e. discordant with PiB positivity, PiB+/CSF-, or

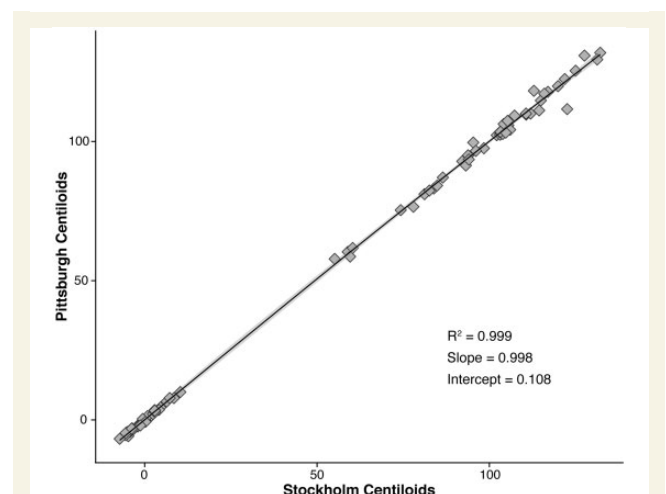


Figure 1 Linear correlation plot showing the relationship between original/recalculated PiB Centiloids. Original (Pittsburgh) and recalculated (Stockholm) PiB Centiloids are shown on the ordinate and abscissa, respectively (YC-0, $n = 34$; AD-100, $n = 45$).

discordant with CSF positivity, PiB–/CSF+). Agreement between visual and Centiloid based classification was assessed using percentage agreement and Fleiss *k*.

Results

Participant characteristics and CSF mixture modelling cut-offs

Subject characteristics according to diagnostic group are presented in Table 1. Groups did not differ in terms of age, sex, education, or in the period between CSF and PiB PET assessments. As expected, MMSE scores were lower in patient groups, relative to cognitively normal subjects (MCI, Alzheimer's disease, FTD, $P < 0.001$; VaD, $P = 0.01$), and differed between patient groups (MCI > Alzheimer's disease and FTD, $P < 0.001$). No significant differences in the prevalence of the APOE $\epsilon 4$ allele were found between groups. Levels of total tau were higher in Alzheimer's disease with respect to cognitively normal subjects ($P < 0.001$), FTD and VaD ($P < 0.01$). Compared to cognitively normal subjects, phosphorylated tau levels were higher in MCI and Alzheimer's disease ($P < 0.01$ and 0.001 , respectively) and, relative to MCI, higher in Alzheimer's disease ($P < 0.001$). Phosphorylated tau was also found to be higher in Alzheimer's disease, compared to FTD and VaD ($P < 0.001$). The unbiased cut points to identify an abnormal concentration of amyloid- β in CSF were <515 pg/ml and 0.72 (MSD amyloid- β_{42} and amyloid- $\beta_{42/40}$, respectively) and <896 pg/ml and 0.76 (MS-RMP amyloid- β_{42} and amyloid- $\beta_{42/40}$, respectively).

Pittsburgh compound B PET findings

The proportion of PiB scans visually rated as positive was greatest in Alzheimer's disease (93%), followed by MCI (62%), FTD (15%), cognitively normal (8%), and VaD (0%). Global Centiloid was higher in patient groups in comparison to cognitively normal (MCI, Alzheimer's disease, $P < 0.001$), in Alzheimer's disease, FTD, and VaD, in comparison to MCI ($P < 0.001$, 0.001 , 0.01 , respectively) and in Alzheimer's disease, relative to FTD and VaD ($P < 0.001$). No difference was found between FTD and VaD.

CSF amyloid- β findings

Locally measured INNOTEST and reanalysed CSF levels (Table 2) were lower in patients, relative to controls ($P < 0.05$), with levels in Alzheimer's disease lower than those in MCI ($P < 0.001$). Using MSD and MS-RMP, amyloid- β_{42} concentrations were lower in MCI ($P < 0.05$) and Alzheimer's disease ($P < 0.001$), with respect to cognitively normal subjects. Further, amyloid- β_{42} values were lower in Alzheimer's disease CSF, compared to MCI ($P < 0.001$), FTD, and VaD ($P < 0.01$). Findings using the ratio of amyloid- β_{42} to amyloid- β_{40} were in agreement with those for amyloid- β_{42} : cognitively normal versus MCI ($P < 0.001$), MCI versus Alzheimer's disease ($P < 0.001$), Alzheimer's disease versus FTD ($P < 0.001$) and VaD ($P < 0.01$). No between-group differences were found for amyloid- β_{40} .

Relative to INNOTEST, amyloid- β_{42} concentrations were lower using MSD, and higher using MS-RMP; this pattern

Table 1 Demographic, clinical and biomarker characteristics according to diagnostic group

	CN (n = 13)	MCI (n = 81)	AD (n = 122)	FTD (n = 20)	VaD (n = 7)
Age, years	67 (69, 60)	64 (70, 58)	65 (72, 59)	64 (70, 60)	61 (74, 57)
Sex, M: F (% F)	6: 7 (54%)	37: 44 (54%)	50: 72 (59%)	9: 11 (55%)	3: 4 (57%)
Education, 1–4	3 (4, 2.8)	3 (4, 2)	3 (3, 2)	2 (3, 2)	2 (3, 1.5)
MMSE, points	29 (30, 28)	27 (28, 26)	23 (26, 20)	23 (27, 20)	26 (27.5, 22.5)
APOE $\epsilon 4$, ≥ 1 allele [†]	4 (0%)	25 (57%)	29 (56%)	2 (33%)	N/A
INNOTEST A β_{42} , pg/ml	843 (900, 732)	535 (698, 409)	413 (530, 308)	641 (726, 433)	491 (675.5, 386.5)
INNOTEST A β_{42} positive	1 (8%)	46 (57%)	96 (79%)	10 (50%)	5 (71%)
INNOTEST T-tau, pg/ml ^a	252 (318, 204)	313 (520, 210)	488 (772, 326)	307 (409, 193)	261 (306, 219)
INNOTEST P-tau, pg/ml ^b	43 (58, 33)	59 (77, 43)	74 (107, 57)	43 (64, 31)	41 (48, 28)
CSF-PiB, months	2.63 (7.05, 1.55)	3.93 (8.30, 1.77)	2.43 (5.14, 0.74)	2.03 (3.87, 1.09)	3.47 (5.20, 2.93)
PiB positive (Visual)	1 (8%)	50 (62%)	114 (93%)	3 (15%)	0 (0%)
PiB, SUVR	1.26 (1.32, 1.21)	1.54 (1.87, 1.24)	1.83 (2.05, 1.67)	1.18 (1.25, 1.09)	1.25 (1.27, 1.11)
PiB, Centiloid	15.1 (20.8, 8.3)	47.5 (87.6, 10.9)	84.1 (110.8, 63.1)	4.6 (13.8, –5.9)	12.8 (14.7, –3.9)
PiB positive (Centiloid)	1 (8%)	47 (58%)	112 (92%)	3 (15%)	0 (0%)

Values are reported as median (quartile3, quartile 1), or as *n* (%). PiB SUVR and Centiloid refer to global cortical composite.

A β_{42} = amyloid- β_{42} ; AD = Alzheimer's disease; APOE $\epsilon 4 = \epsilon 4$ allele of apolipoprotein E; CN = cognitively normal older individuals; MMSE = Mini-Mental State Examination; N/A = not applicable; P-tau = tau hyperphosphorylated at threonine 181; T-tau = total tau.

^aOwing to between country differences, a 4-point scale was used for educational level: 1 = basic schooling, 2 = professional training, 3 = college education, 4 = university degree.

^bData missing for nine cognitively normal, 37 MCI, 70 Alzheimer's disease, 14 FTD, and seven VaD subjects.

[†]Data missing for three MCI, three Alzheimer's disease, one FTD, and one VaD subject.

[‡]Data missing for eight MCI, 16 Alzheimer's disease, and three FTD subjects.

Table 2 Results for INNOTEST and reanalysed CSF according to diagnostic group

	CN (n = 13)	MCI (n = 81)	AD (n = 122)	FTD (n = 20)	VaD (n = 7)
INNOTEST A β_{42} , pg/ml	843 (900, 732)	535 (698, 409)	413 (530, 309)	641 (726, 433)	491 (675.5, 386.5)
A β_{42} positive	1 (8%)	46 (57%)	96 (79%)	10 (50%)	5 (71%)
MSD A β_{40} , pg/ml	5363 (7369, 4425)	5607 (7188, 4536)	5476 (6556, 4333)	5213 (6149, 4225)	5187 (6180, 4499)
A β_{42} , pg/ml	524 (719, 428)	352 (510, 249)	258 (374, 193)	448 (556, 300)	529 (630, 369)
A β_{42} positive	6 (46%)	60 (74%)	115 (94%)	12 (60%)	3 (43%)
A β_{42} /A β_{40}	0.97 (1.17, 0.85)	0.60 (0.89, 0.48)	0.51 (0.57, 0.42)	0.93 (1.05, 0.78)	1.03 (1.05, 0.79)
A β_{42} /A β_{40} positive	3 (23%)	52 (64%)	113 (93%)	7 (35%)	2 (29%)
MS-RMP A β_{40} , pg/ml	9305 (12647, 7301)	8619 (11493, 6324)	8160 (10903, 6353)	7788 (9715, 6590)	7510 (9982, 5762)
A β_{42} , pg/ml	956 (1286, 654)	568 (863, 396)	441 (617, 318)	704 (968, 484)	760 (1060, 498)
A β_{42} positive	6 (46%)	63 (78%)	115 (94%)	14 (70%)	5 (71%)
A β_{42} /A β_{40}	0.96 (1.01, 0.76)	0.61 (0.92, 0.47)	0.5 (0.6, 0.38)	0.91 (1.06, 0.38)	1.02 (1.10, 0.81)
A β_{42} /A β_{40} positive	3 (23%)	58 (72%)	117 (96%)	10 (50%)	2 (29%)

Values are reported as median (quartile3, quartile 1), or as n (%).

A β = amyloid- β ; AD = Alzheimer's disease; CN = cognitively normal older individuals; MMSE = Mini-Mental State Examination.

Cut-offs used to determine positivity were as follows: INNOTEST amyloid- β_{42} , <557 pg/ml; MSD amyloid- β_{42} , <515 pg/ml; MSD amyloid- $\beta_{42/40}$, <0.72; MS amyloid- β_{42} , <896 pg/ml; MS amyloid- $\beta_{42/40}$, <0.76.

held across all groups, save for VaD subjects, where the inverse was found. For all subjects, however, MS-RMP amyloid- β_{40} levels were higher than those for MSD. Only minor differences were seen between platforms when using the ratio of amyloid- β_{42} to amyloid- β_{40} . The correlation between local and centralized CSF measurements was moderate (INNOTEST amyloid- β_{42} and amyloid- β_{42} from MSD and MS-RMP: Spearman's $\rho = 0.74$, $P < 0.001$; Spearman's $\rho = 0.74$, $P < 0.001$, Fig. 2A and B, respectively), but high between the new, centralized measurements (MSD and MS-RMP): (amyloid- β_{42} , Spearman's $\rho = 0.93$, $P < 0.001$, Fig. 2C; amyloid- $\beta_{42/40}$, Spearman's $\rho = 0.91$, $P < 0.001$, Fig. 2D).

Agreement between visual and Centiloid-based Pittsburgh compound B classification

Across groups, agreement between visual and Centiloid based PiB ratings was 97% (235 of 243; Cohen $k = 0.92$). Of the eight instances of disagreement, six (75%) were rated as visually positive, Centiloid negative (four MCI, two Alzheimer's disease), and two as visually negative, Centiloid positive (one cognitively normal, one MCI). While discordance rates in FTD and VaD between visual assessment and Centiloid did not differ, rates in MCI were consistently higher when using Centiloid, with mixed findings in cognitively normal and Alzheimer's disease subjects (Table 3).

Concordance between PiB Centiloid and CSF amyloid- β

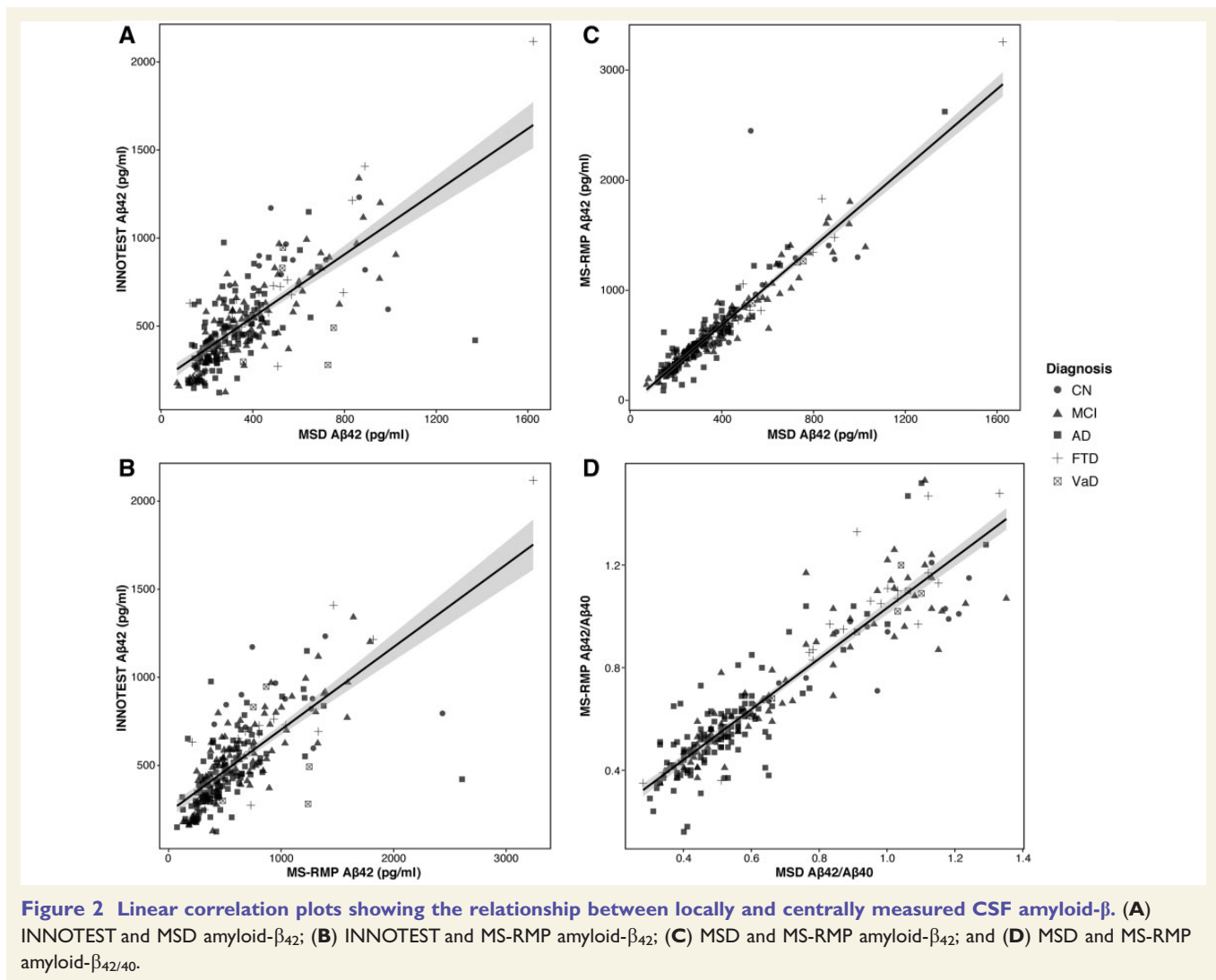
Using the total sample set, the concordance between PiB and CSF amyloid- β_{42} concentrations measured by INNOTEST,

MSD and MS-RMP were 73%, 77% and 76%, respectively (Figs 3, 4A and B). Using the ratio of amyloid- β_{42} to amyloid- β_{40} further improved the concordance (MSD, 90%; MS-RMP 88%, see Fig. 4C and D).

Using reanalysed amyloid- β_{42} , concordance was highest in Alzheimer's disease and MCI, with findings for cognitively normal, FTD, and VaD varying between MSD and MS-RMP techniques. In VaD subjects, discordance was the same as INNOTEST using MS-RMP, but lower using MSD; while across FTD, MCI, and cognitively normal subjects, discordance was higher using MSD and MS-RMP. Across all groups, however, the ratio of amyloid- β_{42} to amyloid- β_{40} was better than when using amyloid- β_{42} alone, with the greatest change seen in the VaD group. When looking at discordance with local amyloid- β_{42} , PET was overall more often abnormal than CSF, with isolated CSF positivity predominant when using the reanalysed data (Fig. 5).

Comparison of concordant and discordant subjects

Comparison of subjects showing concordant and discordant amyloid biomarker results are shown in Supplementary Tables 1–5. Using INNOTEST amyloid- β_{42} , total and phosphorylated tau were found to be higher in concordant positive subjects, relative to those concordant negative and those discordant with abnormal CSF. Tau levels were likewise found to be higher in the discordant PET positive group, relative to both discordant with isolated abnormal CSF and discordant negative subgroups (Supplementary Table 1). When using reanalysed CSF values, the prevalence of the APOE $\epsilon 4$ allele was found to be higher in those concordant positive, relative to those concordant negative. Age differences were noted between groups defined using MSD and MS-RMP amyloid- β_{42} , though findings were not



consistent. Similarly, a greater percentage of females was found in the concordant positive group for MSD amyloid- $\beta_{42/40}$, relative to PiB-/CSF-, though for MS-RMP amyloid- β_{42} , a greater percentage of females was noted in the PiB-/CSF+ group, relative to those concordant positive. Mini-Mental State Examination scores were lower in those with concordant positive findings, relative to those concordant negative; findings, however, varied across platforms in comparison to the other biomarker pairings. With respect to the interval between CSF and PET, only for MS-RMP amyloid- β_{42} was a difference found (PiB+/CSF- > PiB+/CSF+ and PiB-/CSF+, $P < 0.05$). Supplementary Fig. 1 shows the relationship between concordance (INNOTEST amyloid- β_{42} and PiB Centiloids) and CSF tau findings.

Discussion

Here, we report concordance levels between PiB PET and CSF amyloid- β (amyloid- β_{42} and amyloid- $\beta_{42/40}$) using

subjects from the European BIOMARKAPD initiative. Despite good agreement between these measures, discordance was observed in a subset of patients across all groups, using both local and centrally analysed measurements. Given the well-described intra- and intercentre variability in CSF amyloid- β_{42} concentrations using INNOTEST ELISA (Mattsson *et al.*, 2011), it was expected that concordance with PiB would be increased using reanalysed measurements. Comparison of discordance using local and reanalysed CSF, however, yielded variable results, with improved agreement with PiB classification seen only in the Alzheimer's disease patients using reanalysed amyloid- β_{42} —and VaD, when using MSD amyloid- β_{42} —and across patient groups when using amyloid- $\beta_{42/40}$. This pattern may suggest that the reanalysed results are a more faithful approximation of amyloid- β_{42} levels across groups, the centralized analysis component having removed the variance imposed by differences in INNOTEST measurements between laboratories. While it is thus tempting to speculate that MSD and MS-RMP providing more accurate estimates of amyloid- β_{42} concentration levels, this

Table 3 Discordance findings between PiB and CSF across the various platforms investigated and using both visual and Centiloid

	CN (n = 13)		MCI (n = 81)		AD (n = 122)		FTD (n = 20)		VaD (n = 7)	
	Visual	Centiloid	Visual	Centiloid	Visual	Centiloid	Visual	Centiloid	Visual	Centiloid
INNOTEST A β 42, PiB	2 (15%)	3 (23%)	18 (22%)	20 (25%)	29 (24%)	30 (25%)	9 (45%)	9 (45%)	5 (71%)	5 (71%)
PiB + /CSF–	1	2	11	10	23	23	2	2	0	0
PiB–/CSF +	1	1	7	10	6	7	7	7	5	5
MSD A β 42, PiB	7 (54%)	6 (46%)	18 (22%)	21 (26%)	12 (10%)	13 (11%)	9 (45%)	9 (45%)	3 (43%)	3 (43%)
PiB + /CSF–	1	1	4	4	5	5	0	0	0	0
PiB–/CSF +	6	5	14	17	7	8	9	9	3	3
A β 42/A β 40, PiB	2 (15%)	3 (23%)	6 (7%)	12 (15%)	6 (5%)	6 (5%)	4 (20%)	4 (20%)	2 (29%)	2 (29%)
PiB + /CSF–	0	1	2	3	3	3	0	0	0	0
PiB–/CSF +	2	2	4	9	3	3	4	4	2	2
MS-RMP A β 42, PiB	7 (54%)	6 (46%)	21 (26%)	25 (31%)	12 (10%)	13 (11%)	11 (55%)	11 (55%)	5 (71%)	5 (71%)
PiB + /CSF–	1	1	4	4	5	5	0	0	0	0
PiB–/CSF +	6	5	17	21	7	8	11	11	5	5
A β 42/A β 40, PiB	3 (23%)	4 (31%)	10 (12%)	14 (17%)	8 (7%)	9 (7%)	7 (35%)	7 (35%)	2 (29%)	2 (29%)
PiB + /CSF–	0	1	1	1	2	2	0	0	0	0
PiB–/CSF +	3	3	9	13	6	7	7	7	2	2

Values are reported as n or n (%).

AD = Alzheimer's dementia; A β 42 = amyloid- β ₄₂; A β 42/40 = amyloid- β _{42/40}; CN = cognitively normal older individuals; MMSE = Mini-Mental State Examination.

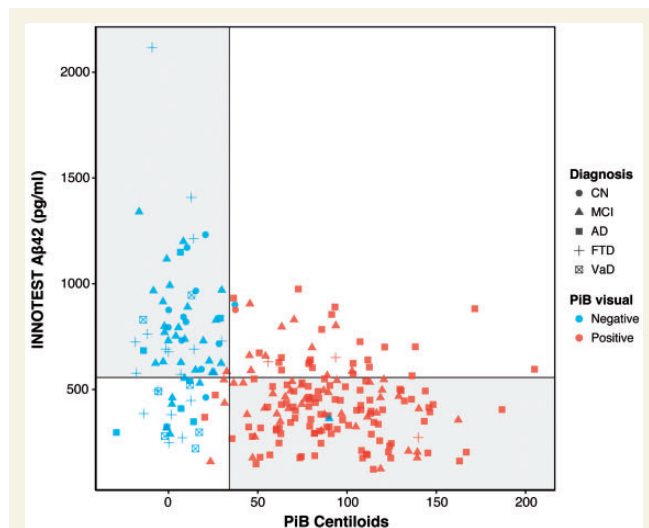


Figure 3 Scatterplot showing concordance between INNOTEST amyloid- β ₄₂ and PiB Centiloids. Circles indicate cognitively normal subjects, triangles MCI, squares Alzheimer's disease, crosses FTD, and crossed squares VaD. The vertical line reflects the Centiloid cut-off of 34; the horizontal line the cut-off of 557 pg/ml for INNOTEST amyloid- β ₄₂. Blue indicates PiB scans were visually rated as negative, red as positive. The grey quadrants indicate concordance between amyloid- β biomarkers (*top left*, concordant negative: PiB–/CSF–; *bottom right*, concordant positive: PiB + /CSF +). The white quadrants indicate discordance between amyloid- β biomarkers (*bottom left*, discordant with isolated CSF positivity: PiB–/CSF +; *top right*, discordant with isolated PiB positivity: PiB + /CSF–).

explanation seems unlikely since these analytical techniques have been shown to correlate tightly—both with one another, and with INNOTEST—when samples are analysed in a single run under standardized conditions (Bjerke *et al.*, 2016).

As expected, concordance rates matched closely across groups using MSD and MS-RMP. Relative to MSD, MS-RMP classified an additional four patients (one in both the MCI and FTD groups, two in VaD group) as CSF positive using amyloid- β ₄₂, and one cognitively normal subject using amyloid- β _{42/40}. Concordance findings using the ratio of amyloid- β ₄₂ to amyloid- β ₄₀—a measure which adjusts for 'high' and 'low' amyloid- β production levels, and thus for false negative (just above the cut-off) and false positive amyloid- β ₄₂ results, respectively (Wiltfang *et al.*, 2007; Lewczuk *et al.*, 2015)—were, as expected, greater than when using amyloid- β ₄₂ alone. This effect was greatest in the FTD and VaD groups. Though based on a relatively small sample size, this finding ties into recent work showing that subcortical changes non-specific to Alzheimer's disease may alter global levels of amyloid- β isoforms (Janelidze *et al.*, 2016; van Westen *et al.*, 2016). This raises the possibility that the amyloid- β _{42/40} ratio may allow for differentiating conditions in which amyloid- β ₄₀ and amyloid- β ₄₂ can be reduced in parallel, from true amyloid-positive cases. Future studies, however, will be needed to better understand the relationship between Alzheimer's disease pathology and vascular changes, as well as matrix effects on amyloid- β ₄₂ measurements.

PiB-PET scans in the present study ranged from 40- to 90-min post-injection, with 20- or 30-min time windows used to normalize global cortical uptake to that within

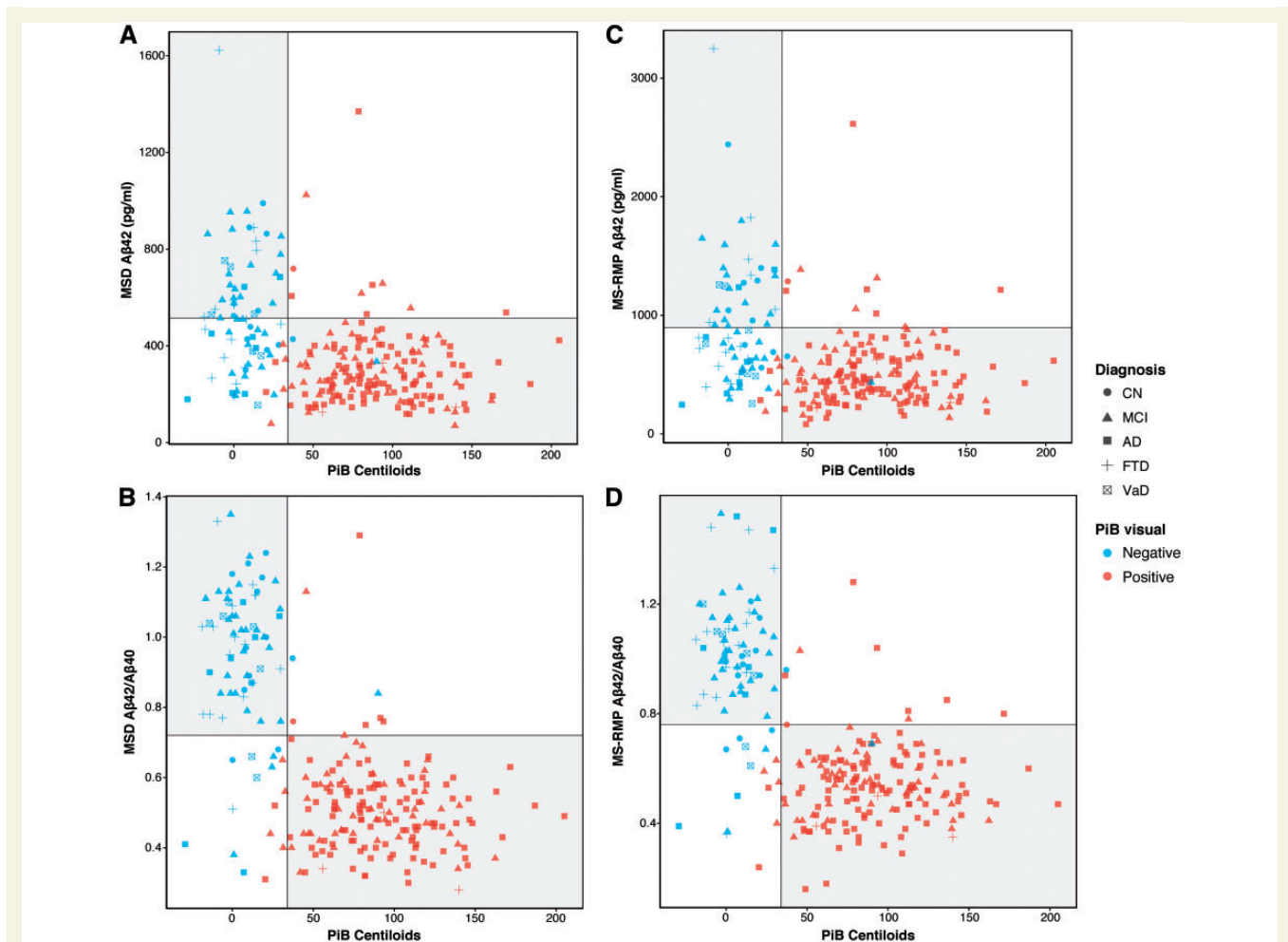


Figure 4 Scatterplots reflecting concordance between PiB Centiloids and reanalysed CSF. (A) MSD amyloid- β_{42} (cut-off < 515 pg/ml). (B) MSD amyloid- $\beta_{42/40}$ (cut-off < 0.72); (C) MS-RMP amyloid- β_{42} (cut-off < 896 pg/ml), and (D) MS-RMP amyloid- β_{42} /amyloid- β_{40} (cut-off < 0.76). Grey circles indicate cognitively normal healthy control subjects, triangles indicate MCI, squares indicate Alzheimer's disease, crosses FTD, and crossed squares VaD. The vertical lines reflect the Centiloid cut-off of 34; the horizontal lines reflect the cut-offs of < 515 pg/ml, < 0.72, < 896 pg/ml, and < 0.76 for MSD (amyloid- β_{42} , amyloid- $\beta_{42/40}$) and MS-RMP (amyloid- β_{42} , amyloid- $\beta_{42/40}$), respectively. Blue indicates PiB scans visually rated as negative, red as positive. The grey quadrants indicate concordance between amyloid- β biomarkers (top left, concordant negative: PiB-/CSF-; bottom right, concordant positive: PiB+/CSF+). The white quadrants indicate discordance between amyloid- β biomarkers (bottom left, discordant with isolated CSF positivity: PiB-/CSF+; top right, discordant with isolated PiB positivity: PiB+/CSF-). A β = amyloid- β .

the cerebellar grey matter. Given that the range of PiB SUVr values using this reference tissue have been shown to vary dynamically over time (Lopresti *et al.*, 2005; McNamee *et al.*, 2009), the Centiloid approach was implemented to ensure appropriate comparability of imaging results. In line with previous studies using visual and quantitative-based reads (Rabinovici *et al.*, 2011; Mountz *et al.*, 2015), high agreement was observed between both classification methods, with all cases rated as visually positive, Centiloid negative, exhibiting values close to the cut-off. Though few in number, these cases highlight that subjects with borderline positive values can prove visually challenging and contribute to discrepant interpretations across assessment approaches. In the two subjects classified as amyloid-positive using Centiloids and negative using visual assessment, the pattern of cortical uptake was

unclear owing to atrophy, high white matter signal, and poor image quality. Though the clinical use of amyloid-PET in routine clinical practice will likely depend on visual assessment alone, our findings suggest that Centiloid ratings can be used independently, or as a valuable adjunct to visual reads in multicentre studies.

The global composite PiB SUVr cut-off of 1.41 was selected for use in the present study owing to it having been previously established using our processing pipeline, and due to the fact that we were restricted to the use of a PiB-PET template owing to limited availability of structural imaging in our study population. Though somewhat more conservative than the only other Centiloid cut-off of 27.78 available in the literature (Ayakta *et al.*, 2016), our higher cut-off reflects differences in populations, method for cut-off selection, and processing pipelines, including

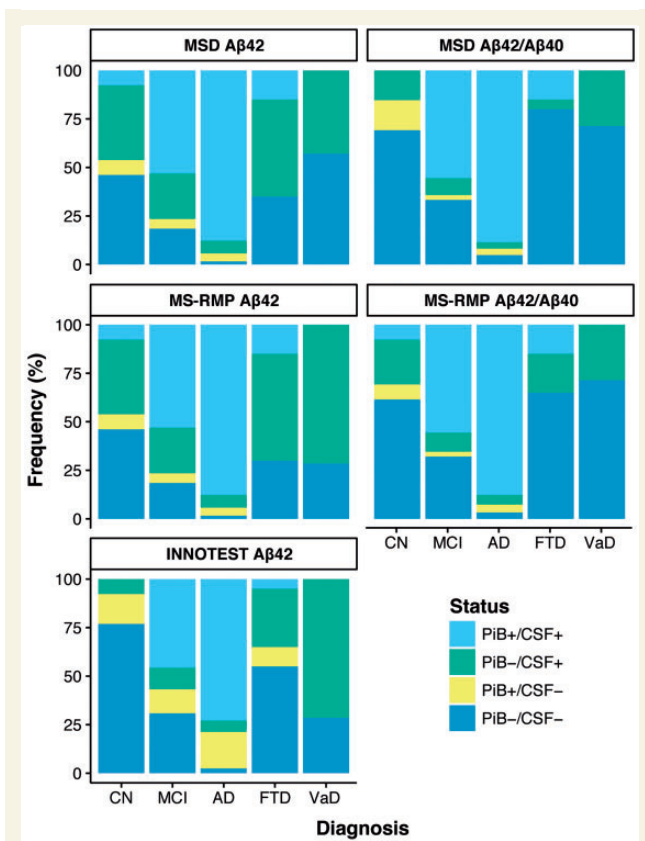


Figure 5 Frequency plots showing different agreement profiles between PiB PET and CSF. Values of <557 pg/ml (INNOTEST amyloid- β_{42}), <515 pg/ml (MSD amyloid- β_{42}), <0.72 (MSD amyloid- $\beta_{42/40}$), <896 pg/ml (MS-RMP amyloid- β_{42}), <0.76 (MS-RMP amyloid- $\beta_{42/40}$), and global Centiloid value >34 were used to classify subjects as concordant positive (PiB+/CSF+), concordant negative (PiB-/CSF-), discordant with CSF positivity (PiB-/CSF+), and discordant with PiB positivity (PiB+/CSF-). A β = amyloid- β .

the choice of scanning window and volumes of interest. For instance, our approach used slope and intercept parameters from a 40–60 min post-injection interval to determine Centiloid equivalence to our SUVr cut-off (in contrast to 50–70 min), itself established using the 95th percentile approach in healthy older subjects [versus receiver operating characteristic (ROC) curve in a mixed patient population, with CERAD score as standard of truth]. Indeed, back calculation of the 27.78 Centiloid cut-off to SUVr using our pipeline yielded an SUVr close to ours (1.38), highlighting the effects of varying analysis methods, the resulting limited comparability of SUVr data across centres, and the attendant importance of Centiloid standardization.

Classification mismatch using PiB and amyloid- β_{42} may be due a number of factors. Most mismatched subjects in our study had isolated low CSF amyloid- β . In some individuals, this was due PiB retention being only somewhat elevated, and thus falling below the cut point for positivity. In addition, since PiB binds only weakly to amorphous amyloid- β plaques (Bacskaï *et al.*, 2007; Ikonovic

et al., 2008), PiB prove unable to detect variants of Alzheimer's disease characterized by the predominance of diffuse (non-fibrillar) plaques (Cairns *et al.*, 2009; Schöll *et al.*, 2012). Of course low amyloid- β_{42} in CSF is also known to occur in isolation in non-Alzheimer's disease conditions (Ewers *et al.*, 2015; Skillbäck *et al.*, 2015) and may also be related to entrapment in interstitial drainage pathways (Weller *et al.*, 2001), epitope masking due to binding of amyloid- β_{42} to carrier proteins (Otto *et al.*, 2000; Wiltfang *et al.*, 2003), or the presence of amyloid as a secondary pathology. In cognitively normal subjects, abnormal CSF amyloid- β_{42} can also be seen, and is thought to possibly represent preclinical Alzheimer's disease (Fagan *et al.*, 2009a; Sperling *et al.*, 2011; Palmqvist *et al.*, 2016). Isolated increased PiB uptake using INNOTEST data likely reflected between-centre variability in INNOTEST results; using reanalysed data, however, this pattern was infrequent and was seen mainly in MCI and Alzheimer's disease. This finding may in some cases be due to cut-off levels but may also reflect the possibility that fibrillar amyloid- β can be detected first in some individuals, as has been reported previously (Koivunen *et al.*, 2008; Forsberg *et al.*, 2010; Landau *et al.*, 2013).

Among the studies that have thus far examined the association between amyloid PET and CSF amyloid- β_{42} (Fagan *et al.*, 2006, 2007, 2009b; Forsberg *et al.*, 2008, 2010; Jagust *et al.*, 2009; Tolboom *et al.*, 2009; Degerman Gunnarsson *et al.*, 2010; Weigand *et al.*, 2011; Landau *et al.*, 2013; Zwan *et al.*, 2014, 2016; Palmqvist *et al.*, 2014, 2016; Mattsson *et al.*, 2015), few have directly compared tau levels in those with concordant and discordant biomarker results. In the studies that have, tau was found to be higher in PET+/CSF+ subjects, relative to those PET-/CSF+ (Palmqvist *et al.*, 2016; Zwan *et al.*, 2016), in PET+/CSF+ subjects, relative to those PET-/CSF+ (Mattsson *et al.*, 2015; Zwan *et al.*, 2016), and in subjects discordant with PET positivity, relative to both those concordant negative and discordant with abnormal CSF (Zwan *et al.*, 2016). Using INNOTEST amyloid- β_{42} , our findings match those reported up to now. Comparison of tau levels between biomarker subgroups defined using reanalysed CSF, however, was not performed, due to differences in CSF methodology (INNOTEST versus xMAP Luminex multiplexing). Though group differences were noted for gender and APOE in the present study, in the aggregate with findings from other studies (Mattsson *et al.*, 2015; Toledo *et al.*, 2015), the precise modulatory role exerted by these variables is not yet clear. Lastly, concordance findings here reported are unlikely to have been much altered by inclusion of CSF tau data, with the majority of abnormal tau values clustered in the concordant positive quadrant. Future studies incorporating new approaches for CSF tau quantification and multivariate modelling are required to further clarify differences between biomarker subgroups.

Though discordance between CSF and amyloid- β PET has thus far been discussed primarily in the context of the increasing use of these two techniques in the diagnosis

of cognitively impaired individuals, an additional area of importance is that of future clinical trials. In one of the largest studies conducted so far on the relationship between amyloid- β biomarkers, it was shown that concordance increased significantly as a function of disease stage (Mattsson *et al.*, 2015), with the highest levels of discordance noted in cognitively normal and subjective memory complaint subjects. In a related study focusing on non-demented subjects diagnosed as cognitively healthy controls or MCI, evidence was shown in support of the hypothesis that CSF may detect amyloid- β accumulation in preclinical Alzheimer's disease earlier than amyloid PET (Palmqvist *et al.*, 2016). There is thus increasing evidence to suggest that discussions surrounding the interchangeability of amyloid- β biomarkers may be less applicable with respect to the detection of cerebral amyloidosis in the preclinical phase of Alzheimer's disease. Specifically, isolated CSF positivity may represent a profile suitable for inclusion in clinical trials of amyloidcentric agents (Hardy *et al.*, 2014), or related interventions targeting modifiable risk factors (Ngandu *et al.*, 2015). Given unresolved differences between current classification schemes (Sperling *et al.*, 2011; Dubois *et al.*, 2014, 2016), further follow-up studies to verify progression to amyloid PET positivity, neurodegenerative changes and cognitive decline, are required.

A possible limitation of this study was the lack of gold standard autopsy confirmation. In addition to confirming or refuting clinical diagnoses for included subjects, post-mortem data would possibly have allowed for an improved understanding of PiB negative discordant cases, including the potential effects of coincident pathologies. Autopsy data would, moreover, have helped clarify the seven cases showing discrepant classifications using visual and Centiloid based methods; at least five of the six visually positive cases, however, would likely have crossed the SUVR/Centiloid threshold for positivity had partial volume correction (PVC) been applied. Though the lack of PVC stands as a potential caveat, its application would likely only have resulted in the relocation of a subset of isolated CSF+ cases to the concordant positive quadrant. Future studies on amyloid biomarkers, however, should examine the effect of this type of correction. As a further caveat, the INNOTEST amyloid- β_{42} ELISA would ideally have been used instead of MSD, though the effect of this is likely to have been minimal (Bjerke *et al.*, 2016). Other possible limitations include the relatively low number of cognitively normal subjects and patients with FTD and VaD, as well as the fact that *APOE* genotype data was not available for all subjects. Lastly, CSF classification cut-offs were established with mixture modelling; while a robust approach for establishing unbiased thresholds, and used in several studies (Buchhave *et al.*, 2012; Palmqvist *et al.*, 2014), these cut-offs cannot be viewed as generalizable beyond the present work.

Overall, the agreement levels between amyloid PET and CSF amyloid- β_{42} here reported are in line with previous studies, and support the strong agreement of these two

metrics in MCI and Alzheimer's disease, with mainly isolated low CSF amyloid- β_{42} in FTD, VaD and cognitively normal subjects. While technical factors cannot be excluded outright as contributing to measured amyloid- β_{42} and amyloid- β_{40} levels in CSF assays, even when run in the same time and place, continued discordance using centrally reanalysed samples suggests that biological factors are also at play. While further studies are required, in particular longitudinal studies on amyloid biomarker trajectories, with a focus on intraindividual change, our findings suggest that, at least in a minority of subjects, these measures may not be interchangeable, reflecting instead distinct but inter-related processes. Future work using standardized amyloid PET and CSF amyloid- β_{42} , as well as post-mortem pathology data, will be critical to gaining an improved understanding of amyloid biomarker discordance.

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Conflict of interest

K.B. reports personal fees from IBL International, Roche Diagnostics and Eli Lilly. C.A.F.V.A. reports personal fees from Desitin Arzneimittel GmbH, Dr Willmar Schwabe GmbH & Co, personal fees and non-financial support from Nutricia GmbH, Lilly Deutschland GmbH, and grants from Roche Diagnostics GmbH, Biologische Heilmittel Heel GmbH, and ViaMed GmbH. J.O.R. serves as a consultant neurologist for Clinical Research Services Turku (CRST) Ltd. A.N. has received grants from GE Healthcare and Bayer Healthcare, served on the scientific advisory boards of GE Healthcare, Avid, and Eli Lilly, and received speaker honorarium from GE Healthcare, Piramal, Novartis, and Bayer Healthcare.

Supplementary material

Supplementary material is available at *Brain* online.

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