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RESEARCH ARTICLE

Amyloid-β PET and CSF in an autopsy-confirmed cohort

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Abstract

Objective: Accumulation of amyloid-β is among the earliest changes in Alzheimer's disease (AD). Amyloid-β positron emission tomography (PET) and $A\beta_{42}$ in cerebrospinal fluid (CSF) both assess amyloid-β pathology in-vivo, but 10–20% of cases show discordant (CSF+/PET− or CSF-/PET+) results. The neuropathological correspondence with amyloid-β CSF/PET discordance is unknown. **Methods:** We included 21 patients from our tertiary memory clinic who had undergone both CSF $A\beta_{42}$ analysis and amyloid-β PET, and had neuropathological data available. Amyloid-β PET and CSF results were compared with neuropathological ABC scores (comprising of Thal (A), Braak (B), and CERAD (C) stage, all ranging from 0 [low] to 3 [high]) and neuropathological diagnosis. **Results:** Neuropathological diagnosis was AD in 11 (52%) patients. Amyloid-β PET was positive in all A3, C2, and C3 cases and in one of the two A2 cases. CSF $A\beta_{42}$ was positive in 92% of ≥A2 and 90% of ≥C2 cases. PET and CSF were discordant in three of 21 (14%) cases: CSF+/PET− in a patient with granulomatosis with polyangiitis (A0B0C0), CSF+/PET− in a patient with FTLD-TDP type B (A2B1C1), and CSF-/PET+ in a patient with AD (A3B3C3). Two CSF+/PET+ cases had a non-AD neuropathological diagnosis, that is FTLD-TDP type E (A3B1C1) and adult-onset leukoencephalopathy with axonal spheroids (A1B1C0). **Interpretation:** Our study demonstrates neuropathological underpinnings of amyloid-β CSF/PET discordance. Furthermore, amyloid-β biomarker positivity on both PET and CSF did not invariably result in an AD diagnosis at autopsy, illustrating the importance of considering relevant comorbidities when evaluating amyloid-β biomarker results.

Introduction

Among the earliest neuropathological events in Alzheimer's disease (AD) is the accumulation and aggregation of amyloid-β, which occurs decades before symptom onset.[1] Amyloid-β can aggregate in the brain parenchyma as plaques or in the cerebral vasculature as cerebral amyloid angiopathy (CAA). Two methods are currently employed to assess amyloid-β pathology in-vivo. $A\beta_{42}$ levels in the cerebrospinal fluid (CSF) reflect the concentrations of soluble amyloid-β, which has been shown to correlate with amyloid-β deposits in the brain.[2] Alternatively, positron emission tomography (PET) with amyloid-β radiotracers can be used to visualize cerebral amyloid-β deposits.[3–5] These two methods are considered interchangeable for the assessment of amyloid pathology in-vivo and for the diagnosis of AD in both clinical practice and research.[6,7]

In the majority of cases amyloid-β PET and CSF are concordant, but in 10–20% of patients they show discordant (CSF+/PET− or CSF-/PET+) results.[8,9] One possible hypothesis for the amyloid-β CSF/PET discordance is that soluble CSF Aβ_{42} decreases before significant fibrillar amyloid-β deposits can be detected by PET.[10,11] Although studies have been performed to compare either amyloid-β PET or CSF Aβ_{42} to neuropathological examination results,[2–5] so far no head-to-head cohort studies have been performed to compare in-vivo amyloid-β CSF and PET results to neuropathological findings. Previously, two case reports of patients with discordant amyloid-β CSF/PET (both CSF+/PET−) and available neuropathology have been published,[12,13] in which the negative PET signal was attributed to the lack of neuritic plaques at autopsy. Further investigating the correspondence between amyloid-β PET, CSF, and neuropathology ("standard of truth") is important to shed light on the underlying cause of amyloid-β CSF/PET discordance. Also, if CSF+/PET- amyloid-β status is an indicator of early amyloid-β pathology, this could be instrumental for future disease modifying therapies.[14] Therefore, the aim of this study was to investigate the concordance between PET and CSF amyloid-β status in a sample with available neuropathological results and to characterize the amyloid-β CSF/PET discordant cases neuropathologically.

Methods

Participants

We retrospectively included 21 autopsy cases from the Amsterdam Dementia Cohort who had undergone both CSF Aβ_{42} analysis and amyloid-β PET during life. Patients visiting our tertiary memory center are screened according to a standardized protocol,[15] including a clinical and neuropsychological evaluation, *APOE* genotyping, magnetic resonance imaging (MRI), and a lumbar puncture (LP) for CSF biomarker analysis. Clinical diagnosis is determined during a multidisciplinary meeting.

Amyloid-β PET and CSF analyses were performed between 2007 and 2016 and neuropathological diagnosis was performed between 2011 and 2019 (Fig. 1). In this sample, LP for CSF analysis always preceded amyloid-β PET and, the median CSF-PET time was 28 [interquartile range (IQR): 18, 56] days. The median time difference between amyloid-β PET and patient death was 3.0 (IQR: 1.7, 6.5) years and the time difference between LP and patient death was 3.3 (IQR: 2.0, 6.7) years.

Cerebrospinal fluid

CSF was obtained during life by LP between L3/4 and L5/S1, using a 25-gauge needle and a syringe.[16] Samples were collected in polypropylene collection tubes and centrifuged at 1800*g* for 10 min at 4°C and thereafter frozen at −20°C until routine biomarker analysis. Manual analyses of Aβ_{42}, total tau (t-tau), and phosphorylated tau (p-tau) were performed using sandwich ELISAs (Innotest assays: β-amyloid1-42, tTAU-Ag, and PhosphoTAU-181p; Fujirebio) in the Neurochemistry Laboratory of the Department of Clinical Chemistry of Amsterdam UMC. If two CSF results were available, we used the result closest to the amyloid-β PET (three cases, all with concordant Aβ_{42} status between two samples). As median CSF Aβ_{42} values of our cohort have been gradually increasing over the years, we were unable to use the original CSF Aβ_{42} values.[17] Therefore, we used CSF Aβ_{42} values that have been adjusted for the longitudinal upward drift with a uniform cutoff of 813 pg/mL (<813 pg/mL considered as CSF amyloid-β positive).[18] Additionally, as it has been previously shown that the ratio of CSF Aβ_{42} with CSF (p)tau is superior to CSF Aβ_{42} in predicting the diagnosis of AD,[19] we also used a CSF p-tau/Aβ_{42} ratio with a previously validated cutoff of 0.054.[20] This cutoff was obtained by mixture modeling of 2711 CSF results of the Amsterdam Dementia Cohort, similar to previous work.[18]

Amyloid-β positron emission tomography

Amyloid-β PET was performed using the following PET scanners: ECAT EXACT HR + scanner (Siemens Healthcare, Germany), Gemini TF PET/CT, Ingenuity TF PET-CT, and Ingenuity PET/MRI (Philips Medical Systems, the Netherlands). We included 15 cases with [^{11}C]Pittsburgh Compound B (PIB),[21] three with [^{18}F]florbetaben,[22] and three with [^{18}F]flutemetamol.[23] Amyloid-β PET status (positive or negative) was determined by a majority visual read of three reads. All scans were initially read by an expert nuclear medicine physician (BvB, from 2007 to 2016, read 1). In addition, in 2019 the scans were reread for this study by BvB (read 2) and LC (with extensive experience in reading amyloid-β PET scans, read 3), while being blinded to the results of other visual reads, CSF, and neuropathological results. The three amyloid-β PET visual reads were concordant (either +/+/+ or −/−/−) in 18 of 21 cases. In the three remaining cases (nr 5, 10, 19), two of the three visual reads were positive, and as such these cases were considered PET positive.

Neuropathology

Autopsies were performed by the Department of Pathology of Amsterdam UMC; location VUmc for the

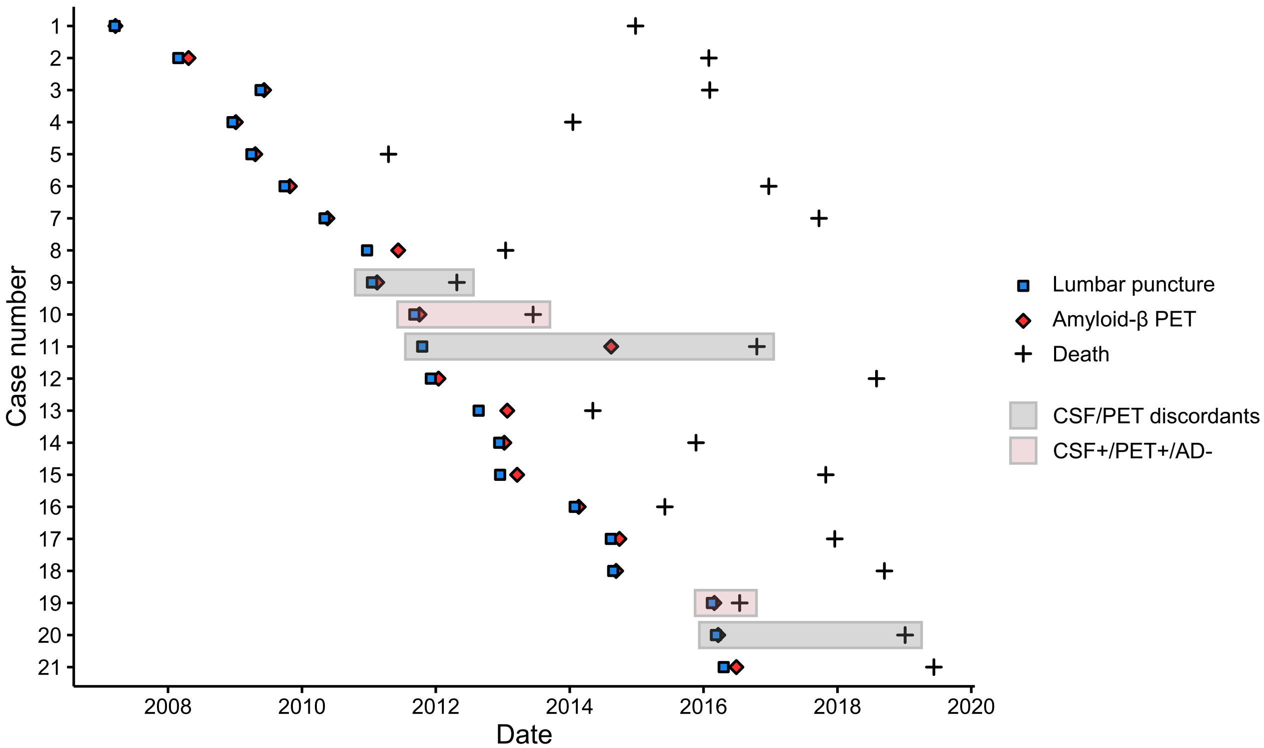


Figure 1. Time between lumbar puncture, amyloid-β PET, and patient death. Abbreviations: AD Alzheimer's disease, CSF cerebrospinal fluid, PET positron emission tomography.

Netherlands Brain Bank or for VUmc. Brain donors or their next of kin signed informed consent regarding the usage of brain tissue and clinical records for research purposes. Brain autopsies and neuropathological diagnosis were performed according to the international guidelines of Brain Net Europe II consortium (http://www.brainnet-europe.org) and the applicable diagnostic criteria.[24,25] For this particular study, every case also without suspicion of AD pathology during life was scored by AR and BB for AD neuropathological changes according to the ABC scoring system by AR and BB,[24] in which the A stands for amyloid-β Thal phase,[26] B for Braak stage for neurofibrillary tangles,[1] and C for CERAD criteria for neuritic plaques.[27] When present, CAA was classified as Type 1 (including capillaries in the parenchyma) or Type 2 (leptomeningeal/cortical without capillary involvement) and staged according to Thal et al.[28]

Statistical analysis

Statistical analysis was conducted using R software (Version 3.6.1). We used descriptive statistics to characterize the sample. We used the Cochrane–Armitage trend test to examine the associations between amyloid-β biomarkers and the neuropathological ABC scores,[29] which allowed us to compare both PET and CSF to neuropathology as we had only binarized results available for amyloid-β PET.

Results

Study population

In our sample of 21 cases, 16 (76%) were male and 10 (48%) were carriers of an *APOE* ε4 allele (Table 1). Mean age at death was 65 ± 8 years and the average last known Mini-Mental State Examination (MMSE, median 2.0 years before death) was 20 ± 6. Eleven (52%) patients had a clinical diagnosis of AD, which was in accordance with neuropathological diagnosis in all AD cases. Two cases (4 and 15, both CSF/PET concordant) carried an autosomal-dominant mutation associated with AD. In 15 (71%) cases CAA (11 CAA-Type 1, 4 CAA-Type 2) was observed at neuropathological examination.

In vivo amyloid-β status

Thirteen (62%) cases were defined as amyloid-β positive based on PET, 14 (67%) based on CSF $A\beta_{42}$, and 11 (52%) based on CSF p-tau/$A\beta_{42}$ ratio. In our sample, CSF $A\beta_{42}$ and amyloid-β PET were concordant in 18 (86%) cases. CSF p-tau/$A\beta_{42}$ ratio was concordant with amyloid-β PET in 17 (81%) cases and with CSF $A\beta_{42}$ in 16 (76%) cases.

Discordance between amyloid-β PET, CSF, and neuropathological diagnosis

Of the three discordant cases between CSF $A\beta_{42}$ and amyloid-β PET, two were CSF+/PET- (case 9, clinical diagnosis: frontotemporal dementia [neuropathological diagnosis: frontotemporal lobar degeneration (FTLD)-TDP type B, ABC score: A2B1C1] and case 20 with vasculitis [granulomatosis with polyangiitis, A0B0C0], and one was CSF-/PET+ (case 11 with AD [AD, A3B3C3], Fig. 2A–C). The three amyloid-β CSF/PET discordant patients all had an *APOE* ε3/ε3 genotype. In addition, there were two CSF+/PET + cases with a non-AD primary neuropathological diagnosis, that is case 10 with semantic dementia [FTLD-TDP type E, A3B1C1; CAA-Type 1 stage 2] and case 20 with adult-onset leukoencephalopathy with axonal spheroids (HDLS) [leukodystrophy due to HDLS, A1B1C0; CAA-Type 1 stage 1] (Fig. 2D–E).

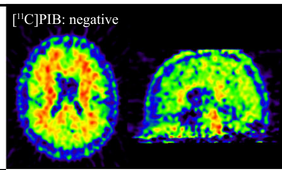
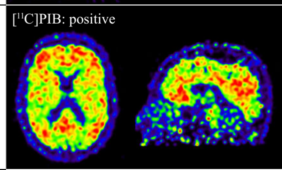
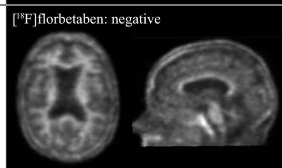
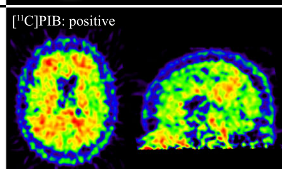
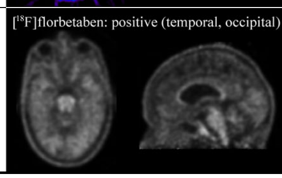
Association between biomarkers and ABC scores

CSF $A\beta_{42}$ (Fig. 3A) was positive in 12 of 13 (92%) and CSF p-tau/$A\beta_{42}$ ratio (Fig. 3B) in 10 of 13 (77%) of the A2/A3 cases. Both CSF $A\beta_{42}$ and p-tau/$A\beta_{42}$ ratio were positive in 10 of 11 (91%) B2/B3 cases and 9 of 10 (90%) C2/C3 cases. Amyloid-β PET (Fig. 3C) was positive in one of the two A2 cases, and in all A3 and/or B2/B3 and/or C2/C3 cases. Cochrane trend analyses showed that there

Table 1. Case characteristics.

									Neuropathology		
	Nr	Sex	Age at death	APOE genotype	Clinical diagnosis	CSF $A\beta_{42}$	Amyloid-β PET	CSF/PET status	ABC	Primary diagnosis	CAA-Type
	1	m	75	E4E4	AD	810	Positive	CSF+/PET+	A3B3C3	AD	2
	2	m	65	E3E3	AD	640	Positive	CSF+/PET+	A3B3C3	AD	2
	3	m	65	E3E3	CBS	940	Negative	CSF-/PET−	A0B1C0	FTLD-TDP type A	–
	4	f	43	E3E3	AD	554	Positive	CSF+/PET+	A3B3C3	AD	1
	5	f	64	E4E4	AD	619	Positive	CSF+/PET+	A2B2C2	AD	1
	6	m	69	E3E4	AD	504	Positive	CSF+/PET+	A3B3C3	AD	1
	7	m	76	E3E4	FTD	1110	Negative	CSF-/PET−	A1B0C0	FTLD-TDP type A	–
	8	m	60	–	Dementia unspecified	1136	Negative	CSF-/PET−	A0B0C0	Autoimmune encephalitis	–
*	9	m	75	E3E3	FTD	787	Negative	CSF+/PET−	A2B1C1	FTLD-TDP type B	–
†	10	m	64	E4E4	SD	739	Positive	CSF+/PET+	A3B1C1	FTLD-TDP type E	1
*	11	m	68	E3E3	AD	828	Positive	CSF-/PET+	A3B3C3	AD	2
	12	m	68	E3E3	Dementia unspecified	1167	Negative	CSF-/PET−	A0B1C0	LBD	–
	13	m	65	E2E3	FTD	1708	Negative	CSF-/PET−	A1B1C0	FTLD/MND-TDP type B	1
	14	m	70	E3E4	AD	755	Positive	CSF+/PET+	A3B3C3	AD	2
	15	f	62	E4E4	AD	681	Positive	CSF+/PET+	A3B3C3	AD	1
	16	f	61	E3E4	FTD	862	Negative	CSF-/PET−	A1B0C0	FTLD-TDP type E	1
	17	m	73	E3E4	AD	644	Positive	CSF+/PET+	A3B2C1	AD	1
	18	m	53	E3E3	AD	587	Positive	CSF+/PET+	A3B3C3	AD	1
†	19	m	50	E3E3	HDLS	676	Positive	CSF+/PET+	A1B1C0	Leukodystrophy due to HDLS	1
*	20	f	65	E3E3	Vasculitis	646	Negative	CSF+/PET−	A0B0C0	Granulomatosis with polyangiitis	–
	21	m	65	E3E4	AD	397	Positive	CSF+/PET+	A3B3C3	AD	1

Asterisks (*) in the first column highlight CSF/PET discordant cases and crosses (†) highlight CSF+/PET + cases with a non-AD neuropathological diagnosis. Values under 813 pg/mL for CSF $A\beta_{42}$ are pathological. Amyloid-β PET positivity was determined by majority visual read. Neuropathological ABC scoring system entails amyloid-β Thal (A) phase, Braak (B) stage for neurofibrillary tangles, and CERAD (C) criteria for neuritic plaques. CAA column indicates the neuropathological type of cerebral amyloid angiopathy: Type 1 (capillary) or Type 2 (leptomeningeal/cortical). Abbreviations: AD Alzheimer's disease, CAA cerebral amyloid angiopathy, CBS corticobasal syndrome, CSF cerebrospinal fluid, FTD frontotemporal dementia, FTLD frontotemporal lobar degeneration, HDLS Adult-onset leukoencephalopathy with axonal spheroids, LBD Lewy body dementia, MND motoneuron disease, PET positron emission tomography, SD semantic dementia.

	Clinical findings	CSF	Amyloid-β PET	Neuropathology	Explanations for discordance
A case 9	74 y/o man was referred for compulsive and anti-social character change. MMSE was 24/30. On MRI, left hippocampal atrophy (MTA 2), and extensive white matter damage (Fazekas III) with two possible small lacunes were seen. Amyloid-β PET was performed for research and was negative. The patient was diagnosed with possible bvFTD.	**Aβ_{42}: 787** t-tau: 279 p-tau: 41	[11C]PIB: negative	Diagnosis: FTLD-TDP type B ABC score: A2B1C1 Additional findings: N/A	**CSF+/PET-/AD-** > CSF detected amyloid co-pathology > CSF Aβ_{42} could be false-positive as it is close to the cut-off (individual CSF Aβ_{42} production or pre-analytical factors)
B case 11	63 y/o man visited our center with memory complaints. MMSE was 30/30 but the neuropsychological exam confirmed memory dysfunction. The MRI was normal and the CSF analysis was inconclusive. Amnestic MCI was diagnosed. During follow-up there was a slow decline in memory and executive functioning and after 3 years amyloid-β PET was requested. The PET scan was read positive and the patient was diagnosed with AD.	Aβ_{42}: 828 **t-tau: 498** **p-tau: 66**	[11C]PIB: positive	Diagnosis: AD ABC score: A3B3C3 Additional findings: CAA-Type 2 (stage 1)	**CSF-/PET+/AD+** > CSF Aβ_{42} is false negative as it is close to the cut-off and CSF tau levels were already increased (individual variation of CSF Aβ_{42} or pre-analytical factors) > The long time difference between CSF analysis and amyloid PET could be an additional contributing factor
C case 20	62 y/o woman was referred because of apathy and tiredness. MMSE was 30/30. White-matter lesions and a lacune in the right thalamus were found on the MRI. Aβ PET was performed and was negative. CSF pleocytosis was found and Aβ_{42} was decreased. An autoimmune cause was suspected. Over the next 2 years there was subacute cognitive decline and progression of vascular damage based on MRI. FDG PET showed increased uptake in the thoracic aorta, indicative of vasculitis.	**Aβ_{42}: 646** t-tau: 251 p-tau: 40	[18F]florbetaben: negative	Diagnosis: Granulomatosis with polyangiitis ABC score: A0B0C0 Additional findings: Aβ-positive axons in the substantia nigra and in the sub-thalamic nucleus, corresponding to leakage of amyloid precursor protein.	**CSF+/PET-/AD-** > Decreased CSF Aβ_{42} due to neuroinflammation
D case 10	53 y/o man was referred because of severe word finding problems and behavioral change. MMSE was 18/30. Family history was positive for early-onset dementia. The CSF analysis was considered normal (prior adjustment for Aβ_{42} longitudinal drift) and MRI showed anteriotemporal atrophy (MTA: left 2, right 1). FDG PET showed bilateral frontal and left temporal hypometabolism. Aβ PET was clinically requested and initially read as negative. Patient was diagnosed with SD.	**Aβ_{42}: 739** t-tau: 362 p-tau: 38	[11C]PIB: positive	Diagnosis: FTLD-TDP type E ABC score: A3B1C1 Additional findings: CAA-Type 1 (stage 2)	**CSF+/PET+/AD-** > Detection of amyloid co-pathology by both CSF and PET > Possible detection of CAA
E case 19	49 y/o man was referred to our memory clinic due to multi-domain (memory, executive functioning, visuospatial, behavioral) cognitive decline over 1-2 years. MMSE was 24/40. Familial history was positive for early-onset dementia. MRI showed extensive parietal atrophy and white matter lesions. Amyloid-β PET was requested for research and was initially read as negative. Genetic analysis found CSF1R mutation and HDLS was diagnosed.	**Aβ_{42}: 676** **t-tau: 528** p-tau: 33	[18F]florbetaben: positive (temporal, occipital)	Diagnosis: Leukodystrophy due to HDLS ABC score: A1B1C0 Additional findings: CAA-Type 1 (stage 1)	**CSF+/PET+/AD-** > Detection of CAA by PET and CSF

Figure 2. Discordance between amyloid-β CSF, PET, and autopsy. Vignettes illustrating amyloid-β CSF/PET discordant cases (A,B,C) and CSF+/PET + cases with a non-AD neuropathological diagnosis (D,E). CSF values for Aβ_{42} < 813 pg/mL, for phosphorylated tau (p-tau) >52 pg/mL, and for total tau (t-tau) >375 pg/mL are pathological (indicated by bold). Amyloid-β PET scans in cases 10 and 19 were initially read as amyloid negative, but for this study the scans were considered amyloid positive based on majority visual read. Abbreviations: AD Alzheimer's disease, CAA cerebral amyloid angiopathy, CSF cerebrospinal fluid, FDG fluorodeoxyglucose, FTD frontotemporal dementia, FTLD frontotemporal lobar degeneration, HDLS Adult-onset leukoencephalopathy with axonal spheroids, MCI Mild cognitive impairment, MMSE Mini-Mental State Examination, MRI Magnetic resonance imaging, MTA - Medial temporal lobe atrophy, PET positron emission tomography, SD sematic dementia, TDP transactive response DNA-binding protein.

is an increasing proportion of biomarker-positive cases from score 0 to 3 across all ABC scores for amyloid-β PET (Z-score = -3.93 for A, $Z = -3.81$ for B, $Z = -3.68$ for C, all $P < 0.001$) and CSF Aβ_{42} ($Z = -2.92$, $P = 0.003$ for A; $Z = -2.46$, $P = 0.014$ for B; $Z = -2.60$, $P = 0.009$ for C). In *APOE* ε4 carriers, both CSF Aβ_{42} and amyloid-β PET were positive in all A2/A3 and/or B2/B3 and/or C2/C3 cases. In *APOE* ε4 noncarriers, CSF Aβ_{42} was positive in 80% of A2/A3, 75% of B2/B3, and 75% of C2/C3 cases, and amyloid-β PET was positive in 80% A2/A3 cases and all B2/B3 and/or C2/C3 cases.

Association between biomarkers and neuropathological diagnosis

Finally, we investigated the association between binarized biomarker results and neuropathological diagnosis. Amyloid-β PET was positive in all AD cases, but also indicated amyloid-β pathology in two cases without AD as neuropathological diagnosis (Fig. 4). Both CSF Aβ_{42} and p-tau/Aβ_{42} were positive in 10 of 11 AD cases. Decreased CSF Aβ_{42} with a normal CSF p-tau/Aβ_{42} ratio was seen in three non-AD cases (HDLS [A1B1C0]. FTLD-TDP type B [A2B1C1], FTLD-TDP type E [A3B1C1]), and one AD case (A3B3C3). There were three cases with a non-AD neuropathological diagnosis (HDLS, FTLD-TDP type E, and FTLD/MND-TDP type B) with normal levels of CSF p-tau but with increased CSF t-tau.

Discussion

The primary aim of this study was to investigate the concordance between PET and CSF amyloid-β status in a

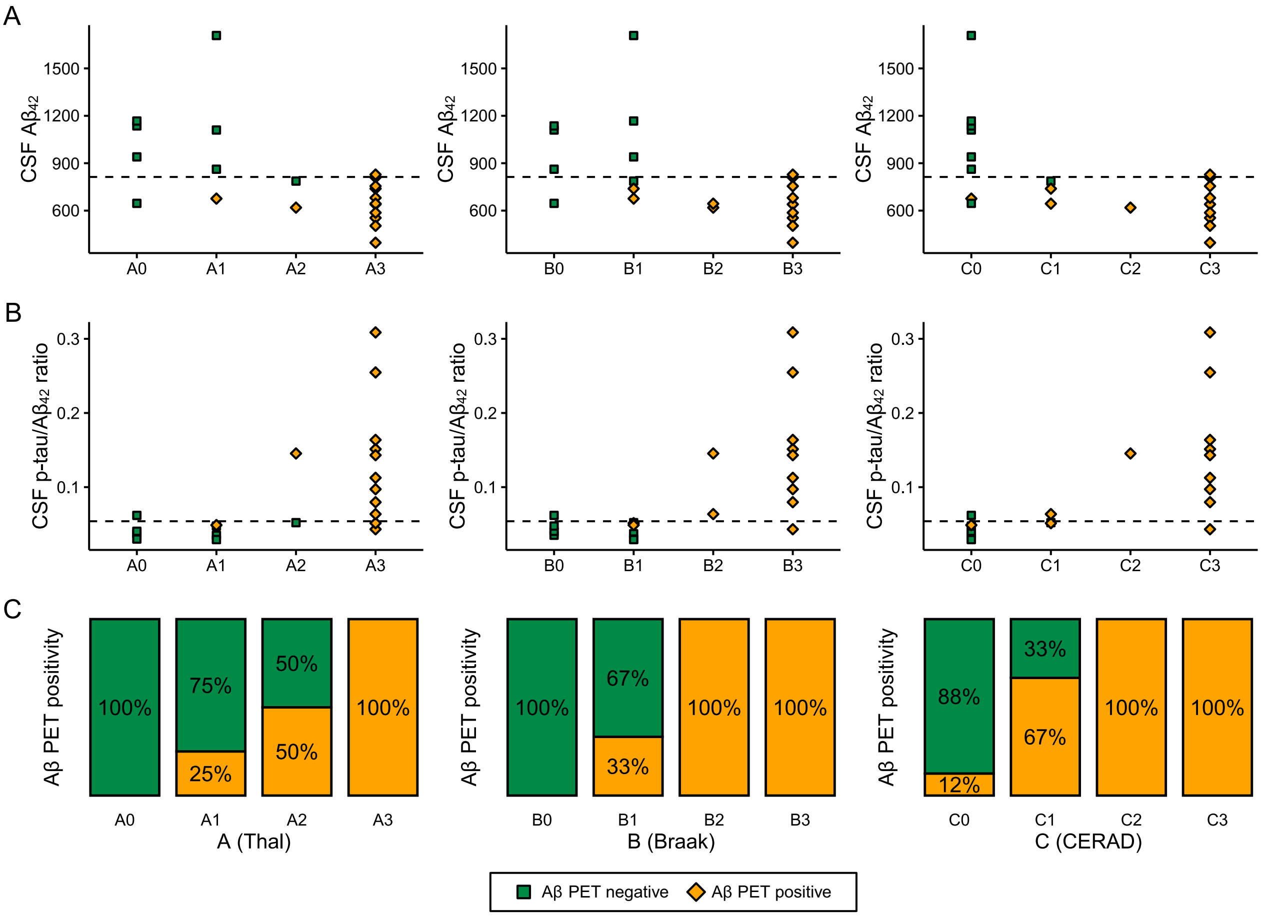


Figure 3. Correspondence of CSF Aβ_{42} (A), CSF p-tau/Aβ_{42} ratio (B), and amyloid-β (Aβ) PET (C) to neuropathological ABC scoring. Neuropathological ABC scoring system entails amyloid-β Thal phase (A0-A3), Braak stage for neurofibrillary tangles (B0-B3), and CERAD criteria for neuritic plaques (C0-C3). Dashed lines represent cutoffs for CSF Aβ_{42} (813 pg/mL) and CSF p-tau/Aβ_{42} ratio (0.054).

sample with available neuropathological results in order to enhance our understanding of the amyloid-β CSF/PET discordant cases. We found that although both CSF and PET generally captured AD pathological change, there was still 14% (3/21) discordance between the two modalities. In our sample, possible reasons for amyloid-β CSF/PET discordance included neuroinflammation (CSF+/PET− in a case of granulomatosis with polyangiitis, A0B0C0), detection of amyloid-β co-pathology (CSF+/PET− in FTLD-TDP type B, A2B1C1) and additional factors influencing CSF Aβ_{42} levels (CSF-/PET+ in AD, A3B3C3). Additionally, we described two CSF+/PET+ non-AD cases illustrating that amyloid-β biomarker positivity on both PET and CSF does not invariably result in an AD diagnosis at autopsy. This highlights that it is important to consider other comorbidities when evaluating the results of amyloid-β biomarkers, especially since molecular biomarkers for non-AD neurodegenerative diseases are currently lacking.

Although in the majority of cases, amyloid-β PET and CSF Aβ_{42} show concordant results, 10-20% discordant CSF/PET status has repeatedly been shown.[8,9,30] As amyloid-β CSF/PET discordance rates are highest in patients with early disease, it has been hypothesized that CSF/PET discordance might be partly explained by early decreases of CSF Aβ_{42} that precede amyloid-β depositions visible by PET.[10,11] On the other hand, amyloid-β CSF/PET discordance in patients with dementia could be explained by one modality detecting beginning amyloid-β co-pathology in non-AD cases.[8] To our knowledge, this is the first serial study including patients who have both amyloid-β PET and CSF Aβ_{42} in addition to neuropathological data available. In line with previous in vivo studies, we found a 14% (3/21) CSF/PET discordance rate. We reported a CSF+/PET- patient with A2B1C1 FTLD-TDP type B, where it is feasible that the reduction of CSF Aβ_{42} is caused by concomitant amyloid-β pathology. However, as the Aβ_{42} value was relatively close to the cutoff, it is not

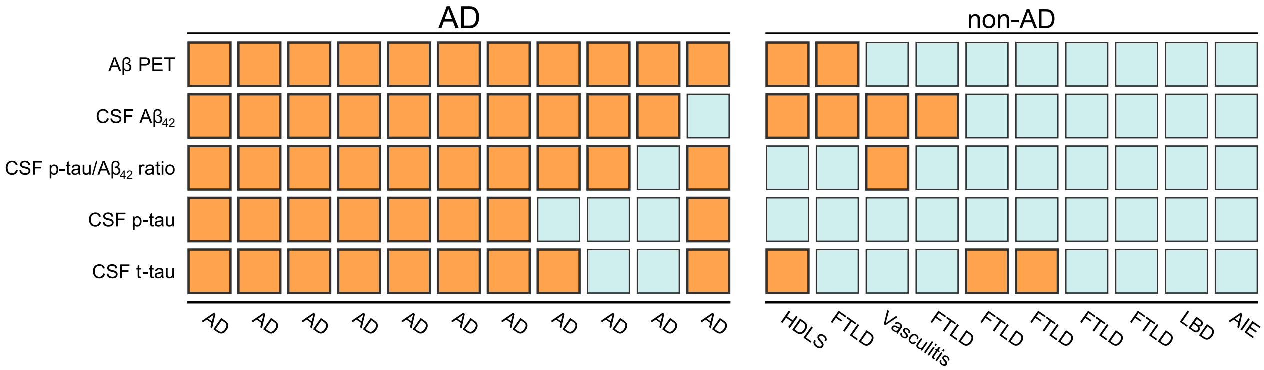


Figure 4. Biomarker status by primary neuropathological diagnosis. Colors indicate binarized status of biomarkers: orange for biomarker positive, blue for biomarker negative. Abbreviations: A*β* Amyloid-*β*, AD Alzheimer's disease, AIE autoimmune encephalitis, CSF cerebrospinal fluid, FTLD frontotemporal lobar degeneration, HDLS Adult-onset leukoencephalopathy with axonal spheroids, PET positron emission tomography.

possible to entirely exclude individual CSF $A\beta_{42}$ dynamics (i.e., this patient intrinsically producing less $A\beta_{42}$)[31] or preanalytical factors.[32] Previously, two CSF+/PET- case reports with available neuropathology have been published. First, a negative PIB PET scan was reported in a 91-year-old patient with abnormal CSF $A\beta_{42}$ and tau biomarkers with sporadic AD.[12] The negative amyloid-*β* PET status was attributed to the absence of a significant amount of fibrillar plaques (i.e., with a fibrillar core that the tracer binds to), although diffuse plaques were present. Second, low PIB PET retention with decreased CSF $A\beta_{42}$ was reported in a familial AD case with arctic amyloid precursor protein (APP) mutation, thought to be caused by the lack of fibrillar amyloid-*β* plaques characteristic for this mutation.[13] Future studies with neuropathological data are needed to further validate whether amyloid-*β* CSF+/PET- status is caused by beginning amyloid-*β* depositions and explore additional neuropathological substrates for CSF/PET discordance, such as differences in distribution, load, and morphology of amyloid-*β* plaques and possible influences of co-pathologies.

In our sample, there were two cases with amyloid-*β* CSF+/PET+ biomarker status who did not meet neuropathological criteria for AD. The first had a diagnosis of FTLD-TDP type E with a high Thal score but only sparse neuritic plaques (A3B1C1). It is feasible that in this case both biomarkers detected concomitant amyloid-*β* co-pathology as increased PIB PET signal has been shown to be related to fibrillar plaque load even in case of sparse neuritic plaques.[33,34] The patient was also diagnosed with CAA-Type 1 stage 2, which could also contribute to the amyloid positivity, as CAA has been shown to affect both amyloid-*β* PET tracer uptake[35] and CSF $A\beta_{42}$ levels.[36] The second CSF+/PET+ patient with a low score for AD pathology (A1B1C0) was diagnosed with HDLS, an autosomal-dominant white matter disease due to mutations in the gene encoding colony-stimulating factor 1 receptor (CSF1R).[37] Previous case reports of HDLS including CSF analyses have provided no evidence for alterations in $A\beta_{42}$ levels.[38,39] It is also unlikely that preanalytical assay effects caused the decrease of CSF $A\beta_{42}$ in this case as the patient had a separate CSF $A\beta_{42}$ sample with decreased $A\beta_{42}$ 4 months earlier. Similar to the previous patient, CAA-Type 1 was present and might have contributed to the positive amyloid-*β* biomarker status, especially since PET tracer uptake was seen predominantly in the occipital region, a predilection site for CAA pathology.[35] This illustrates that even concordant positivity of two amyloid-*β* biomarkers does not always result in a neuropathological diagnosis of AD, and relevant co-pathologies should always be considered.

There were four cases where CSF $A\beta_{42}$ was decreased without a neuropathological diagnosis of AD. In three of them there was neuropathological evidence for amyloid-*β* co-pathology, but we also described a CSF+/PET- case with granulomatosis with polyangiitis (formerly known as Wegener's granulomatosis), which is in line with literature, as neuroinflammation[40,41] as well as infection[42,43] have been previously shown to cause decreased CSF $A\beta_{42}$ without the presence of AD pathology. This highlights that in select cases, there might be unspecific decreases in CSF $A\beta_{42}$ levels without AD, although these cases might be distinguished from AD pathology based on clinical findings and MR imaging. In our particular case, after A*β* immunostaining, A*β* immunoreactive axons were seen, which can be attributed to the leakage of APP that is reported in various conditions such as ischemia, traumatic brain injury and – similar to this case – inflammation.[44] The possible connection of this finding with the decrease in CSF $A\beta_{42}$ is unclear, although it is tempting to hypothesize that the loss of APP leads to the interruption of the APP pathway and the reduction of its product

$A\beta_{42}$ in the CSF. We were unable to find previous case reports of vasculitis with available CSF $A\beta_{42}$ analysis, but primary angiitis of the central nervous system has been associated with decreased APP in the CSF,[45] lending support to that speculative theory.

CSF p-tau/$A\beta_{42}$ ratio was slightly more specific than CSF $A\beta_{42}$ for capturing the neuropathological diagnosis of AD, which has been previously shown in studies involving living subjects.[19] In the CSF-/PET+ discordant case we presented, the patient with a clinically advanced AD dementia had a CSF $A\beta_{42}$ value just above the cutoff, but CSF p-tau/$A\beta_{42}$ ratio was in the pathological range. In this case, CSF $A\beta_{42}$ was likely false negative, possibly due to individual differences in CSF dynamics, as both CSF t-tau and p-tau were already increased. This also highlights the advantage of using continuous measurements as opposed to binarized data, as the distance from cutoff includes additional information. Although (p)tau/$A\beta_{42}$ ratio may be superior to $A\beta_{42}$ when predicting clinically advanced disease with increased (p)tau levels, this may hamper the detection of merely amyloid-β pathology, where tau tangle pathology has not yet begun. This may become clinically significant if anti-amyloid treatment arrives in the future. Finally, we reported an isolated increase in CSF t-tau with normal CSF p-tau levels in three non-AD cases (two FTLD and one HDLS). Although CSF t-tau and p-tau are highly correlated, this finding supports the notion that CSF t-tau can increase in other brain pathologies[46] and CSF p-tau is more AD specific.[47]

The primary strength of our study is the availability of two amyloid-β biomarkers and a neuropathological assessment in a relatively large patient cohort that allowed us to compare the two in vivo amyloid-β biomarkers to neuropathological change. Although PET and CSF were usually performed close in time, there was a median 3-year delay between the amyloid-β biomarkers and autopsy, as is often the case with studies involving in-vivo biomarkers and autopsy data. While this might have impacted our results, a major change over 3 years is unlikely, given the remarkably slow course of AD.[48] We used standardized uptake value ratio images for PET visual read, which could have an impact on our results as non-displaceable binding potential images have been shown to be more reliable in detecting early amyloid-β pathology.[49,50] Another limitation is that we included subjects from the year 2006, and over time technologic advancement has taken place, leading to both increased image quality of PET scans and understanding of preanalytical factors influencing CSF (leading to longitudinal drift of median values, in our cohort). Finally, correcting CSF $A\beta_{42}$ values with CSF $A\beta_{40}$ has been shown to account for the individual variation in the production of amyloid-β.[51] As CSF $A\beta_{40}$ values were only available for seven patients (and none of them were among the discordant cases), we did not include $A\beta_{42/40}$ ratio in our analyses.

In conclusion, our findings illustrate a range of reasons for the amyloid-β CSF/PET discordance, and that even concordant amyloid-β biomarker positivity accurately reflecting amyloid-β pathology does not always equal a definite neuropathological diagnosis of AD. Thus, it is important to consider comorbidities as well as other neurodegenerative diseases when using amyloid-β biomarkers for clinical diagnosis, especially since molecular biomarkers for non-AD neurodegenerative diseases are currently lacking.

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Authors' Contributions

JR, RO, FB, and PS conceived the study and designed the protocol. JR performed statistical analysis, analyzed/interpreted data, and drafted the manuscript. RO and FB provided overall study supervision. JR, BB, LC, RO, and FB participated in writing the manuscript. LC and BvB did the visual reads of the amyloid-β PET scans. BB and AR were responsible for neuropathological evaluation. CT, AR, BvB, and PS had a major role in the acquisition of data, and critically revised and edited the manuscript for intellectual content. All the authors read and approved the final version of the manuscript.

Conflict of Interest

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References

1. Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol 1991;82:239–259. https://doi.org/10.1007/BF00308809
2. Strozyk D, Blennow K, White LR, Launer LJ. CSF Abeta 42 levels correlate with amyloid-neuropathology in a population-based autopsy study. Neurology 2003;60:652–656. https://doi.org/10.1212/01.WNL.0000046581.81650.D0
3. Ikonomovic MD, Klunk WE, Abrahamson EE, et al. Post-mortem correlates of in vivo PiB-PET amyloid imaging in a typical case of Alzheimer's disease. Brain 2008;131:1630–1645. https://doi.org/10.1093/brain/awn016
4. Curtis C, Gamez JE, Singh U, et al. Phase 3 trial of flutemetamol labeled with radioactive fluorine 18 imaging and neuritic plaque density. JAMA Neurol 2015;72:287–294. https://doi.org/10.1001/jamaneurol.2014.4144
5. Sabri O, Sabbagh MN, Seibyl J, et al. Florbetaben PET imaging to detect amyloid beta plaques in Alzheimer's disease: phase 3 study. Alzheimer's Dement 2015;11:964–974. https://doi.org/10.1016/j.jalz.2015.02.004
6. Jack CR, Bennett DA, Blennow K, et al. NIA-AA research framework: toward a biological definition of Alzheimer's disease. Alzheimer's Dement 2018;14:535–562. https://doi.org/10.1016/j.jalz.2018.02.018
7. McKhann GM, Knopman DS, Chertkow H, et al. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimer's Dement 2011;7:263–269. https://doi.org/10.1016/j.jalz.2011.03.005
8. de Wilde A, Reimand J, Teunissen CE, et al. Discordant amyloid-β PET and CSF biomarkers and its clinical consequences. Alzheimers Res Ther 2019;11:78. https://doi.org/10.1186/s13195-019-0532-x
9. Ewers M, Mattsson N, Minthon L, et al. CSF biomarkers for the differential diagnosis of Alzheimer's disease: a large-scale international multicenter study. Alzheimer's Dement. 2015;11:1306–1315. https://doi.org/10.1016/j.jalz.2014.12.006
10. Palmqvist S, Schöll M, Strandberg O, et al. Earliest accumulation of β-amyloid occurs within the default-mode network and concurrently affects brain connectivity. Nat Commun 2017;8: 1214. https://doi.org/10.1038/s41467-017-01150-x
11. Vlassenko AG, McCue L, Jasielec MS, et al. Imaging and cerebrospinal fluid biomarkers in early preclinical Alzheimer disease. Ann Neurol 2016;80:379–387. https://doi.org/10.1002/ana.24719
12. Cairns NJ, Ikonomovic MD, Benzinger T, et al. Absence of Pittsburgh compound B detection of cerebral amyloid β in a patient with clinical, cognitive, and cerebrospinal fluid markers of Alzheimer disease. Arch Neurol 2009;66:1557–1562. https://doi.org/10.1001/archneurol.2009.279
13. Scholl M, Wall A, Thordardottir S, et al. Low PiB PET retention in presence of pathologic CSF biomarkers in Arctic APP mutation carriers. Neurology 2012;79:229–236. https://doi.org/10.1212/WNL.0b013e31825fdf18
14. Hardy J, Bogdanovic N, Winblad B, et al. Pathways to Alzheimer's disease. J Intern Med 2014;275:296–303. https://doi.org/10.1111/joim.12192
15. Van Der Flier WM, Pijnenburg YAL, Prins N, et al. Optimizing patient care and research: the Amsterdam dementia cohort. J Alzheimer's Dis 2014;41:313–327. https://doi.org/10.3233/JAD-132306
16. Engelborghs S, Niemantsverdriet E, Struyfs H, et al. Consensus guidelines for lumbar puncture in patients with neurological diseases. Alzheimer's Dement Diagnosis, Assess Dis Monit 2017;8:111–126. https://doi.org/10.1016/j.dadm.2017.04.007
17. Schindler SE, Sutphen CL, Teunissen C, et al. Upward drift in cerebrospinal fluid amyloid-β 42 assay values for more than 10 years. Alzheimer's Dement 2018;14:62–70. https://doi.org/10.1016/j.jalz.2017.06.2264
18. Tijms BM, Willemse EAJ, Zwan MD, et al. Unbiased approach to counteract upward drift in cerebrospinal fluid amyloid-β 1–42 analysis results. Clin Chem 2018;64:576–585. https://doi.org/10.1373/clinchem.2017.281055
19. Duits FH, Teunissen CE, Bouwman FH, et al. The cerebrospinal fluid "Alzheimer profile": easily said, but what does it mean? Alzheimer's Dement 2014;10:713–723.e2. https://doi.org/10.1016/j.jalz.2013.12.023
20. Willemse EAJ, Tijms BM, van Berckel BNM, Scheltens P, Teunissen CE. Optimal use of cerebrospinal fluid amyloid-beta(1–42) in the memory clinic: use of biomarker ratios across immunoassays. (in prep).
21. Ossenkoppele R, Prins ND, Pijnenburg YAL, et al. Impact of molecular imaging on the diagnostic process in a memory clinic. Alzheimer's Dement 2013;9:414–421. https://doi.org/10.1016/j.jalz.2012.07.003
22. de Wilde A, van der Flier WM, Pelkmans W, et al. Association of amyloid positron emission tomography

with changes in diagnosis and patient treatment in an unselected memory clinic cohort. JAMA Neurol 2018;75:1062. https://doi.org/10.1001/jamaneurol.2018.1346
23. Zwan MD, Bouwman FH, Konijnenberg E, et al. Diagnostic impact of [18F]flutemetamol PET in early-onset dementia. Alzheimers Res Ther 2017;9:2. https://doi.org/10.1186/s13195-016-0228-4
24. Montine TJ, Phelps CH, Beach TG, et al. National Institute on Aging–Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease: a practical approach. Acta Neuropathol 2012;123:1–11. https://doi.org/10.1007/s00401-011-0910-3
25. Mackenzie IRA, Neumann M, Bigio EH, et al. Nomenclature and nosology for neuropathologic subtypes of frontotemporal lobar degeneration: an update. Acta Neuropathol 2010;119:1–4. https://doi.org/10.1007/s00401-009-0612-2
26. Thal DR, Rüb U, Orantes M, Braak H. Phases of Aβ-deposition in the human brain and its relevance for the development of AD. Neurology 2002;58:1791–1800. https://doi.org/10.1212/WNL.58.12.1791
27. Mirra SS, Heyman A, McKeel D, et al. The consortium to establish a registry for Alzheimer's disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. Neurology 1991;41:479. https://doi.org/10.1212/wnl.41.4.479
28. Thal DR, Ghebremedhin E, Rüb U, et al. Two types of sporadic cerebral amyloid angiopathy. J Neuropathol Exp Neurol 2002;61:282–293. https://doi.org/10.1093/jnen/61.3.282
29. Armitage P. Tests for linear trends in proportions and frequencies. Biometrics 1955;11:375. https://doi.org/10.2307/3001775
30. Reimand J, Collij L, Scheltens P, et al. Amyloid-beta CSF/PET discordance vs tau load 5 years later: it takes two to tangle. medRxiv [preprint]. 2020. https://doi.org/10.1101/2020.01.29.20019539
31. Hansson O, Lehmann S, Otto M, et al. Advantages and disadvantages of the use of the CSF Amyloid β (Aβ) 42/40 ratio in the diagnosis of Alzheimer's Disease. Alzheimers Res Ther 2019;11:34. https://doi.org/10.1186/s13195-019-0485-0
32. Hansson O, Mikulskis A, Fagan AM, et al. The impact of preanalytical variables on measuring cerebrospinal fluid biomarkers for Alzheimer's disease diagnosis: a review. Alzheimer's Dement 2018;14:1313–1333. https://doi.org/10.1016/j.jalz.2018.05.008
33. Lowe VJ, Lundt ES, Albertson SM, et al. Neuroimaging correlates with neuropathologic schemes in neurodegenerative disease. Alzheimer's Dement 2019;15:927–939. https://doi.org/10.1016/j.jalz.2019.03.016
34. Murray ME, Lowe VJ, Graff-Radford NR, et al. Clinicopathologic and 11 C-Pittsburgh compound B implications of Thal amyloid phase across the Alzheimer's disease spectrum. Brain 2015;138:1370–1381. https://doi.org/10.1093/brain/awv050
35. Johnson KA, Gregas M, Becker JA, et al. Imaging of amyloid burden and distribution in cerebral amyloid angiopathy. Ann Neurol 2007;62:229–234. https://doi.org/10.1002/ana.21164
36. Charidimou A, Friedrich JO, Greenberg SM, Viswanathan A. Core cerebrospinal fluid biomarker profile in cerebral amyloid angiopathy. Neurology 2018;90:e754–e762. https://doi.org/10.1212/WNL.0000000000005030
37. Zhuang L-P, Liu C-Y, Li Y-X, et al. Clinical features and genetic characteristics of hereditary diffuse leukoencephalopathy with spheroids due to CSF1R mutation: a case report and literature review. Ann Transl Med 2020;8:11. https://doi.org/10.21037/atm.2019.12.17
38. Levin J, Tiedt S, Arzberger T, et al. Diffuse leukoencephalopathy with spheroids: biopsy findings and a novel mutation. Clin Neurol Neurosurg 2014;122:113–115. https://doi.org/10.1016/j.clineuro.2014.04.022
39. Spitzer P, Kohl Z, Golitz P, et al. Biochemical markers of neurodegeneration in hereditary diffuse leucoencephalopathy with spheroids. Case Rep 2014;2014: bcr2012008510. https://doi.org/10.1136/bcr-2012-008510
40. Trysberg E, Höglund K, Svenungsson E, et al. Decreased levels of soluble amyloid beta-protein precursor and beta-amyloid protein in cerebrospinal fluid of patients with systemic lupus erythematosus. Arthritis Res Ther 2004;6:129–136. https://doi.org/10.1186/ar1040
41. Mori F, Rossi S, Sancesario G, et al. Cognitive and cortical plasticity deficits correlate with altered amyloid-β CSF levels in multiple sclerosis. Neuropsychopharmacology 2011;36:559–568. https://doi.org/10.1038/npp.2010.187
42. Krut JJ, Zetterberg H, Blennow K, et al. Cerebrospinal fluid Alzheimer's biomarker profiles in CNS infections. J Neurol 2013;260:620–626. https://doi.org/10.1007/s00415-012-6688-y
43. Mattsson N, Bremell D, Anckarsäter R, et al. Neuroinflammation in Lyme neuroborreliosis affects amyloid metabolism. BMC Neurol 2010;10:51. https://doi.org/10.1186/1471-2377-10-51
44. Hayashi T, Ago K, Nakamae T, et al. Two different immunostaining patterns of beta-amyloid precursor protein (APP) may distinguish traumatic from nontraumatic axonal injury. Int J Legal Med 2015;129:1085–1090. https://doi.org/10.1007/s00414-015-1245-8
45. Ruland T, Wolbert J, Gottschalk MG, et al. Cerebrospinal fluid concentrations of neuronal proteins are reduced in primary angiitis of the central nervous system. Front Neurol 2018;9:1–9. https://doi.org/10.3389/fneur.2018.00407
46. Schöll M, Maass A, Mattsson N, et al. Biomarkers for tau pathology. Mol Cell Neurosci 2019;97:18–33. https://doi.org/10.1016/j.mcn.2018.12.001

47. Olsson B, Lautner R, Andreasson U, et al. CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis. Lancet Neurol 2016;15:673–684. https://doi.org/10.1016/S1474-4422(16)00070-3
48. Villemagne VL, Burnham S, Bourgeat P, et al. Amyloid β deposition, neurodegeneration, and cognitive decline in sporadic Alzheimer's disease: a prospective cohort study. Lancet Neurol 2013;12:357–367. https://doi.org/10.1016/S1474-4422(13)70044-9
49. Collij LE, Konijnenberg E, Reimand J, et al. Assessing amyloid pathology in cognitively normal subjects using 18 F-flutemetamol PET: comparing visual reads and quantitative methods. J Nucl Med 2019;60:541–547. https://doi.org/10.2967/jnumed.118.211532
50. van Berckel BNM, Ossenkoppele R, Tolboom N, et al. Longitudinal amyloid imaging using 11C-PiB: methodologic considerations. J Nucl Med 2013;54:1570–1576. https://doi.org/10.2967/jnumed.112.113654
51. Lewczuk P, Matzen A, Blennow K, et al. Cerebrospinal fluid Aβ42/40 corresponds better than Aβ42 to amyloid PET in Alzheimer's disease. J Alzheimer's Dis 2016;55:813–822. https://doi.org/10.3233/JAD-160722

