BRAIN
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Biomarker A+T−: is this Alzheimer's disease or not? A combined CSF and pathology study

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The biological definition of Alzheimer's disease using CSF biomarkers requires abnormal levels of both amyloid (A) and tau (T). However, biomarkers and corresponding cutoffs may not always reflect the presence or absence of pathology. Previous studies suggest that up to 32% of individuals with autopsy-confirmed Alzheimer's disease show normal CSF p-tau levels *in vivo*, but these studies are sparse and had small sample sizes. Therefore, in three independent autopsy cohorts, we studied whether or not CSF A+T− excluded Alzheimer's disease based on autopsy.

We included 215 individuals, for whom ante-mortem CSF collection and autopsy had been performed, from three cohorts: (i) the Amsterdam Dementia Cohort (ADC) [$n = 80$, 37 (46%) Alzheimer's disease at autopsy, time between CSF collection and death 4.5 ± 2.9 years]; (ii) the Antwerp Dementia Cohort (DEM) [$n = 92$, 84 (91%) Alzheimer's disease at autopsy, time CSF collection to death 1.7 ± 2.3 years]; and (iii) the Alzheimer's Disease Neuroimaging Initiative (ADNI) [$n = 43$, 31 (72%) Alzheimer's disease at autopsy, time CSF collection to death 5.1 ± 2.5 years]. Biomarker profiles were based on dichotomized CSF $A\beta_{1\text{-}42}$ and p-tau levels. The accuracy of CSF AT profiles to detect autopsy-confirmed Alzheimer's disease was assessed. Lastly, we investigated whether the concordance of AT profiles with autopsy diagnosis improved when CSF was collected closer to death in 9 (10%) DEM and 30 (70%) ADNI individuals with repeated CSF measurements available.

In total, 50–73% of A+T− individuals and 100% of A+T+ individuals had Alzheimer's disease at autopsy. Amyloid status showed the highest accuracy to detect autopsy-confirmed Alzheimer's disease (accuracy, sensitivity and specificity in the ADC: 88%, 92% and 84%; in the DEM: 87%, 94% and 12%; and in the ADNI cohort: 86%, 90% and 75%, respectively). The addition of CSF p-tau did not further improve these estimates. We observed no differences in demographics or degree of Alzheimer's disease neuropathology between A+T− and A+T+ individuals with autopsy-confirmed Alzheimer's disease. All individuals with repeated CSF measurements remained stable in $A\beta_{1\text{-}42}$ status during follow-up. None of the Alzheimer's disease individuals with a normal p-tau status changed to abnormal; however, four (44%) DEM individuals and two (7%) ADNI individuals changed from abnormal to normal p-tau status over time, and all had Alzheimer's disease at autopsy.

In summary, we found that up to 73% of A+T− individuals had Alzheimer's disease at autopsy. This should be taken into account in both research and clinical settings.

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Introduction

Recently, the National Institute on Aging and Alzheimer's Association (NIA-AA) introduced a research framework to biologically define Alzheimer's disease (AD) based on biomarkers, rather than clinical symptoms.[1] This biological definition for AD requires abnormal biomarkers for both amyloid (A) and tau (T), which can be measured in CSF with amyloid-$\beta_{1\text{-}42}$ ($A\beta_{1\text{-}42}$) and phosphorylated tau (p-tau), by PET imaging or in blood. Previous criteria using biomarker evidence for AD required at least an abnormal amyloid marker, regardless of the tau measure.[2] The AT classification system assumes that the combination of both abnormal amyloid and tau biomarkers most accurately reflects the presence of AD pathology, and that individuals who have only abnormal amyloid do not have AD but instead have Alzheimer's pathological change.

However, this approach is potentially problematic in two ways. First, it requires the dichotomization of amyloid and tau biomarkers. Ideally, cutoff values for dichotomization should be determined against the gold standard of autopsy-proven diagnoses. However, only a few studies have done this, since pathological data are scarce. As an alternative, cutoff points have often been determined based on distinguishing between clinical diagnosis of AD dementia versus controls,[3,4] which may not accurately reflect the presence of neuropathological changes.[1] This introduces uncertainty in determining the AT status of individuals. Secondly, one previous study reported that up to 32% of individuals with autopsy-confirmed AD can show normal p-tau levels in CSF *in vivo*,[5] which suggests that the criteria requiring both abnormal amyloid and abnormal tau status may be too strict and would miss a substantial number of individuals with AD neuropathology who have only abnormal amyloid. Still, the literature on the association between ante-mortem CSF biomarker status and post-mortem verified AD is sparse. Previous studies investigating biomarker accuracy in the context of autopsy-confirmed AD were often limited in sample size, did not include clinical diagnoses other than AD or only investigated single biomarkers and not combined markers in the context of the AT(N) framework.[5–7]

In this study, using three independent cohorts, we investigated whether individuals with abnormal CSF amyloid and normal p-tau levels (i.e. A+T− biomarker profile) had AD at autopsy and whether CSF p-tau status improved detection of autopsy-confirmed AD over CSF amyloid status alone. In addition, for individuals with repeated CSF measurements available, we investigated whether the concordance of CSF amyloid and tau status with autopsy diagnosis improved when CSF was collected closer to death.

Materials and methods

Participants and cohorts

We selected individuals from the Amsterdam Dementia Cohort (ADC), the Antwerp Dementia Cohort (DEM) and the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu) who had CSF $A\beta_{1\text{-}42}$and p-tau measurements available at first visit and subsequent post-mortem neuropathological examination. The institutional review boards of all participating institutions approved the procedures for this study. Written informed consent was obtained from all participants and/or their legal representatives. The ADC is comprised of individuals who visited the Alzheimer Center of the Amsterdam UMC, location VUmc.[8,9] Individuals visiting the memory clinic receive an extensive diagnostic workup for dementia, of which the results are discussed in weekly multidisciplinary meetings in order to reach a consensus clinical diagnosis according to the diagnostic and research guidelines of all major neurodegenerative diseases.[10–21] In the ADC, patients were selected regardless of clinical diagnosis. The DEM was selected from the Institute Born-Bunge Neurobiobank based on a neuropathological diagnosis of AD. Patients were recruited through the Memory Clinic of Hospital Network Antwerp (ZNA) Middelheim and Hoge Beuken and through other centres referring to the Neurobiobank of the Institute Born-Bunge.[22,23] ADNI is a public-private partnership led by Principal Investigator Michael W. Weiner MD and was started in 2003. ADNI's main aim is to validate AD biomarkers and investigate the use of these biomarkers in combination with clinical and neuropsychological examination to assess the clinical progression of AD individuals with mild cognitive impairment or early dementia. For up-to-date information, see www.adni-info.org.

CSF biomarker analyses

Lumbar puncture was performed using a standardized protocol with a small-bore atraumatic needle, and CSF was collected in sterile polypropylene collection tubes.[24] The mean time between CSF collection and death was 4.5 ± 2.9 years in the ADC, 1.7 ± 2.3 years in the DEM and 5.1 ± 2.5 years in the ADNI cohort. For both the ADC and DEM, $A\beta_{1\text{-}42}$ and p-tau concentrations were determined using enzyme-linked immunosorbent assays (ELISAs) [Innotest® β-AMYLOID(1-42) and Innotest PHOSPHO-TAU(181P), Fujirebio]. For the ADC, a drift-corrected cutoff of <813 pg/ml was applied to determine $A\beta_{1\text{-}42}$ abnormality,[25] and the clinically-validated cutoff

of >52 pg/ml was applied to determine p-tau abnormality.[4] For the DEM, the cutoffs were <638.5 pg/ml and >56.5 pg/ml, respectively (autopsy-based).[26] For the ADNI cohort, $A\beta_{1-42}$ and p-tau levels were analysed using the Roche Elecsys immunoassay platform as described previously.[27,28] A cutoff of <977 pg/ml was applied to determine $A\beta_{1-42}$ abnormality and >24 pg/ml for p-tau abnormality (clinically-validated cutoffs).[29] Biomarker profiles were based on dichotomized CSF $A\beta_{1-42}$ with p-tau (AT), resulting in four possible combinations for each profile: A−T−, A+T−, A−T+ and A+T+. For the ADC, a cutoff of >0.08 pg/ml was used to determine abnormality of the ratio of $A\beta_{1-42}$/p-tau,[6] and in the ADNI cohort, the cutoff was >0.025 pg/ml.[29]

Neuropathological examination

Neuropathological assessment of AD was performed according to the NIA-AA guidelines.[30,31] The ABC scoring system was used to describe AD neuropathological changes. The A score is based on the location of Aβ deposition (plaques) according to Thal phases 0 to 5,[32,33] which are converted to an A score of 0 (phase 0), 1 (phase 1 and 2), 2 (phase 3) or 3 (phase 4 and 5). The B score is based on the location of neurofibrillary tangles according to Braak stages 0–VI,[34,35] which are converted to a B score of 0 (phase 0), 1 (phase 1 and 2), 2 (phase 3 and 4) or 3 (phase 5 and 6). The C score is based on the density of neuritic plaques according to the CERAD stages,[31,36] which is converted to a C score of 0 (none), 1 (sparse), 2 (moderate) or 3 (frequent). Based on this score a diagnosis was given of 'no AD' or 'low', 'intermediate' or 'high' AD neuropathologic change. For a small subset of ADC participants ($n = 13$, 16%) criteria from the National Institute of Aging and Reagan Institute for the neuropathological assessment of AD were used.[37] For our analyses both 'intermediate' and 'high' AD neuropathologic change scores were considered as pathologically confirmed AD, while 'low' AD neuropathologic change scores were considered as absence of pathologically confirmed AD. In the ADC, of all individuals with available neuropathological diagnosis, a subset of 39 (49%) individuals had all A, B and C scores available. In the DEM, eight patients were found to have an 'ABC' score that would not be considered sufficient to explain dementia (i.e. seven had a score of 'low' AD neuropathologic change and one a score of 'no AD') but were diagnosed with definite AD by the exclusion of all other causes based on neuropathology.

Statistical analysis

Baseline demographic features were analyzed and compared using χ^2 tests, unpaired *t*-tests and Mann–Whitney U-tests, depending on type and distribution of the variable. We assessed the concordance, diagnostic accuracy, i.e. the proportion of correctly classified subjects [true positive (TP) + true negative (TN)] among all subjects (TP + TN + FP + FN), sensitivity and specificity of AT profiles to detect autopsy-confirmed AD. In order to analyse the additional value of p-tau to CSF $A\beta_{1-42}$ status, analyses were performed between all A+ and all A− individuals and between A+T+ individuals and individuals with other AT profiles. We used Spearman's rho (ρ) to determine correlations between CSF amyloid and p-tau levels with pathological scores A, B and C. We investigated differences in clinical diagnosis, demographic characteristics and in degree of AD neuropathology between A+T+ and A+T− individuals with and without autopsy-confirmed AD. Finally, a subset of individuals from the DEM ($n = 9$) and the ADNI cohort ($n = 30$) had repeated CSF measurements available (mean time between first and last CSF measurement 2.1 ± 1.8 and 2.9 ± 1.7 years, respectively), and for these we investigated changes in CSF $A\beta_{1-42}$ and p-tau status over time and whether these were in concordance with their autopsy diagnosis. All analyses were performed using R version 3.6.1 —'Action of the Toes'.[38]

Data availability

The data that support the findings of this study are available on request from the corresponding author. The data from the ADC and DEM are not publicly available due to privacy restrictions. ADNI data can be requested through their website.

Results

Cohort demographics

In total, we included 215 individuals with CSF collection and autopsy from the ADC [$n = 80$, median (IQR) age 62 (58–66) years, 29 (36%) female, 37 (46%) with AD at autopsy], the DEM [$n = 92$, median (IQR) age 76 (71–84) years, 45 (49%) female, 84 (91%) with AD at autopsy] and the ADNI cohort [$n = 43$, median (IQR) age 78 (73–83) years, 9 (21%) female, 31 (72%) with AD at autopsy] (Tables 1 and 2). The ADC individuals with AD at autopsy were older at time of death [median (IQR): 69 (66–74) years versus 65 (61–69) years, $P = 0.004$], more likely to be diagnosed with AD dementia ($P < 0.001$) and had a longer period between CSF collection and death [median (IQR) 5.2 (3.1–7.4) versus 3 (1.8–5.6) years, $P = 0.006$] compared with ADC individuals without AD at autopsy. The DEM individuals with AD at autopsy did not show differences in these characteristics from those without AD at autopsy. ADNI autopsy-confirmed AD individuals more frequently carried an *APOE* ε4 allele compared with those without AD at autopsy [21 (68%) versus 0 (0%), $P = 0.001$].

Concordance of AT and AN with Alzheimer's disease pathology

First, we investigated the concordance of the CSF AT profiles with the neuropathological diagnosis of AD. Of individuals with an A+T− profile, in the ADC 7/14 (50%) had AD at autopsy, in the DEM 19/26 (73%) and in the ADNI cohort 5/8 (63%). All individuals with an A+T+ profile had AD at autopsy ($n = 27$ in the ADC, $n = 60$ in the DEM, $n = 23$ in the ADNI cohort) (Fig. 1). Overall, amyloid status by itself showed the highest accuracy to detect the pathological diagnosis of AD (Table 3). When using only p-tau, accuracy decreased compared with using only amyloid status (respectively, 0.77–0.82 compared with 0.86–0.88); however, given the overlapping 95% confidence interval, the superiority of the amyloid biomarker should be taken with caution. Compared with amyloid alone, the combination of amyloid and p-tau did not improve the accuracy to detect the pathological diagnosis of AD. A+T+ status showed a higher specificity but also lower sensitivity compared with amyloid status alone. Using the ratio of p-tau/$A\beta_{1-42}$in the ADC and the ADNI cohort resulted in the highest accuracy to detect pathological AD.

Next, we studied relationships between CSF $A\beta_{1-42}$ and p-tau levels with separate pathological scores (ABC). In the subset of the ADC with available pathological scores ($n = 39$, 49%), lower $A\beta_{1-42}$ and higher p-tau levels were strongly correlated with higher pathological scores A, B and C (all $P < 0.001$) (Supplementary Table 1). In the ADNI cohort, the correlations between $A\beta_{1-42}$ and p-tau markers and pathological scores were similar to those of the ADC, albeit slightly weaker (all $P < 0.001$), and in the DEM only higher p-tau levels were correlated with higher pathological scores (all $P \leq 0.01$).

Table 1 Cohort demographics

	Total			ADC		DEM		ADNI	
	ADC	DEM	ADNI	No AD	AD	No AD	AD	No AD	AD
n	80	92	43	43	37	8	84	12	31
Demographics									
Sex (female)	29 (36%)	45 (49%)	9 (21%)	17 (40%)	12 (32%)	6 (75%)	39 (46.4%)	2 (16.7%)	7 (23%)
Age at baseline, years	62 (58–66)	76 (71–84)	78 (73–83)	61 (56–65)	63 (59–68)	81 (77–83)	76 (71–84)	80 (76–83)	77 (73–83)
Age at death, years	67 (64–70)	78 (73–86)	83 (78–86)	65 (61–69)	69 (66–74)**	83 (78–85)	77 (72–86)	84 (81–86)	81 (77–86)
Time between CSF collection and death, years	4 (2–6.1)	0.5 (0.1–2.8)	4.9 (3.1–7.1)	3 (1.8–5.6)	5.2 (3.1–7.4)**	0.1 (0.1–1.6)	0.6 (0.1–2.8)	4.5 (3.5–6.7)	5 (2.7–7.1)
APOE ε4 carrier	36 (51%)	35/64 (55%)	21 (49%)	16 (43%)	20 (61%)	1/4 (25%) *n* = 7	34/60 (56.7%) *n* = 74	0 (0%)	21 (68%)***
Clinical diagnosis									
Cognitively normal	3 (4%)	0	7 (16%)	1 (2%)	2 (5%)	0	0	4 (33.3%)	3 (10%)
Mild cognitive impairment	3 (4%)	2 (2.5%)	21 (49%)	1 (2%)	2 (5%)	0 (0%)	2 (2.7%)	8 (66.7%)	13 (42%)
AD dementia	28 (35%)	53 (65.4%)	15 (35%)	3 (7%)	25 (68%)***	2 (28.6%)	51 (68.9%)	0 (0%)	15 (48%)*
Non-AD dementia	35 (44%)[a]	14 (17.3%)[b]	—	30 (70%)	5 (14%)***	5 (71.4%)	9 (12.2%)**	—	—
Other	11 (14%)[c]	12 (14.8%)[d]	—	8 (19%)	3 (8%)	0 (0%)	12 (16.2%)	—	—

Cohort demographics of the ADC, DEM and ADNI cohort with comparisons made between the three cohorts and stratified for presence of pathological AD at autopsy. Results presented in *n* (%) or median (IQR).

[a]Including dementia with Lewy bodies (no AD *n* = 1; AD *n* = 2), frontotemporal lobar degeneration (no AD *n* = 19; AD *n* = 2), primary progressive aphasia (no AD *n* = 2), vascular dementia (no AD *n* = 1) and other dementias (no AD *n* = 7; AD *n* = 1).

[b]Including dementia with Lewy bodies (AD *n* = 1), frontotemporal lobar degeneration spectrum (no AD *n* = 1, AD *n* = 6), vascular dementia (no AD *n* = 1), Creutzfeldt-Jakob disease (no AD *n* = 2, AD *n* = 1), Parkinson's disease (no AD *n* = 1) and Charles Bonnet syndrome (AD *n* = 1).

[c]Diagnosis either psychiatric disorder, neurologic non-neurodegenerative disorder or postponed diagnosis due to clinical uncertainty.

[d]Diagnosis either mixed dementia (*n* = 3), unspecified dementia (*n* = 2) or clinical doubt between AD dementia and non-AD dementia (*n* = 7).

*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$ for AD versus no AD within cohort.

Table 2 Cohort biomarker concentrations

	ADC		DEM		ADNI	
	no AD	AD	no AD	AD	no AD	AD
n	43	37	8	84	12	31
$A\beta_{1\text{-}42}$ pg/ml	977 (861–1143)	640 (561–752)	475 (332–583)	442 (306–495)	1275 (1068–1703)	594 (445–726)
abnormal	7 (16%)	34 (92%)	7 (87.5%)	79 (94%)	3 (25%)	28 (90%)
P-tau pg/ml	39 (32–44)	78 (60–100)	48 (39–54)	75 (57–97)	18 (15–22)	36 (26–44)
abnormal	6 (14%)	29 (78%)	0 (0%)	63 (75%)	3 (25%)	25 (81%)
T-tau pg/ml	340 (258–449)	594 (453–830)	427 (302–796)	584 (375–1003)	198 (171–252)	330 (275–453)
abnormal	17 (40%)	31 (84%)	6 (75%)	71 (84.5%)	2 (17%)	25 (81%)

Baseline biomarker concentrations in the ADC, DEM and ADNI cohort, stratified for presence of pathological AD at autopsy. Results presented in median (IQR) or *n* (%). For ADC and ADNI all $P \leq 0.002$. For DEM p-tau all $P \leq 0.001$, for DEM $A\beta_{1\text{-}42}$ and t-tau all *P* not significant.

Comparison of A+T+ and A+T− individuals

We next studied if A+T− individuals with autopsy-confirmed AD differed in their clinical diagnosis, demographic characteristics or degree of AD neuropathology from A+T+ individuals with autopsy-confirmed AD (Supplementary Table 2). We did not find any differences, but this may also reflect that the group sizes were small. On a trend level, A+T− individuals with autopsy-confirmed AD were more often diagnosed with non-AD dementia, including dementia with Lewy bodies and frontotemporal dementia, compared to A+T+ individuals. Within the A+T− group, individuals with autopsy-confirmed AD did not differ from those with another autopsy diagnosis in terms of clinical and demographical information. In the ADC and the ADNI cohort, A+T− individuals without AD at autopsy had lower neuropathological B and C scores, and in the DEM they had lower A and B scores compared with A+T− individuals with AD at autopsy.

Stability over time in AT classification

For a subset of 9 (10%) DEM and 30 (70%) ADNI individuals with repeated CSF measurements (mean ± SD time between first and last measurement 2.1 ± 1.8 and 2.9 ± 1.7 years, respectively), we investigated the stability of AT profiles over time. Over the repeated measurements, all individuals showed a stable $A\beta_{1\text{-}42}$ status. None changed from A+T− to A+T+. In the DEM, four individuals changed from A+T+ to A+T− over time (all pathological diagnosis of AD and clinical diagnosis of dementia at first CSF measurement). In the ADNI cohort, the majority of individuals showed a stable tau status [*n* = 28 (93%)]. Two individuals changed from A+T+ to A+T− (all pathological diagnosis of AD, one with clinical diagnosis of dementia at first CSF measurements and one with mild cognitive impairment at first measurement and progression to dementia at last CSF measurement).

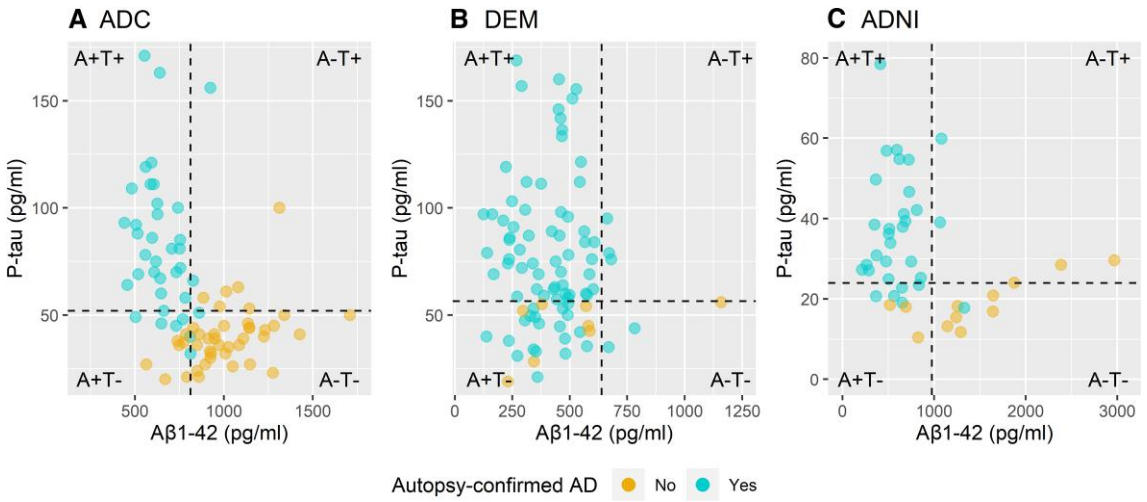

Figure 1 Neuropathological confirmation of AD and biomarker AT classification. (**A**) ADC, (**B**) DEM and (**C**) ADNI. The dotted vertical lines correspond to the $A\beta_{1\text{-}42}$ cutoffs of <813 pg/ml for ADC, <638.5 pg/ml for DEM and <977 pg/ml for ADNI. The dotted horizontal lines correspond to the p-tau cutoffs of >52 pg/ml for ADC, >56.5 pg/ml for DEM and >24 pg/ml for ADNI.

Discussion

In the current biomarker-based definition of AD, individuals with an A+T– profile are considered to have 'AD pathologic change', but not AD, with the suggestion that a T– profile reflects a lack of tangles. However, biomarkers and their corresponding cutoffs may not always accurately reflect the presence or absence of pathology, and here we studied whether a CSF biomarker A+T– profile indeed excluded AD based on autopsy. We found that up to 73% of A+T– individuals did have AD at autopsy. Furthermore, we found that CSF amyloid abnormality (i.e. A+ status), irrespective of p-tau abnormality (i.e. T+ status), showed the highest accuracy to detect a neuropathological diagnosis of AD. Adding p-tau increased the specificity, but at the cost of decreased sensitivity over amyloid status by itself. Our results imply that a biomarker profile with abnormal CSF amyloid and normal p-tau levels does not exclude the presence of AD at autopsy.

In our study, 19 to 25% of all autopsy-confirmed AD individuals showed normal CSF p-tau levels *in vivo* (while $A\beta_{1\text{-}42}$ was abnormal), which is in line with previously reported sensitivities of p-tau to detect AD pathology, which range between 66 and 85% (see Supplementary material). These previous studies[5,6,22,39–44] also show that $A\beta_{1\text{-}42}$by itself is the most sensitive predictor for the post-mortem pathological diagnosis of AD. In the current AD research framework, these A+T– individuals would be considered to show AD pathological change and might be incorrectly labelled as not having AD (at time of measurement). For research purposes, it may be preferable to label these cases as biological AD, since this profile does not exclude pathological AD and may represent a distinct subtype of AD with possibly different biological characteristics. Alternatively, the ratio of p-tau/$A\beta_{1\text{-}42}$ may be used for the diagnosis of AD, since our study showed that the application of this ratio improved accuracy compared with amyloid status alone. Still, in the ADNI cohort, but not in the ADC, this ratio was more likely to be abnormal in A+T– individuals with autopsy-confirmed AD. While using only the amyloid marker resulted in a lower specificity, nearly all A+ cases without pathologically-confirmed AD did show some degree of plaques and/or tangles, indicating that these individuals too could potentially benefit from amyloid and tau modifying treatments. It should be noted that depending on the research goal, sensitivity and specificity should be weighed up, and when the intention is to reduce false positives, A+T+ is preferred over amyloid status alone.

One explanation of negative tau markers in individuals with pathologically-confirmed AD could be that CSF p-tau levels may rise later. However, our comparison of last known AT profiles before death to first AT profile measurements showed that the majority of individuals remained stable, and those who changed, changed from T+ to T–, even though they had AD at autopsy. This suggests that the diagnostic value of CSF tau status varies depending on the clinical stage of AD, with lower accuracy later in the disease.[45] Possibly, the change from T+ to T– closer to autopsy may reflect a change in tau metabolism, as Mattsson-Carlgren *et al.*[46] showed that altered tau metabolism and subsequent CSF p-tau increases occur early in AD pathogenesis, preceding tau deposition. Together with our findings, this suggests that *in vivo* CSF p-tau measures may reflect alterations in other processes in response to amyloid and in addition to the presence of insoluble tau aggregates. Recent research has shown that tau is physiologically secreted[47] and that tau production is enhanced by amyloid pathology.[48] Possibly, individuals with A+T– profiles have physiologically low tau processing, which may result in low CSF p-tau levels despite the presence of amyloid and tangle pathology. A recent CSF proteomics study identified different AD subtypes of which one, characterized by blood–brain barrier dysfunction and hypoplasticity, showed lower p-tau and t-tau levels.[49] This has also been reported in a combined proteomics and genetic study by Visser.[50] For individuals with an A+T– profiles but without AD at autopsy, it could be possible that all biomarkers are lower due to a CSF flow issue, as is frequently seen in individuals with hydrocephalus.[51] However, these studies[49,50] also noted specific proteins to be increased, including e.g. neurofilament light.

Our results showing normal tau profiles in AD urge us to re-examine the definition of T status when based on CSF in AD. Possibly, CSF p-tau cutoffs need recalibration, as in our study some AD individuals with A+T– profiles had CSF p-tau concentrations close to the cutoffs, and not all cutoffs were validated on autopsy-confirmed cases. Still, in general dichotomous cutoffs

Table 3 Accuracy of CSF biomarkers for each cohort

	ADC							DEM							ADNI						
CSF biomarker	TP	FN	FP	TN	A (95% CI)	Se	Sp	TP	FN	FP	TN	A (95% CI)	Se	Sp	TP	FN	FP	TN	A (95% CI)	Se	Sp
$A\beta_{1-42}$ status	34	3	7	36	0.88 (0.78–0.94)	0.92	0.84	79	5	7	1	0.87 (0.78–0.93)	0.94	0.12	28	3	3	9	0.86 (0.72–0.95)	0.9	0.75
P-tau status	29	8	6	37	0.82 (0.72–0.9)	0.78	0.86	63	21	0	8	0.77 (0.67–0.85)	0.75	1	25	6	3	9	0.79 (0.64–0.9)	0.81	0.75
$A\beta_{1-42}$ and p-tau status	27	10	0	43	0.88 (0.78–0.94)	0.73	1	60	24	0	8	0.74 (0.64–0.83)	0.71	1	23	8	0	12	0.81 (0.67–0.92)	0.74	1
P-tau/$A\beta_{1-42}$ ratio	28	9	0	43	0.89 (0.8–0.95)	0.76	1								30	1	2	10	0.93 (0.81–0.99)	0.97	0.83

A = Accuracy; CI = confidence interval; FN = false negative; FP = false positive; Se = sensitivity; Sp = specificity; TN = true negative; TP = true positive.

will never be 100% accurate, and a recent study showed that CSF tau shows four subgroups rather than a bimodal distribution.[52] Cutoffs allowing for a grey zone may therefore have added value for the diagnosis of AD.

A potential limitation of this study is that sample sizes remained relatively small per cohort, which may have resulted in limited statistical power for subgroup comparisons on clinical and demographical characteristics. Furthermore, the small sample sizes may have attributed to overlapping 95% confidence intervals of biomarker accuracies, and so superiority of the CSF amyloid biomarker over CSF p-tau should be interpreted with caution. Regardless, we were able to replicate our main results in three independent cohorts. Future studies with larger samples of both *in vivo* AD biomarkers and post-mortem autopsy are needed in order to better investigate differences between A+T+ and A+T− AD subgroups. Another potential limitation is that the DEM was selected based on a neuropathological diagnosis of AD according to the pathologists' conclusion and contained only a limited number of individuals without AD according to our criteria, thus evoking the risk of selection bias. Out of eight DEM individuals that did not fulfill our criteria for pathological AD, seven had 'low' AD pathological change in combination with an abnormal CSF amyloid marker and no other neuropathological diagnosis, which might explain the low specificity found for CSF amyloid in this cohort. Clinically these individuals were often diagnosed with non-AD dementia, and so these individuals may represent atypical AD cases or clinically mild AD cases with comorbidities affecting cognition. Finally, we studied AT definitions based on CSF markers only, and so it remains unclear whether these results generalize to other methods to measure amyloid and tau, including plasma p-tau markers and tau PET. Studies have shown that both CSF and plasma p-tau markers become abnormal earlier than tau PET[53,54] and are rather markers in response to early amyloidosis and/or altered tau metabolism,[45] while tau PET correlates better with tangle pathology. On the other hand, tau PET becomes abnormal only in more advanced disease stages when more widespread tau pathology is present[55,56] and may therefore be less sensitive to earlier pathological changes. Different T markers seem to (partially) provide independent information, and so in order to choose the best fitting definition for T for each study, these biomarker properties need to be taken into account. Future studies should further aim to include all biomarker modalities to investigate and directly compare their accuracy to detect pathology in autopsy-confirmed studies. A strength of this study is that this is one of few studies that also had autopsy confirmation for individuals with intact cognition as well as with other clinical non-AD dementia diagnoses including dementia with Lewy bodies and frontotemporal dementia. Furthermore, since the cohorts used two different assay platforms, we have been able to determine that our results reflect general processes, rather than peculiarities of a specific assay.

In conclusion, our results indicate that normal CSF p-tau levels in combination with an abnormal amyloid marker do not exclude the presence of AD at autopsy. This should be taken into account in both research and clinical settings.

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Supplementary material

Supplementary material is available at *Brain* online.

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