

Changes in five cerebrospinal fluid and three plasma Alzheimer's disease biomarkers across increasing brain amyloid-beta and tau pathology burden

Andrea Mastrangelo^{1,2}, Simone Baiardi², Edoardo Ruggeri², Giuseppe Mario Bentivenga¹, Claudia Marina Vargiu², Angela Mammana², Marco Sbriccoli³, Barbara Polischi², Benedetta Carlà², Sabina Capellari^{1,2}, Piero Parchi^{1,2}

¹Department of Biomedical and Neuromotor Sciences, University of Bologna, Bologna, Italy

²IRCCS, Istituto delle Scienze Neurologiche di Bologna, Bologna, Italy

³Department of Neuroscience, Istituto Superiore di Sanità, Rome, Italy

Correspondence to Piero Parchi:

Email: piero.parchi@unibo.it

Phone number: +39-051496674

SUPPLEMENTARY TABLES

Supplementary Table 1 - Demographic and basic neuropathological features in the non-prion subgroup with complete CSF biomarker availability

	Non-prion CSF cohort with complete CSF biomarker availability (n=61)
Age at CSF sampling^a	74.1 (63.1-78.6)
Age at death^a	74.6 (66.0-80.0)
Females	35 (57.4)
APOE ε4 carriers	10 (16.4)
Time CSF sampling-death^b	1.0 (0.2-7.8)
Thal phase	
0	17 (27.9)
1-2	15 (24.6)
3	16 (26.2)
4-5	13 (21.3)
Braak stage	
0	24 (39.3)
1-2	23 (37.7)
3-4	12 (19.7)
5-6	2 (3.3)
ADNC level	
Not AD	17 (27.9)
Low	30 (49.2)
Intermediate	12 (19.7)
High	2 (3.3)
Aβ score quartiles	
Reference	15 (24.6)
1st quartile	14 (22.9)
2 nd quartile	14 (22.9)
3 rd quartile	8 (13.1)
4 th quartile	10 (16.4)
Tau score quartiles	
Reference	14 (22.9)
1st quartile	17 (27.9)
2 nd quartile	10 (16.4)
3 rd quartile	13 (21.3)
4 th quartile	7 (11.5)

a: expressed in years; b: expressed in months.

Continuous variables are expressed as median (interquartile range). Categorical variables are expressed as number (%). Abbreviations: AD, Alzheimer's disease; ADNC, Alzheimer's disease neuropathologic change; CSF, cerebrospinal fluid

Supplementary Table 2 - Details on primary neuropathological diagnoses of participants included in the study

	Entire cohort (N=250)	CSF cohort (n=230)	Plasma cohort (n=101)
Prion disease	162	159	41
sCJD MM(V)1	63	61	19
sCJD MM(V1) + 2C	28	27	4
sCJD VV2	19	19	6
sCJD MV2K	17	17	4
sCJD MV2K+2C	3	3	0
sCJD MM(V)2C	4	4	0
sCJD MM(V)2C+1	5	5	1
sCJD MM2T+2C	1	1	0
sCJD VV1	2	2	1
sCJD VM1	1	1	0
VPSPr	1	1	1
gCJD, M1	15	15	4
gCJD, atypical	1	1	1
FFI	2	2	0
Non-prion disease	88	71	60
AD, High ADNC	6	4	6
AD, Intermediate ADNC	9	9	5
AD, Low ADNC	10	8	8
LBD, neocortical subtype	7	4	5
FTLD, TDP-43	5	3	5
FTLD, tau	2	2	0
MSA	7	5	5
PART	3	2	1
Encephalitis	6	5	5
IRIS encephalitis	2	2	2
Vascular disease ^a	10	8	8
Neoplastic disease ^b	6	5	2
Wernicke's encephalopathy	4	3	1
Other ^c	11	11	7

a: including subcortical ischemic vascular disease (n=6), cerebral infarction (n=3), haemorrhagic subacute encephalopathy (n=1); b: including primary central nervous system lymphoma (n=2), intravascular lymphoma (n=2), neoplastic meningitis (n=2); c: including lymphocytic meningitis (n=2), progressive multifocal leukoencephalopathy (n=1), not otherwise specified leukoencephalopathy (n=1), non-specific neuropathologic changes (n=7)

Abbreviations: AD, Alzheimer's disease; ADNC, Alzheimer's disease neuropathologic change; CSF, cerebrospinal fluid; FFI, fatal familial insomnia; FTLD, frontotemporal lobar degeneration; gCJD, genetic Creutzfeldt-Jakob disease; IRIS, immune reconstitution inflammatory syndrome; LBD, Lewy Body disease; MSA, multiple system atrophy; PART, primary age-related tauopathy; sCJD, sporadic Creutzfeldt-Jakob disease; VPSPr, variably protease-sensitive prionopathy

Supplementary Table 3 - Associations of biofluid biomarkers with continuous values of A β and tau pathology scores

		Aβ score	Tau score	Combined model	
				Aβ score	Tau score
CSF cohort					
CSF Aβ ₄₂ /Aβ ₄₀	β coefficient	-0.01 (-0.02 to -0.01)	-0.02 (-0.02 to -0.02)	-0.01 (-0.01 to -0.01)	-0.004 (-0.01 to -0.001)
	p value	<0.001	<0.001	<0.001	0.021
Non-prion CSF cohort					
CSF Aβ ₄₂ /Aβ ₄₀	β coefficient	-0.02 (-0.02 to -0.01)	-0.02 (-0.03 to -0.01)	-0.01 (-0.02 to -0.01)	-0.001 (-0.01 to 0.003)
	p value	<0.001	<0.001	<0.001	0.495
CSF p-tau ₁₈₁	β coefficient	0.02 (0.01 to 0.03)	0.04 (0.03 to 0.05)	0.01 (-0.002 to 0.02)	0.03 (0.01 to 0.05)
	p value	<0.001	<0.001	0.098	0.002
CSF Aβ ₄₂ /p-tau ₁₈₁	β coefficient	-0.03 (-0.04 to -0.03)	-0.06 (-0.07 to -0.05)	-0.01 (-0.02 to -0.004)	-0.04 (-0.06 to -0.03)
	p value	<0.001	<0.001	0.006	<0.001
CSF p-tau ₂₁₇	β coefficient	0.04 (0.03 to 0.05)	0.08 (0.06 to 0.10)	0.02 (0.005 to 0.03)	0.05 (0.02 to 0.08)
	p value	<0.001	<0.001	0.008	<0.001
CSF Aβ ₄₂ /p-tau ₂₁₇	β coefficient	-0.05 (-0.06 to -0.04)	-0.09 (-0.11 to -0.07)	-0.03 (-0.04 to -0.01)	-0.05 (-0.08 to -0.03)
	p value	<0.001	<0.001	<0.001	<0.001
Plasma cohort					
Plasma Aβ ₄₂ /Aβ ₄₀	β coefficient	-0.001 (-0.003 to 0.001)	0.001 (-0.002 to 0.005)	-0.003 (-0.006 to -0.0004)	0.005 (0.001 to 0.01)
	p value	0.234	0.533	0.026	0.047
Non-prion plasma cohort					
Plasma Aβ ₄₂ /Aβ ₄₀	β coefficient	-0.0005 (-0.003 to 0.002)	0.002 (-0.002 to 0.006)	-0.003 (-0.008 to 0.0009)	0.006 (-0.0005 to 0.01)
	p value	0.702	0.333	0.116	0.071
Plasma p-tau ₂₁₇	β coefficient	0.02 (0.01 to 0.03)	0.03 (0.02 to 0.04)	0.007 (-0.01 to 0.02)	0.02 (0.004 to 0.04)
	p value	<0.001	<0.001	0.303	0.019
Plasma p-tau ₂₁₇ /Aβ ₄₂	β coefficient	0.02 (0.01 to 0.03)	0.04 (0.02 to 0.05)	0.004 (-0.01 to 0.02)	0.03 (0.01 to 0.05)
	p value	<0.001	<0.001	0.558	<0.001

Results are derived from multivariable linear regression models with log-transformed biomarker values as dependent variables and continuous values of A β and tau score, both separately and as simultaneous predictors as independent variables. Separate models were fitted for each biomarker-pathology association. All models were adjusted for age at CSF/plasma sampling, sex and time between CSF/plasma sampling and death; models including participants with prion disease were additionally adjusted for prion disease status. Analyses on CSF p-tau₂₁₇ and A β_{42} /p-tau₂₁₇ were performed in a subgroup (n=61). β coefficients are reported as value (95% confidence interval). P values of statistically significant associations are shown in bold. Abbreviations: CSF, cerebrospinal fluid

Supplementary Table 4 - Association of A β and tau pathology score levels with changes in CSF and plasma biomarkers

A β pathology score					
	β coefficient	p value		β coefficient	p value
CSF cohort (n=230)			Plasma cohort (n=101)		
CSF A β_{42} /A β_{40}			Plasma A β_{42} /A β_{40}		
1 st quartile	-0.02 (-0.10 to 0.05)	0.567	1 st quartile	-0.007 (-0.12 to 0.11)	0.896
2 nd quartile	-0.11 (-0.19 to -0.04)	0.001	2 nd quartile	-0.04 (-0.15 to 0.08)	0.527
3 rd quartile	-0.42 (-0.51 to -0.34)	<0.001	3 rd quartile	-0.14 (-0.26 to -0.02)	0.025
4 th quartile	-0.62 (-0.71 to -0.54)	<0.001	4 th quartile	-0.02 (-0.14 to 0.09)	0.670
Non-prion CSF cohort (n=71)			Non-prion plasma cohort (n=60)		
CSF A β_{42} /A β_{40}			Plasma A β_{42} /A β_{40}		
1 st quartile	-0.05 (-0.18 to 0.09)	0.488	1 st quartile	-0.07 (-0.25 to 0.10)	0.390
2 nd quartile	-0.23 (-0.36 to -0.09)	0.001	2 nd quartile	-0.02 (-0.19 to 0.15)	0.849
3 rd quartile	-0.57 (-0.73 to -0.41)	<0.001	3 rd quartile	0.03 (-0.17 to 0.23)	0.760
4 th quartile	-0.80 (-0.95 to -0.65)	<0.001	4 th quartile	-0.06 (-0.24 to 0.12)	0.504
CSF p-tau ₁₈₁			Plasma p-tau ₂₁₇		
1 st quartile	0.03 (-0.38 to 0.44)	0.886	1 st quartile	-0.13 (-0.70 to 0.44)	0.653
2 nd quartile	0.23 (-0.18 to 0.65)	0.261	2 nd quartile	-0.06 (-0.63 to 0.51)	0.828
3 rd quartile	0.64 (0.15 to 1.13)	0.011	3 rd quartile	0.42 (-0.25 to 1.08)	0.213
4 th quartile	1.20 (0.73 to 1.67)	<0.001	4 th quartile	0.86 (0.26 to 1.46)	0.005
CSF A β_{42} p-tau ₁₈₁			Plasma p-tau ₂₁₇ /A β_{42}		
1 st quartile	-0.05 (-0.50 to 0.40)	0.830	1 st quartile	-0.07 (-0.64 to 0.50)	0.794
2 nd quartile	-0.36 (-0.82 to 0.09)	0.113	2 nd quartile	-0.06 (-0.63 to 0.50)	0.822
3 rd quartile	-1.33 (-1.86 to -0.79)	<0.001	3 rd quartile	0.32 (-0.34 to 0.98)	0.333
4 th quartile	-1.66 (-2.19 to -1.15)	<0.001	4 th quartile	0.91 (0.32 to 1.51)	0.003
CSF p-tau ₂₁₇					
1 st quartile	0.23 (-0.30 to 0.77)	0.387			
2 nd quartile	0.50 (-0.05 to 1.04)	0.074			
3 rd quartile	1.19 (0.52 to 1.86)	<0.001			
4 th quartile	2.18 (1.53 to 2.82)	<0.001			
CSF A β_{42} p-tau ₂₁₇					
1 st quartile	-0.19 (-0.73 to 0.35)	0.478			
2 nd quartile	-0.65 (-1.20 to -0.11)	0.020			
3 rd quartile	-1.55 (-2.22 to -0.87)	<0.001			
4 th quartile	-2.57 (-3.21 to -1.92)	<0.001			
Tau pathology score					
Non-prion CSF cohort (n=71)			Non-prion plasma cohort (n=60)		
CSF p-tau ₁₈₁			Plasma p-tau ₂₁₇		
1 st quartile	-0.15 (-0.55 to 0.26)	0.472	1 st quartile	-0.29 (-0.81 to 0.23)	0.271
2 nd quartile	0.18 (-0.30 to 0.67)	0.448	2 nd quartile	0.15 (-0.51 to 0.82)	0.641
3 rd quartile	0.82 (0.32 to 1.32)	0.001	3 rd quartile	-0.27 (-0.95 to 0.41)	0.430
4 th quartile	1.12 (0.63 to 1.62)	<0.001	4 th quartile	0.68 (0.06 to 1.29)	0.032
CSF A β_{42} p-tau ₁₈₁			Plasma p-tau ₂₁₇ /A β_{42}		
1 st quartile	-0.13 (-0.53 to 0.26)	0.501	1 st quartile	-0.09 (-0.58 to 0.39)	0.698
2 nd quartile	-0.36 (-0.83 to 0.11)	0.128	2 nd quartile	0.11 (-0.51 to 0.73)	0.718
3 rd quartile	-1.16 (-1.64 to -0.67)	<0.001	3 rd quartile	-0.03 (-0.66 to 0.60)	0.924
4 th quartile	-2.14 (-2.62 to -1.65)	<0.001	4 th quartile	1.05 (0.48 to 1.62)	<0.001
CSF p-tau ₂₁₇					
1 st quartile	-0.27 (-0.85 to 0.30)	0.348			
2 nd quartile	0.03 (-0.65 to 0.72)	0.921			
3 rd quartile	1.12 (0.41 to 1.83)	0.002			
4 th quartile	1.90 (1.11 to 2.69)	<0.001			
CSF A β_{42} p-tau ₂₁₇					
1 st quartile	-0.02 (-0.60 to 0.54)	0.928			
2 nd quartile	-0.21 (-0.89 to 0.46)	0.529			
3 rd quartile	-1.44 (-2.14 to -0.74)	<0.001			
4 th quartile	-2.71 (-3.49 to -1.93)	<0.001			

Results are derived from multivariable linear regression models with log-transformed biomarker values as dependent variables and categorical quartiles of A β or tau pathology scores as independent variables. Separate models were fitted for each biomarker-pathology association. All models were adjusted for age at CSF/plasma sampling, sex and time between CSF/plasma sampling and death; models including participants with prion disease were additionally adjusted for prion disease status. In each model, the reference category consisted of participants with a corresponding pathology score of 0. β coefficients are reported as value (95% confidence interval). Analyses on CSF p-tau₂₁₇ and A β ₄₂/p-tau₂₁₇ were performed in a subgroup (n=61). β coefficients and p values corresponding to pathology levels showing statistically significant differences from the reference group are indicated in bold. Abbreviations: CSF, cerebrospinal fluid

Supplementary Table 5 - Association of A β and tau pathology score levels with changes in CSF biomarkers in the subgroup of non-prion participants with complete CSF biomarker availability

A β pathology score		
	β coefficient	p value
Non-prion CSF cohort (n=61)		
CSF A β_{42} /A β_{40}		
1 st quartile	-0.05 (-0.20 to 0.10)	0.528
2 nd quartile	-0.25 (-0.41 to -0.10)	0.002
3 rd quartile	-0.56 (-0.75 to -0.37)	<0.001
4 th quartile	-0.80 (-0.99 to -0.62)	<0.001
CSF p-tau ₁₈₁		
1 st quartile	0.12 (-0.32 to 0.57)	0.573
2 nd quartile	0.36 (-0.09 to 0.81)	0.118
3 rd quartile	0.65 (0.09 to 1.20)	0.023
4 th quartile	1.21 (0.68 to 1.74)	<0.001
CSF A β_{42} p-tau ₁₈₁		
1 st quartile	-0.08 (-0.52 to 0.35)	0.706
2 nd quartile	-0.51 (-0.96 to -0.07)	0.024
3 rd quartile	-1.01 (-1.55 to -0.46)	<0.001
4 th quartile	-1.60 (-2.13 to -1.08)	<0.001
Tau pathology score		
Non-prion CSF cohort (n=61)		
CSF p-tau ₁₈₁		
1 st quartile	-0.20 (-0.64 to 0.24)	0.369
2 nd quartile	0.03 (-0.49 to 0.55)	0.903
3 rd quartile	0.82 (0.29 to 1.36)	0.003
4 th quartile	1.05 (0.45 to 1.66)	<0.001
CSF A β_{42} p-tau ₁₈₁		
1 st quartile	-0.10 (-0.52 to 0.32)	0.637
2 nd quartile	-0.21 (-0.72 to 0.29)	0.402
3 rd quartile	-1.14 (-1.66 to -0.62)	<0.001
4 th quartile	-1.86 (-2.45 to -1.28)	<0.001

Results are derived from multivariable linear regression models with log-transformed biomarker values as dependent variables and categorical quartiles of A β or tau pathology scores as independent variables. Separate models were fitted for each biomarker-pathology association. All models were adjusted for age at CSF sampling, sex and time between CSF sampling and death. In each model, the reference category consisted of participants with a corresponding pathology score of 0. β coefficients are reported as value (95% confidence interval). β coefficients and p values corresponding to pathology levels showing statistically significant differences from the reference group are indicated in bold. Abbreviations: CSF, cerebrospinal fluid

Supplementary Table 6 - Association of tau pathology score levels with changes in CSF and plasma biomarkers in the subgroup of participants without PART

Tau pathology score					
Non-prion CSF cohort, non-PART (n=65)			Non-prion plasma cohort, non-PART (n=57)		
CSF <i>p</i> -tau ₁₈₁			Plasma <i>p</i> -tau ₂₁₇		
1 st quartile	-0.15 (-0.55 to 0.25)	0.451	1 st quartile	-0.27 (-0.81 to 0.27)	0.319
2 nd quartile	0.34 (-0.21 to 0.90)	0.220	2 nd quartile	0.15 (-0.61 to 0.91)	0.697
3 rd quartile	0.84 (0.34 to 1.34)	0.001	3 rd quartile	-0.27 (-0.98 to 0.43)	0.443
4 th quartile	1.14 (0.65 to 1.63)	<0.001	4 th quartile	0.68 (0.04 to 1.32)	0.037
CSF Aβ ₄₂ <i>p</i> -tau ₁₈₁			Plasma <i>p</i> -tau ₂₁₇ /Aβ ₄₂		
1 st quartile	-0.13 (-0.53 to 0.26)	0.504	1 st quartile	-0.16 (-0.63 to 0.30)	0.494
2 nd quartile	-0.38 (-0.93 to 0.18)	0.181	2 nd quartile	0.16 (-0.49 to 0.82)	0.614
3 rd quartile	-1.21 (-1.71 to -0.71)	<0.001	3 rd quartile	0.02 (-0.59 to 0.63)	0.944
4 th quartile	-2.15 (-2.64 to -1.65)	<0.001	4 th quartile	1.09 (0.53 to 1.64)	<0.001
CSF <i>p</i> -tau ₂₁₇					
1 st quartile	-0.29 (-0.88 to 0.30)	0.323			
2 nd quartile	0.26 (-0.52 to 1.06)	0.503			
3 rd quartile	1.13 (0.40 to 1.86)	0.003			
4 th quartile	1.89 (1.09 to 2.69)	<0.001			
CSF Aβ ₄₂ <i>p</i> -tau ₂₁₇					
1 st quartile	-0.02 (-0.60 to 0.57)	0.949			
2 nd quartile	-0.31 (-1.09 to 0.48)	0.437			
3 rd quartile	-1.49 (-2.22 to -0.76)	<0.001			
4 th quartile	-2.69 (-3.49 to -1.89)	<0.001			

Results are derived from multivariable linear regression models with log-transformed biomarker values as dependent variables and categorical quartiles of tau pathology scores as independent variables. Separate models were fitted for each biomarker. All models were adjusted for age at CSF/plasma sampling, sex and time between CSF/plasma sampling and death. In each model, the reference category consisted of participants with a tau pathology score of 0. β coefficients are reported as value (95% confidence interval). Analyses on CSF *p*-tau₂₁₇ and Aβ₄₂/*p*-tau₂₁₇ were performed in a subgroup (n=56). β coefficients and p values corresponding to pathology levels showing statistically significant differences from the reference group are indicated in bold. Abbreviations: CSF, cerebrospinal fluid; PART, primary age-related tauopathy

Supplementary Table 7 - Association of tau pathology score levels with changes in CSF and plasma biomarkers after correction for A β pathology score

Tau pathology score					
Non-prion CSF cohort (n=71)			Non-prion plasma cohort (n=60)		
CSF p-tau ₁₈₁			Plasma p-tau ₂₁₇		
1 st quartile	-0.17 (-0.57 to 0.22)	0.388	1 st quartile	-0.34 (-0.84 to 0.17)	0.185
2 nd quartile	0.11 (-0.36 to 0.59)	0.632	2 nd quartile	0.03 (-0.61 to 0.67)	0.922
3 rd quartile	0.58 (0.02 to 1.13)	0.041	3 rd quartile	-0.67 (-1.40 to 0.06)	0.071
4 th quartile	0.72 (0.07 to 1.37)	0.031	4 th quartile	0.08 (-0.68 to 0.85)	0.822
CSF A β ₄₂ p-tau ₁₈₁			Plasma p-tau ₂₁₇ /A β ₄₂		
1 st quartile	-0.10 (-0.47 to 0.28)	0.606	1 st quartile	-0.12 (-0.60 to 0.36)	0.607
2 nd quartile	-0.27 (-0.72 to 0.18)	0.242	2 nd quartile	0.03 (-0.58 to 0.65)	0.918
3 rd quartile	-0.82 (-1.35 to -0.30)	0.002	3 rd quartile	-0.29 (-0.99 to 0.41)	0.410
4 th quartile	-1.58 (-2.20 to -0.96)	<0.001	4 th quartile	0.67 (-0.06 to 1.40)	0.073
CSF p-tau ₂₁₇					
1 st quartile	-0.34 (-0.87 to 0.19)	0.204			
2 nd quartile	-0.15 (-0.79 to 0.48)	0.625			
3 rd quartile	0.56 (-0.16 to 1.29)	0.125			
4 th quartile	0.92 (0.003 to 1.85)	0.049			
CSF A β ₄₂ p-tau ₂₁₇					
1 st quartile	0.05 (-0.43 to 0.54)	0.815			
2 nd quartile	0.02 (-0.56 to 0.61)	0.937			
3 rd quartile	-0.74 (-1.41 to -0.07)	0.030			
4 th quartile	-1.49 (-2.34 to -0.64)	<0.001			

Results are derived from multivariable linear regression models with log-transformed biomarker values as dependent variables and categorical quartiles of tau pathology scores as independent variables. Separate models were fitted for each biomarker. All models were adjusted for age at CSF/plasma sampling, sex, time between CSF/plasma sampling and death and continuous A β pathology score. In each model, the reference category consisted of participants with a tau pathology score of 0. β coefficients are reported as value (95% confidence interval). Analyses on CSF p-tau₂₁₇ and A β ₄₂/p-tau₂₁₇ were performed in a subgroup (n=61). β coefficients and p values corresponding to pathology levels showing statistically significant differences from the reference group are indicated in bold. Abbreviations: CSF, cerebrospinal fluid

Supplementary Table 8 - Association of Thal phase levels with changes in CSF and plasma biomarkers

Thal phase					
	β coefficient	p value		β coefficient	p value
CSF cohort (n=230)			Plasma cohort (n=101)		
CSF $A\beta_{42}/A\beta_{40}$			Plasma $A\beta_{42}/A\beta_{40}$		
1-2	-0.07 (-0.14 to -0.01)	0.026	1-2	-0.02 (-0.12 to 0.08)	0.706
3	-0.31 (-0.39 to -0.24)	<0.001	3	-0.05 (-0.17 to 0.06)	0.372
4-5	-0.63 (-0.72 to -0.55)	<0.001	4-5	-0.10 (-0.21 to 0.01)	0.077
Non-prion CSF cohort (n=71)			Non-prion plasma cohort (n=60)		
CSF $A\beta_{42}/A\beta_{40}$			Plasma $A\beta_{42}/A\beta_{40}$		
1-2	0.009 (-0.12 to 0.13)	0.885	1-2	-0.04 (-0.20 to 0.12)	0.640
3	-0.34 (-0.48 to -0.21)	<0.001	3	-0.01 (-0.20 to 0.17)	0.880
4-5	-0.71 (-0.84 to -0.57)	<0.001	4-5	-0.08 (-0.25 to 0.09)	0.349
CSF p-tau ₁₈₁			Plasma p-tau ₂₁₇		
1-2	-0.02 (-0.41 to 0.36)	0.899	1-2	-0.13 (-0.66 to 0.41)	0.634
3	0.32 (-0.10 to 0.74)	0.137	3	0.06 (-0.56 to 0.67)	0.853
4-5	1.01 (0.59 to 1.43)	<0.001	4-5	0.69 (0.12 to 1.27)	0.019
CSF $A\beta_{42}$ p-tau ₁₈₁			Plasma p-tau ₂₁₇ / $A\beta_{42}$		
1-2	0.07 (-0.33 to 0.48)	0.713	1-2	-0.07 (-0.60 to 0.47)	0.797
3	-0.59 (-1.03 to -0.15)	0.010	3	0.13 (-0.49 to 0.74)	0.677
4-5	-1.60 (-2.03 to -1.16)	<0.001	4-5	0.75 (0.17 to 1.32)	0.012
CSF p-tau ₂₁₇					
1-2	0.16 (-0.34 to 0.67)	0.520			
3	0.58 (0.04 to 1.13)	0.035			
4-5	1.96 (1.38 to 2.55)	<0.001			
CSF $A\beta_{42}$ p-tau ₂₁₇					
1-2	-0.09 (-0.60 to 0.42)	0.716			
3	-0.81 (-1.36 to -0.27)	0.004			
4-5	-2.35 (-2.93 to -1.76)	<0.001			

Results are derived from multivariable linear regression models with log-transformed biomarker values as dependent variables and categorical Thal phases (1-2, 3 and 4-5) as independent variables. Separate models were fitted for each biomarker. All models were adjusted for age at CSF/plasma sampling, sex and time between CSF/plasma sampling and death; models including participants with prion disease were additionally adjusted for prion disease status. In each model, the reference category consisted of participants with Thal phase of 0. Analyses on CSF p-tau₂₁₇ and $A\beta_{42}$ /p-tau₂₁₇ were performed in a subgroup (n=61). β coefficients are reported as value (95% confidence interval). β coefficients and p values corresponding to pathology levels showing statistically significant differences from the reference group are indicated in bold. Abbreviations: CSF, cerebrospinal fluid

Supplementary Table 9 - Association of Braak stage levels with changes in CSF and plasma biomarkers

Braak stage					
	β coefficient	p value		β coefficient	p value
Non-prion CSF cohort (n=71)			Non-prion plasma cohort (n=60)		
CSF $p\text{-tau}_{181}$			Plasma $p\text{-tau}_{217}$		
1-2	0.28 (-0.06 to 0.61)	0.106	1-2	-0.14 (-0.58 to 0.29)	0.515
3-4	1.19 (0.76 to 1.61)	<0.001	3-4	0.17 (-0.38 to 0.71)	0.541
5-6	1.38 (0.84 to 1.93)	<0.001	5-6	1.20 (0.64 to 1.76)	<0.001
CSF $A\beta_{42}/p\text{-tau}_{181}$			Plasma $p\text{-tau}_{217}/A\beta_{42}$		
1-2	-0.37 (-0.72 to -0.03)	0.034	1-2	0.25 (-0.16 to 0.66)	0.224
3-4	-1.74 (-2.17 to -1.31)	<0.001	3-4	0.69 (0.17 to 1.20)	0.009
5-6	-2.09 (-2.65 to -1.54)	<0.001	5-6	1.56 (1.04 to 2.09)	<0.001
CSF $p\text{-tau}_{217}$					
1-2	0.37 (-0.09 to 0.84)	0.115			
3-4	1.91 (1.31 to 2.51)	<0.001			
5-6	2.69 (1.65 to 3.73)	<0.001			
CSF $A\beta_{42}/p\text{-tau}_{217}$					
1-2	-0.45 (-0.93 to 0.03)	0.065			
3-4	-2.29 (-2.90 to -1.67)	<0.001			
5-6	-3.05 (-4.12 to -1.98)	<0.001			
Non-prion CSF cohort, non-PART (n=65)			Non-prion plasma cohort, non-PART (n=57)		
CSF $p\text{-tau}_{181}$			Plasma $p\text{-tau}_{217}$		
1-2	0.32 (-0.03 to 0.67)	0.074	1-2	-0.17 (-0.63 to 0.29)	0.455
3-4	1.23 (0.82 to 1.64)	<0.001	3-4	0.16 (-0.40 to 0.71)	0.573
5-6	1.41 (0.88 to 1.94)	<0.001	5-6	1.19 (0.62 to 1.77)	<0.001
CSF $A\beta_{42}/p\text{-tau}_{181}$			Plasma $p\text{-tau}_{217}/A\beta_{42}$		
1-2	-0.39 (-0.76 to -0.02)	0.039	1-2	0.17 (-0.23 to 0.58)	0.402
3-4	-1.76 (-2.20 to -1.32)	<0.001	3-4	0.70 (0.21 to 1.19)	0.006
5-6	-2.11 (-2.67 to -1.54)	<0.001	5-6	1.57 (1.06 to 2.08)	<0.001
CSF $p\text{-tau}_{217}$					
1-2	0.45 (-0.04 to 0.94)	0.072			
3-4	1.93 (1.33 to 2.53)	<0.001			
5-6	2.74 (1.70 to 3.79)	<0.001			
CSF $A\beta_{42}/p\text{-tau}_{217}$					
1-2	-0.50 (-1.02 to 0.01)	0.056			
3-4	-2.29 (-2.92 to -1.65)	<0.001			
5-6	-3.06 (-4.16 to -1.95)	<0.001			

Results are derived from multivariable linear regression models with log-transformed biomarker values as dependent variables and categorical Braak stages (1-2, 3-4 and 5-6) as independent variables. Separate models were fitted for each biomarker. All models were adjusted for age at CSF/plasma sampling, sex and time between CSF/plasma sampling and death; models including participants with prion disease were additionally adjusted for prion disease status. In each model, the reference category consisted of participants with Braak stage of 0. Analyses were repeated after exclusion of subjects with PART (i.e., showing tau pathology without A β deposits). Analyses on CSF $p\text{-tau}_{217}$ and $A\beta_{42}/p\text{-tau}_{217}$ were performed in a subgroup (n=61 including PART, n=56 excluding PART). β coefficients are reported as value (95% confidence interval). β coefficients and p values corresponding to pathology levels showing statistically significant differences from the reference group are indicated in bold. Abbreviations: CSF, cerebrospinal fluid

Supplementary Table 10 - Association of ADNC levels with changes in CSF and plasma biomarkers

ADNC levels					
	β coefficient	p value		β coefficient	p value
CSF cohort (n=230)			Plasma cohort (n=101)		
CSF $A\beta_{42}/A\beta_{40}$			Plasma $A\beta_{42}/A\beta_{40}$		
Low	-0.18 (-0.25 to -0.12)	<0.001	Low	-0.05 (-0.15 to 0.04)	0.254
Intermediate	-0.67 (-0.77 to -0.56)	<0.001	Intermediate	-0.09 (-0.21 to 0.04)	0.168
High	-0.62 (-0.83 to -0.41)	<0.001	High	0.06 (-0.10 to 0.21)	0.477
Non-prion CSF cohort (n=71)			Non-prion plasma cohort (n=60)		
CSF $A\beta_{42}/A\beta_{40}$			Plasma $A\beta_{42}/A\beta_{40}$		
Low	-0.15 (-0.28 to -0.02)	0.025	Low	-0.06 (-0.21 to 0.08)	0.385
Intermediate	-0.73 (-0.91 to -0.55)	<0.001	Intermediate	-0.06 (-0.24 to 0.13)	0.545
High	-0.61 (-0.84 to -0.38)	<0.001	High	0.07 (-0.13 to 0.26)	0.492
CSF p-tau ₁₈₁			Plasma p-tau ₂₁₇		
Low	0.09 (-0.23 to 0.41)	0.561	Low	-0.08 (-0.53 to 0.37)	0.718
Intermediate	1.07 (0.64 to 1.51)	<0.001	Intermediate	0.23 (-0.35 to 0.81)	0.435
High	1.28 (0.72 to 1.84)	<0.001	High	1.22 (0.62 to 1.83)	<0.001
CSF $A\beta_{42}$ p-tau ₁₈₁			Plasma p-tau ₂₁₇ / $A\beta_{42}$		
Low	-0.18 (-0.51 to 0.15)	0.287	Low	-0.05 (-0.48 to 0.38)	0.811
Intermediate	-1.62 (-2.07 to -1.18)	<0.001	Intermediate	0.44 (-0.11 to 0.99)	0.117
High	-1.99 (-2.57 to -1.42)	<0.001	High	1.34 (0.76 to 1.92)	<0.001
CSF p-tau ₂₁₇					
Low	0.35 (-0.07 to 0.77)	0.104			
Intermediate	1.91 (1.32 to 2.49)	<0.001			
High	2.70 (1.66 to 3.74)	<0.001			
CSF $A\beta_{42}$ p-tau ₂₁₇					
Low	-0.41 (-0.85 to 0.02)	0.065			
Intermediate	-2.27 (-2.88 to -1.67)	<0.001			
High	-3.05 (-4.12 to -1.98)	<0.001			

Results are derived from multivariable linear regression models with log-transformed biomarker values as dependent variables and categorical ADNC levels (Low, Intermediate, High) as independent variables. Separate models were fitted for each biomarker. All models were adjusted for age at CSF/plasma sampling, sex and time between CSF/plasma sampling and death; models including participants with prion disease were additionally adjusted for prion disease status. In each model, the reference category consisted of participants classified as not AD. Analyses on CSF p-tau₂₁₇ and $A\beta_{42}$ /p-tau₂₁₇ were performed in a subgroup (n=61). β coefficients are reported as value (95% confidence interval). β coefficients and p values corresponding to pathology levels showing statistically significant differences from the reference group are indicated in bold. Abbreviations: ADNC, Alzheimer's disease neuropathologic change; CSF, cerebrospinal fluid

Supplementary Table 11 – Discriminatory performance of CSF and plasma biomarkers across increasing levels of A β pathology burden

	AUC	Maximized Youden-index cut-off			$\approx$ 95% specificity cut-off
		Value	Sensitivity	Specificity	Value
CSF cohort (n=230)					
CSF Aβ₄₂/Aβ₄₀					
>0	0.829 (0.777-0.881)	<0.080	69.4 (61.8-76.0)	85.7 (75.7-92.0)	<0.074
>8	0.886 (0.843-0.929)	<0.074	69.9 (60.9-77.6)	93.2 (87.1-96.5)	<0.071
>16	0.937 (0.903-0.971)	<0.069	78.6 (68.6-86.0)	96.6 (92.2-98.5)	<0.070
>24	0.966 (0.943-0.989)	<0.066	91.5 (81.6-96.3)	94.7 (90.3-97.2)	<0.066
>32	0.950 (0.921-0.979)	<0.066	95.1 (83.9-99.1)	87.3 (81.8-91.3)	<0.052
>40	0.935 (0.895-0.974)	<0.066	95.6 (79.0-99.8)	80.2 (74.2-85.0)	<0.046
Non-prion CSF cohort (n=71)					
CSF Aβ₄₂/Aβ₄₀					
>0	0.873 (0.792-0.954)	<0.078	74.1 (61.1-83.9)	100 (81.6-100)	<0.078
>8	0.939 (0.879-0.999)	<0.071	84.6 (70.3-92.7)	96.9 (84.3-99.8)	<0.074
>16	0.984 (0.964-1.000)	<0.070	93.7 (79.8-98.9)	92.3 (79.7-97.3)	<0.069
>24	0.964 (0.926-1.000)	<0.054	88.0 (70.0-95.8)	95.6 (85.5-99.2)	<0.054
>32	0.977 (0.949-1.000)	<0.054	100 (83.2-100)	90.4 (79.4-95.8)	<0.052
>40	0.952 (0.904-0.999)	<0.054	100 (77.2-100)	81.0 (69.1-89.1)	<0.046
CSF p-tau₁₈₁					
>0	0.710 (0.573-0.846)	>36.5	57.4 (44.2-69.7)	82.3 (59.0-93.8)	>79.0
>8	0.782 (0.675-0.890)	>37.5	69.2 (53.6-81.4)	81.2 (64.7-91.1)	>79.0
>16	0.833 (0.737-0.929)	>37.5	81.2 (64.7-91.1)	82.0 (67.3-91.0)	>79.0
>24	0.819 (0.720-0.918)	>37.5	84.0 (65.3-93.6)	73.9 (59.7-84.4)	>103.5
>32	0.889 (0.810-0.968)	>46.5	89.5 (68.6-98.1)	80.8 (68.1-89.2)	>103.5
>40	0.887 (0.807-0.967)	>46.5	92.3 (66.7-99.6)	74.1 (61.6-83.6)	>103.5
CSF Aβ₄₂/p-tau₁₈₁					
>0	0.791 (0.678-0.903)	<13.36	74.1 (61.1-83.9)	82.3 (59.0-93.8)	<9.31
>8	0.835 (0.745-0.925)	<14.91	87.2 (73.3-94.4)	62.5 (45.2-77.1)	<7.99
>16	0.909 (0.846-0.973)	<13.36	96.9 (84.3-99.8)	69.2 (53.6-81.4)	<7.99
>24	0.914 (0.848-0.980)	<8.50	80.0 (60.9-91.1)	91.3 (79.7-96.6)	<6.68
>32	0.954 (0.907-1.000)	<8.50	94.7 (75.4-99.7)	88.5 (77.0-94.6)	<6.68
>40	0.900 (0.822-0.979)	<7.24	92.3 (66.7-99.6)	86.2 (75.1-92.8)	<2.52
CSF p-tau₂₁₇					
>0	0.799 (0.680-0.917)	>9.50	47.8 (34.1-61.9)	100 (79.6-100)	>8.95
>8	0.838 (0.741-0.935)	>4.90	84.4 (68.2-93.1)	65.5 (47.3-80.1)	>13.85
>16	0.886 (0.805-0.967)	>8.95	74.1 (55.3-86.8)	88.2 (73.4-95.3)	>13.85
>24	0.901 (0.824-0.978)	>8.95	85.0 (64.0-94.8)	82.9 (68.7-91.5)	>14.15
>32	0.941 (0.868-1.000)	>9.65	93.3 (70.2-99.7)	84.8 (71.8-92.4)	>14.15
>40	0.953 (0.901-1.000)	>9.65	100 (74.1-100)	80.0 (67.0-88.8)	>17.05
CSF Aβ₄₂/p-tau₂₁₇					
>0	0.852 (0.756-0.949)	<129.3	84.8 (71.8-92.4)	80.0 (54.8-92.9)	<63.97
>8	0.888 (0.810-0.966)	<52.90	68.7 (51.4-82.0)	93.1 (78.0-98.8)	<52.90
>16	0.934 (0.877-0.990)	<51.69	74.1 (55.3-86.8)	97.1 (85.1-99.8)	<52.27
>24	0.971 (0.953-1.000)	<48.35	90.0 (69.9-98.2)	95.1 (83.9-99.1)	<48.35
>32	0.980 (0.952-1.000)	<44.94	100 (79.6-100)	91.3 (79.7-96.6)	<31.96
>40	0.956 (0.908-1.000)	<44.94	100 (74.1-100)	84.0 (71.5-91.7)	<22.16
Plasma cohort (n=101)					
Plasma Aβ₄₂/Aβ₄₀					
>0	0.603 (0.489-0.718)	<0.076	44.9 (34.3-55.9)	87.0 (67.9-95.5)	<0.068
>8	0.628 (0.518-0.738)	<0.076	51.7 (39.3-63.8)	82.9 (68.7-91.5)	<0.065
>16	0.655 (0.543-0.766)	<0.076	57.1 (43.3-70.0)	80.8 (68.1-89.2)	<0.065
>24	0.600 (0.477-0.722)	<0.075	55.0 (39.8-69.3)	77.0 (65.1-85.8)	<0.063
>32	0.529 (0.385-0.674)	<0.073	46.4 (29.5-64.2)	76.7 (65.8-84.9)	<0.062
>40	0.556 (0.395-0.718)	<0.073	52.6 (31.7-72.7)	75.6 (65.3-83.6)	<0.060

Non-prion plasma cohort (n=60)					
Plasma Aβ₄₂/Aβ₄₀					
>0	0.635 (0.490-0.780)	<0.079	59.6 (45.3-72.4)	76.9 (49.7-91.8)	<0.072
>8	0.565 (0.418-0.713)	<0.076	51.3 (35.9-66.5)	78.3 (58.1-90.3)	<0.063
>16	0.613 (0.466-0.759)	<0.076	56.2 (39.3-71.8)	78.6 (60.5-89.8)	<0.063
>24	0.580 (0.421-0.739)	<0.073	50.0 (32.1-67.9)	79.4 (63.2-89.6)	<0.063
>32	0.543 (0.369-0.718)	<0.073	52.4 (32.4-71.7)	76.9 (61.7-87.3)	<0.063
>40	0.611 (0.418-0.804)	<0.073	64.3 (38.8-83.7)	76.1 (62.1-86.1)	<0.060
Plasma p-tau₂₁₇					
>0	0.719 (0.575-0.864)	>0.359	42.5 (29.5-56.7)	100 (77.2-100)	>0.340
>8	0.761 (0.639-0.882)	>0.213	81.1 (65.8-90.5)	60.9 (40.8-77.8)	>0.531
>16	0.802 (0.693-0.911)	>0.267	75.0 (57.9-86.7)	75.0 (55.6-87.3)	>0.531
>24	0.789 (0.671-0.907)	>0.379	61.5 (42.5-77.6)	91.2 (77.0-96.9)	>0.581
>32	0.902 (0.823-0.981)	>0.379	76.2 (54.9-89.4)	92.3 (79.7-97.3)	>0.629
>40	0.840 (0.724-0.956)	>0.379	78.6 (52.4-92.4)	82.6 (69.3-90.9)	>0.808
Plasma p-tau₂₁₇/Aβ₄₂					
>0	0.731 (0.567-0.894)	>0.014	55.3 (41.2-68.6)	84.6 (57.8-97.3)	>0.024
>8	0.759 (0.628-0.890)	>0.008	83.8 (68.9-92.3)	60.9 (40.8-77.8)	>0.026
>16	0.815 (0.705-0.926)	>0.014	71.9 (54.6-84.4)	82.1 (64.4-92.1)	>0.026
>24	0.806 (0.698-0.914)	>0.011	80.8 (62.1-91.5)	67.6 (50.8-80.9)	>0.026
>32	0.893 (0.815-0.970)	>0.014	90.5 (71.1-98.3)	76.9 (61.7-87.3)	>0.026
>40	0.856 (0.755-0.956)	>0.014	92.9 (68.5-99.6)	67.4 (53.0-79.1)	>0.034

A β score thresholds were defined using 8-unit intervals. Subjects with a score of zero were included in the lowest interval, whereas subjects with values above the 90th percentile of the entire cohort were grouped in the highest interval. Analyses on CSF p-tau₂₁₇ and A β ₄₂/p-tau₂₁₇ were performed in a subgroup (n=61). AUC, sensitivity and specificity are expressed as value (95% confidence interval). Cut-off values for CSF p-tau₁₈₁ and p-tau₂₁₇ and plasma p-tau₂₁₇ are expressed in pg/ml. Abbreviations: AUC, area under the curve; CSF, cerebrospinal fluid

Supplementary Table 12 - Discriminatory performance of CSF and plasma biomarkers across increasing levels of tau pathology burden

	AUC	Maximized Youden-index cut-off			≈95% specificity cut-off
		Value	Sensitivity	Specificity	Value
Non-prion CSF cohort (n=71)					
CSF p-tau ₁₈₁					
>0	0.664 (0.528-0.799)	>46.5	45.4 (33.0-58.5)	87.5 (64.0-97.8)	>79.0
>5	0.795 (0.688-0.902)	>46.5	68.7 (51.4-82.0)	87.2 (73.3-94.4)	>79.0
>10	0.900 (0.819-0.977)	>37.5	95.2 (77.3-99.8)	74.0 (60.4-84.1)	>79.0
>15	0.949 (0.902-0.996)	>46.5	100 (81.6-100)	81.5 (69.2-89.6)	>79.0
>20	0.902 (0.832-0.973)	>53.0	100 (77.2-100)	77.6 (65.3-86.4)	>115.5
>25	0.855 (0.766-0.945)	>53.0	100 (67.6-100)	71.4 (59.3-81.1)	>150.5
CSF Aβ ₄₂ /p-tau ₁₈₁					
>0	0.818 (0.715-0.922)	<13.87	76.4 (63.6-85.6)	81.2 (57.0-93.4)	<11.19
>5	0.866 (0.779-0.953)	<7.68	62.5 (45.2-77.1)	97.4 (86.8-99.9)	<7.99
>10	0.952 (0.899-1.000)	<7.24	85.7 (65.4-95.0)	96.0 (86.5-99.3)	<7.68
>15	0.992 (0.979-1.000)	<7.24	100 (81.6-100)	94.4 (84.9-98.5)	<6.68
>20	0.971 (0.937-1.000)	<5.98	100 (77.2-100)	93.1 (83.6-97.3)	<3.20
>25	0.950 (0.896-1.000)	<5.98	100 (67.6-100)	85.7 (75.0-92.3)	<2.57
CSF p-tau ₂₁₇					
>0	0.691 (0.557-0.826)	>9.50	46.8 (33.3-60.8)	100 (78.5-100)	>9.30
>5	0.853 (0.754-0.953)	>9.50	70.4 (51.5-84.1)	91.2 (77.0-96.9)	>11.25
>10	0.961 (0.917-1.000)	>9.50	94.1 (73.0-99.7)	86.4 (73.3-93.6)	>13.10
>15	0.994 (0.979-1.000)	>15.25	92.3 (66.7-99.6)	100 (92.6-100)	>13.85
>20	0.969 (0.923-1.000)	>17.05	100 (70.1-100)	96.1 (87.0-99.3)	>15.25
>25	0.934 (0.863-1.000)	>17.05	100 (51.0-100)	87.7 (76.7-93.9)	>50.90
CSF Aβ ₄₂ /p-tau ₂₁₇					
>0	0.801 (0.679-0.922)	<62.77	61.7 (47.4-74.2)	92.9 (68.5-99.6)	<62.77
>5	0.893 (0.809-0.978)	<62.77	88.9 (71.9-96.1)	82.3 (66.5-91.6)	<48.35
>10	0.979 (0.949-1.000)	<48.35	94.1 (73.0-99.7)	90.9 (78.8-96.4)	<43.41
>15	0.995 (0.984-1.000)	<31.96	100 (77.2-100)	97.9 (89.1-99.9)	<39.02
>20	0.972 (0.934-1.000)	<22.16	100 (70.1-100)	94.2 (84.4-98.4)	<22.16
>25	0.930 (0.859-1.000)	<22.16	100 (51.1-100)	86.0 (74.7-92.7)	<6.77
Non-prion plasma cohort (n=60)					
Plasma p-tau ₂₁₇					
>0	0.728 (0.594-0.863)	>0.299	55.1 (41.3-68.1)	100 (74.1-100)	>0.277
>5	0.797 (0.685-0.909)	>0.288	73.3 (55.5-85.8)	76.7 (59.1-88.2)	>0.670
>10	0.821 (0.710-0.931)	>0.359	68.2 (47.3-83.6)	86.8 (72.7-94.2)	>0.693
>15	0.879 (0.794-0.963)	>0.359	75.0 (53.1-88.8)	87.5 (73.9-94.5)	>0.693
>20	0.861 (0.765-0.956)	>0.288	93.3 (70.2-99.7)	66.7 (52.1-78.6)	>0.808
>25	0.912 (0.827-0.997)	>0.531	88.9 (56.5-99.4)	88.2 (76.6-94.5)	>0.886
Plasma p-tau ₂₁₇ /Aβ ₄₂					
>0	0.740 (0.584-0.897)	>0.011	61.2 (47.2-73.6)	81.8 (52.3-96.7)	>0.021
>5	0.819 (0.713-0.925)	>0.011	80.0 (62.7-90.5)	73.3 (55.5-85.8)	>0.024
>10	0.877 (0.790-0.965)	>0.014	86.4 (66.7-95.2)	76.3 (60.8-87.0)	>0.024
>15	0.919 (0.852-0.985)	>0.014	95.0 (76.4-99.7)	77.5 (62.5-87.7)	>0.026
>20	0.921 (0.853-0.988)	>0.014	100 (79.6-100)	71.1 (56.6-82.3)	>0.026
>25	0.928 (0.848-1.000)	>0.021	88.9 (56.5-99.4)	82.3 (69.7-90.4)	>0.034

Tau score thresholds were defined using 5-unit intervals. Subjects with a score of zero were included in the lowest interval, whereas subjects with values above the 90th percentile of the entire cohort were grouped in the highest interval. Analyses on CSF p-tau₂₁₇ and Aβ₄₂/p-tau₂₁₇ were performed in a subgroup (n=61). AUC, sensitivity and specificity are expressed as value (95% confidence interval). Cut-off values for CSF p-tau₁₈₁ and p-tau₂₁₇ and plasma p-tau₂₁₇ are expressed in pg/ml. Abbreviations: AUC, area under the curve; CSF, cerebrospinal fluid

Supplementary Table 13 - Discriminatory performance of CSF and plasma biomarkers across increasing Thal phases

	AUC	Maximized Youden-index cut-off			≈95% specificity cut-off
		Value	Sensitivity	Specificity	Value
CSF cohort (n=230)					
CSF Aβ₄₂/Aβ₄₀					
>0	0.829 (0.777-0.882)	<0.080	70.3 (62.7-76.9)	84.0 (74.1-90.6)	<0.074
>1	0.862 (0.814-0.911)	<0.073	65.5 (56.6-73.5)	94.6 (88.7-97.5)	<0.073
>2	0.925 (0.891-0.959)	<0.070	77.6 (67.7-85.2)	93.8 (88.6-96.7)	<0.069
>3	0.951 (0.924-0.978)	<0.066	95.0 (83.5-99.1)	86.8 (81.3-90.9)	<0.051
>4	0.915 (0.874-0.957)	<0.055	91.3 (73.2-98.4)	87.4 (82.2-91.3)	<0.044
Non-prion CSF cohort (n=71)					
CSF Aβ₄₂/Aβ₄₀					
>0	0.840 (0.746-0.933)	<0.076	73.1 (59.7-83.2)	94.7 (75.4-99.7)	<0.076
>1	0.916 (0.845-0.987)	<0.076	86.0 (72.7-93.4)	92.9 (77.3-98.7)	<0.074
>2	0.976 (0.948-1.000)	<0.070	91.2 (77.0-96.9)	94.6 (82.3-99.0)	<0.070
>3	0.957 (0.915-0.999)	<0.054	94.4 (74.2-99.7)	86.8 (75.2-93.4)	<0.047
>4	0.911 (0.844-0.978)	<0.054	100 (75.7-100)	79.7 (67.7-88.0)	<0.041
CSF p-tau₁₈₁					
>0	0.701 (0.566-0.835)	>25.5	78.8 (66.0-87.8)	57.9 (36.4-76.9)	>79.0
>1	0.766 (0.650-0.881)	>25.5	88.4 (75.5-94.9)	60.7 (42.4-76.4)	>81.0
>2	0.809 (0.708-0.910)	>37.5	76.5 (60.0-87.6)	81.1 (65.8-90.5)	>81.0
>3	0.872 (0.787-0.956)	>37.5	94.4 (74.2-99.7)	69.8 (56.5-80.5)	>93.5
>4	0.877 (0.791-0.964)	>46.5	91.7 (64.6-99.6)	72.9 (60.4-82.6)	>115.5
CSF Aβ₄₂/p-tau₁₈₁					
>0	0.757 (0.641-0.873)	<13.36	73.1 (59.7-83.2)	73.7 (51.2-88.2)	<9.31
>1	0.797 (0.694-0.900)	<14.91	83.7 (70.0-91.9)	64.3 (45.8-79.3)	<7.99
>2	0.882 (0.808-0.956)	<9.03	67.6 (50.8-80.9)	91.9 (78.7-97.2)	<7.99
>3	0.922 (0.852-0.993)	<7.99	88.9 (67.2-98.0)	88.7 (77.4-94.7)	<5.98
>4	0.907 (0.827-0.986)	<7.24	91.7 (64.6-99.6)	84.7 (73.5-91.8)	<2.57
CSF p-tau₂₁₇					
>0	0.784 (0.661-0.907)	>9.50	47.7 (33.7-62.1)	94.1 (73.0-99.7)	>9.50
>1	0.844 (0.747-0.941)	>3.30	94.4 (81.9-99.0)	56.0 (37.1-73.3)	>13.10
>2	0.861 (0.772-0.950)	>8.95	69.0 (50.8-82.7)	87.5 (71.9-95.0)	>13.10
>3	0.952 (0.899-1.000)	>8.95	100 (77.2-100)	77.1 (63.5-86.7)	>15.25
>4	0.934 (0.859-1.000)	>9.65	100 (67.6-100)	75.5 (62.4-85.1)	>39.65
CSF Aβ₄₂/p-tau₂₁₇					
>0	0.822 (0.718-0.926)	<129.30	84.1 (70.6-92.1)	70.6 (46.9-86.7)	<56.49
>1	0.874 (0.787-0.962)	<129.30	94.4 (81.9-99.0)	68.0 (48.4-82.8)	<52.90
>2	0.908 (0.839-0.977)	<51.69	69.0 (50.8-82.7)	96.9 (84.3-99.8)	<52.27
>3	0.968 (0.928-1.000)	<44.94	100 (77.2-100)	87.5 (75.3-94.1)	<22.16
>4	0.915 (0.843-0.988)	<44.94	100 (67.6-100)	79.2 (66.5-88.0)	<8.26
Plasma cohort (n=101)					
Plasma Aβ₄₂/Aβ₄₀					
>0	0.595 (0.481-0.709)	<0.076	45.4 (34.8-56.5)	87.5 (69.0-95.7)	<0.068
>1	0.616 (0.505-0.728)	<0.076	48.4 (36.6-60.4)	81.1 (65.8-90.5)	<0.063
>2	0.642 (0.531-0.753)	<0.076	56.0 (42.3-68.8)	80.4 (67.5-89.0)	<0.063
>3	0.637 (0.506-0.769)	<0.075	61.3 (43.8-76.3)	75.7 (64.5-84.2)	<0.063
>4	0.563 (0.406-0.719)	<0.073	52.6 (31.7-72.7)	75.6 (65.3-83.6)	<0.060
Non-prion plasma cohort (n=60)					
Plasma Aβ₄₂/Aβ₄₀					
>0	0.621 (0.478-0.764)	<0.076	47.8 (34.1-61.9)	85.7 (60.1-97.5)	<0.072
>1	0.561 (0.409-0.712)	<0.079	60.0 (44.6-73.6)	65.0 (43.3-81.9)	<0.063
>2	0.615 (0.469-0.762)	<0.075	56.2 (39.3-71.8)	82.1 (64.4-92.1)	<0.063
>3	0.630 (0.464-0.796)	<0.073	57.1 (36.5-75.5)	79.5 (64.5-89.2)	<0.063
>4	0.592 (0.410-0.773)	<0.073	57.1 (32.6-78.6)	73.9 (59.7-84.4)	<0.056

Plasma p-tau₂₁₇					
>0	0.724 (0.587-0.862)	>0.359	43.5 (30.2-57.7)	100 (78.5-100)	>0.340
>1	0.761 (0.639-0.884)	>0.359	47.5 (32.9-62.5)	95.0 (76.4-99.7)	>0.359
>2	0.793 (0.682-0.904)	>0.359	56.2 (39.3-71.8)	92.9 (77.3-98.7)	>0.531
>3	0.837 (0.733-0.941)	>0.379	66.7 (45.4-82.8)	87.2 (73.3-94.4)	>0.670
>4	0.824 (0.704-0.944)	>0.379	71.4 (45.3-88.3)	80.4 (66.8-89.3)	>0.808
Plasma p-tau₂₁₇/Aβ₄₂					
>0	0.742 (0.588-0.896)	>0.014	56.5 (42.2-69.8)	85.7 (60.1-97.5)	>0.024
>1	0.780 (0.649-0.910)	>0.014	62.5 (47.0-75.8)	85.0 (64.0-94.8)	>0.026
>2	0.801 (0.688-0.914)	>0.014	68.7 (51.4-82.0)	78.6 (60.5-89.8)	>0.026
>3	0.842 (0.746-0.939)	>0.011	90.5 (71.1-98.3)	66.7 (51.0-79.4)	>0.028
>4	0.807 (0.696-0.919)	>0.011	92.9 (68.5-99.6)	58.7 (44.3-71.7)	>0.043

Analyses on CSF p-tau₂₁₇ and A β ₄₂/p-tau₂₁₇ were performed in a subgroup (n=61). AUC, sensitivity and specificity are expressed as value (95% confidence interval). Cut-off values for CSF p-tau₁₈₁ and p-tau₂₁₇ and plasma p-tau₂₁₇ are expressed in pg/ml. Abbreviations: AUC, area under the curve; CSF, cerebrospinal fluid

Supplementary Table 14 - Discriminatory performance of CSF and plasma biomarkers across increasing Braak stages

	AUC	Maximized Youden-index cut-off			≈95% specificity cut-off
		Value	Sensitivity	Specificity	Value
Non-prion CSF cohort (n=71)					
CSF p-tau ₁₈₁					
>0	0.762 (0.653-0.872)	>37.5	65.1 (50.2-77.6)	82.1 (64.4-92.1)	>79.0
>1	0.814 (0.701-0.926)	>46.5	74.1 (55.3-86.8)	84.1 (70.6-92.1)	>79.0
>2	0.913 (0.830-0.995)	>46.5	94.4 (74.2-99.7)	81.1 (68.6-89.4)	>79.0
>3	0.892 (0.817-0.967)	>53.0	100 (75.7-100)	76.3 (64.0-85.3)	>147.5
>4	0.894 (0.814-0.974)	>81.0	100 (56.5-100)	84.8 (74.3-91.6)	>150.5
>5	0.986 (0.958-1.000)	>178.0	100 (5.1-100)	98.6 (92.3-99.9)	>150.5
CSF Aβ ₄₂ /p-tau ₁₈₁					
>0	0.839 (0.747-0.930)	<13.36	83.7 (70.0-91.9)	75.0 (56.6-87.3)	<7.99
>1	0.889 (0.808-0.970)	<7.24	70.4 (51.5-84.1)	97.7 (88.2-99.9)	<7.68
>2	0.969 (0.920-1.000)	<7.24	94.4 (74.2-99.7)	94.3 (84.6-98.5)	<7.24
>3	0.956 (0.911-1.000)	<5.98	100 (75.7-100)	91.5 (81.6-96.3)	<3.20
>4	0.945 (0.880-1.000)	<5.23	100 (56.5-100)	83.3 (72.6-90.4)	<2.49
>5	0.957 (0.910-1.000)	<1.94	100 (5.1-100)	95.7 (88.1-98.8)	<2.14
CSF p-tau ₂₁₇					
>0	0.804 (0.697-0.911)	>9.50	56.8 (40.9-71.3)	95.8 (79.8-99.8)	>9.50
>1	0.871 (0.763-0.980)	>9.50	81.8 (61.5-92.7)	89.7 (76.4-95.9)	>11.60
>2	0.971 (0.924-1.000)	>15.25	85.7 (60.1-97.5)	100 (92.4-100)	>13.85
>3	0.965 (0.920-1.000)	>17.05	100 (67.6-100)	94.3 (84.6-98.5)	>17.05
>4	0.983 (0.950-1.000)	>55.80	100 (17.8-100)	98.3 (91.0-99.9)	>50.90
>5	na	na	na	na	na
CSF Aβ ₄₂ /p-tau ₂₁₇					
>0	0.855 (0.761-0.948)	<93.88	83.9 (68.9-92.3)	70.8 (50.8-85.1)	<48.35
>1	0.908 (0.828-0.988)	<54.70	86.4 (66.7-95.2)	84.6 (70.3-92.7)	<43.41
>2	0.986 (0.963-1.000)	<31.96	92.9 (68.5-99.6)	97.9 (88.9-99.9)	<39.02
>3	0.965 (0.920-1.000)	<22.16	100 (67.6-100)	92.4 (82.1-97.0)	<12.74
>4	0.949 (0.880-1.000)	<8.26	100 (17.8-100)	91.5 (81.6-96.3)	<6.03
>5	na	na	na	na	na
Non-prion plasma cohort (n=60)					
Plasma p-tau ₂₁₇					
>0	0.748 (0.618-0.878)	>0.299	58.5 (43.4-72.2)	84.2 (62.4-94.5)	>0.359
>1	0.782 (0.666-0.899)	>0.359	61.5 (42.5-77.5)	88.2 (73.4-95.3)	>0.693
>2	0.842 (0.737-0.948)	>0.359	71.4 (50.0-86.2)	87.2 (73.3-94.4)	>0.693
>3	0.908 (0.831-0.984)	>0.288	100 (78.5-100)	67.4 (53.0-79.1)	>0.736
>4	0.912 (0.820-1.000)	>0.531	87.5 (52.9-99.3)	86.5 (74.7-93.3)	>0.808
>5	0.767 (0.480-1.000)	>0.299	100 (17.8-100)	56.9 (44.1-68.8)	>1.031
Plasma p-tau ₂₁₇ /Aβ ₄₂					
>0	0.819 (0.704-0.934)	>0.011	70.7 (55.5-82.4)	84.2 (62.4-94.5)	>0.021
>1	0.817 (0.712-0.922)	>0.011	80.8 (62.1-91.5)	67.6 (50.8-80.9)	>0.024
>2	0.891 (0.807-0.975)	>0.014	90.5 (71.1-98.3)	76.9 (61.7-87.3)	>0.024
>3	0.948 (0.894-1.000)	>0.017	92.9 (68.5-99.6)	84.8 (71.8-92.4)	>0.026
>4	0.939 (0.855-1.000)	>0.028	87.5 (52.9-99.4)	92.3 (81.8-97.0)	>0.034
>5	0.724 (0.507-0.941)	>0.014	100 (17.8-100)	55.2 (42.4-67.2)	>0.050

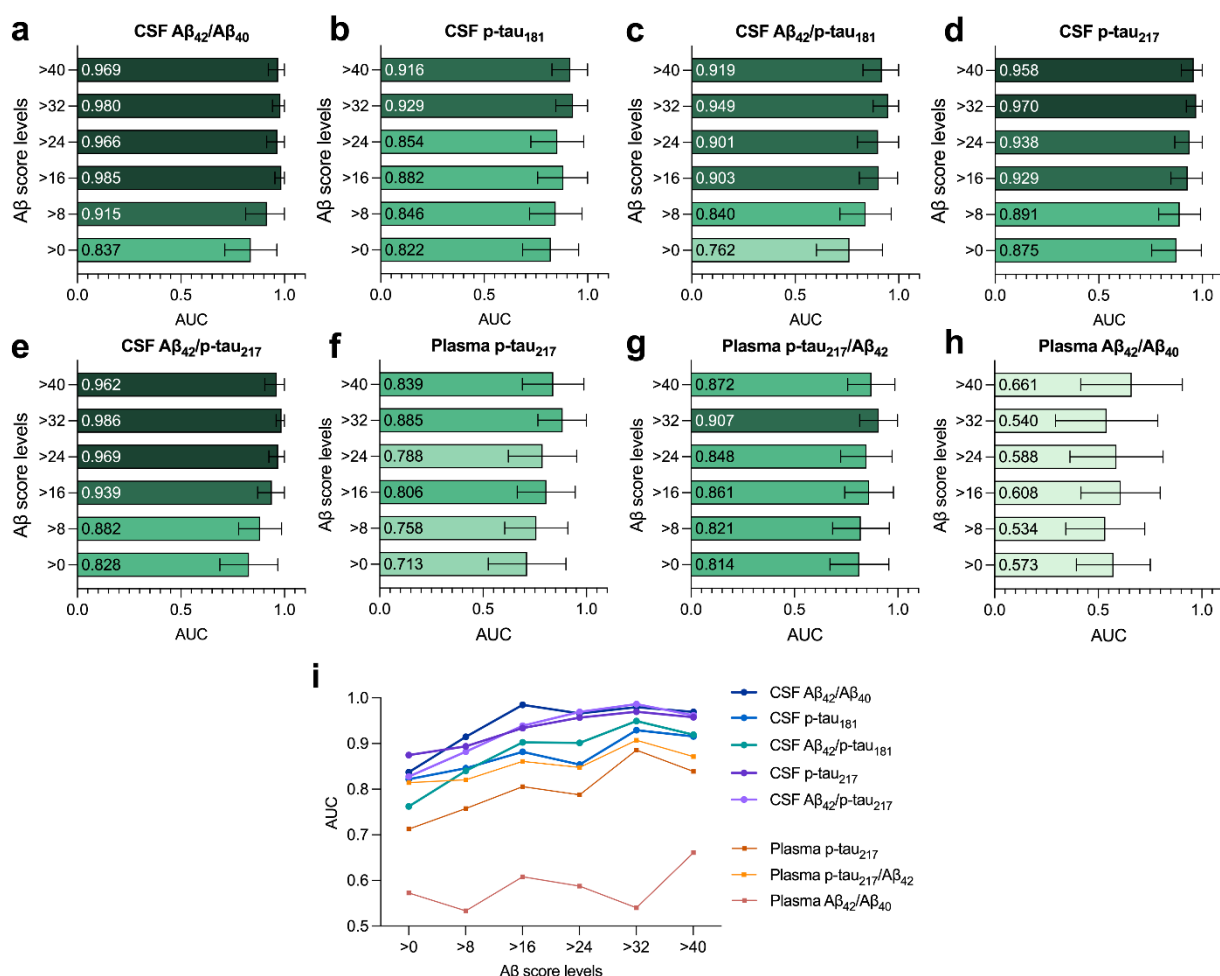
Analyses on CSF p-tau₂₁₇ and Aβ₄₂/p-tau₂₁₇ were performed in a subgroup (n=61). AUC, sensitivity and specificity are expressed as value (95% confidence interval). Cut-off values for CSF p-tau₁₈₁ and p-tau₂₁₇ and plasma p-tau₂₁₇ are expressed in pg/ml. Abbreviations: AUC, area under the curve; CSF, cerebrospinal fluid; na, not available

Supplementary Table 15 - Discriminatory performance of CSF and plasma biomarkers across increasing ADNC levels

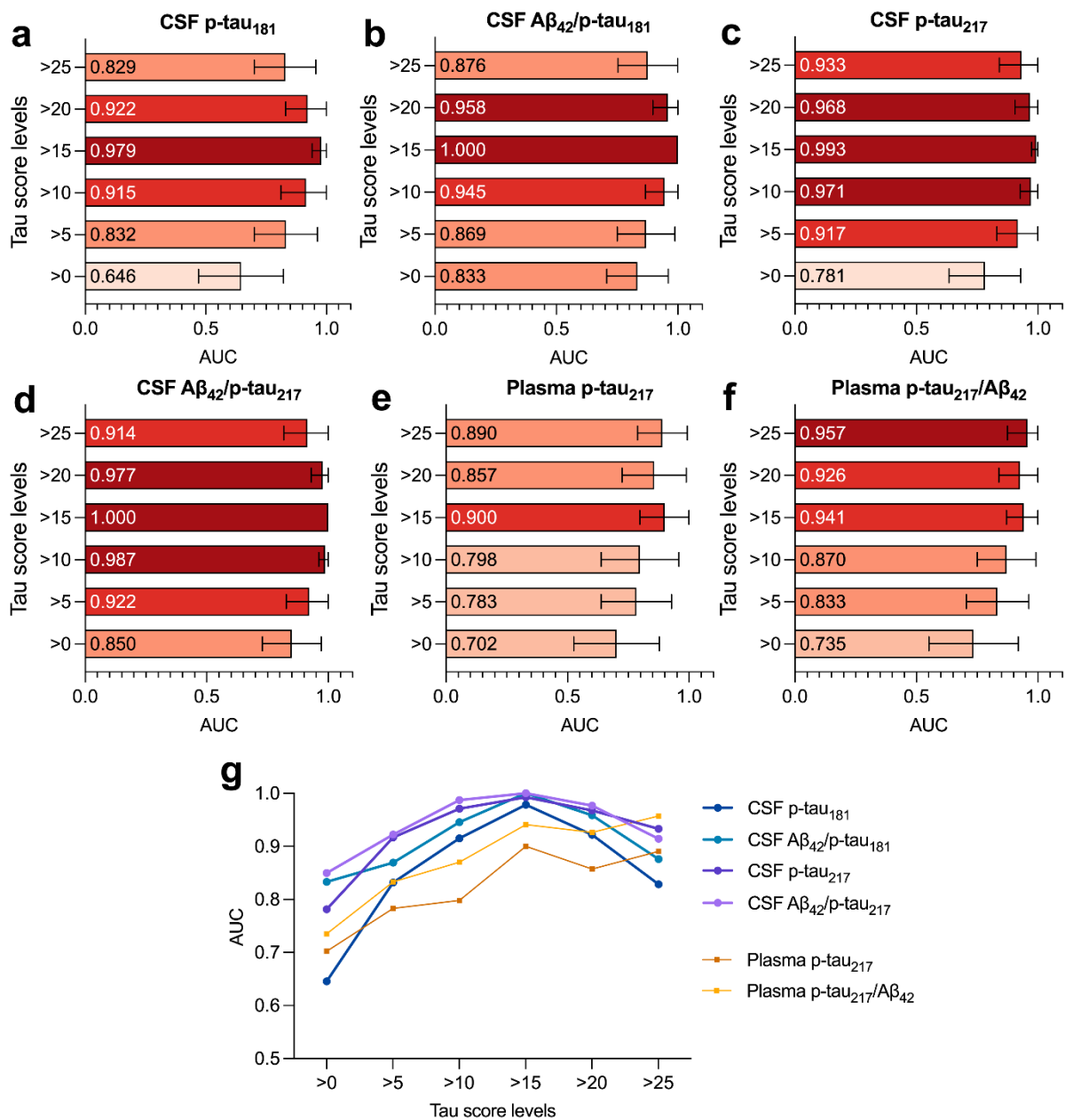
	AUC	Maximized Youden-index cut-off			≈95% specificity cut-off
		Value	Sensitivity	Specificity	Value
CSF cohort (n=230)					
CSF Aβ ₄₂ /Aβ ₄₀					
>Not AD	0.829 (0.777-0.882)	<0.080	70.3 (62.7-76.9)	84.0 (74.1-90.6)	<0.074
>Low	0.938 (0.897-0.980)	<0.058	93.9 (80.4-98.9)	88.3 (83.1-92.1)	<0.049
>Int.	0.902 (0.843-0.960)	<0.054	100 (56.5-100)	82.2 (76.7-86.7)	<0.037
Non-prion CSF cohort (n=71)					
CSF Aβ ₄₂ /Aβ ₄₀					
>Not AD	0.840 (0.746-0.933)	<0.076	73.1 (59.7-83.2)	94.7 (75.4-99.7)	<0.076
>Low	0.971 (0.938-1.000)	<0.054	100 (82.4-100)	88.7 (77.4-94.7)	<0.047
>Int.	0.838 (0.730-0.946)	<0.054	100 (56.5-100)	71.2 (59.4-80.7)	<0.031
CSF p-tau ₁₈₁					
>Not AD	0.701 (0.566-0.835)	>25.5	78.8 (66.0-87.8)	57.9 (36.3-76.9)	>79.0
>Low	0.913 (0.830-0.995)	>46.5	94.4 (74.2-99.7)	81.1 (68.6-89.4)	>79.0
>Int.	0.894 (0.814-0.974)	>81.0	100 (56.5-100)	84.8 (74.3-91.6)	>150.5
CSF Aβ ₄₂ /p-tau ₁₈₁					
>Not AD	0.757 (0.641-0.873)	<13.36	73.1 (59.7-83.2)	73.7 (51.2-88.2)	<9.31
>Low	0.969 (0.920-1.000)	<7.24	94.4 (74.2-99.7)	94.3 (84.6-98.5)	<7.24
>Int.	0.945 (0.880-1.000)	<5.23	100 (56.5-100)	83.3 (72.6-90.4)	<2.49
CSF p-tau ₂₁₇					
>Not AD	0.784 (0.661-0.907)	>9.50	47.7 (33.7-62.1)	94.1 (73.0-99.7)	>9.50
>Low	0.971 (0.924-1.000)	>15.25	85.7 (60.1-97.5)	100 (92.4-100)	>13.85
>Int.	0.983 (0.950-1.000)	>55.80	100 (17.8-100)	98.3 (91.0-99.9)	>50.90
CSF Aβ ₄₂ /p-tau ₂₁₇					
>Not AD	0.822 (0.718-0.926)	<129.30	84.1 (70.6-92.1)	70.6 (46.9-86.7)	<56.49
>Low	0.986 (0.963-1.000)	<31.96	92.9 (68.5-99.6)	97.9 (88.9-99.9)	<39.02
>Int.	0.949 (0.880-1.000)	<8.26	100 (17.7-100)	91.5 (81.6-96.3)	<6.03
Plasma cohort (n=101)					
Plasma Aβ ₄₂ /Aβ ₄₀					
>Not AD	0.595 (0.481-0.709)	<0.076	45.4 (34.8-56.5)	87.5 (69.0-95.7)	<0.068
>Low	0.561 (0.422-0.700)	<0.073	50.0 (32.6-67.4)	78.1 (67.3-86.0)	<0.062
>Int.	0.571 (0.318-0.824)	>0.096	37.5 (13.7-69.4)	90.3 (82.6-94.8)	>0.108
Non-prion plasma cohort (n=60)					
Plasma Aβ ₄₂ /Aβ ₄₀					
>Not AD	0.621 (0.478-0.764)	<0.076	47.8 (34.1-61.9)	85.7 (60.1-97.5)	<0.072
>Low	0.522 (0.351-0.694)	<0.073	50.0 (29.9-70.1)	75.0 (59.8-85.8)	<0.063
>Int.	0.594 (0.346-0.841)	>0.095	37.5 (13.7-69.4)	92.3 (81.8-97.0)	>0.112
Plasma p-tau ₂₁₇					
>Not AD	0.724 (0.587-0.862)	>0.359	43.5 (30.2-57.7)	100 (78.5-100)	>0.340
>Low	0.831 (0.722-0.941)	>0.288	85.0 (64.0-94.8)	70.0 (54.6-81.9)	>0.693
>Int.	0.912 (0.820-1.000)	>0.531	87.5 (52.9-99.4)	86.5 (74.7-93.3)	>0.808
Plasma p-tau ₂₁₇ /Aβ ₄₂					
>Not AD	0.742 (0.588-0.896)	>0.014	56.5 (42.2-69.8)	85.7 (60.1-97.5)	>0.024
>Low	0.879 (0.790-0.967)	>0.014	90.0 (69.9-98.2)	75.0 (59.8-85.8)	>0.026
>Int.	0.939 (0.855-1.000)	>0.028	87.5 (52.9-99.4)	92.3 (81.8-97.0)	>0.034

Analyses on CSF p-tau₂₁₇ and A β ₄₂/p-tau₂₁₇ were performed in a subgroup (n=61). AUC, sensitivity and specificity are expressed as value (95% confidence interval). Cut-off values for CSF p-tau₁₈₁ and p-tau₂₁₇ and plasma p-tau₂₁₇ are expressed in pg/ml. Abbreviations: AD, Alzheimer's disease; ADNC, Alzheimer's disease neuropathologic change; AUC, area under the curve; CSF, cerebrospinal fluid; Int, intermediate

SUPPLEMENTARY FIGURES



Supplementary Fig. 1 Discriminatory performance of biofluid biomarkers across increasing Aβ pathology burden in the subgroup of non-prion participants with complete biomarker data available (n=38). a-h: Bar plots of AUC values for each biomarker for discriminating progressively higher Aβ burden, defined by increasing Aβ score thresholds (y-axis). Error bars represent 95% confidence intervals. Increasing shades of green reflect higher AUC values. i: Line plot showing AUC values across Aβ score thresholds for all biomarkers. 95% confidence intervals are not shown for graphical reasons. Abbreviations: AUC, area under the curve



Supplementary Fig. 2 Discriminatory performance of biofluid biomarkers across increasing tau pathology burden in the subgroup of non-prion participants with complete biomarker data available (n=38). a-f: Bar plots of AUC values for each biomarker for discriminating progressively higher tau burden, defined by increasing tau score thresholds (y-axis). Error bars represent 95% confidence intervals. Increasing shades of red reflect higher AUC values. g: Line plot showing AUC values across tau score thresholds for all biomarkers. 95% confidence intervals are not shown for graphical reasons. Abbreviations: AUC, area under the curve