Statistical Supplement – Table of contents:

Tables

Figures

Table 1: Data distribution analysis

Data distribution was analyzed using D´Agostino-Pearson omnibus test and Quantile-quantile plot analysis (QQ plot analysis) at α = 0.05. None of the tested inflammatory marker data was normal distributed according to both tests. Most of the data followed a distribution slightly skewed towards lower values. SAA and CRP levels followed a lognormal distribution.

Parameter	D'Agostino-Pearson omnibus test		QQ plot analysis	
	p-value	Data normal distributed?	Fit to normal distribution (R^2)	Type of distribution
Age	0.0002	No	0.95	normal
Storage Time	<0.0001	No	0.94	skewed
Aß42	<0.0001	No	0.88	skewed
Tau	<0.0001	No	0.89	skewed
p-tau	<0.0001	No	0.91	skewed
Aß42/40	0.0001	No	0.95	normal
Aß42/ptau	<0.0001	No	0.88	skewed
CSF protein	<0.0001	No	0.85	skewed
CSF albumin	<0.0001	No	0.86	skewed
CSF IgG	<0.0001	No	0.71	skewed
MIF	<0.0001	No	0.70	skewed
VEGF	<0.0001	No	0.90	skewed
Flt-1	<0.0001	No	0.96	normal
Il-8	<0.0001	No	0.88	skewed
Il-6	<0.0001	No	0.75	skewed
MCP-1	<0.0001	No	0.81	inconclusive
IP-10	<0.0001	No	0.74	skewed
ICAM-1	<0.0001	No	0.92	skewed
VCAM-1	<0.0001	No	0.84	skewed
SAA	<0.0001	No	0.16	lognormal
CRP	<0.0001	No	0.01	lognormal
C1q	<0.0001	No	0.69	inconclusive,
C3a	<0.0001	No	0.72	skewed
Il1RAcP	<0.0001	No	0.83	skewed
TREM2	<0.0001	No	0.84	skewed

Table 2: Outlier analysis

Samples with high values of at least one inflammatory protein marker were considered as outliers (value sorted by decreasing size; values at least 2-fold higher than next value in list considered outliers). Medical records of respective patients were searched for conditions with potential influence on markers.

Potential Outlier No.	Suspicious values	Medical Record	Conclusion
1	MCP-1	History of stroke, but no acute comorbidities	Sample not excluded
2	CRP	No indication of comorbidities	Sample not excluded
3	High erythrocytes	Blood contamination during LP in first fractions, research sample obtained from “clear” fraction	Sample not excluded as aliquots used for study where free of blood
4	Il1-RAcP	No indication of comorbidities	Sample not excluded
5	Il-8	No indication of comorbidities	Sample not excluded
6	Il-8	No indication of comorbidities	Sample not excluded
7	Il-8	No indication of comorbidities	Sample not excluded
8	Il-8	No indication of comorbidities	Sample not excluded
9	Il-8, sICAM	No indication of comorbidities	Sample not excluded

Table 3: Description of main cohorts

This table is redundant to table 1 from the main text, but includes the exact p values of cohort comparisons. Overview of the main patient cohorts (ND, MCI and AD cases), potential confounders such as age, sex and biobank storage time, as well as standard AD biomarker and neuropsychology findings. Results are described by median ± standard deviation, range and coverage in the dataset (in percent), and statistical results (p values of Kruskal-Wallis test and significant post-hoc comparisons adjusted by the Bonferroni method). Consistent with the difference in diagnosis, CSF biomarkers and neuropsychological results were highly discriminative. Age, sex and storage time varied between cohorts and were therefore considered as confounders in subsequent covariate testing.

32

Feature	Main Clinical Cohorts			p-value (Significant cohort-wise comparisons)
	ND	MCI	AD	
Number (n)	85	130	116	-
Age (yrs.)	67 ± 11 43 - 81; 100%	71 ± 8 48 - 85; 100%	74 ± 8 50 - 88; 100%	**$1x10E^{-10}$** (ND vs. MCI, AD)
Sex (% male)	65	65	45	**$Chi^2 = 2x10E^{-3}$** (AD Vs. ND, MCI)
Storage Time (mth.)	27 ± 21 0.3 - 95.0; 100%	36 ± 23 4.0 - 91.0; 100%	22 ± 19 4.0 - 94.0; 100%	**$7x10E^{-5}$** (MCI Vs. ND, AD)
Aβ42 pg/ml	552 ± 315 138 – 1653; 100%	432 ± 302 87 – 1326; 100%	319 ± 191 91 – 1505; 100%	**$1x10E^{-9}$** (All cohorts)
Aβ42/40	0.10 ± 0.03 0.02 – 0.25; 100%	0.07 ± 0.04 0.02 – 0.23; 100%	0.05 ± 0.02 0.03 – 0.15; 100%	**$1x10E^{-23}$** (All cohorts)
t-tau pg/ml	289 ± 228 36 – 1210; 100%	419 ± 290 71 – 1528; 100%	680 ± 344 126 – 2157; 100%	**$3x10E^{-14}$** (All cohorts)
p-tau-181 pg/ml	43 ± 25 16 – 157; 100%	59 ± 30 18 – 158; 100%	76 ± 36 23 – 248; 100%	**$1x10E^{-12}$** (All cohorts)
Aβ42/p-tau-181	17.0 ± 9.2 2.0 – 42.8; 100%	9.7 ± 9.1 1.5 – 48.8; 100%	4.5 ± 3.8 1.3 – 25.2; 100%	**$6x10E^{-27}$** (All cohorts)
CSF protein (mg/l)	488 ± 167 257 – 999; 93%	458 ± 148 248 – 1090; 98%	453 ± 144 245 – 1055; 99%	0.423
CSF Albumin (mg/l)	268 ± 137 103 – 688; 88%	256 ± 116 100 – 724; 98%	260 ± 116 118 – 803; 98%	0.485
CSF IgG (mg/l)	31 ± 17 8 – 94; 88%	28 ± 13 9 – 108; 98%	28 ± 16 11 – 128; 98%	0.263
CSF leukocytes (cells/µl)	1 ± 2 0 – 11; 93%	1 ± 1 0 – 7; 98%	1 ± 1 0 – 7; 99%	0.942
MMSE	29 ± 1 27 – 30; 73%	26 ± 3 18 – 30; 91%	21 ± 4 6 – 29; 96%	**$1x10E^{-48}$** (All cohorts)
Education (yrs.)	15 ± 2 11 – 18; 18%	14 ± 4 8 – 22; 82%	13 ± 4 8 – 22; 72%	0.129
Word list recall	20 ± 3 16 – 23; 18%	16 ± 4 5 – 27; 81%	13 ± 4 3 – 19; 69%	**$1x10E^{-11}$** (All cohorts)
Word list delayed recall	6 ± 1 4 – 9; 18%	4 ± 2 0 – 10; 78%	2 ± 2 0 – 7; 60%	**$6x10E^{-16}$** (All cohorts)
Word list discrimination (%)	100 ± 3 90 – 100; 18%	95 ± 15 0 – 100; 81%	85 ± 21 0 – 100; 69%	**$7x10E^{-6}$** (All cohorts)
Figure recall	8 ± 2 2 – 11; 16%	5 ± 3 0 – 11; 81%	3 ± 3 0 – 10; 60%	**$4x10E^{-10}$** (All cohorts)
TMT A	52 ± 27 20 – 119; 16%	57 ± 33 26 – 180; 80%	84 ± 46 29 – 180; 69%	**$4x10E^{-3}$** (AD Vs. ND, MCI)
TMT B	115 ± 50 53 – 245; 16%	166 ± 80 52 – 300; 75%	300 ± 71 76 – 300; 60%	**$5x10E^{-11}$** (All cohorts)
Semantic Fluency	22 ± 7 14 – 39; 18%	16 ± 6 5 – 34; 81%	12 ± 5 3 – 26; 72%	**$2x10E^{-10}$** (All cohorts)
Phonetic Fluency	12 ± 4 7 – 19; 16%	10 ± 5 1 – 26; 79%	9 ± 4 2 – 22; 70%	**$7x10E^{-3}$** (MCI Vs. AD)
FCSRT free	32 ± 8 14 – 40; 14%	19 ± 8 0 – 40; 63%	9 ± 8 0 – 33; 42%	**$2x10E^{-12}$** (All cohorts)
FCSRT Sum	48 ± 3 45 – 57; 14%	47 ± 8 0 – 48; 63%	36 ± 15 2 – 48; 42%	**$6x10E^{-10}$** (AD Vs. ND, MCI)

33

Table 4: Description of supplementary cohort

The table provides an overview of the supplementary patient cohorts (PD, DLB, FTD and ALS cases), potential confounders such as age, sex and biobank storage time, as well as standard AD biomarker and NPT findings. Each feature is described with median value ± standard deviation, Minimum to maximum value and coverage of data within the dataset (in %). N.A.: Data not available for at least one patient. Respective data for the larger main cohort is described in the article.

42

Feature	PD	DLB	FTD	ALS
Number (n)	16	11	20	21
Age (yrs.)	73 ± 7 57 - 85; 100%	71 ± 8 56 - 83; 100%	62 ± 9 42 - 77; 100%	67 ± 10 42 - 83; 100%
Gender (% male)	75	73	65	57
Storage Time (mth.)	28 ± 22 4.0 - 72.0; 100%	38 ± 22 2.0 - 69.0; 100%	51 ± 22 4.0 - 85.0; 100%	42 ± 22 4.0 – 80.0; 100%
Aβ42 pg/ml	522 ± 323 141 - 1160; 100%	346 ± 202 200 - 869; 100%	488 ± 198 187 - 901; 100%	583 ± 214 247 - 917; 100%
Aβ42/40	0.09 ± 0.02 0.04 – 0.11; 100%	0.06 ± 0.02 0.03 – 0.11; 100%	0.09 ± 0.02 0.06 – 0.12; 100%	0.09 ± 0.02 0.06 – 0.13; 100%
t-tau pg/ml	249 ± 306 98 - 1404; 100%	407 ± 184 54 - 666; 100%	437 ± 296 81 - 1039; 100%	250 ± 151 48 - 581; 100%
p-tau-181 pg/ml	45 ± 28 17 - 137; 100%	54 ± 22 30 - 92; 100%	40 ± 30 20 - 140; 100%	39 ± 13 21 - 83; 100%
Aβ42/p-tau-181	11.2 ± 6.1 0.1 – 21.9; 100%	8.7 ± 5.4 2.8 – 22.7; 100%	12.7 ± 6.4 3.5 – 24.2; 100%	13.6 ± 6.5 0.1 – 26.3; 100%
CSF protein (mg/l)	501 ± 173 362 - 968; 94%	545 ± 188 437 - 982; 91%	464 ± 109 269 - 675; 100%	446 ± 181 233 - 1047; 100%
CSF Albumin (mg/l)	309 ± 130 141 - 608; 88%	320 ± 153 218 - 676; 91%	266 ± 96 133 - 477; 100%	216 ± 154 113 - 726; 90%
CSF IgG (mg/l)	32 ± 17 14 - 70; 88%	34 ± 13 27 - 65; 91%	25 ± 10 11 - 47; 100%	27 ± 18 14 - 66; 90%
CSF leukocytes (cells/µl)	2 ± 2 0 – 9; 94%	2 ± 1 1 – 4; 91%	1 ± 2 0 – 10; 100%	1 ± 2 0 – 7; 100%
MMSE	25 ± 4 16 - 27; 38%	21 ± 5 13 - 27; 82%	24 ± 4 15 - 30; 95%	30 5% (single value)
Education (yrs.)	15 ± 1 11 – 18; 13%	13 ± 4 8 – 22; 64%	16 ± 4 8 – 22; 80%	N.A.
Word list recall	13 ± 8 7 – 18; 13%	11 ± 4 4 - 15; 64%	19 ± 7 3 - 23; 70%	N.A.
Word list delayed recall	4 6% (single value)	4 ± 3 1 - 7; 45%	6 ± 2 2 - 9; 60%	N.A.
Word list discrimination (%)	83 ± 25 65 - 100; 13%	95 ± 10 75 - 100; 55%	93 ± 17 50 - 100; 70%	N.A.
Figure recall	8 6% (single value)	7 ± 1 6 - 8; 36%	7 ± 3 2 - 11; 55%	N.A.
TMT A	113 ± 95 46 - 180; 13%	127 ± 51 57 - 180; 64%	58 ± 36 30 - 142; 65%	N.A.
TMT B	213 ± 123 126 - 300; 13%	300 ± 44 201 - 300; 45%	137 ± 85 62 - 300; 60%	N.A.
Semantic Fluency	14 ± 7 9 - 19; 13%	11 ± 4 2 - 14; 64%	10 ± 5 2 - 22; 75%	N.A.
Phonetic Fluency	9 6% (single value)	6 ± 3 1 - 10; 55%	7 ± 5 1 - 18; 65%	N.A.
FCSRT free	16 6% (single value)	15 ± 13 5 - 24; 18%	18 ± 7 12 - 32; 45%	N.A.
FCSRT Sum	48 6% (single value)	39 ± 13 29 - 48; 18%	48 ± 5 32 - 48; 45%	N.A.

Table 5: Inflammatory protein levels in main cohort

The table shows the results of cohort-wise comparisons for the inflammatory protein panel in the main cohort of ND, MCI and AD patients. For each protein, median, standard deviation, minimum and maximum values are provided. Significant results are highlighted bold. For pairwise inter-cohort comparisons, Bonferroni adjusted p values are reported and significant at $\alpha = 0.05$. In this setting, CRP and sTREM2 showed significant differences between the ND cohort on the one hand and MCI as well as AD cohorts on the other. A comparison including the supplementary cohort is included in the article.

Protein	ND	MCI	AD	Kruskal-Wallis p	Pairwise adjusted p
VEGF (pg/ml)	6.0 ± 3.1 0.0 - 18.6	6.0 ± 2.3 2.0 - 19.8	5.6 ± 3.3 0.0 - 25.3	0.168	-
sVEGFR-1 (pg/ml)	47.3 ± 23.7 18.2 - 139.4	49.3 ± 16.7 21.8 - 102.5	51.9 ± 15.9 21.0 - 102.6	0.276	-
MCP-1 (pg/ml)	323 ± 112 169 - 753	342 ± 102 204 - 948	342 ± 96 183 - 644	0.541	-
IP-10 (pg/ml)	283 ± 173 76 - 901	290 ± 172 66 - 953	306 ± 295 69 - 1662	0.549	-
Il-6 (pg/ml)	0.9 ± 0.8 0.0 - 6.0	1.1 ± 1.0 0.0 - 6.6	1.0 ± 0.8 0.0 - 5.3	0.127	-
Il-8 (pg/ml)	42.9 ± 15.8 18.2 - 147.9	45.1 ± 32.8 26.1 - 346.5	44.7 ± 13.9 24.9 - 102.7	0.354	-
SAA (pg/ml)	1377 ± 9100 383 - 64597	1264 ± 2405 148 - 22212	1342 ± 3994 205 - 37163	0.790	-
CRP (pg/ml)	5410 ± 12840 262 - 67633	3111 ± 9868 135 - 59843	2750 ± 10767 372 - 64675	**$4x10E^{-4}$****	ND-MCI: 0.003 ND-AD: $5x10E^{-4}$
sICAM-1 (pg/ml)	2730 ± 1105 1138 - 6684	3061 ± 1272 1287 - 10583	3005 ± 1024 1377 - 6107	0.321	-
sVCAM-1 (pg/ml)	8188 ± 3108 4116 - 23498	8946 ± 3069 3631 - 21950	8753 ± 2473 4700 - 15076	0.321	-
MIF (pg/ml)	1932 ± 1227 745 - 8530	2030 ± 1432 970 - 12779	2021 ± 846 856 - 6681	0.421	-
C1q (ng/ml)	197 ± 80 82 - 383	209 ± 91 73 - 642	215 ± 84 62 - 567	0.463	-
C3aDesArg (ng/ml)	26.9 ± 38.4 0 - 197.6	29.9 ± 28.9 6.1 - 185.6	28.3 ± 21.4 4.6 - 151.5	0.295	-
sIl-1RAcP (pg/ml)	2982 ± 2035 1228 - 10469	3503 ± 2604 519 - 15714	3589 ± 3039 1363 - 26852	0.351	-
sTREM2 (pg/ml)	2994 ± 2269 690 - 13746	4071 ± 2544 795 - 12950	4315 ± 2235 756 - 11965	**0.001****	ND-MCI: 0.004 ND-AD: 0.002

Table 6: Inflammatory protein levels in supplementary cohort

The table shows the distribution of inflammatory marker levels in the cohorts of PD; DLB, FTD and ALS patients. Significant results in Kruskal-Wallis tests are highlighted bold. For pairwise comparisons, Bonferroni adjusted p values are reported and significant at $\alpha = 0.05$ (adjusted for pairwise comparisons of each cohort against each other). In this setting, CRP and sTREM2 and their ratio still differentiated between the main cohorts. Several other markers were significant in comparisons based on the supplemental cohorts with different strength of the respective adjusted pairwise comparisons. “None significant” indicates that pairwise comparisons did not sustain Bonferroni adjustment.

Protein	PD	DLB	FTD	ALS	Kruskal-Wallis p	Pairwise adjusted p
Number (n)	16	11	20	21		
VEGF (pg/ml)	6.1 ± 2.1 3.5 – 11.0	5.9 ± 1.8 3.4 – 8.4	5.0 ± 1.2 2.9 – 7.2	4.6 ± 3.9 3.1 – 17.9	**0.038**	None significant
sVEGFR-1 (pg/ml)	41.1 ± 13.2 21.4 – 70.6	40.4 ± 9.7 30.0 – 60.1	36.4 ± 11.1 25.2 – 76.6	36.0 ± 10.4 28.8 – 69.0	**1x10E^{-6}**	FTD-ND: 0.003 FTD-MCI: 0.003 FTD-AD: 7x10E^{-6} ALS-ND: 0.046 ALS-MCI: 0.047 ALS-AD: 0.002
MCP-1 (pg/ml)	335 ± 73 217 - 473	309 ± 110 194 - 565	336 ± 112 202 - 722	335 ± 112 194 - 637	0.862	-
IP-10 (pg/ml)	217 ± 79 81 - 363	285 ± 185 84 - 728	198 ± 126 48 - 638	198 ± 108 44 - 528	**5x10E^{-4}**	FTD-ND: 0.037 FTD-MCI: 0.038 FTD-AD: 0.006 ALS-AD: 0.033
Il-6 (pg/ml)	1.3 ± 0.3 1-0 – 2.3	1.2 ± 1.1 0.9 – 4.7	1.1 ± 0.4 0.6 – 1.8	1.3 ± 0.4 0.8 – 2.5	**4x10E^{-5}**	ALS-ND: 0.006 PD-ND: 0.005 PD-AD: 0.036
Il-8 (pg/ml)	49.1 ± 9.1 37.9 – 67.1	51.4 ± 8.7 37.6 – 64.7	41.4 ± 7.8 26.9 – 55.3	48.7 ± 11.9 33.3 – 80.8	**0.038**	None significant
SAA (pg/ml)	1415 ± 1707 478 - 7173	1664 ± 13463 533 - 43319	851 ± 1097 244 - 5009	1071 ± 758 524 - 3538	0.087	-
CRP (pg/ml)	3906 ± 6556 984 - 20099	4735 ± 11074 521 - 28337	2910 ± 6794 370 - 26426	5792 ± 6567 458 - 29691	**0.001**	ND-MCI: 0.022 ND-AD: 0.003
sICAM-1 (pg/ml)	3465 ± 858 2457 - 5704	4129 ± 748 3053 - 5849	2909 ± 1098 1599 - 6156	3427 ± 835 1988 - 5442	**0.005**	DLB-ND: 0.006 DLB-MCI: 0.040
sVCAM-1 (pg/ml)	9055 ± 3188 5228 - 15110	9479 ± 2111 6535 - 13866	7818 ± 2214 4008 - 13119	8619 ± 1831 5163 - 11832	0.481	-
MIF (pg/ml)	1966 ± 961 827 - 4586	1837 ± 515 1279 - 2708	1611 ± 1362 935 - 7176	1432 ± 368 958 - 2683	**5x10E^{-4}**	ALS-ND: 0.005 ALS-MCI: 2x10E^{-4} ALS-AD: 0.002
C1q (ng/ml)	233 ± 73 101 - 386	212 ± 57 127 - 330	189 ± 48 92 - 280	192 ± 70 120 - 360	0.555	-
C3aDesArg (ng/ml)	33.2 ± 23.5 17.0 – 101.9	37.2 ± 38.3 16.4 – 141.5	30.8 ± 30.3 11.3 – 141.2	23.7 ± 24.7 12.4 – 83.9	0.272	-
sIl-1RAcP (pg/ml)	5925 ± 3424 3053 - 15887	6705 ± 3152 3839 - 13051	4310 ± 2422 2213 - 11079	4382 ± 2341 1587 - 9897	**2x10E^{-7}**	PD-ND: 2x10E^{-4} PD-MCI: 0.001 PD-AD: 0.003 DLB-ND: 5x10E^{-4} DLB-MCI: 0.002 DLB-AD: 0.005
sTREM2 (pg/ml)	4186 ± 2243 1796 - 8978	4222 ± 1675 2853 - 8785	3343 ± 2031 525 - 8826	3623 ± 2456 1245 - 9680	**0.017**	ND-MCI: 0.025 ND-AD: 0.016

Table 7: Association between inflammatory and AD biomarkers in main cohort

The table shows the results of cohort-wise comparisons for the inflammatory protein panel if samples of the main cohort are divided by pathological vs. non-pathological levels of amyloid or tau biomarkers. Discrimination was based on the following cutoff values: Aβ42 350 pg/ml, Aβ42/40 0.07, t-tau 470 pg/ml, p-tau-181 56 pg/ml. Significant results are highlighted bold. Values are provided with Mann-Whitney U test p values and, for significant findings, Spearman correlation p – and r values derived from the correlation matrix. In this setting, there were a large number of markers consistently associated with tau pathology. Some of these were likewise associated with pathological amyloid values.

Protein	By Aβ42	By Aβ42/40	By t-tau	By p-tau-181
VEGF	0.419	**0.044** p = 0.007 r = 0.148	**0.027** p = 0.005 r = -0.156	**0.008** p = 0.004 r = -0.160
sVEGFR-1	**0.003** p = $1x10E^{-4}$ r = 0.208	**0.033** p = 0.019 r = -0.129	**$2x10E^{-12}$** p = $6x10E^{-21}$ r = 0.485	**$1x10E^{-15}$** p = $3x10E^{-28}$ r = 0.556
MCP-1	0.963	0.190	**0.006** p = 0.001 r = 0.185	0.101
IP-10	0.951	0.773	**0.024** p = 0.001 r = 0.185	**0.034** p = 0.004 r = 0.157
Il-6	0.832	0.876	0.513	0.644
Il-8	0.290	0.336	0.053	0.232
SAA	0.079	0.104	0.443	0.102
CRP	**0.005** p = 0.013 r = 0.136	**$3x10E^{-5}$** p = $9x10E^{-5}$ r = 0.214	**0.005** p = 0.001 r = -0.175	**0.002** p = 0.003 r = -0.162
sICAM-1	0.118	**0.033** p = 0.075 r = -0.098	**$3x10E^{-4}$** p = $8x10E^{-5}$ r = 0.215	**$2x10E^{-4}$** p = $8x10E^{-7}$ r = 0.267
sVCAM-1	**0.015** p = 0.095 r = 0.086	**0.042** p = 0.131 r = -0.083	**$2x10E^{-6}$** p = $3x10E^{-8}$ r = 0.300	**$1x10E^{-8}$** p = $5x10E^{-15}$ r = 0.413
MIF	0.699	0.067	**$2x10E^{-11}$** p = $2x10E^{-15}$ r = 0.419	**$3x10E^{-11}$** p = $1x10E^{-20}$ r = 0.481
C1q	**0.017** p = 0.063 r = 0.102	0.500	**$3x10E^{-8}$** p = $3x10E^{-11}$ r = 0.354	**$2x10E^{-10}$** p = $2x10E^{-14}$ r = 0.403
C3aDesArg	0.422	0.606	0.888	0.611
sIl-1RAcP	0.810	**0.036** p = 0.117 r = -0.086	**0.046** p = 0.019 r = 0.128	0.109
sTREM2	0.070	**0.002** p = 0.037 r = -0.115	**$2x10E^{-12}$** p = $4x10E^{-15}$ r = 0.414	**$9x10E^{-11}$** p = $2x10E^{-17}$ r = 0.444

Table 8: Influence of ApoE genotype in the main cohort

ApoE genotype data was available for 213 samples of the main cohort (18 ND, 102 MCI and 93 AD cases). Comparisons were based either on the exact genotype (2/2, 2/3, 2/4, 3/3, 3/4, 4/4) or the number of ApoE4 alleles (0, 1, or 2 alleles). Significant results from Kruskal-Walli-tests are highlighted bold. For pairwise comparisons, Bonferroni-adjusted p values are reported and significant at $\alpha = 0.05$. CRP was the only inflammatory marker significantly associated with ApoE genotype with a tendency of lower values associated with increasing number of ApoE4 alleles. Homozygous 4/4 carriers compared to non-carriers drove this effect.

Protein/Marker	By exact ApoE genotype		By number of ApoE4 alleles	
	Kruskal-Wallis p	Pairwise adjusted p	Kruskal-Wallis p	Pairwise adjusted p
Aβ42	**0.040**	4/4 – 3/3: 0.023	**0.004**	2-1: 0.022 2-0: 0.003
Aβ42/40	**$9x10E^{-5}$**	4/4 – 3/3: $1x10E^{-4}$ 4/4 – 2/3: 0.042	**$1x10E^{-3}$**	2-0: $5x10E^{-3}$ 1-0: 0.008
t-tau	0.053	-	**0.019**	2-0: 0.022
p-tau-181	**0.008**	4/4 – 2/3: 0.007	**0.004**	2-0: 0.005
Aβ42/p-tau-181	**$2x10E^{-4}$**	4/4 – 3/3: 0.001 4/4 – 2/3: 0.034	**$4x10E^{-5}$**	2-0: $6x10E^{-5}$ 1-0: 0.012
VEGF	0.516	-	0.403	-
sVEGFR-1	0.194	-	0.505	-
MCP-1	0.571	-	0.146	-
IP-10	0.435	-	0.737	-
Il-6	0.571	-	0.524	-
Il-8	0.416	-	0.267	-
SAA	0.108	-	0.104	-
CRP	**0.007**	4/4 – 3/3: 0.019	**0.009**	2-0: 0.014
sICAM-1	0.061	-	0.294	-
sVCAM-1	0.316	-	0.404	-
MIF	0.231	-	0.991	-
C1q	0.216	-	0.602	-
C3aDesArg	0.065	-	0.150	-
sIl-1RAcP	0.330	-	0.157	-
sTREM2	0.119	-	0.071	-

Table 9: Influence of sex on inflammatory protein levels

Levels of several inflammatory proteins varied significantly between male and female subjects. Significant results from Mann-Whitney-U tests are highlighted bold. For those markers that showed a significant test result, the tendency was always that levels were higher in males compared to females.

Protein	Mann-Whitney-U p	Median (male) pg/ml	Median (female) pg/ml
VEGF	**0.004***	6.1	5.5
sVEGFR-1	0.226	48.5	51.6
MCP-1	**0.001****	361	319
IP-10	0.327	299	277
Il-6	**0.005***	1.1	0.9
Il-8	**0.002***	46.1	42.8
SAA	0.983	1341	1348
CRP	**0.036***	3724	2775
sICAM-1	**5x10E^{-7}****	3216	2583
sVCAM-1	**1x10E^{-8}****	9607	7642
MIF	0.177	2045	1953
C1q	**1x10E^{-7}****	233x10E^{3}	189 x10E^{3}
C3aDesArg	**9x10E^{-5}****	31.9x10E^{3}	24.4 x10E^{3}
sIl-1RAcP	**0.003****	3612	2969
sTREM2	**0.004***	2650	2373

Table 10: Homogeneity of the ND (non-demented) cohort

The ND cohort consisted of patients classified by medical diagnosis as follows: Depression (DEP, n = 6), excluded neuroborreliosis (EN, n = 5), polyneuropathy (PNP, n = 28), other movement disorders (MD, n = 2), subjective cognitive impairment without pathological neuropsychological results (SCI, n = 16), normal pressure hydrocephalus without dementia (NPH, n = 8), idiopathic intracranial hypertension (IIH, n = 3), others (O, n = 17). Classes were based on existence of at least 2 identical clinical diagnoses within the total of ND. Significant results from Kruskal-Wallis tests are highlighted bold. For pairwise comparisons, Bonferroni-adjusted p values are reported and significant at $\alpha = 0.05$ (adjusted for pairwise comparisons of each cohort against each other). For several markers, there was a tendency of subcohort variances that was not strong enough to be traceable in pairwise comparisons. For 4 markers (VEGF, VEGFR, Il-6 and sIl1-RAcP) the NPH subcohort showed significant differences to at least one other subcohort. Among these, the most robust (in terms of cohort sizes) was the difference of VEGFR levels between NPH and PNP.

Protein	Kruskal-Wallis p	Pairwise adjusted p
VEGF	**0.047**	NPH-EN; p = 0.042
sVEGFR-1	**0.001**	NPH-EN; p = 0.006 NPH-PNP; p = 0.008 NPH-MD; p = 0.043
MCP-1	0.090	-
IP-10	**0.023**	None after adjustment
Il-6	**0.012**	NPH-Depression; p = 0.028
Il-8	**0.024**	None after adjustment
SAA	0.182	-
CRP	0.260	-
sICAM-1	**0.041**	None after adjustment
sVCAM-1	0.095	-
MIF	0.325	-
C1q	0.180	-
C3aDesArg	**0.019**	None after adjustment
sIl-1RAcP	**0.003**	NPH-IIH; p = 0.007 IIH-O; p = 0.040
sTREM2	**0.046**	None after adjustment

Table 11: Protein ratios

Based on effects observed in the cohort comparison data, we tested how ratios of proteins with inverse effects or tendencies (VEGF and its soluble receptor sVEGFR; CRP and sTREM2) perform in cohort-wise comparisons by Kruskal-Wallis test. The ratio VEGF/sVEGFR did not improve discriminative testing in comparison to sVEGFR alone. The ratio CRP/sTREM2 was a slightly better discriminator than its single components in comparisons based on clinical diagnosis and amyloid. For tau, the ratio was stronger than CRP alone but weaker than sTREM2. Noteworthy, other variants of this ratio (e.g., CRP/sTREM2 or lg CRP/sTREM2) did not result in significant test results.

Ratio	Cohorts	p value
VEGF/sVEGFR	Main Cohorts	0.297
	Amyloid	0.076
	Tau	**$3x10E^{-7}$**
CRP/TREM2	Main Cohorts	**$1x10E^{-6}$** ND - MCI: $3x10E^{-5}$ ND – AD: $4x10E^{-6}$
	Amyloid	**$2x10E^{-7}$**
	Tau	**$2x10E^{-8}$**

Table 12: Confounder-adjusted comparisons

Based on correlation and cohort comparison analyses, we considered patient age, sex and biobank storage time as the statistically most influential covariates. These variables differed significantly between clinical cohorts while being in many cases correlated to inflammatory protein levels. For CRP and sTREM2, we also included ApoE genotype. Analysis was restricted to those inflammatory proteins with the most significant findings in previous cohort comparisons. To test adjustments for pathological cohorts, we focused on the highly discriminative ratio Aβ42/40 for amyloid and on t-tau as both tau isoforms were highly correlated. Log-transformed protein concentrations were used to ensure compatibility with parametric ANCOVA testing. Cohorting refers to the comparison under investigation. For each comparison, the covariate p value indicates if the covariate is of significant influence. The adjusted p value is the result of the cohort comparison adjusted for the influence of the covariate and is only reported when covariates had significant influence. Significant findings are highlighted bold. When of significant influence, adjustment for age and, in most cases, biobank storage time lead to less significant test results. In contrast, adjusting for sex frequently improved p values of cohort comparisons. Of the tested proteins, CRP was less strongly affected by age, sex or storage time, but strongly by ApoE genotype, leading to less significant findings after adjustment.

Protein	Cohort	Cohort p value	Covariance feature	Covariates			
				Age	Gender	Storage Time	ApoE Alleles
sTREM2	Clinical	0.001	Covariate p	**$1x10E^{-11}$**	**0.003**	0.328	0.098
			Adjusted p	**0.270**	**$4x10E^{-4}$**	-	-
	Amyloid	0.004	Covariate p	**$3x10E^{-12}$**	**0.003**	0.226	0.059
			Adjusted p	**0.730**	**0.001**	-	-
	Tau	$2x10E^{-12}$	Covariate p	**$4x10E^{-9}$**	**0.002**	0.133	**0.032**
			Adjusted p	**$3x10E^{-7}$**	**$5x10E^{-13}$**	-	**$9x10E^{-8}$**
CRP	Clinical	$5x10E^{-4}$	Covariate p	0.095	0.072	0.753	**0.017**
			Adjusted p	-	-	-	**0.581**
	Amyloid	$4x10E^{-5}$	Covariate p	0.083	0.084	0.906	**0.030**
			Adjusted p	-	-	-	**0.048**
	Tau	0.019	Covariate p	0.333	**0.038**	0.755	**0.010**
			Adjusted p	-	**0.023**	-	**0.546**
VEGF	Amyloid	0.078	Covariate p	0.121	**0.004**	**$3x10E^{-6}$**	-
			Adjusted p	-	**0.158**	**0.164**	-
	Tau	0.006	Covariate p	0.066	**0.003**	**$3x10E^{-6}$**	-
			Adjusted p	-	**0.008**	**0.013**	-
sVEGFR	Amyloid	0.032	Covariate p	**0.016**	0.407	**$3x10E^{-4}$**	-
			Adjusted p	**0.243**	-	**0.062**	-
	Tau	$8x10E^{-11}$	Covariate p	0.308	0.382	**$4x10E^{-4}$**	-
			Adjusted p	-	-	**$2x10E^{-10}$**	-
sICAM-1	Amyloid	0.064	Covariate p	**$8x10E^{-11}$**	**$5x10E^{-7}$**	0.361	-
			Adjusted p	**0.585**	**0.012**	-	-
	Tau	0.001	Covariate p	**$2x10E^{-9}$**	**$7x10E^{-7}$**	0.322	-
			Adjusted p	**0.157**	**$2x10E^{-4}$**	-	-
sVCAM-1	Aymloid	0.044	Covariate p	**$5x10E^{-9}$**	**$7x10E^{-10}$**	0.199	-
			Adjusted p	**0.879**	**0.004**	-	-
	Tau	$5x10^{-6}$	Covariate p	**$4x10E^{-7}$**	**$5x10E^{-10}$**	0.149	-
			Adjusted p	**0.005**	**$4x10E^{-7}$**	-	-
sIl-1RAcP	Amyloid	0.010	Covariate p	**0.003**	**0.003**	0.613	-
			Adjusted p	**0.165**	**0.003**	-	-
	Tau	0.025	Covariate p	**0.002**	**0.006**	0.668	-
			Adjusted p	**0.267**	**0.017**	-	-
C1q	Tau	$4x10^{-8}$	Covariate p	**$4x10^{-10}$**	**$2x10^{-9}$**	0.566	-
			Adjusted p	**0.001**	**$2x10^{-9}$**	-	-
MIF	Tau	0.028	Covariate p	0.425	0.608	**$2x10^{-6}$**	-
			Adjusted p	-	-	**0.010**	-
MCP-1	Tau	0.008	Covariate p	**$2x10^{-7}$**	**$4x10^{-4}$**	0.389	-
			Adjusted p	**0.375**	**0.004**	-	-
IP-10	Tau	0.041	Covariate p	0.439	0.289	0.262	-
			Adjusted p	-	-	-	-

Table 13: Discriminative power of significant proteins

The table summarizes the results of discriminative power analysis for proteins with significant results in inter-cohort comparisons. Sensitivity and specificity were equally weighted to determine cutoff values. In average, the sensitivity / specificity reached was around 60%.

Protein	Cohorts to Discriminate	Cutoff Value	Sensitivity / Specificity (%)
sTREM2	ND Vs. MCI	3542 pg/ml	58.5
	ND Vs. AD	3531 pg/ml	58.6
	Amyloid	3664 pg/ml	58.3
	Tau	3852 pg/ml	63.5
CRP	ND Vs. MCI	3975 pg/ml	59.5
	ND Vs. AD	3858 pg/ml	58.6
	Amyloid	3524 pg/ml	60.4
	Tau	3197 pg/ml	57.2
CRP/sTREM2	ND Vs. MCI	1.15	60.0
	ND Vs. AD	1.09	60.3
	Amyloid	1.01	62.0
	Tau	0.90	64.2
VEGF	Amyloid	5.9 pg/ml	53.7
	Tau	5.9 pg/ml	54.7
sVEGFR	Amyloid	49.9 pg/ml	55.1
	Tau	50.9 pg/ml	69.8
sICAM-1	Amyloid	2985 pg/ml	53.5
	Tau	2965 pg/ml	57.2
sVCAM-1	Aymloid	8650 pg/ml	55.1
	Tau	8691 pg/ml	61.0
sIl-1RAcP	Amyloid	3472 pg/ml	52.9
	Tau	3472 pg/ml	52.2
C1q	Tau	206 ng/ml	62.3
MIF	Tau	2026 pg/ml	65.4
MCP-1	Tau	341 pg/ml	55.3
IP-10	Tau	297 pg/ml	56.6

Table 14: Association of inflammatory proteins with neuropsychological performance

The table lists significant findings obtained by Spearman correlation analysis of inflammatory proteins that showed at least one significant association with NPT results. Significant correlations are highlighted bold. Across the main cohorts (ND, MCI and AD patients), there were 16 significant correlations with low strength ($r < 0.3$). In the MCI cohort alone, there were 7 significant correlations of similar strength, of which 3 where also found in the main cohort. The strongest correlations were found in the AD cohort, which showed a total of 10 significant correlations of which 6 matched the main cohort.

Protein	Correlate	Main Cohort		MCI cohort		AD cohort	
		p	r	p	r	p	r
MIF	FCSRT free	0.249	-0.097	0.487	-0.078	**0.001**	**-0.458**
	FCSRT sum	0.537	-0.052	0.963	0.005	**$3x10E^{-4}$**	**-0.498**
VEGF	Wordlist delayed recall	**0.007**	**0.197**	0.133	0.151	0.496	0.083
	Semantic Fluency	0.275	-0.077	0.237	-0.116	**0.015**	**-0.265**
	Phonetic Fluency	**0.035**	**-0.150**	0.414	-0.081	**0.003**	**-0.331**
Il-6	Figure Recall	0.111	0.116	**0.048**	**0.193**	0.389	0.105
Il-8	TMTB	0.254	0.085	**0.038**	**-0.209**	0.490	-0.084
	FCSRT free	**0.020**	**-0.194**	0.323	-0.110	**0.041**	**-0.293**
SAA	TMTA	**0.030**	**0.154**	**0.025**	**0.220**	0.374	0.101
	Phonetic Fluency	**0.010**	**-0.182**	0.146	-0.144	**0.001**	**-0.348**
CRP	Semantic Fluency	0.227	-0.085	0.376	-0.087	**0.036**	**-0.230**
	Phonetic Fluency	**0.005**	**-0.197**	0.120	-0.154	**$3x10E^{-4}$**	**-0.391**
	FCSRT free	**0.004**	**0.241**	0.051	0.217	0.270	0.161
	FCSRT sum	**0.008**	**0.222**	**0.022**	**0.253**	0.458	0.109
sICAM-1	MMSE	**0.004**	**-0.168**	0.096	-0.154	0.698	-0.057
	Phonetic Fluency	**0.011**	**-0.180**	0.309	0.100	**0.014**	**-0.273**
sVCAM-1	MMSE	**0.023**	**-0.133**	0.106	-0.150	0.639	-0.045
C1q	MMSE	**0.035**	**-0.124**	0.513	-0.061	0.478	-0.068
C3aDesArg	Semantic Fluency	0.142	-0.104	**0.033**	**-0.208**	0.103	-0.180
	Phonetic Fluency	0.126	-0.109	0.340	-0.095	**0.044**	**-0.224**
sIl-1RAcP	Wordlist Discrimination	0.379	0.063	**0.048**	**0.193**	0.426	0.090
	TMTA	**0.001**	**0.233**	**0.032**	**0.210**	0.082	0.196
	TMTB	**0.005**	**0.208**	0.061	0.190	0.119	0.188
sTREM2	MMSE	**$3x10E^{-4}$**	**-0.210**	0.390	-0.08	0.919	-0.010
	FCSRT free	**0.019**	**-0.196**	0.479	-0.079	0.161	-0.203

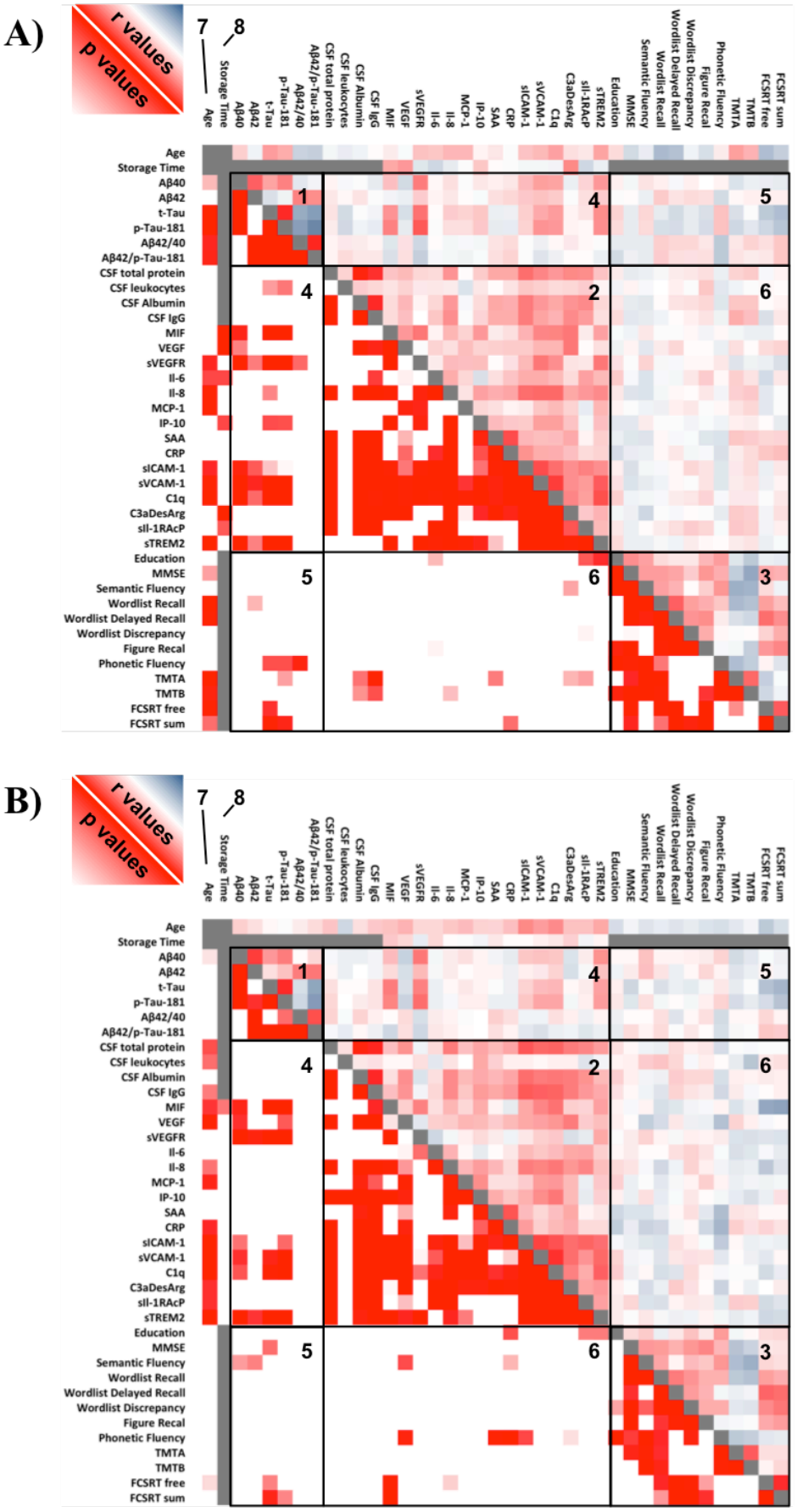
Table 15: Regression analysis of significant correlations with cognition

Regression analysis for significant correlations between inflammatory markers and NPT results, controllig for the influuence of age, sex and education. Significant p values and associated standardized coefficients β are highlighted bold. Significant associations were predominantly found for phonetic fluency in the AD group.

Protein	Correlate	Group	Spearman p	Regression p	Regression β
MIF	FCSRT free	AD	0.001	0.078	-0.264
	FCSRT sum	AD	$3x10E^{-4}$	**0.001**	**-0.469**
VEGF	Semantic fluency	AD	0.015	**0.022**	**-0.256**
	Phonetic fluency	AD	0.003	**0.005**	**-0.298**
Il-6	Figure recall	MCI	0.048	0.468	0.069
Il-8	FCSRT free	AD	0.041	0.083	-0.265
	TMTB	MCI	0.038	0.720	0.035
SAA	Phonetic fluency	AD	0.001	0.055	-0.205
	TMTA	MCI	0.025	**0.039**	**0.197**
CRP	Phonetic fluency	AD	$3x10E^{-4}$	**0.007**	**-0.294**
	Semantic fluency	AD	0.036	0.131	-0.176
	FCSRT sum	MCI	0.022	0.161	0.160
sICAM-1	Phonetic fluency	AD	0.014	**0.033**	**-0.248**
C3aDesArg	Phonetic fluency	AD	0.044	**0.025**	**-0.232**
	Semantic fluency	MCI	0.033	0.327	-0.088
sIl-1RAcP	Wordlist Discrimination	MCI	0.048	0.096	0.165
	TMTA	MCI	0.032	**<0.001**	**0.334**

Figure 1: Correlation Matrix of MCI and AD cohorts

The figure is complementary to figure 3 from the main text but depicts the correlation matrix calculated only for the MCI cohort (A) and the AD cohort (B). Most striking were changes in the correlations to NPT results (box 6, which were more pronounced and robust in the AD cohort (compare figure 3 and 4 from main text and table 15 within this supplement).


A)
B)
r values
p values
7
8
Age
Storage Time
Aβ40
Aβ42
t-Tau
p-Tau-181
Aβ42/40
Aβ42/p-Tau-181
CSF total protein
CSF leukocytes
CSF Albumin
CSF IgG
MIF
VEGF
sVEGFR
Il-6
Il-8
MCP-1
IP-10
SAA
CRP
sICAM-1
sVCAM-1
C1q
C3aDesArg
sIl-1RAcP
sTREM2
Education
MMSE
Semantic Fluency
Wordlist Recall
Wordlist Delayed Recall
Wordlist Discrepancy
Figure Recal
Phonetic Fluency
TMTA
TMTB
FCSRT free
FCSRT sum
1
2
3
4
5
6
Color Code:
Correlation not applicable
Spearman p
1
0
Spearman r
-1
0
1