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Neurological and inflammatory biomarkers in CSF along the Alzheimer's disease spectrum

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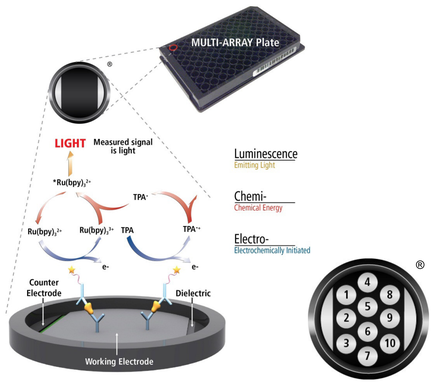
❶ Abstract

Background
Alzheimer's disease (AD) is a heterogeneous neurodegenerative disease with a decades-long prodromal period. Monitoring of sequential pathological changes in neurodegeneration, inflammation, neurovascular dysfunction, oxidative stress and metabolic stress may provide the opportunity for intervention before symptom onset. Assessment with multiple biomarkers may also inform more tailored therapeutic intervention.
Methods
Using MULTI-ARRAY technology, 53 biomarkers were measured using less than 200 µL of CSF from individuals with AD dementia (n=100), mild cognitive impairment (MCI) with progression to dementia during the following 5-year follow-up (n=100), MCI non-progressors (n=100), and subjective cognitive decline (SCD) (n=93), collected by ACE Alzheimer Center Barcelona (ACE). Biomarkers were selected to cover multiple putative disease mechanisms such as neurovascular dysfunction, inflammation, neurodegeneration, tissue injury, and metabolic stress. One-way ANOVA with Bonferroni correction was applied to determine groupwise differences. Area under the curve (AUC) for receiver operating characteristic curves was calculated to assess biomarker utility for predicting dementia progression.
Results
For 43 assays, more than 80% of samples provided concentrations within the dynamic range of the assay. We found concentration differences of 30 CSF biomarkers to be statistically significant across cognitive groups, with the most significant groupwise comparisons between the AD and MCI progressor groups relative to the MCI non-progressor and SCD groups. There were 17 analytes for which mean comparisons were statistically different between MCI progressor and non-progressor groups. Ten proteins, pTau217, total tau, Nf-L, GFAP, MIF, MMP-10, YKL-40, NfH, MIP-1α, and IL-15, demonstrated an AUC > 0.7 for differentiation of MCI progressors and non-progressors, showing promise for differentiating MCI individuals at risk of progressing to dementia, with ptau217 being the most significant (AUC > 0.99).
Conclusions
Here we present an exploratory study with quantitative immunoassays where we identified several CSF biomarkers indicative of dementia or progression to dementia covering multiple pathological mechanisms. Further successful integration into a biomarker panel could help personalize treatment, stratify individuals for therapeutic studies and provide a better understanding of how these early pathologies impact disease progression.

❷ Methods

MSD® electrochemiluminescence detection technology uses SULFO-TAG™ labels that emit light upon electrochemical stimulation initiated at the electrode surfaces of MULTI-ARRAY® and MULTI-SPOT® microplates.
All assays presented here were tested with 25µL assay diluent + 25µL sample, calibrator or control per well.



Electrochemiluminescence Technology

- Minimal non-specific background and strong responses to analyte yield high signal-to-background ratios.
- The stimulation mechanism (electricity) is decoupled from the response (light signal), minimizing matrix interference.
- Only labels bound near the electrode surface are excited, enabling non-washed assays.
- Labels are stable, non-radioactive, and directly conjugated to biological molecules.
- Emission at ~620 nm eliminates problems with color quenching.
- Multiple rounds of label excitation and emission enhance light levels and improve sensitivity.
- Carbon electrode surface has 10X greater binding capacity than polystyrene wells.
- Surface coatings can be customized.

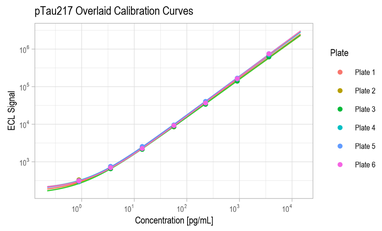
❸ Samples

Samples and associated deidentified data (Table 1) were provided by ACE Alzheimer Center Barcelona (ACE). Aliquots were selected from the ACE biorepository based on diagnosis as determined by ACE Diagnostic Unit via initial and follow up clinical assessments and amyloid, tau and pTau181 CSF measurements. Samples were subaliquoted and shipped to MSD where all testing was conducted blinded.

Table 1: Sample characteristics. Alzheimer's disease (AD) dementia at the time of lumbar puncture (LP; n=99), MCI progressor (MCI+) had mild cognitive impairment at time of LP and progressed to AD dementia within a 5 year follow up window (n=101), MCI non-progressors (MCI-) had mild cognitive impairment at time of LP and did not progress to AD dementia within a 5 year follow up window (n=100), and subjective cognitive decline (SCD) (n=93). Diagnoses were determined by the ACE Diagnostic Unit.

Group		AD	MCI +	MCI -	SCD	p value
Sex	Female	50 (50.5)	50 (49.5)	50 (50.0)	55 (59.1)	0.494
	Male	49 (49.5)	51 (50.5)	50 (50.0)	38 (40.9)	
Age	Mean (SD)	73.6 (8.4)	75.7 (5.9)	68.5 (8.8)	65.9 (7.0)	<0.001
ATN	A+T+N+	96 (97.0)	95 (94.1)	-	7 (7.6)	<0.001
	A+T+N-	3 (3.0)	6 (5.9)	-	1 (1.1)	
	A-T-N-	-	-	100 (100.0)	62 (67.4)	
	A+T-N-	-	-	-	13 (14.1)	
	A-T+N+	-	-	-	7 (7.6)	
	A-T+N-	-	-	-	2 (2.2)	
APOE	e2e3	1 (1.0)	5 (5.0)	11 (11.2)	16 (18.6)	<0.001
	e2e4	3 (3.1)	1 (1.0)	-	3 (3.5)	
	e3e3	47 (48.0)	35 (35.0)	77 (78.6)	47 (54.7)	
	e3e4	43 (43.9)	46 (46.0)	10 (10.2)	17 (19.8)	
	e4e4	4 (4.1)	13 (13.0)	-	3 (3.5)	
CDR	0	-	-	-	93 (100.0)	<0.001
	0.5	-	101 (100.0)	100 (100.0)	-	
	1	87 (87.9)	-	-	-	
	2	12 (12.1)	-	-	-	
MMSE	Mean (SD)	20.3 (4.6)	23.8 (3.4)	27.0 (2.5)	29.4 (0.8)	<0.001
GDS	2	-	-	-	93 (100.0)	<0.001
	3	-	101 (100.0)	100 (100.0)	-	
	4	86 (86.9)	-	-	-	
	5	13 (13.1)	-	-	-	

❹ Assay Performance



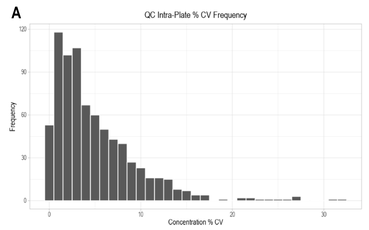


Figure 1: Samples were tested in singlicate over 6 plate runs by one operator per assay. Duplicate calibrator curves were run on each plate.

Figure 2: Histograms showing distribution of %CV for three quality control (QC) samples spanning the analytical range for each assay. (A) Intra-plate %CV between duplicates for each assay and (B) inter-plate %CV between mean concentrations of each control.

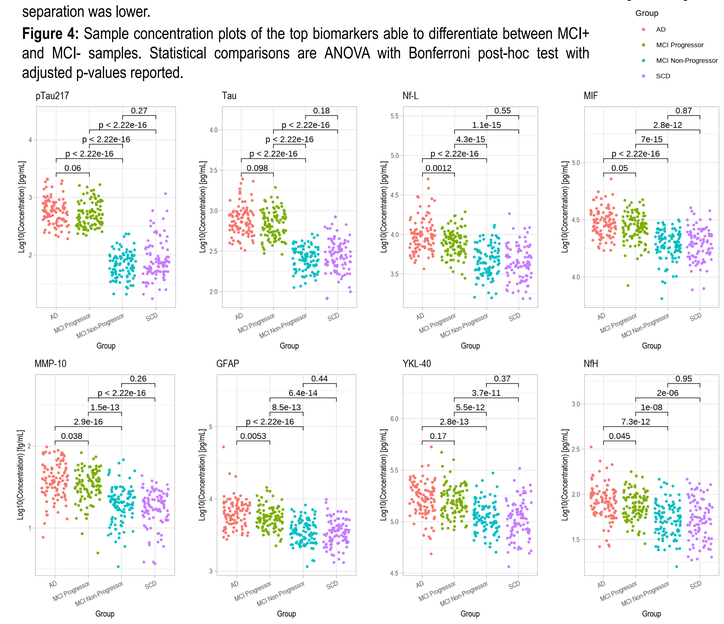
Table 2: Assay list, units, in-well analytical range, in-well sample volume with dilution factor, and percent of samples detected.

Analyte	In-well TOC (pg/mL)	In-well LOD (pg/mL)	CSF Sample Dilution Factor	MSD Panel	% Detectable
YKL-40	2,435	0.18	1,000	U-PLEX® Human YKL-40	100
GFAP	880	0.28	100	S-PLEX® Neurology Panel 1	100
Nf-L	4,330	1.17			100
Tau	153	0.059			100
ASC	30	0.0035	100	Custom	100
IP-10	22,800	0.57	100	V-PLEX® Chemokine Panel 1 (human) Gen. B (subplex)	100
MCP-1	4,512	0.11			100
IL-6	6.4	0.0012	100	S-PLEX Human IL-6	100
NPTX-1	4,000	0.31	100	Custom U-PLEX	100
S100B	2,500	0.13			100
pTau217	3,630	0.18	25	S-PLEX Human Tau (pT217)	100
NfH	5,000	0.20	10	R-PLEX® Human Neurofilament H	100
IL-8	1,202	0.091	10	V-PLEX Proinflammatory Panel 1 Human (subplex)	100
MAG	50	0.0023	10	Custom	100
MMP-2	40,000	47.0	10	Custom U-PLEX	100
IGFBP-2	70,000	88.5			100
TNFR1	1,000	0.14			100
MIF	27,000	3.06			100
SCFR/Kit	40,000	3.49			100
ErbB2	10,000	1.43			100
REG-4	4,000	0.48			100
MMP-9	75,000	5.81			99
Ca15.3	3,000	1.17			99
S100A6	500,000	52.4			100
MMP-1	100,000	3.99	5	Human MMP 3-Plex Ultrasensitive Kit (subplex)	30
MMP-3	100,000	2.14			100
MMP-10	6,500	0.30	5	U-PLEX Human MMP-10 (total)	100
MOG	5,000	0.21	5	Custom	100
TREM2	25,000	0.63	5	Custom	100
Eotaxin	1,480	0.24	2	V-PLEX Chemokine Panel 1 (human) Gen. B (subplex)	99
MIP-1β	606	0.39			100
Eotaxin-3	17,900	1.71			7
TARC/CCL17	744	0.10			94
MIP-1α	614	0.052			100
MDC	4,710	1.07			18
MCP-4	383	0.057			85
GM-CSF	1,180	0.10	2	V-PLEX Cytokine Panel 1 Human (subplex)	0
IL-1α	422	0.063			15
IL-5	888	0.049			99
IL-7	913	0.10			80
IL-12/23p40	3,450	0.27			99
IL-15	840	0.079			100
IL-16	2,620	0.57			25
TNF-β	717	0.086			14
VEGF-A	1,280	0.085			100
IL-10	167	0.024	1	S-PLEX Proinflammatory Panel 1 (human) (subplex)	98
IL-12p70	505	0.059			24
IL-4	55	0.012			1
TNF-a	49.2	0.016			96
IL-2	78.6	0.026			87
IL-1B	160	0.075			7
IFN-γ	40.5	0.009			94
IL-17A	216	0.11			2

❺ Biomarker Data

pTau217 and total Tau in CSF show excellent separation between the AD dementia, MCI+ groups, and the MCI-, SCD groups. Highly significant differences between these groups were also observed with other neural and inflammatory markers such as Nf-L, MIF, MMP-10, GFAP, YKL-40, NfH, MIP-1α, IL-15, MCP-1, and S100A6, although the degree of separation was lower.

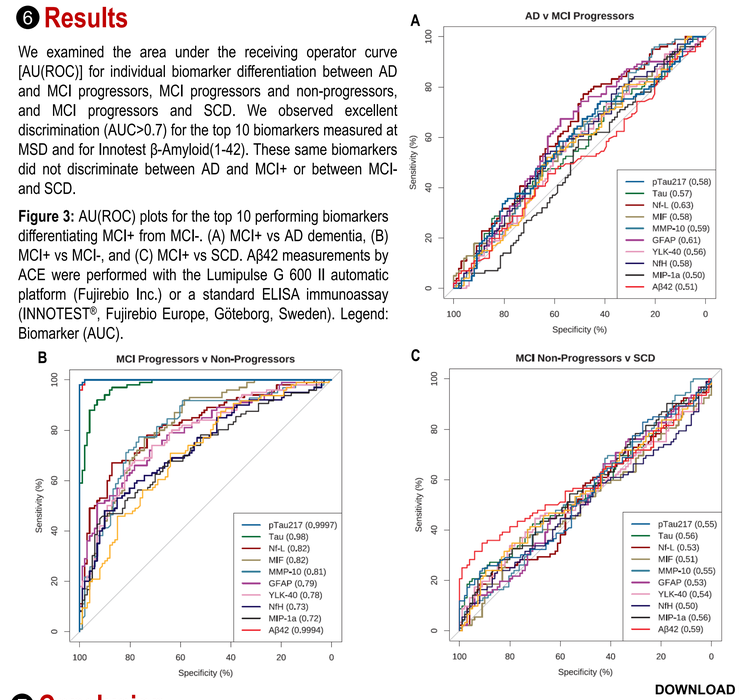
Figure 4: Sample concentration plots of the top biomarkers able to differentiate between MCI+ and MCI- samples. Statistical comparisons are ANOVA with Bonferroni post-hoc test with adjusted p-values reported.



❻ Results

We examined the area under the receiving operator curve [AU(ROC)] for individual biomarker differentiation between AD and MCI progressors, MCI progressors and non-progressors, and MCI progressors and SCD. We observed excellent discrimination (AUC>0.7) for the top 10 biomarkers measured at MSD and for Innotest β-Amyloid(1-42). These same biomarkers did not discriminate between AD and MCI+ or between MCI- and SCD.

Figure 3: AU(ROC) plots for the top 10 performing biomarkers differentiating MCI+ from MCI-. (A) MCI+ vs AD dementia, (B) MCI+ vs MCI-, and (C) MCI+ vs SCD. Aβ42 measurements by ACE were performed with the Lumipulse G 600 II automatic platform (Fujirebio Inc.) or a standard ELISA immunoassay (INNOTEST®, Fujirebio Europe, Göteborg, Sweden). Legend: Biomarker (AUC).



❼ Conclusion

Here we present an exploratory study with quantitative immunoassays where we identified several CSF biomarkers indicative of dementia or progression to dementia covering multiple pathological mechanisms. Assay performance shows excellent detectability with sample-sparing immunoassays, and strong concordance to comparable assays.
Our identification of AD biomarkers associated with different pathological processes could allow for identification of different AD sub-classes to advance research towards personalized treatment, stratify individuals for therapeutic studies and provide a better understanding of how these early pathologies impact disease progression.

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❽ Acknowledgements

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