Cross-sectional investigation of synaptic markers Neurogranin and BACE1 in CSF from the AIBL study

Christopher J Fowler[1], Erik Stoops[2] , Eugeen Vanmechelen[2], Jeroen Vanbrabant[2], Nele Dewit[3], Kimberley Mauroo[3], Christopher C. Rowe[4], Jurgen Fripp[5], Qiao-Xin Li[1], Pierrick Bourgeat[5], Steven J Collins[6], Ralph N Martins[7,8], Colin L. Masters[1], Paul Maruff[9], Hugo Marcel Vanderstichele[10] and James D Doecke[11]

(1)The Florey Institute of Neuroscience and Mental Health, Melbourne, VIC, Australia, (2)ADx NeuroSciences NV, Ghent, Belgium, (3)ADx NeuroSciences NV, Technologiepark 94, 9052, Ghent, Belgium, (4)Department of Molecular Imaging, Austin Health, Melbourne, VIC, Australia, (5)CSIRO Health and Biosecurity, Australian E-Health Research Centre, Brisbane, QLD, Australia, (6)St Vincent's Hospital Melbourne, Fitzroy, VIC, Australia, (7)Centre of Excellence for Alzheimer's Disease Research and Care, School of Medical and Health Sciences, Edith Cowan University, Joondalup, Western Australia, Australia, (8)Macquarie University, Sydney, NSW, Australia, (9)University of Melbourne, Melbourne, VIC, Australia, (10)Biomarkable bv, Gent, Belgium, (11)The Australian e-Health Research Centre, CSIRO, Brisbane, QLD, Australia

Poster number #78084

Background

- Synaptic disfunction is a feature of Alzheimer's disease (AD). Measurement of synaptic proteins may assist with disease staging and can be investigated in relation to amyloid accumulation and the A/T/N framework.
- BACE1 is located presynaptically and cleaves APP into Aβ fragments. BACE1 has been variably reported to increase or decrease in concentration in CSF along the spectrum of AD progression.[1,2]
- Neurogranin (Ng) is located post-synaptically and is found in dendritic spines. Ng is involved with calmodulin signalling and long-term potentiation. Ng is reported to decrease in brain tissue, but increase in CSF during AD.[3] The Ng/BACE1 ratio is suggested to change in AD and be prognostic for future cognitive decline.[4]
- Alpha-synuclein is located presynaptically, and aggregates are linked with Parkinson's disease and other neurodegenerative diseases. Alpha-synuclein comprises a non-amyloid component of amyloid plaques.
- Additionally, soluble Aβ measurement is fraught with pre-analytical handling and inter-laboratory testing variability. Alternate protein measures to Aβ may help with diagnostic accuracy.

Methodology

Participants

- n=106 with CSF and matching PET-amyloid scans from the Australian Imaging, Biomarkers and Lifestyle (AIBL) study were analysed for CSF synaptic markers BACE1, Ng and alpha-synuclein using commercially available ELISA assays (EUROIMMUN).

Assay

- BACE1 was developed with ADx401 (5G7) and ADx402 (10B8) antibodies.
- Neurogranin C-terminal fragment truncated at proline 75 (trunc p75) assay used monoclonal antibodies ADx403 (ADxNGCI2) and ADx451 (ADxNGCT1).
- Alpha-synuclein assay used ADx301 and ADx302 antibodies.
- Total-tau and Aβ42 were measured using the Roche Elecsys® Gen1 on frozen aliquots.

Biospecimen sample

- Fasting CSF was collected by either gravity or aspiration into 15mL PP falcon tubes on wet ice using a graduated 22/27G needle. CSF was spun at 2000g for 10min, 4ºC, transferred to a new PP tube, inverted to remove any gradient effect, aliquoted and frozen in NUNC CryoBankIt tubes and stored in LN2 vapour phase tanks.

Imaging and ATN Criteria

- PET-amyloid status (A+) was defined using a Centiloid of 20.
- In ATN criteria T+ defined by CSF pTau181 status, and N+ was CSF tTau.

Results

Cohort demographics

- 106 CSF samples were analysed retrospectively in people who were CN, MCI or AD and were recruited as part of the AIBL cohort.
- AIBL cohort ethnicity is >98% northern European Caucasian.

	Total Sample	PET Aβ-	PET Aβ+	p-value
N (%)	106	72 (68%)	34 (32%)	
Gender Male, N (%)	53 (50%)	33 (46%)	20 (59%)	0.21
Mean Age, years (SD)	72.9 (5.7)	72.6 (5.9)	73.6 (5.5)	0.41
APOE ε4 Carriage, N (%)	32 (30%)	15 (21%)	17 (50%)	0.0023
Median MMSE, (MAD)	28 (1.5)	29 (1.5)	27 (3)	0.01
Median CDR SB, (MAD)	0 (0)	0 (0)	0.5 (0.7)	0.00012
Mean AIBL PACC Score (SD)	-0.5 (1.2)	-0.1 (0.6)	-1.2 (1.6)	0.00027
Diagnosis CN N %	11 (10%)	67 (63%)	11 (10%)	
Diagnosis MCI N %	84 (79%)	5 (5%)	17 (16%)	
Diagnosis AD N %	11 (10%)	0	6 (6%)	<0.0001

SD: Standard deviation; MAD: Median absolute deviation; MMSE: Mini mental state exam; CDR-SB: Clinical dementia rating sum of boxes; PACC: Preclinical Alzheimer cognitive composite; CN: Cognitively normal; MCI: Mild cognitive impairment.

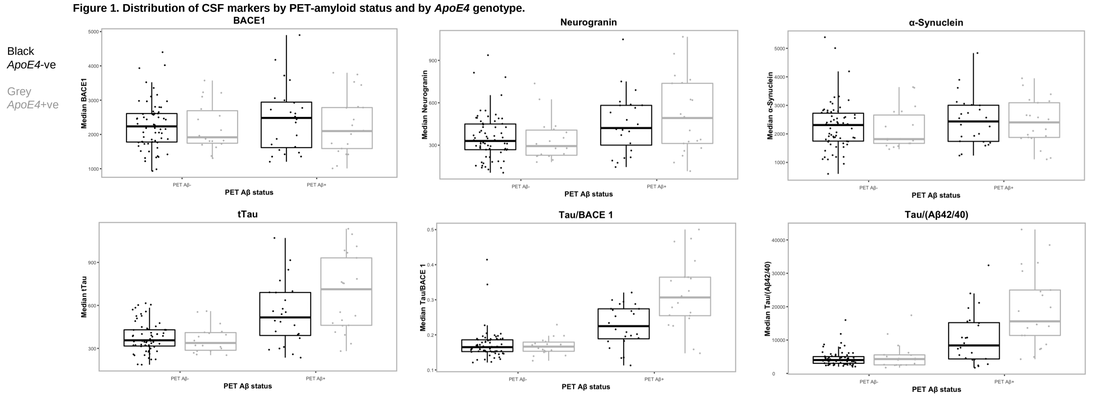
Results cont.

Synaptic marker levels in relation to PET-amyloid status

- Cross-sectional separation between PET-amyloid NEG and POS was significant for CSF Ng (p<0.05) but not BACE1 or alpha-synuclein.
- CSF total-Tau had the best separation of a single marker, and this signal was improved using a ratio of tTau/BACE1. *ApoE4* carriage saw elevated signals for all measures involving tTau in the amyloid POS group.
- The tTau/BACE1 ratio was not significantly different to tTau/Aβ(42/40) (p<0.5).

	AUC 95%CI	p-value	Sens	Spec	PPV	NPV	Accuracy
tTau	0.86 (0.79 - 0.93)	1.12E-09	56.76	98.55	95.45	80.95	83.96
tTau/BACE1	0.90 (0.82 - 0.97)	2.27E-11	72.97	97.1	93.1	87.01	88.68
tTau/AB(42/40)	0.89 (0.82 - 0.96)	3.73E-11	89.19	73.91	64.71	92.73	79.25

Figure 1. Distribution of CSF markers by PET-amyloid status and by *ApoE4* genotype.

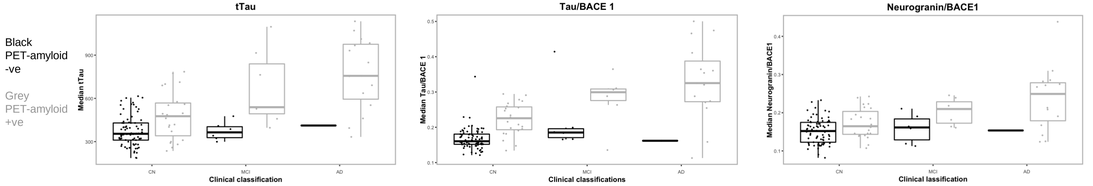


Results cont.

Biomarker levels in relation to increasing clinical severity

- In the amyloid positive group tTau (p<0.01), tTau/BACE1 (p<0.05) and neurogranin/BACE1 (p<0.05) ratio increased in the presence of worsening clinical symptoms between CN and AD.
- In the CN subgroup, separation of PET-amyloid POS (preclinical AD) from NEG was improved by the CSF tTau/BACE1 ratio (p<0.0001) compared to tTau alone (p<0.01). The neurogranin/BACE1 ratio trended elevated (p=0.056) in CN POS.

Figure 2. Distribution of CSF markers by clinical classification and PET-amyloid status.

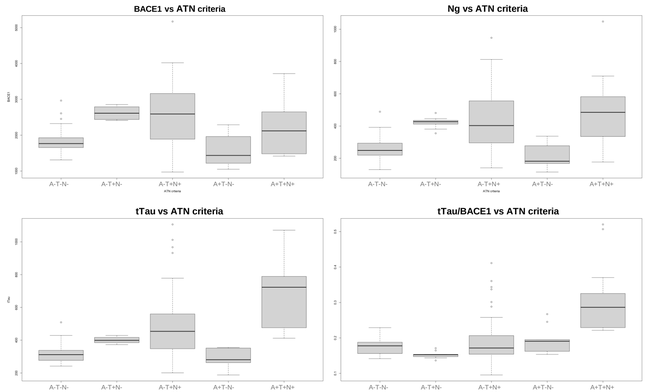


Results cont.

Synaptic marker levels in relation to ATN criteria

- Ng was elevated in all cases where T+ was present, even in absence of amyloid positivity.
- BACE1 was elevated in the presence of T+, and trended reduced in preclinical AD (A+T-)
- The tTau/BACE1 ratio was elevated only in the presence of A+T+.

Figure 3. Distribution of CSF markers by A/T/N criteria



Conclusions

- Neurogranin, while able to weakly separate PET-amyloid POS from NEG, was found using A/T/N criteria to be elevated in all T+ groups, independent of amyloid, thus consistent with recent literature concluding that Ng is non-specific for Alzheimer's disease and is a marker of synaptic dysfunction and closely correlated with Tau.[5]
- Matching another report, BACE1 was found to be reduced in preclinical AD (A+T-).[1] BACE1 was elevated in all instances of T+.
- Total-Tau was elevated in all cases of T+. When used in conjunction with BACE1 the ratio tTau/BACE1 became specific for AD (A+T+N+) only.
- The Ng/BACE1 ratio was weakly elevated in CN A+ and increased as clinical symptoms worsened. It remains to be tested in the AIBL cohort whether this measure has prognostic ability in regard to longitudinal cognitive decline.
- Aβ 40 and 42 measurements have wide inter-lab assay variability. The ability of CSF tTau/BACE1 to distinguish PET-amyloid NEG and POS was equivalent to the tTau/Aβ(42/40) ratio and may indicate an alternative measure to Aβ for predicting PET-amyloid positivity.

References

1. Kirsebom et al 2022. Stable cerebrospinal fluid neurogranin and β-site amyloid precursor protein cleaving enzyme 1 levels differentiate predementia Alzheimer's disease patients. *Brain Commun.* 2022 Sep 24;4(5)
2. Zhong et al (2007) Levels of beta-secretase (BACE1) in cerebrospinal fluid as a predictor of risk in mild cognitive impairment. *Arch Gen Psychiatry.* 2007 Jun;64(6):718-26.
3. De Vos (2015) C-terminal neurogranin is increased in cerebrospinal fluid but unchanged in plasma in Alzheimer's disease. *Alz & Dem* Vol 11, Issue 12, Dec 2015, 1461-1469
4. Kirsebom et al (2018) Cerebrospinal fluid neurogranin/β-site APP-cleaving enzyme 1 predicts cognitive decline in preclinical Alzheimer's disease. *Alz Dem* 2018; 4: 617–627.
5. Willemse et al (2021) Neurogranin as biomarker in CSF is non-specific to Alzheimer's disease dementia. *Neurobiol Aging.* 2021 Dec;108:99-109.

Disclosures

ADx NeuroSciences is now owned by Fujirebio. C Fowler, J Doecke, Jurgen Fripp, Qiao-Xln Li, Pierrick Bourgeat, S Collins, R Martins and C Masters have no COI to declare. Eugeen Vanmechelen is cofounder and shareholder of ADx NeuroSciences. Erik Stoops is employee and shareholder of ADx NeuroSciences. Jeroen Vanbrabant, Nele Dewit, and Kimberley Mauroo are employees of ADx NeuroSciences. Paul Maruff is a full-time employee of Cogstate Ltd. Christopher C. Rowe has received research grants from NHMRC, Enigma Australia, Biogen, Eisai, and Abbvie; he is on the scientific advisory board for Cerveau Technologies; and consulted for Prothena, Eisai, Roche, and Biogen Australia. Hugo Vanderstichele is a cofounder of ADx NeuroSciences and a founder of Biomarkable.