

Clinical utility of Cerebrospinal fluid A β 42 and tau measures in diagnosing mild cognitive impairment in early onset dementia

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ABSTRACT

Background: The differentiation of a preclinical or prodromal Alzheimer's disease (AD) is challenging particularly in patients with Early Onset Alzheimer's or Related Dementias (EOARD). We report our experience on diagnostic lumbar puncture to diagnose EOARD at a tertiary neurocognitive referral centre in Nottingham, England from March 2018 to October 2020.

Methods: Thirty participants aged 32-68 years old (mean 59 years; 57% female) were included. Clinical diagnosis was based on clinical presentation, neurocognitive profile, neuroradiological features (MRI, FDG-PET CT) and cerebrospinal fluid (CSF) amyloid- β 42 (A β 42), total and Thr181-phosphorylated tau (p-tau) measurements.

Results: Patients with Mild Cognitive Impairment (MCI) who progressed to AD (prodromal AD) had significantly higher CSF total (797.63 pg/ml) and p-tau (82.31 pg/ml), and lower A β 42 levels (398.94 pg/ml) in comparison to their counterparts with stable MCI (sMCI) (total tau 303.67 pg/ml, p-tau 43.56 pg/ml, A β 42 873.44 pg/ml) ($p < 0.001$ for CSF total and p-tau measures and $p < 0.00001$ for CSF A β 42 measures). None of the CSF biomarkers correlated with any of the cognitive performance measures. Principal component analysis confirmed that the clinical diagnosis of MCI secondary to Alzheimer's disease, namely prodromal AD (as per NIA-AA criteria) in younger adults, was associated with decreased CSF A β 42.

Conclusion: In Early Onset Alzheimer's disease, low levels of CSF A β 42 appear to be more sensitive than total and p-tau measures in differentiating Alzheimer's MCI from other forms of dementia. Further work on larger samples of EOARD in clinical practice will address the cost effectiveness of making an earlier diagnosis.

KEYWORDS

Early Onset Alzheimer's disease, Mild Cognitive Impairment, AD, CSF, MCI, Amyloid- β

ABBREVIATIONS

A β 42: Amyloid- β 42

AD: Alzheimer's disease

CSF: cerebrospinal fluid

EOARD: Early Onset Alzheimer's or Related Dementia

MCI: Mild Cognitive Impairment

p-tau: 181 Threonine phosphorylated tau

sMCI: stable MCI

INTRODUCTION

The differentiation of preclinical or prodromal Alzheimer's disease (AD) can be challenging particularly in patients with Early Onset Alzheimer's and Related Dementias (EOARD). The first clinical symptoms of AD during the prodromal phase are subjective mild cognitive impairment, that, in some, may present with features consistent with depression [1], or anxiety, [2] which can cause hindrance in diagnosing EOARD at the early stage. After screening for objective cognitive impairment and appropriate investigations to exclude clinical mimics, some will be diagnosed with mild cognitive impairment (MCI).

On average, the conversion from Alzheimer's MCI (i.e. MCI secondary to prodromal AD) to dementia takes five years, although some patients may remain stable over a prolonged period of time [3]. During this period, patients may be monitored annually by clinical services without the support that is appropriate for their employment and advanced planning, and these provisions are particularly relevant for those below the age of 65. Depending on the regional clinical services, patients with MCI may have multiple referrals between their General Practitioners, Memory Clinics, and Neurology Services until they eventually progress to dementia. The long prodromal phase of uncertainties has an impact on patients' lives as well as financial bearing upon the NHS resources due to multiple assessments and investigations to differentiate normal aging from a prodromal AD.

During this prodromal phase, however, there are biochemical changes in the brain which are also reflected in the cerebrospinal fluid (CSF). Decreased levels of Amyloid- β 42 (A β 42) or A β 42/A β 40 ratio in the CSF are well-established markers of AD that can be identified up to 15 years before the onset of Alzheimer's dementia [4]. Elevations of tau in the CSF, a marker of axonal damage, is another AD biomarker [5]. The presence of A β 42 and tau abnormalities in

the CSF has 95% sensitivity and 87% specificity to predict conversion of MCI to Alzheimer's dementia [6].

The initial studies on CSF A β 42 and tau proteins reported similar lower levels of A β 42 between early and late onset established AD, but significantly higher total and phosphorylated tau (p-tau) protein in the Early Onset Alzheimer's disease [7]. However, studies of the early stages of AD, i.e. prodromal AD with mild cognitive impairment, are underreported in younger adults. In a study of 308 people with MCI (<70 years), including mixed early and late onset MCI, a great overlap was reported in measurements across different diagnostic states [8], but no attempt was made to address whether any of the analysed CSF biomarkers were related to the age of presentation with MCI. Thus, it remains largely unknown whether the CSF biomarker profile is similar between the early onset and the late onset AD. Since the progression and cognitive impairment are more expeditious in the early onset AD dementia compared to the late onset dementia, it is reasonable to expect that they may differ at a molecular and biomarker level.

In the current study, we address the CSF biomarker profile in early onset MCI patients who attended a tertiary neurocognitive referral centre. In the absence of firm data regarding the CSF expression of A β 42 and tau in early onset MCI secondary to AD, we analysed these biomarker measures in relation to patients' cognitive performance and age at the time of the MCI diagnosis and compared them with those of age-matched adults with stable MCI (sMCI).

MATERIALS AND METHODS

PARTICIPANT RECRUITMENT

All participants in the current study came from the EOARD clinic at the Queen's Medical Centre in Nottingham. This NHS tertiary referral Centre consists of a multidisciplinary team

of Specialist Consultants in neurology, psychiatry, psychology, neuroradiology and nuclear medicine, as well as allied healthcare professionals, such as Occupational Therapists, Dementia Nurses and Support Workers. The Centre serves patients from primary and secondary health Care services, Memory Clinics, Psychiatry, Clinical Psychology and General Neurology Services.

On average, 450 patients with cognitive symptoms in the absence of known causes (i.e. alcohol abuse, brain trauma, major depression, etc.) are annually referred and assessed at the Centre. A multi-disciplinary approach is used for uncertain or complex cases to consider further investigations including a diagnostic lumbar puncture for CSF studies. Patients with overt dementia are deemed unnecessary or non-capacitated to have a diagnostic lumbar puncture unless a neuroinflammatory, prion or infectious cause is suspected according to the NICE guidelines [9].

For this study, we included 30 patients with the onset of cognitive symptoms under the age of 65 years, who attended the Service from March 2018 to October 2020. The clinical diagnosis of AD or other dementia syndrome was based on clinical and neuroradiological assessments, including neurocognitive examinations, multimodal MRI, Positron Emission Tomography with 2-[fluorine-18] fluoro-2-deoxy-D-glucose (FDG-PET CT) of the brain and CSF studies of A β 42, total tau, and Thr181-phosphorylated tau (p-tau) to characterise MCI aetiology. Further follow up under the clinical service monitored the stability or progression of cognitive decline to confirm an underlying neurodegeneration.

The study was reviewed and approved by the East Midlands-Derby Research Ethics Committee (Ref:18/EM/0292) and also had an approval from the UK Health Research Authority. Informed consent was obtained from all participants to include and analyse their clinical data.

CLINICAL AND COGNITIVE ASSESMENTS

26 out of the 30 participants who had diagnostic lumbar puncture also had neurocognitive testing results available from local hospital records (Table 1). 4 patients did not undergo further cognitive assessment in the clinic due to previous assessments at other centres. Of these 26 patients, 21 agreed to have an additional neurocognitive battery for assessments of memory, attention, language, praxis and number abilities with the Birmingham Cognitive Screen (BCoS) for research purposes [10]. 5 participants did not opt into lengthy BCoS assessment due to multiple previous cognitive tests in the primary and secondary care settings. For these 5 patients, other clinical neurocognitive assessments were used for analysis, including Addenbrooke's Cognitive Examination, ACE-R version III [11], Montreal Cognitive Assessment, MoCA [12], Repeatable Battery for the Assessment of Neuropsychological Status, RBANS [13], and Warrington's Recognition memory task [14].

Patients were classified into three groups according to their clinical diagnosis:

- a) Prodromal AD - NIA-AA Research Framework Alzheimer's Disease (n=16) [15]
- b) sMCI, impairment in cognitive battery with no evidence of neurodegeneration on physical examination, neuroimaging studies, or abnormal levels of A β 42/tau in the CSF (n=9); and
- c) Other neurodegenerative conditions included: Frontotemporal Dementia, Corticobasal Degeneration, Lewy Body Dementia and mental health disorder (n=5).

All patients with prodromal AD provided consent to have dementia gene panel including mutations of APP, PSEN 1 and PSEN2. None of them had a known inherited abnormal mutation, and therefore all the cases in this study were classified as early onset sporadic AD. Patients remained under the care of the clinical service for at least 5 months (up to 30 months, Table 1) to ensure appropriate support is provided. Cognitive assessments were repeated during the follow-up to monitor their progression and match patients' clinical need and support. The final clinical and neurocognitive assessment was used as the measure of progression.

CSF STUDIES

The participants had a lumbar puncture at the Neuroscience's Day Case Unit, Queen's Medical Centre. Standard NHS protocols were followed to obtain CSF samples [16]. The analysis of cell count, protein, and infectious screening of the CSF samples was performed in the local clinical pathology laboratory. Aliquoted frozen CSF was transferred to an NHS approved laboratory (The CSF Dementia Biomarker Service at the NHS Neuroimmunology and CSF Laboratory, University College London) to measure A β 42, total tau and p-tau levels, using the INOTEST platform [17]. The reference laboratory [18] set up their own normal levels of A β 42 (627 – 1322 pg/ml), total tau (146 -595 pg/ml), Thr181p-tau (below 68 pg/ml), and these were used as control/normal values in absence of a control clinical group in the current study. At the time of the study, the approved CSF Dementia Biomarker Service laboratory did not offer CSF A β 40 measures, and the A β 42/A β 40 ratio, use as a measure of cerebral amyloid deposition, could not be established.

STATISTICAL ANALYSIS

Differences in demographics and cognitive domains between three groups of patients including Prodromal AD, sMCI and other neurodegenerative disorders were analysed employing the analysis of covariance (ANOVA), To determine the relationship between CSF analytes and age, Pearson Correlation analysis and Polynomial regression analysis were used. These statistical analyses were performed using SPSS for Windows (version 28.0; SPSS, Chicago, IL); $p < 0.05$ was considered significant.

Principal Component Analysis (PCA) was performed to study the relationship between clinical measures and the CSF total tau, p-tau and A β 42 levels. This descriptive analysis helps observe clusters which can have a biological meaning. The aim was to find separate clusters for patients with prodromal AD and those with stable MCI and use the obtained data for the diagnosis of

each individual patient. The data that was obtained from the PCA plot was used to produce formulas (an algorithm, Appendix I) in order to possibly segregate similar patients between the diagnostic groups.

The PCA analysis utilised the data for a total of 30 patients (summarised in Table 1) including 9 patients described as stable MCI, 16 patients diagnosed with prodromal AD, and 5 patients classified in the “Other” diagnostic group. The latter represented Dementia with Lewy Body, frontotemporal dementia, corticobasal degeneration and mental health disorder with autism background (Figure 1).

The PCA scores (Figure 1) corresponded to the patients’ CSF total tau and A β 42 levels minus the mean of all the patients analysed in the PCA multiplied by the rotation. The analysis was carried out in Rstudio using the prcomp method [19-21].

To differentiate patients with prodromal AD from stable MCI in this study, minimal sample sizes were calculated to obtain meaningful differences between the means of the A β 42 and total tau.

RESULTS

CLINICAL MEASURES OF COGNITIVE CHANGES

Although none of the patients in the sMCI group showed impaired language or attention, over 70% of patients had memory impairment, irrespective of their diagnosis, with similar levels of executive dysfunction in prodromal AD and stable MCI (16.7% and 14.3%, respectively) (Table 1). Thus, in this study population, patients with early-stage or prodromal AD could not be clearly distinguished from those with stable MCI based on initial cognitive testing alone.

As in older people, the CSF tau and A β 42 measures followed the well-known distribution of lower A β 42 and higher total and p-tau levels in patients with prodromal AD (Table 2, Appendix II). These were significantly different from the sMCI group, with only the p-tau that did not reach statistical significance ($p=0.065$).

LINEAR REGRESSION BETWEEN CSF BIOMARKERS

Correlation analysis was carried out to assess if there was a significant correlation between the CSF measures. As expected, p-tau and total tau had a strong positive linear correlation. There was also a statistically significant negative correlation between A β 42 and both total tau and p-tau (Figure 1). CSF biomarker results for all study participants can be seen in Table 3.

CSF BIOMARKERS AND AGE

The linear regression analysis on the total group of 29 participants (age range 55 to 68 years of age) did not show any significant relationship of CSF measures with age, though indicated a nonsignificant decline in p-tau and total tau measures with age ($r=-0.416$, $p=0.467$ and $r=-0.021$, $p=0.914$, respectively; Figure 2a and 2b). In contrast, the CSF A β 42 and total CSF protein measures tended to increase with age, albeit not reaching statistical significance ($r=0.116$, $p=0.550$ and $r=0.267$, $p=0.198$, respectively; Figure 2c and 2d).

The low R^2 values of the linear regression model indicated that the response between the predictor value (age) and the response variables (CSF A β 42 and tau protein measures) are more complex than just a simple linear relationship, and that polynomial regression analysis may be required. Indeed, quadratic regression analysis better demonstrated the nonlinear relationship between the analysed variables, and showed a significance between age and CSF total, p-tau and A β 42 CSF measures (Figure 2, 1c). In contrast to the linear analysis, the polynomial regression analysis indicated that >29% of the analysed tau values were explained by participants' age in this new model.

COGNITION IS NOT RELATED TO CSF BIOMARKERS IN EOARD

Detailed cognitive assessments were carried out on 26 of the 30 patients with available CSF examination data. Of these, 21 had BCoS, whereas the remainder had different cognitive tests. None of the correlation analyses between cognitive and CSF measures yielded a significant p-value (Table 4). In general, there was little correlation between the CSF biomarkers of AD and cognitive assessment in adults with prodromal AD and/or stable MCI.

CSF PCA ANALYSIS

In order to summarise the CSF biomarker findings in relation to their clinical use, we carried out a PCA analysis. For this, the PCA plot was based on both CSF A β 42 and total tau levels to show differences in clusters of prodromal AD and stable MCI (Figure 3). The data for CSF A β 42 and total tau levels produced a sufficient segregation between the described diagnostic groups. However, the total tau data on the PCA plot did not reach the required sample size for 95% confidence. The fact that A β 42 sample size calculation produced smaller sample sizes for 95% confidence level indicated that for this population, the CSF A β 42 might have more discriminatory value to differentiate the patients. P-tau levels were considered for PCA analysis but finally it was discarded as a variable as it did not help discriminating the different groups.

DETERMINATION OF SAMPLE SIZE BASED ON CSF MEASURES

The two main groups analysed in this study included the prodromal AD and stable MCI groups. To determine meaningful sample sizes required for the CSF biomarkers (A β 42, total and p-tau) for the early diagnosis of prodromal AD in younger adults, we used the following formula:

Based on this formula, the CSF A β 42 measures were conducted on an appropriate number of participants in both groups, whereas the use of CSF total and p-tau measures was underpowered, especially the numbers needed for the p-tau (Table 5).

DISCUSSION

We report the benefits of diagnostic lumbar puncture for patients with early onset mild cognitive impairment in a clinical setting, and its contribution to differentiate a progressive cognitive decline (prodromal AD) from patients with stable MCI. The use of CSF A β 42 can be predictive of progressive cognitive decline and determinant of the early onset AD in clinical practice, whereas baseline cognitive assessments and neuroimaging have been inconclusive. In this study, insignificant correlation between abnormal CSF biomarkers and impaired cognition suggests that the initial cognitive testing is not sufficient for prognostication of patients with MCI and identification of individuals at risk of progression to prodromal AD against those who are anticipated to remain stable. Thus, the use of CSF biomarker analysis, particularly CSF A β 42, is clinically useful for prompt diagnosis of early onset sporadic AD. During this early phase of the disease, Alzheimer's pathology is evident in the CSF before significant AD-related cognitive impairment becomes apparent [17]. Further, identifying prodromal AD will be the key in the event of new disease-modifying treatments for AD.

Adults with early onset AD have cognitive impairment profile that is not identical to that seen in older adults with late onset dementia [22]. Various cognitive assessments prior to referral to our service is reflective of difficulties in establishing the correct diagnoses for these young adults. An early and accurate diagnosis can help these individuals to make decisions on planning extended family, provide them with appropriate support to continue employment in a safe environment, and potentially consider Amyloid- or tau-targeting/disease-modifying

treatments before emerging dementia. Since brain atrophy on MRI and changes in other imaging modalities for the diagnosis of neurodegenerative disorders (i.e. FDG-PET) are not necessarily an early marker to differentiate individuals with prodromal AD (with progressive MCI) from those with stable MCI, the use of CSF A β 42 can reliably help for diagnostic work up in selected individuals. We also confirm that the depletion of CSF A β 42 is an early change in the AD cascade, as suggested in the ATN framework for established AD [15]. Furthermore, a smaller sample with 95% confidence interval was produced by using the A β 42 for the calculation, and this suggests that the CSF A β 42 may be more indicative for the diagnosis of early onset AD.

In a recent study, both Thr181p-tau and Thr217p-tau levels were significantly elevated in older individuals with MCI and distinguished prodromal AD from the MCI without AD (AUC = 82.6%–90.5%) [23]. However, a recent systematic review suggests that the lack of CSF A β 42 and tau abnormalities in patients with MCI, has a great utility in excluding AD, whereas abnormal levels of A β 42 and tau in the CSF does not necessarily rule in Alzheimer's disease [22]. Our findings, on the other hand, suggest that in young adults it is the lower CSF A β 42 levels that discriminate between the prodromal AD and stable MCI.

Our study has some limitations. Although there were sufficient patients to meet the requirements in sample size for the CSFA β 42 analysis, it had fewer than ideal patients for 95% confidence level for both CSF total and p-tau. Hence, a larger data set is needed to obtain more conclusive data. Further, we used a PCA analysis and took into account both A β 42 and total tau data to show differences in clusters of prodromal AD and stable MCI. Based on this analysis, it was the loss of A β 42, but not changes in the CSF tau, that had a diagnostic advantage in the preclinical stages of AD. In addition, a confirmed memory impairment alone in the absence of A β 42 abnormality seems to be consistent with stable MCI.

There is evidence that performance on verbal fluency tasks is able to discriminate between patients with stable MCI and those that will progress to AD [24], and our findings do show that the AD group had lower language test scores than those in the stable MCI group. However, because the majority of participants in our study had cognitive assessment with BCoS, the data from the 5 participants who had ACE-R III cognitive assessment are not sufficient to explore differences in semantic and phonemic fluency between the stable MCI and AD groups. Further studies into language assessment sub-score differences in stable MCI and AD would need to be conducted.

Our pilot findings now need to be confirmed in a larger longitudinal study to determine the conversion from prodromal early onset AD to AD dementia. Further, we examined CSF A β 42 and p-tau that were made available to the NHS by the reference laboratory during this study, meaning measures of Thr217p-tau were not available. Since then, the laboratory service has been upgraded to enable measuring A β 42/A β 40 ratio which our patients in this study were lacking. This could have provided further support of CSF amyloid decrease in preclinical AD in younger people, since CSF A β 42/40 corresponds better than A β 42 with Amyloid PET (AUC 0.936 versus 0.814, respectively, $p < 0.0001$) [25].

The cognitive assessment, BCoS, is an assessment tool lasting 1-2 hours and examines cognitive functions including memory, attention, speech and language, executive function, numeric abilities and action planning in order to build up a detailed cognitive profile. Similar to other detailed scales used in research studies, this scale addresses distinct aspects of cognition for the diagnosis of prodromal AD. However, memory problems alone were equally common in those with stable MCI. Since BCoS is not routinely used in Memory clinic services, our findings need confirmation by standard cognitive tools used in clinical practice.

CONCLUSION

Although the initial aim of the study was to present the clinical value of diagnostic lumbar puncture in the differential diagnosis of early onset AD in routine clinical setting, the analysis of clinical, cognitive and biochemical data provided a wealth of information and confirmed the heterogeneous outcomes of prodromal AD in younger people. Although the majority of our findings fell short of being ‘statistically significant’, we were able to determine the minimum size of participants required for similar studies in the future. Furthermore, our results helped identify the possible combination of cognitive and CSF biomarkers (CSF A β 42) to predict the conversion to prodromal AD or stable MCI. A recent study suggested that impairment on at least two memory tests for MCI diagnosis can improve the prediction of medial temporal atrophy and conversion to AD [26]. Future studies in young people with stable MCI will provide further information as to whether our pilot findings may also represent the minimum criteria for diagnosing prodromal AD.

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Conflict of interest: None

Author contributions: AAH and EML conceptualised and designed the study. AAH applied for ethical approval of the study involving human participants (Ref:18/EM/0292). TB and AAH obtained clinical information from medical records. AAH and KJ described clinical diagnosis. AAH, TB, LM and EML wrote the manuscript. LM and EML performed statistical analysis. AAH and EML edited versions and revised the final manuscript. All co-authors edited the manuscript.

Ethic statement: The studies involving human participants were reviewed and approved by East Midlands-Derby Research Ethics Committee (Ref:18/EM/0292).

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APPENDIX

Appendix I: Algorithm scheme

Appendix II: Scatter plots

Parameters	All (n=30)	AD (n=16)	Stable MCI (n=9)	Other neurodegenerative conditions (n=5)
Gender, female (%)	56.67	56.25	77.78	20
Age at diagnosis: mean [range], (years)	58 [31-67]	56 [52-64]	58 [51-66]	53 [31-67]
Years in education: mean [range]	13 [8-19]	14 [10-18]	14 [11-19]	12 [8-16]
Smokers/ex-smokers [smokers/ex-smokers %]	3 [10]	1 [6]	0 [0]	2 [40]
Previous alcohol excess [Previous alcohol excess %]	2 [6.67]	2 [12.5]	0 [0]	0 [0]
Mental health symptoms* (%)	50	43.75	66.66	40
Cardiovascular disorders (%)	26.66	25	33.33	20
Diabetes (%)	10	0	22.22	20
Time between symptom onset and presentation to clinic: mean [range], (years)	3 [0-6]	2 [0-4]	4 [1-6]	3 [2-4]
Memory Impairment (%)	73.1	71.4	71.4	80
Attention Impairment (%)	34.6	42.9	0	60
Language Impairment (%)	23.1	35.7	0	20
Executive Dysfunction (%)	20.8	16.7	14.3	40
Patients with disease progression from first to most recent clinic (%)	46.67	56.25	22.22	60
Months between initial clinic appointment and most recent follow up appointment [range]	17 [5-30]	17 [5-30]	17 [5-30]	20 [10-28]

Measures	MCI-AD (n=16)	Stable MCI (n=9)	Other (n=4 ^a)	F	p value
Age(<68y)	58.56+1.19	57.67+2.24	59.50+3.38	0.489	0.783
Total protein*	487.93+53.04	537.50+45.72	528.25+34.22	0.205	0.817
Total tau (146-595 pg/ml)	797.63+95.66	303.67+51.99	315.00+45.24	10.414	0.005 (MCI-AD vs stable MCI p=0.001)
p-tau (<68 pg/ml)	82.31+8.34	43.56+4.32	54.00+5.51	11.557	0.003 MCI-AD vs stable MCI p=0.001; MCI- AD vs Other, p=0.065
A- β_{1-42} (627-1322 pg/ml)*	398.94+31.70	873.44+68.58	669.75+244.54	13.340	0.0001 (MCI-AD vs stable MCI 0.0001)

ID	P-Tau (pg/ml)	Total Tau (pg/ml)	A β_{1-42} (pg/ml)
AD1	100	927	339
AD2	95	682	205
AD3	139	1188	176
AD4	32	228	612
AD5	72	890	323
AD6	57	485	529
AD7	76	628	404
AD8	74	660	242
AD9	94	903	507
AD10	122	1111	397
AD11	143	1447	347
AD12	47	361	454
AD13	69	758	460
AD14	31	248	423
AD15	74	737	378
AD16	92	1509	587
s-MCI1	32	178	671
s-MCI2	40	273	781
s-MCI3	52	542	1192
s-MCI4	49	345	644
s-MCI5	54	130	1142
s-MCI6	35	264	942
s-MCI7	68	557	692
s-MCI8	31	293	1008
s-MCI9	31	151	789
OC1	44	293	739
OC2	63	*	205
OC3	*	128	783
OC4	55	402	410
OC5	*	250	1325

BCoS Cognitive domains/CSF measures		Total protein	Total tau	Phosphorylated tau	A- β 42
Total score	- β	-0.055	0.985	-0.879	0.443
	P	0.864	0.410	0.406	0.309
Language	- β	0.105	1.268	-1.018	0.530
	P	0.735	0.263	0.320	0.211
Praxis	- β	-0.013	1.005	-0.905	0.343
	P	0.968	0.398	0.402	0.437
Memory	- β	-0.028	0.304	-0.351	0.462
	P	0.927	0.781	0.724	0.268
Attention	- β	-0.128	0.385	-0.528	0.228
	P	0.700	0.743	0.623	0.603
Number and calculation abilities	- β	-0.109	-1.206	-1.028	0.460
	P	0.738	0.306	0.336	0.295

CSF Variable	Mean Stable MCI (pg/mL)	Mean prodromal AD (mean, pg/mL)	SD	Stable MCI (mean, pg/mL) sample size needed for 95% confidence	AD sample size needed for 95% confidence
Total tau	303.7	878	386.8	10	17
A β_{1-42}	873.4	402	307.3	9	16
P-tau	43.6	80.9	32.2	16	27

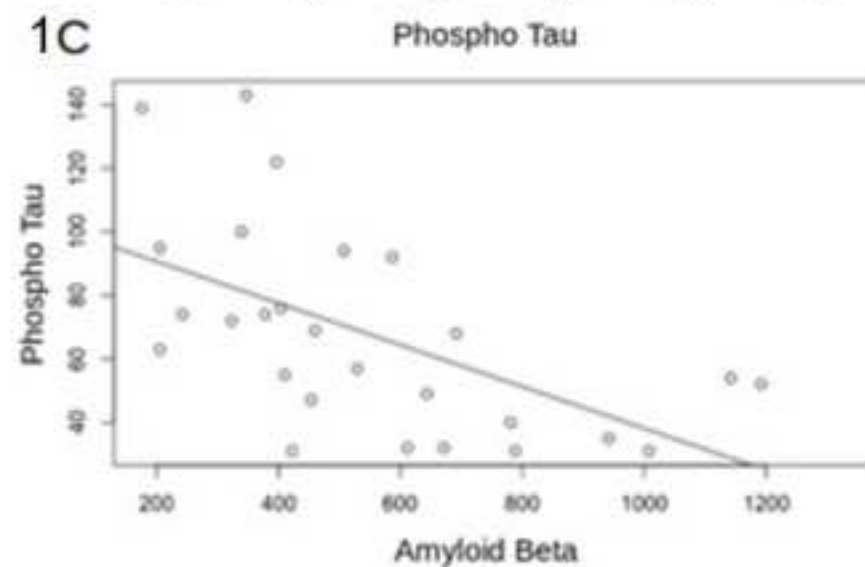
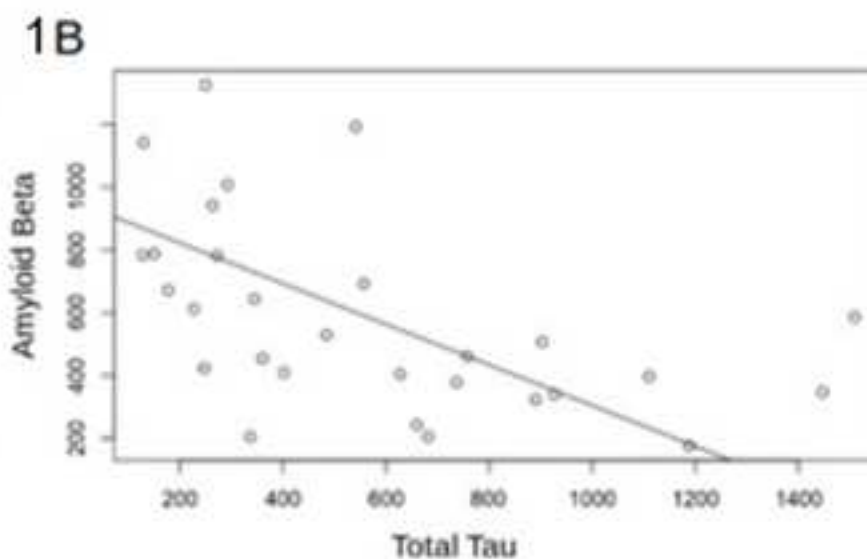
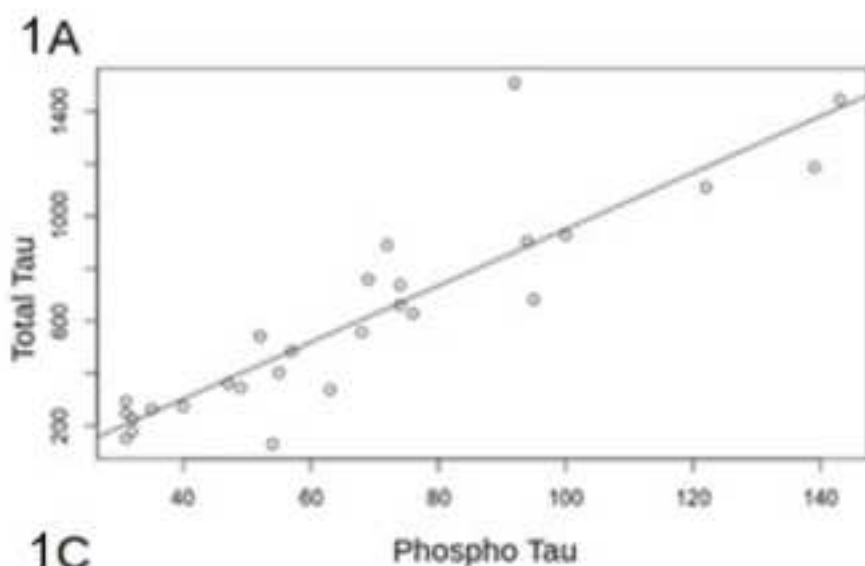
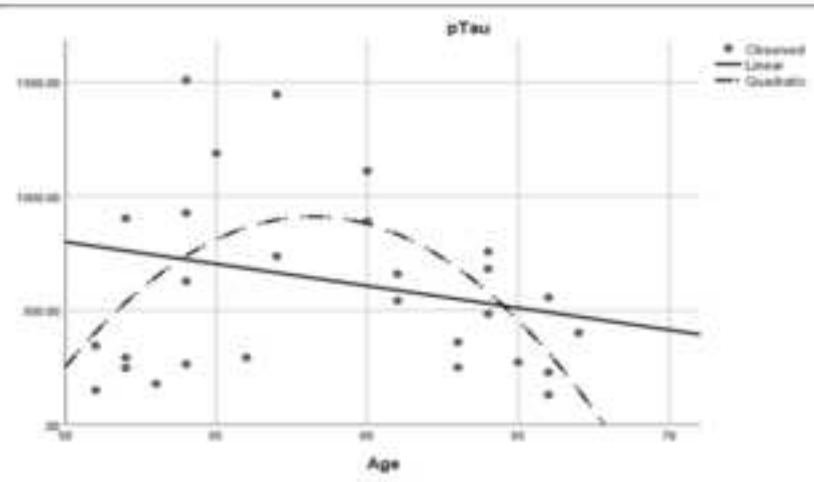
Figure 1: Linear regression analysis between protein biomarkers levels.

Figure A. Phospho Tau to Total Tau correlation: P-value = $2.973e-10$; correlation coefficient 0.895; R-squared: 0.801

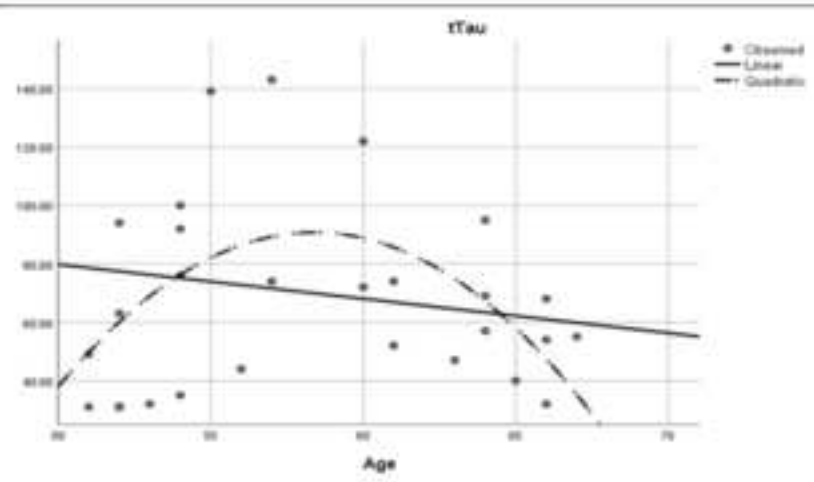
Figure B. Amyloid Beta to Total Tau correlation: P-value = 0.004554; correlation coefficient = -0.511; R-squared: 0.2344

Figure C. Amyloid Beta to Phospho Tau correlation: P-value = 0.001903; correlation coefficient = -0.570; R-squared = 0.3251

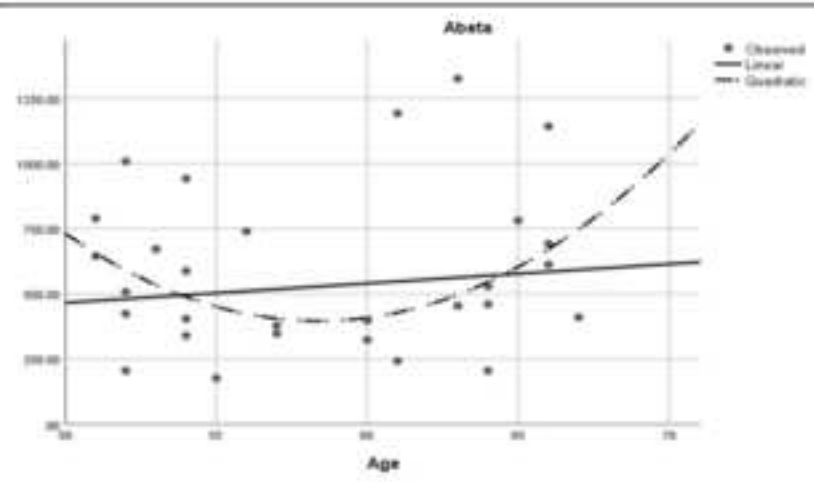
2A CSF Phosphorylated tau protein



2B CSF Total tau protein



2C CSF A β protein



2D CSF Total protein content

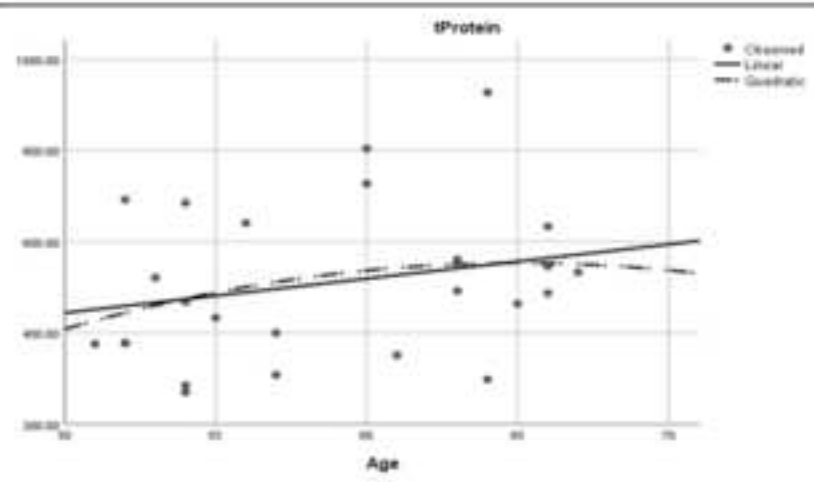
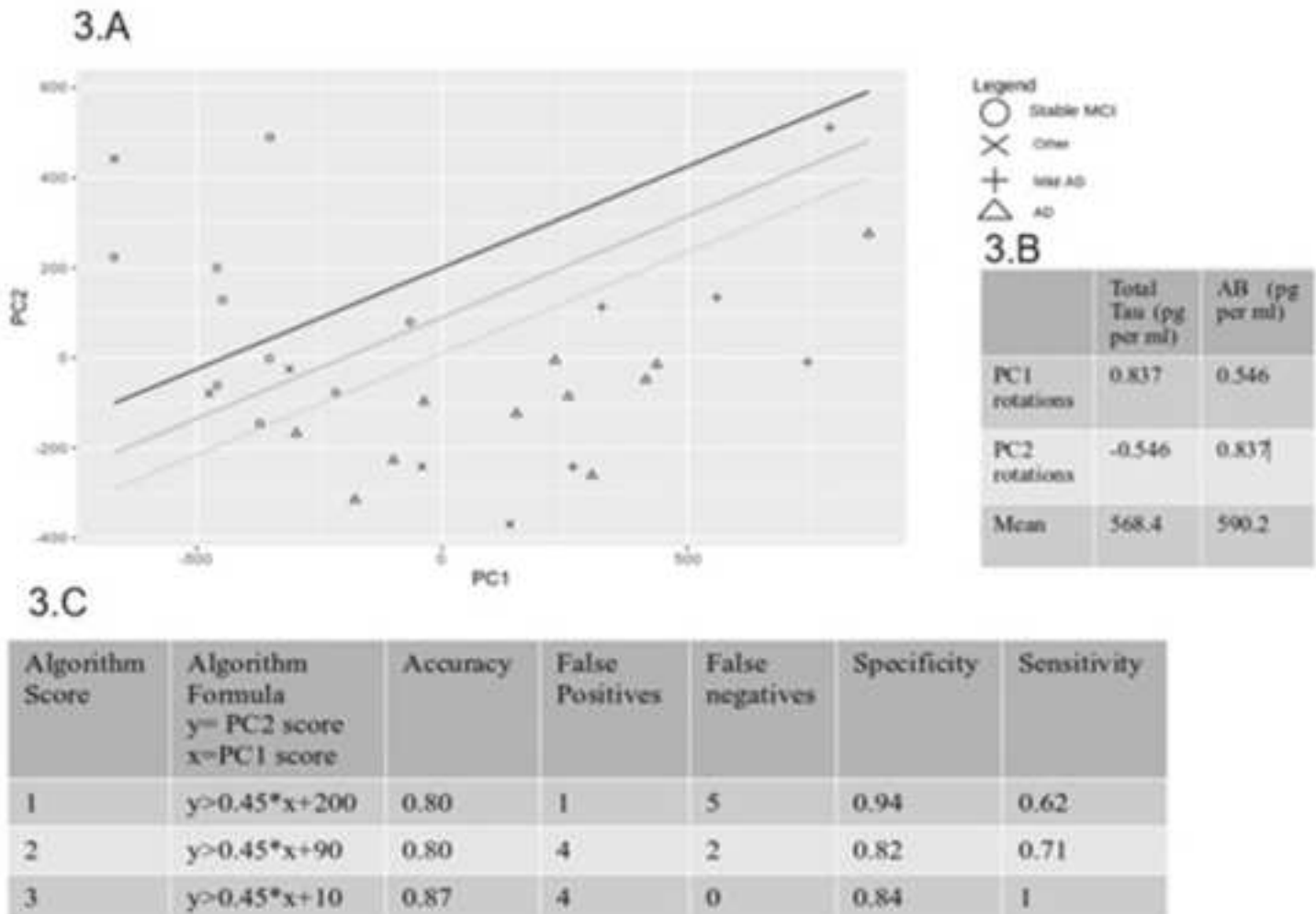


Figure 3: PCA plot using CSF total tau and A- β 

This plot includes the 3 lines that could be used as an example to segregate prodromal AD from the stable MCI patients with different levels of sensitivity and specificity. In addition to the prodromal AD and sMCI, the “Other” group was added to the plot to inform about the efficacy of the separation when more practical data are used. Figure 3b shows the data that are required for PC1 and PC2 calculations to produce the algorithm. Figure 3b shows the specificity and sensitivity values for an algorithm using the PCA graph with different equations to distinguish between the groups. It would appear that the best cut-off line would be the line for score 3. Realistically, better equations will be uncovered by using a larger data set.



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Appendix I.tif





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