UNIVERSITY OF GOTHENBURG

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A head-to-head comparison of Alzheimer's disease plasma biomarkers as predictors of one-year decline in cognitive subdomains

Degree Project in Medicine

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Table of Contents

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1 Abstract

Objective

There is scarce evidence how plasma biomarkers of Alzheimer's disease (AD) relate cross-sectionally and longitudinally to cognitive subdomains. In this study, we investigated these features in a one-year prospective single-center memory clinic research cohort.

Methods

Individuals with AD dementia, mild cognitive impairment (MCI), non-AD neurodegenerative diseases (Non-AD) and community-dwelling cognitively unimpaired (CU) controls from the Translational Biomarkers of Aging and Dementia (TRIAD) cohort (McGill University, Canada) were included. Plasma and cerebrospinal fluid (CSF) (in a subset) biomarkers were measured at baseline. Positron emission tomography (PET) was used stratify groups for β-amyloid pathology. Measures of memory, language, executive function and global cognition were obtained at baseline and after one year (in a subset).

Results

210 individuals (median age, % female) were included in the study, and comprised CU (n = 127; 71, 66), MCI (n = 48; 71, 54), AD (n = 18; 64, 61), and 17 (69; 47) individuals with non-

AD neurodegenerative dementias. Phosphorylated (p)-tau 181 and p-tau231 in both CSF and plasma, and glial fibrillary acidic protein (GFAp) in plasma, increases along the AD continuum (defined as β-amyloid positive by PET) were seen compared to non-AD and CU without β-amyloid pathology. CU performed better on several neuropsychological measures after one year, whereas most were unaltered in cognitively impaired individuals. In CU, neuropsychological performance largely associated with age and years of education. However, for cognitively impaired individuals with β-amyloid pathology, associations were seen with plasma p-tau181, p-tau231 and GFAp in memory and global cognition. No associations were seen with baseline biomarker levels and subsequent cognitive decline in any of the measures.

Conclusion

Biomarkers in plasma reflect AD-specific pathophysiology, and significantly associate with severity of global cognitive and memory impairment. Furthermore, to investigate the prognostic capabilities of biomarker levels in cognitive subdomains, larger and longer studies are warranted.

2 Background

2.1 Alzheimer's disease

In 1907, the German psychiatrist and neuroanatomist Alois Alzheimer described a new disease entity, which he thought was a rare dementia disorder affecting relatively young individuals. Today, it is well known that Alzheimer's disease (AD) is the most common form of dementia, causing 50-75% of all dementia cases, currently affecting approximately 50 million people worldwide[1,2] approximately 100 000 in Sweden alone.[3] Due to an aging population, it is estimated that the number of people affected by AD will increase exponentially – with the prevalence of all-cause dementia expected to nearly double each 20 years – to almost 75 million people 2030 and roughly 130 million 2050. The large share of this increase will most likely occur in low- and middle-income countries.[4]

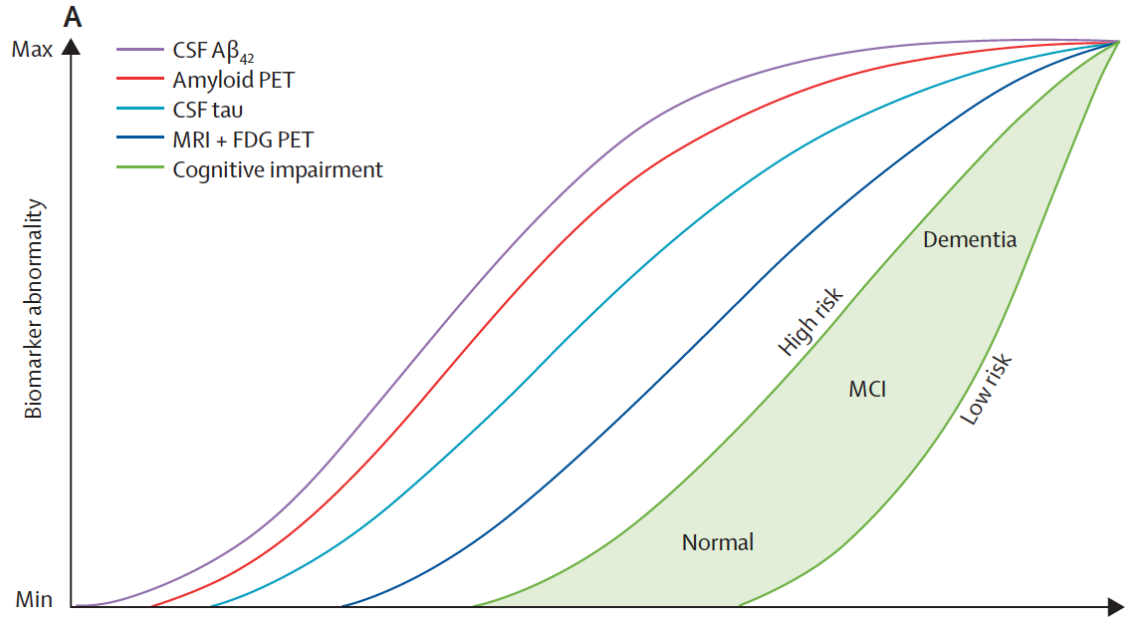
At the autopsy of Auguste Deter, the index case of the disease, Alois Alzheimer found three distinct features: plaques, neurofibrillary tangles and neuronal cell loss. These were presented in a paper entitled: *"Über eine eigenartige Erlranliung der Hirnrinde"* (*Eng.* On an Unusual Illness of the Cerebral Cortex).[5] These features have since then been established as the hallmarks of AD. In the 1980's, it was discovered that plaques consist mainly of amyloid-β (Aβ),[6] and that tangles contain filaments of hyperphosphorylated tau protein.[7] In the early 90s, the amyloid cascade hypothesis on the pathogenesis of AD was presented. This is still the leading theory as to why AD arises in the aging brain. The transmembrane amyloid precursor protein (APP), which naturally undergoes proteolytic cleavage also as a part of normal physiology, but increased generation of APP longer metabolites (42-43 amino acid (aa) long Aβ peptides instead of the 38-40 aa long peptides that normally dominate) causes dominantly inherited AD through mutations in either one of the cleaving enzymes (*PSEN1* or *PSEN2*) or in

the *APP* gene itself. In Down syndrome, trisomy 21, which is the chromosome where *APP* is located, the development of symptomatic AD is nearly inevitable in those who survive to their seventies.[8] In the much more common sporadic form, representing almost 99 % of all cases, the presence of one or two copies of the *APOE ε4* allele increases the lifetime risk of AD. This in particular, and aging in general, likely decreases clearance of Aβ which are then aggregated into oligomers, and later extracellular plaques.[9] Conversely, the *APOE ε2* genotype and other rare mutations in *APP* are protective against this process.[10] This then induces overt phosphorylation of tau, which are later aggregated into intracellular tau tangles and spreads from the medial temporal lobe across the neocortex.[11] These processes likely contribute to a complex cellular response—*the cellular phase* of AD—about which evidence is quickly accumulating, and includes microglial, astrocytic, vascular and likely also oligodendrocytic responses that may be both harmful or protective against the neuronal and synaptic dysfunction, network disturbance and neuronal cell loss which eventually occur. When the different compensatory mechanisms fail, clinical onset of the disease can be observed.[12]

For almost a century, the diagnosis of AD during life has been based purely on clinical symptoms, with definitive diagnosis at autopsy.[1] Towards the end of the 20th, biomarkers in cerebrospinal fluid (CSF) accurately reflecting the three hallmarks: Aβ pathology (Aβ42), altered tau metabolism (phosphorylated (P)-tau181) and neurodegeneration (total (T)-tau or neurofilament light (NfL) were developed, which will be discussed in greater detail below.[1] These diagnostic tests were first measured using standard manual enzyme-linked immunosorbent assays (ELISA), but fully-automatic clinical-grade assays have been developed and validated since then.[13] In addition, during the last decade, accurate positron emission tomography (PET) tracers have been developed reflecting neuronal dysfunction/loss,

aggregated tau[14] and amyloid.[15] Apart from being tools allowing for etiological diagnoses of cognitive impairment, research utilizing these biomarkers has provided much greater understanding of the natural course of AD during life. In 2007[16] and 2011[17], respectively, novel diagnostic criteria included biomarkers as a part of the diagnostic process. A few years later, *Jack et. al* presented a model of AD proposing a temporal order from the earliest neurochemical/imaging signs of disease, to clinically manifest disease (Figure 1), where decreases in CSF Aβ42 and shortly thereafter amyloid PET, reflecting fibrillar Aβ being deposited into plaques are the first biomarker signs of pathology. Thereafter, changes in CSF tau concentrations can be detected, possibly reflecting amyloid-related changes in tau metabolism.[18] Lastly, altered brain glucose metabolism and grey matter volume become evident on fluorodeoxyglucose (FDG) PET and magnetic resonance imaging (MRI), respectively.

Figure 1. Adapted from Jack *et. al.* 2013[19]



Abbreviations: MCI, mild cognitive impairment; CSF, cerebrospinal fluid; MRI, magnetic resonance imaging; FDG PET, fluorodeoxyglucose positron emission tomography; Aβ, amyloid beta.

In 2018, a novel framework for research on AD suggested that the disease should be considered as a pure biological construct, reflected by disease-specific biomarker evidence of Aβ (A) and tau (T) pathology, as well as neurodegeneration ([N], placed in parenthesis, since these changes occur also in other conditions), and that these can be present both in cognitively unimpaired (CU), those with mild cognitive impairment (MCI), or dementia.[20] Based on the accumulating evidence that the abnormalities occur in a sequence described above, this process is referred to as the Alzheimer's *continuum* (Table 2).[20]

Table 1. Adapted from Jack *et al.* 2018.[20]

		Cognitive stage		
		Cognitively unimpaired	**Mild cognitive Impairment**	**Dementia**
Biomarker profile	A-T-(N)-	Normal AD biomarkers. Cognitively unimpaired	Normal AD biomarkers with MCI	Normal AD biomarkers with dementia
	A+T-(N)-	Preclinical Alzheimer's pathologic change	Alzheimer's pathologic change with MCI	Alzheimer's pathologic change with dementia
	A+T+(N)-	Preclinical Alzheimer's disease	Preclinical Alzheimer's disease with MCI (prodromal AD)	Alzheimer's disease with dementia
	A+T+(N)+			
	A+T-(N)+	Alzheimer's and concomitant suspected non-Alzheimer's pathologic change, cognitively unimpaired	Alzheimer's and concomitant suspected non-Alzheimer's pathologic change with MCI	Alzheimer's and concomitant suspected non-Alzheimer's pathologic change with dementia
	A-T+(N)-	Non-Alzheimer's pathologic change, cognitively unimpaired	Non-Alzheimer's pathologic change with MCI	Non-Alzheimer's pathologic change with dementia
	A-T-(N)+			
	A-T+(N)+			

Abbreviations; A, Amyloid-β; T, tau; N, neurodegeneration

However, according to the recent iteration of the international work group (IWG) diagnostic criteria, this definition is challenging in the clinical setting, since having evidence of AD pathophysiology does not always imply when and if symptoms will occur, and that the presence or absence of co-pathologies such as TAR DNA-binding protein 43 (TDP-43) and α-synuclein proteinopathies as well as cerebrovascular lesions contribute to cognitive symptoms in elderly. In addition, other protective or risk-factors modify this course. Thus, their suggestion is that the

diagnosis of AD is clinico-biological and that a combination of symptoms and biomarker evidence must be present for sufficient precision.[21]

2.2 Established biomarkers for Alzheimer's disease

2.2.1 A: Aβ PET and CSF Aβ42

Since the first studies reported that ELISAs using two monoclonal antibodies specifically detecting CSF concentrations of 42 amino acids long Aβ peptides (Aβ1-42) might be a sensitive biomarker of incipient AD pathology,[22] it has now been established that CSF Aβ1-42 in individuals with AD is depleted to around 50 % of concentrations found in healthy individuals.[23] This change reflects Aβ1-42 being deposited into plaques, which is supported by close agreement of lower CSF Aβ1-42 and higher plaque load in brain biopsy studies[24] and *post mortem* autopsy studies.[25] However, in recent years, most studies suggest that a ratio of Aβ1-42, and the concentration of more abundant (but less disease-related) 40 amino acids long peptides (Aβ1-40) is more accurate in identifying Aβ pathology, since it is capable of accounting for interindividual differences in Aβ metabolism and possible preanalytical confounders.[26] The other principle for quantifying Aβ pathology in living individuals is positron emission tomography (PET), measuring the uptake of a radiolabel binding pathological Aβ in brain tissue. The first label was a C^{11}-labeled modified Thioflavin-T molecule known to bind Aβ histologically, called Pittsburgh compound B (PiB).[27] Tracer uptake was shown to be larger in individuals with AD dementia compared with controls. Since then, a multitude of tracers have been developed, which are instead labeled with F^{18}, due to the simplified logistics of a longer half-life. One common way to assess cortical Aβ quantity is to use cerebellum (which is known to be relatively unaffected by Aβ pathology) as a reference. A standardized value uptake ratio (SUVR) is then generated, which can be assessed either visually or calculated

quantitively.[28] Studies have later shown that there is excellent agreement between Aβ PET tracer uptake and neuritic plaque pathology at autopsy[29] and in brain biopsy studies[30] similar to that of CSF Aβ. In addition, the concordance between Aβ PET and CSF Aβ is encouraging (~90 %). In the cases where the results are discordant, it has been suggested that individuals with abnormal CSF Aβ later become Aβ PET positive and thus that CSF Aβ may be more sensitive.[31] Combined results using PET and CSF to study both autosomal dominant and sporadic AD [32] have established that Aβ becomes fully abnormal years before symptom onset, and thus, that it is of limited value to track disease progression as an individual biomarker in symptomatic individuals, but has large negative predictive value of AD.

2.2.2 T: CSF P-tau and Tau PET

The second biomarker category reflecting AD pathophysiology is that of altered tau metabolism and deposition into neurofibrillary tangles. As with Aβ, the first evidence that tau pathology could be reflected by biomarker evidence was through ELISA assays capturing tau protein phosphorylated threonine 181 (p-tau181) and 231 (p-tau231).[33] Recent research suggest that concentrations phosphorylated tau starts to increase in the preclinical phase of AD,[34] and then continues to rise in the early clinical phase of the disease.[35] P-tau species predict conversion from MCI to dementia[36] and are accurate in the differential diagnosis between AD and other important mimics[37], but the common issue of mixed pathologies likely decreases the diagnostic precision.[38] According to the NIA-AA research framework and novel IWG criteria, the other biomarker up to this point which is proposed to reflect tau pathology is PET imaging targeting AD-specific tau deposits, which do not occur in other neurodegenerative diseases with tau pathology.[14] Its diagnostic accuracy is comparable to that of p-tau, although the correlation is somewhat lower than between Aβ measurements,[39] possibly mirroring that CSF p-tau increases

in response to Aβ pathology, and tau PET measures actual neurofibrillary tangles. The predictive ability is likely better than Aβ PET, and is more closely correlated with the neuroanatomical distribution of symptoms[40], but there is insufficient evidence of the magnitude of the predictive ability of tau PET.[21]

2.2.3 (N): T-tau, MRI, FDG PET and NfL

The third biomarker category which is proposed as a part of the Alzheimer's *continuum* is the presence of neurodegeneration. These changes are not *per se* unique to AD, although typical patterns can be seen. Alterations on structural MRI occur relatively late in the phase, and the earliest alteration is atrophy of temporal structures such as the hippocampus, which occurs as a part of aging, but at a greater extent in AD.[41] Albeit not sufficient as a diagnostic marker alone, rate of change predicts disease progression in an accurate fashion.[41] In addition, it is an important measure to exclude co-pathologies such as cerebrovascular disease.[41] FDG PET gives complementary information, as it reflects decreased glucose metabolism. This provides a measure of functional as well as frank neuronal loss, typically in posterior cingulate and temporo-parietal regions, and the typical patterns seen in different neurodegenerative diseases makes it a useful biomarker of differential diagnosis.[42] Fluid biomarkers that are proposed to reflect neurodegeneration include CSF NfL, which is a major component of the neuroaxonal cytoskeleton.[43] Modest increases are seen in AD (around two-fold compared to normal controls), but in analogy to MRI, although not diagnostic, higher concentrations could suggest other causes of neurodegeneration, such as frontotemporal dementia or cerebrovascular disease.[44] Conversely, normal concentrations suggest non-neurodegenerative causes of cognitive symptoms, such as depression. Finally, CSF T-tau has been proposed to be a biomarker of intensity of neuronal loss, since it increases both in AD, even more prominently

in Creutzfeldt Jakob disease (CJD)[45] as well as transiently after acute conditions such as stroke and traumatic brain injury (TBI).[46] However, due to the fact that the levels are not increased in other slowly progressing neurodegenerative diseases and that the ability to differentiate between AD and other mimics is not vastly different from that of P-tau[47], T-tau might be a marker of amyloid-related altered tau-metabolism in AD, as supported by experimental studies, and thus that mechanisms of T-tau release are different in AD compared with CJD, TBI and stroke.[18,48]

2.2.4 Novel biomarkers of AD pathophysiology

In addition to the AT(N) pathologies, and consistent with the complex cellular and molecular interplay, biomarkers that reflect neuroinflammation, synaptic pathology and other relevant pathologies now exist.[49] These will likely gain a larger importance in the future, both in the characterization of AD pathophysiology, as selection tools for pharmaceutical intervention, and possibly also in clinical routine.

2.3 The need for cheaper and less invasive biomarkers

Due to the perceived invasiveness of lumbar puncture (LP) and the large cost of PET imaging, neither technique is suitable for extensive use in primary care where most dementia diagnoses are currently made. Studies indicate that a large proportion of patients with cognitive disorders that are based purely on clinical criteria are not accurately diagnosed not only in primary care,[50] but also specialized clinics.[51,52] This, and the advent of clinical trials aiming to modify the disease at the preclinical stage of AD, highlights the need for less invasive biomarkers that can simplify primary care diagnostics and the pre-screening procedures in clinical trials.

2.4 Single molecule array (Simoa)

ELISA has long been a key method in immunochemistry to quantify specific peptide concentrations in different matrices, and is the method that has been used for the core biomarkers in CSF mentioned above. However, as previously mentioned, large interest in blood biomarkers sparked the development of novel, more sensitive methods. In 2010, a new technique was introduced by *Rissin et al*., called single molecule array (Simoa).[53] This technique shares the basic principles of sandwich ELISA – it uses one antibody to capture the antigen of interest, and a detector antibody connected to biotin. This is then bound strongly to streptavidin-β-galactosidase (SBG). To prevent unspecific binding of antibodies to the antigen and unbound SBG, repeated wash cycles are performed. Lastly, a fluorophore is added, which is cleaved by the galactosidase and generates fluorescence. The intensity of this fluorescence in the sample well is then measured using spectrophotometry compared to the signal of a standard curve with known concentration, and from this, a concentration can be calculated. The difference between standard ELISA and Simoa is that—whereas the fluorescence generated by a standard sandwich ELISA is equally distributed in the entire sample well—Simoa uses antibody-coated microscopic beads to capture its antigen, which are then loaded into wells in which there is only room for one bead. Due to the abundance of beads in comparison to the target analyte at low concentrations, a Poisson distribution can be assumed.[53] Thus, at low concentrations, each well only contains one molecule or none. This entails a very high resolution, and that analytes can be quantified at sub-femtomolar concentrations (< 1 pg/mL).

2.5 Transition to blood

The development of ultrasensitive assays, such as Simoa described above, has enabled translating the CSF biomarkers reflecting AT(N) pathologies to blood. Assays reflecting Aβ

pathology now exist, and those which employ liquid chromatography coupled with mass-spectrometry have shown the most promising results.[54,55] Moderate associations are seen with CSF and PET Aβ measures, likely due to matrix effects and expression of Aβ in peripheral tissues.[54,55] This likely also contributes to the small fold-changes (around 15 %) that are seen between Aβ positive and negative subjects.[54] The food and drug administration (FDA) has approved one of these tests for clinical use, which is an important landmark, although much more standardization work is still needed before their usefulness is proven. Even more promising, perhaps, is the translation of assays targeting p-tau in blood. More specifically, recent studies suggest that tau phosphorylated at either threonine 181 (p-tau181),[56] 217 (p-tau217)[57] or 231 (p-tau231)[58] detect AD-specific early changes in tau metabolism decades before symptom onset, both in sporadic and dominantly inherited forms and reflect CSF and PET measures of tau and Aβ. In most studies, which are conducted in well-characterized research cohorts, the diagnostic accuracy (area under the curve; AUC) between healthy elderly without Aβ pathology and AD is approximately 0.9, which is true also when comparing with other neurodegenerative diseases without Aβ pathology.[56,58,59] This has been confirmed in neuropathological confirmed cases.[60] In addition, they predict conversion from CU, to MCI and AD. Lastly, also NfL has been translated to blood. It has emerged as an easily accessible and dynamic biomarker of general neuronal injury, and moderate increases are seen in AD.[61] It correlates well with CSF NfL[62], which means that it can be used to evaluate the severity of the neurodegenerative process, supported by the fact that levels start to diverge from normal when approaching clinical onset in dominantly inherited AD.[63] Another useful property is its ability to exclude non-neurodegenerative mimics of AD.[64] This is also supported by a recent study, suggesting that neurologists perceive NfL to be useful to rule in or out neurodegeneration in

patients with subjective cognitive complaints and psychiatric disorders.[65] Although scarcely evaluated, the most recent blood biomarker candidate of AD pathology is GFAp—an important component of the astrocytic cytoskeleton[66]—which has proven to differentiate between healthy subjects with and without Aβ pathology (especially when combined with Aβ42/40) [67], and is further increased in AD dementia, where it predicts grey matter atrophy.[68]

2.6 The neuropsychological features of Alzheimer's disease

It is now well established that the first signs of cognitive impairment seen in individuals who later develop AD with dementia are deficits in episodic memory, which is supported by population-based studies of aging.[69,70] This is due to encoding deficiencies, which in turn occurs because of functional and structural alterations in the hippocampus, the region which is known to be affected very early by tau deposition[11] and grey matter atrophy.[41] Thus, the phrase "amnestic syndrome of the hippocampal type" is often used to describe the neuropsychological deficits of AD.[71] Furthermore, defining the concept of MCI, as described by Petersen *et al.*[72] has enabled the characterization of the earliest clinical stages of AD. According to Petersen *et al.*, the most prominent differences according between individuals with dementia and MCI is that the latter tend to be have a single-domain cognitive impairment, and thus largely preserved global cognition as well as normal functions of daily life.[72] In addition, the subtle changes that can be seen with objective tests, are more accurately reflected by a close relative (a spouse or similar) than the subjective changes of the affected.[72] However, MCI is a heterogenous condition and—consistent with the definition of the typical AD neuropsychological profile described above—individuals with amnestic MCI are more likely to progress to AD dementia compared with healthy elderly and those initially presenting with impairment in other cognitive domains.[72] Nonetheless, the primary evaluation of individuals with cognitive symptoms often

include the use of screening tests, such as the Mini Mental Stage Examination (MMSE)—and more recently—the Montreal cognitive assessment (MoCA), which has been suggested to be more sensitive to detect MCI or early dementia,[73] deviations from normal global cognition can be detected.[74] Both of these tests are commonly used to assess disease severity. Another common way to assess disease severity is the clinical dementia rating (CDR) scale, which also assesses the functional component associated with a dementia diagnosis.[75] To detect changes in specific cognitive domains, tests that are validated for that purpose need to be used. Deficits in memory encoding that occur in AD are typically assessed using tests that most often include auditory or visual learning tasks, which are then repeated with a slight delay, and common tests include California Verbal learning test or Rey auditory verbal learning test (RAVLT), with the latter consisting of a 15-word list which the subject learns five times. After an intrusion list is presented, the subject is requested to recall the initial list immediately and after a delay of 20-30 minutes.[76] The language performance can be assessed with tests of verbal fluency— such as naming as many animals as possible within a certain time frame as with the animal fluency test,[77] or confrontational naming, which assess the ability to name pictures being presented to the subject, such as the Boston naming test (BNT).[78] In this test, a number of pictures (generally between 15-60) of objects are presented to the subject, which is then asked to name these, and if difficulties occur, after a cue. Furthermore, executive function comprises several abilities, but important functions include problem-solving and mental flexibility, but also attention and processing speed.[79] Several tests can be used to assess these functions, but common tasks include Wisconsin card-sorting task[80] and part B of the trail making test, in which the subject alternates between numbers and letters to draw a trail.[81] Lastly, visuospatial functions are commonly assessed, which if impaired lead to poor discrimination of shapes, contrasts and lack

of orientation, to mention a few examples.[79] In light of this, less prevalent syndromes associated with AD pathology in areas relevant for executive function (dysexecutive/behavioral variant of AD), language (logopenic primary progressive aphasia) and visuospatial function (posterior cortical atrophy) exist, and is relatively more common among patients with early onset AD.[82]

3 Aim

So far, it is not clear how AD blood biomarkers predict progression in specific cognitive domains, if there is a difference in predictive profile of the major biomarker candidates, and if their predictive power is comparable to CSF biomarkers in these domains.

Therefore, the primary aim is to characterize the association between disease-relevant cognitive domains and blood biomarker concentrations in prodromal AD and AD dementia. Secondly, we will assess putative plasma biomarkers in their ability to predict short-term disease progression, defined as worsening performance in neuropsychological sub-scores. Additionally, we aimed to investigate if the predictive power is comparable to their corresponding biomarkers in CSF.

4 Methods

4.1 Participants

Participants in this study were recruited from the single-center memory clinic-based Translational Biomarkers of Aging and Dementia (TRIAD) cohort, McGill University. The participants included in this study were recruited between May 2017 and March 2020. At baseline, participants were clinically and cognitively evaluated. Tests including MMSE, the CDR, Hachinski Ischemic to identify the risk of vascular dementia[83] and a battery of

neuropsychological tests. Cognitively normal controls had a CDR of 0. MCI patients had a CDR score of 0.5, subjective as well as objective cognitive impairments, but preserved activities of daily living.[84] Alzheimer's disease with dementia (hereafter referred to as AD, if not otherwise stated) patients had CDR scores of ≥ 0.5 and met the National Institute on Aging and the Alzheimer's Association criteria (NIA-AA) for probable AD, as assessed by a physician.[17] Exclusion criteria were: inadequately treated illness or active substance abuse, recent head trauma or major surgery, or if there were contraindications against PET or MRI. The cognitive tests included as continuous outcomes in this study were: the words correctly recalled at the free immediate and delayed recall (after 20 minutes) of RAVLT test was chosen as a measure of verbal memory function (score in each subtest);[76] TMT-B was chosen as a test for executive function (where longer time in seconds indicate a worse score);[81] the 30-item BNT for language fluency (number of items correctly identified including cues);[78] and MoCA for general cognition (total score).[73] Individuals with cognitive impairment, but without Aβ pathology on PET were considered non-AD neurodegenerative diseases and consisted of individuals with MCI, frontotemporal dementia (FTD), progressive supranuclear palsy (PSP), hippocampal sclerosis (HS) and vascular cognitive impairment (VCI). For individuals in the AD continuum, the nomenclature being used is presented in Table 1. Participants were included based on availability of plasma biomarkers and neuropsychological assessments.

4.2 Imaging and biochemical analyses

All participants included in this study underwent amyloid-β PET with fluorine 18–labeled [^{18}F] AZD4694, and Aβ status (positive or negative) was based on visual interpretation of the SUVR map (with cerebellum as the reference region), performed by two neurologists blinded to the clinical data. The plasma and CSF biomarkers included in this study were p-tau231, p-tau181,

GFAp and NfL, and were measured using Simoa (Quanterix, Bilerica, MA) assays on an HD-X analyzer, either *in-house* (plasma and CSF p-tau181[85] and plasma p-tau231[56,58]), or kits (NfL and GFAp) according to the instructions of the manufacturer (Quanterix, Bilerica, MA). CSF P-tau231 was also measured, but using an *in-house* sandwich ELISA which had the same antibody combination as the plasma Simoa method, as previously described.[85] All quality control samples had inter- and intra-assay variability below 15%. Determination of *APOE* genotype was performed using a polymerase chain reaction amplification technique, and presence or absence of at least one *APOE ε4* copy was recorded.[86]

4.3 Statistical analysis

Since the biomarker data was expected to be mostly non-normally distributed, non-parametric statistical tests were used. Baseline characteristics were assessed using Kruskal-Wallis tests with *post hoc* Dunn-Bonferroni adjustment for multiple comparisons to compare continuous variables between more than two groups (not used in demographics table). Comparisons between categorical variables were made using Fisher exact test or χ^2 tests, were appropriate. Since neuropsychological tests were mostly normally distributed, paired t-tests were used to estimate change over a year. Correlations between biomarkers and neuropsychological tests variables were assessed using Spearman correlations. Linear regression models were used to test the relationship between baseline biomarkers as predictor and rate of change (Δcognitive score) in cognition as outcome variable, including age, sex, and years of education in the models to allow for adjustment of these factors. For these analyses, biomarker data were log-transformed to achieve near-normal distribution of the residuals. Analyses were performed using Graphpad prism (v. 9.0) or SPSS (v. 27.0). Tests with a two-sided $P < 0.05$ were considered significant.

4.4 Ethical considerations

Since this study is performed on human participants, and a significant share of these are cognitively healthy elderly, it is especially important to consider the potential risks with the radiation exposure that accompanies a PET scan. However, the effective radiation dose is comparable to the average background radiation (~3.5 mSv), and it is generally considered safe. However, it is not advisable to communicate the amyloid-β status in healthy individuals, since this entails that the subject becomes aware of its higher risk of a detrimental disease with no cure. This study adhered to those guidelines. Furthermore, lumbar puncture is an invasive procedure, but the very low frequency (apart from bothersome, but benign post-puncture headache) of complications makes this a safe procedure. However, this was not mandatory for inclusion in the study. Furthermore, the study has been approved by the Douglas Mental Health University Institute Research Ethics board and the Montreal Neurological Institute PET working committee. A written informed consent was obtained for all participants.

5 Results

5.1 Participants' characteristics

The cohort consisted of 210 individuals, and included CU (n = 127), MCI (n = 48), AD (n = 18), and n = 17 individuals non-AD neurodegenerative dementias. Among these, 129 (61 %) were women. The median (interquartile range [IQR]) age was 71 (69-75) in the whole cohort. The sex and age distributions were not significantly different across groups (Table 2). The median (interquartile range) number years of education was 15 (12-17), which was also similar between groups. 68 (39 %) participants had at least one copy of the *APOE ε4* genotype. Among CU and MCI individuals, 30 (24 %) and 38 (79 %) were Aβ+. In the whole sample, plasma NfL

(rho=0.282, *P*<0.001), as well as CSF (rho=0.192, *P*<0.05) and plasma GFAp (rho=0.281, *P*<0.001) correlated with increasing age. Plasma, but not CSF GFAp was significantly higher in women compared to men (*P*<0.05).

Table 2. Baseline patients' characteristics.

Characteristic	CU	MCI	AD	Non-AD	p-value
No.	127	48	18	17	
Age. median years (IQR)	70.5 (66.8–73.9)	71.0 (65.9–76.4)	63.6 (59.3–68.4)	69.2 (66.2–71.8)	.08
Sex, female/male (% females)	84/43 (66)	26/22 (54)	11/7 (61)	8/9 (47)	.291
Education, median years (IQR)	15.0 (12.3–17.0)	15.0 (11.0–18.0)	15.0 (13.0–16.0)	15.0 (12.0–17.0)	.844
APOE ε4 status, pos./neg. (% pos.)	35/88 (28)	24/23 (51)	7/9 (43)	2/14 (13)	.008
MMSE score, median (IQR)	29.0 (28.3–30)	29.0 (26.0–29.0)	20.0 (16.0–26.0)	13.0 (8.5–24.0)	<.001
MoCA score, median (IQR)	28.0 (27.0-29.0)	22.0 (20.0–24.0)	13.0 (8.00–13.0)	26.0 (23.0–27.0)	.000
Amyloid PET SUVR, median (IQR)	1.28 (1.21–1.47)	1.96 (1.44–2.29)	2.16 (2.05–2.39)	1.20 (1.19–1.29)	<.001
Amyloid PET, pos (%)	30 (23.6)	38 (79.2)	18 (100)	17 (0)	
Biomarker, median [pg/mL] (IQR)					<.001
Plasma P-tau181	10.0 (7.51–13.6)	12.4 (10.4–21.2)	20.6 (15.6–21.4)	9.9 (9.29–13.4)	
CSF P-tau181*	298 (219–419)	561 (319–1067)	1470 (868–2200)	248 (203–329)	<.001
Plasma P-tau231	14.1 (9.40–19.4)	21.2 (12.8–23.9)	27.6 (17.7–30.8)	9.62 (6.15–13.5)	<.001
CSF P-tau231*	9.95 (7.62–13.5)	13.1 (8.00-23.3)	41.6 (23.5–66.6)	7.52 (7.10–10.6)	<.001
Plasma GFAp	174 (141-275)	270 (176–345)	357 (316–531)	128 (96–203)	<.001
CSF GFAp*	12400 (9170–15300)	15600 (10900-20700)	13900 (12800-16400)	11900 (7450–15600)	.015
Plasma NfL	20.5 (14.9–26.3)	20.5 (12.6–27.9)	30.0 (26.5–32.6)	23.7 (19.3–31.7)	.019
CSF NfL**	864 (682–1150)	1050 (907–1320)	1520 (1170–1850)	1020 (781–1720)	.069
Neuropsychological test, median (IQR)					
RAVLT-I, words recalled	11.5 (8.00–13.0)	6.00 (3.00–8.00)	1.00 (0.00–4.00)	7.00 (3.00–10.0)	<.001
RAVLT-D, words recalled	11.0 (8.00-12.0)	5.00 (1.00–8.00)	0.00 (0.00-0.00)	6.00 (3.00–11.0)	<.001
TMT-B, seconds	82.5 (62.5–105)	106 (73.0–150)	196 (151–303)	91.0 (90.0–110)	.002
BNT, figures named	29.0 (29.0-30.0)	28.0 (27.0–30.0)	28.0 (25.0–29.0)	27.0 (26.0–28.0)	<.001

Abbreviations: GFAp, glial fibrillary acidic protein; P-tau181, phosphorylated tau 181; P-tau231, phosphorylated tau 231; NfL, neurofilament light; MoCA, Montreal cognitive assessment; RAVLT-D/I, Rey auditory verbal learning test, delayed/immediate recall; BNT, boston naming test; TMT-B, part B of trail making test; CSF, cerebrospinal fluid; CU, cognitively unimpaired; MCI, mild cognitive impairment; AD, Alzheimer's disease; Non-AD, non-AD neurodegenerative diseases, MMSE, mini mental state examination; IQR, interquartile range. *only of n = 125 subjects. **only for n = 83 subjects. P-values are derived from Kruskal-Wallis tests across groups for continuous variables, and $\chi 2$ tests for categorical variables.

5.2 Plasma biomarkers

To test the hypothesis that biomarkers reflect AD-specific alterations, we examined the plasma biomarker concentrations across groups. After stratifying for Aβ PET status, p-tau231 demonstrated stepwise increases along the AD *continuum* (Figure 2A); Aβ+ CU (*P*<0.001), prodromal AD (*P*<0.0001), and AD dementia groups (*P*<0.0001) had higher concentrations of plasma p-tau231 compared to Aβ- CU. Furthermore, all groups in the AD continuum (Aβ+ CU,

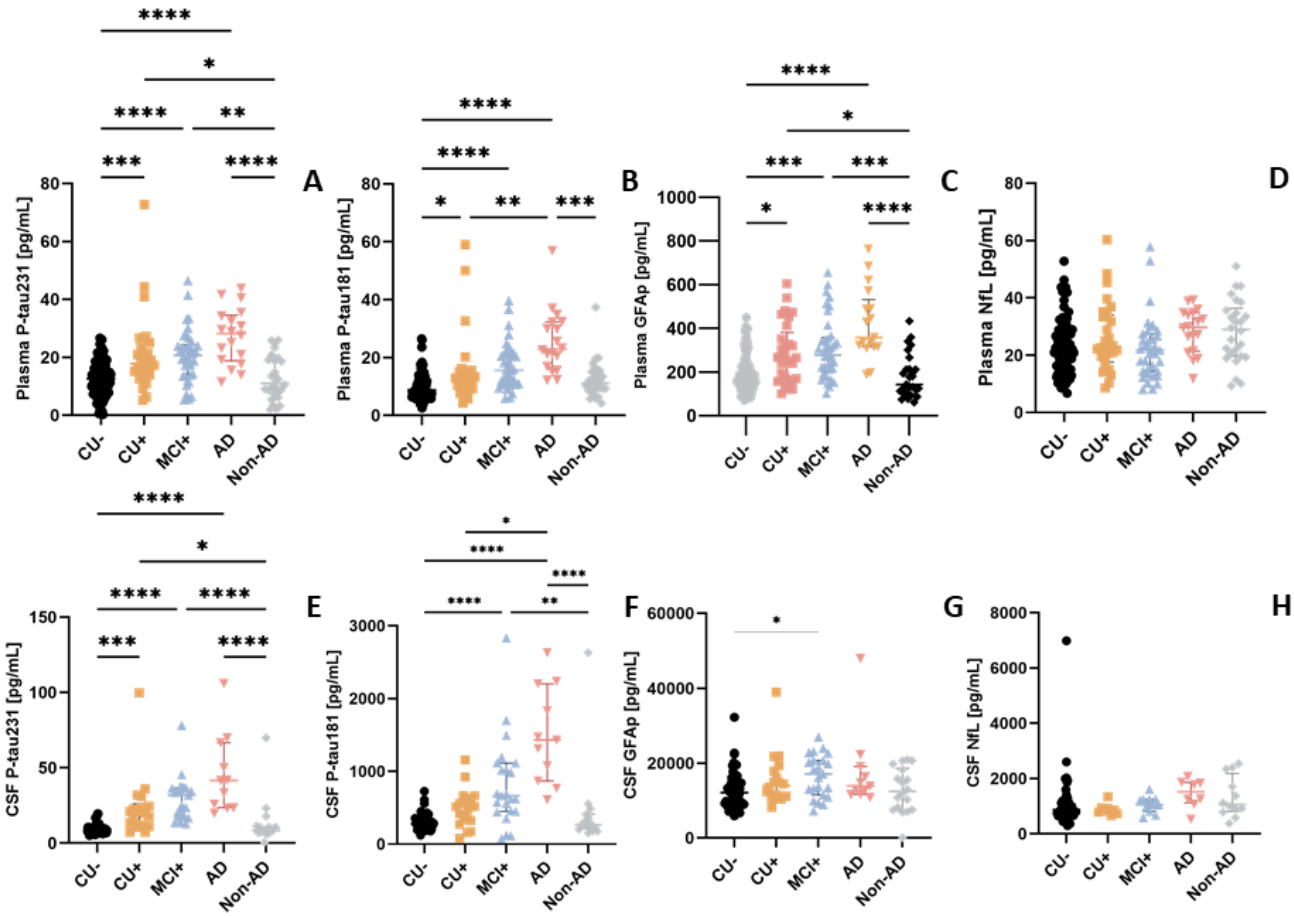
P<0.05; prodromal AD, *P*<0.01; AD, *P*<0.0001) had higher concentrations of plasma p-tau231 compared with non-AD neurodegenerative diseases and Aβ- MCI (analyzed as one group when comparing plasma and CSF biomarkers). For plasma p-tau181, all groups in the AD *continuum* had higher concentrations of p-tau181 as compared to Aβ- CU (Aβ+ CU, *P*<0.05; prodromal AD, *P*<0.0001; AD, *P*<0.0001) (Figure 2B). However, plasma p-tau181 only discriminated between individuals with AD pathology and non-AD neurodegenerative diseases at the dementia stage (*P*<0.001). Furthermore, p-tau181 demonstrated a significant stage-dependent increase, reflected in higher p-tau181 in AD compared with Aβ+ CU (*P*<0.01). Similar patterns were observed for plasma GFAp (Figure 2C), which was higher in Aβ+ CU (P<0.05), prodromal AD (P<0.001) and AD dementia (P<0.001) compared with CU Aβ-. The same pattern was observed when comparing Aβ+ CU (*P*<0.05), prodromal AD (*P*<0.001), and AD dementia (*P*<0.0001) with non-AD neurodegenerative diseases. No significant group differences were seen for plasma NfL (Figure 2D).

5.3 CSF biomarkers

In the subgroup with CSF examination (n = 125), similar patterns were seen for CSF P-tau 231 (Figure 2E); Aβ+ CU (*P*<0.001), prodromal AD (*P*<0.0001), and AD groups (*P*<0.0001) had higher concentrations of plasma p-tau231 compared to Aβ- CU. Furthermore, all groups in the AD continuum (Aβ+ CU, *P*<0.05; prodromal AD, *P*<0.0001; AD, *P*<0.0001) had higher concentrations of CSF p-tau231 compared with non-AD neurodegenerative diseases. Contrary to p-tau231, levels of CSF p-tau181 only significantly increased compared with Aβ- CU when AD pathological change was accompanied with MCI (prodromal AD) (*P*<0.0001) (Figure 2F). However, similarly to plasma p-tau181, CSF p-tau181 was significantly increased at the dementia stage of AD compared to Aβ+ CU (*P*<0.05). Additionally, all groups within the AD

continuum had significantly increased concentrations of CSF p-tau181 compared with non-AD neurodegenerative diseases (Aβ+ CU, $P<0.05$; prodromal AD, $P<0.01$; AD, $P<0.0001$). CSF GFAp was significantly higher only in prodromal AD compared with Aβ- CU ($P<0.05$) (Figure 2G), but no significant group differences were seen for CSF NfL (Figure 2H).

Figure 2. Cross-sectional group-wise comparisons between biomarkers.



Abbreviations: GFAp, glial fibrillary acidic protein; P-tau181, phosphorylated tau 181; P-tau231, phosphorylated tau 231; NfL, neurofilament light; CSF, cerebrospinal fluid; CU, cognitively unimpaired; MCI, mild cognitive impairment; AD, Alzheimer's disease; Non-AD, non-AD neurodegenerative diseases.

*$P<0.05$, ** $P<0.01$, *** $P<0.001$, ****$P<0.0001$, derived from Kruskal-Wallis test with *post hoc* Dunn-Bonferroni for multiple comparisons when significant differences were seen across groups.

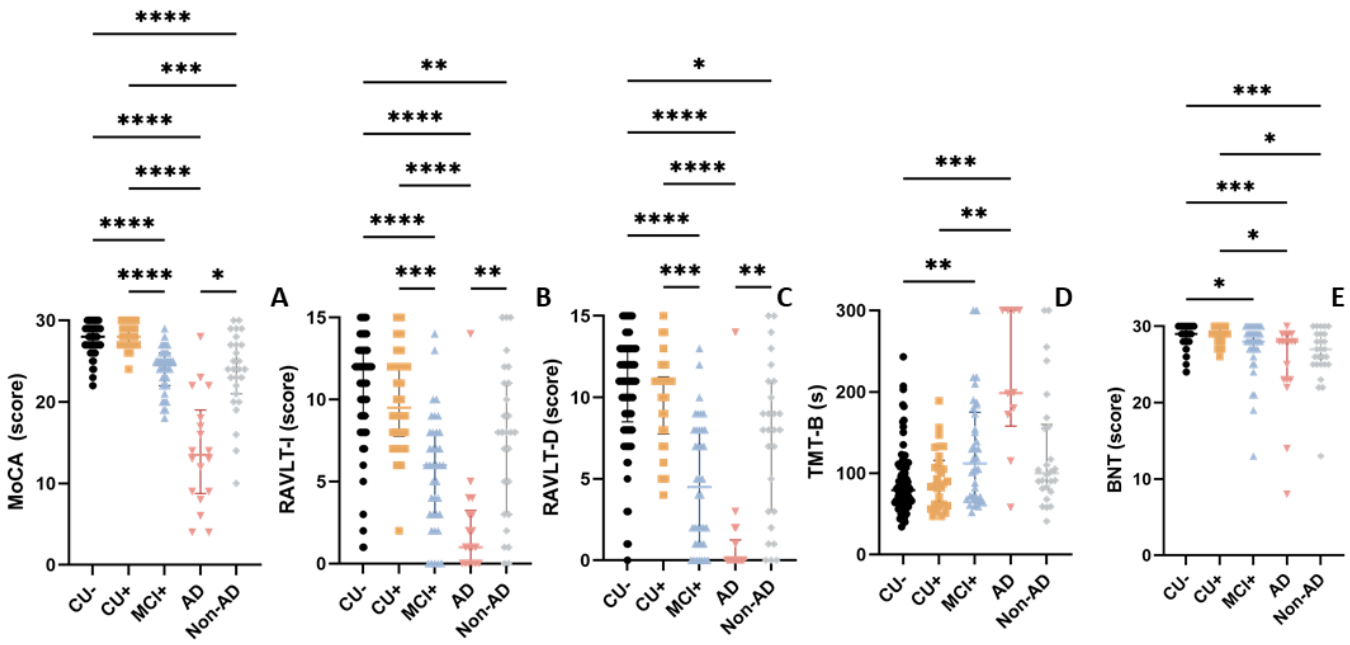
A +/- indicates Aβ PET positivity and negativity, respectively.

5.4 Neuropsychology

Next, we examined the neuropsychological patterns across the same groups. As expected, the scores on a cognitive screening test MoCA (Fig 3A), were lower in the cognitively impaired groups (prodromal AD, AD and non-AD neurodegenerative diseases; all $P<0.001$) compared with cognitively unimpaired, irrespective of Aβ status. In addition, the AD group performed worse compared with those with non-AD neurodegenerative diseases ($P<0.05$). When investigating the cognitive subdomains, evidence of memory impairment as tested with the RAVLT immediate recall (Fig 3B), prodromal AD ($P<0.001$), AD dementia ($P<0.001$) and individuals with non-AD diseases ($P<0.05$) recalled significantly fewer words compared with Aβ- CU. Similar differences were seen for those with cognitive impairment due to AD pathology when comparing with Aβ+ CU (prodromal AD and AD dementia; both $P<0.001$), but not for those with non-AD neurodegenerative diseases. Individuals with AD dementia performed significantly worse compared with the group having non-AD neurodegenerative diseases. These differences were preserved when assessing the number of words that were recalled after 20 minutes (Figure 3C). Furthermore, worse language functions as indexed with BNT were also observed in the groups with cognitively impaired individuals compared with Aβ- CU (Figure 3E). In addition, individuals with AD dementia and non-AD neurodegenerative diseases performed worse compared with Aβ+ CU. Signs of impaired executive function were seen in AD when comparing to both Aβ+/- CU (Aβ- CU, $P<0.001$; Aβ+ CU, $P<0.01$) (Figure 3D). Prodromal AD performed significantly worse than Aβ- CU ($P<0.01$). To achieve an understanding if a cognitive change could be seen over the short time-period of this study, the subset with available neuropsychological data at one year were divided into groups of cognitively impaired (CI) and unimpaired, irrespective of Aβ+ status (CU: n = 74; CI, n = 28).

Both participants with and without cognitive impairment at baseline performed worse on the MoCA after one year (P<0.05) (Figure 4A and F). In the CU group, participants performed significantly better on both the immediate and delayed recall in the RAVLT after one year (P<0.001) (Figure 4G and H), as well as the TMT-B (P<0.01) (Figure 4I). No improvement was seen for CI individuals (Figure 4D). Language performance as indexed with BNT did not change in any of the groups (Figure 4E and J).

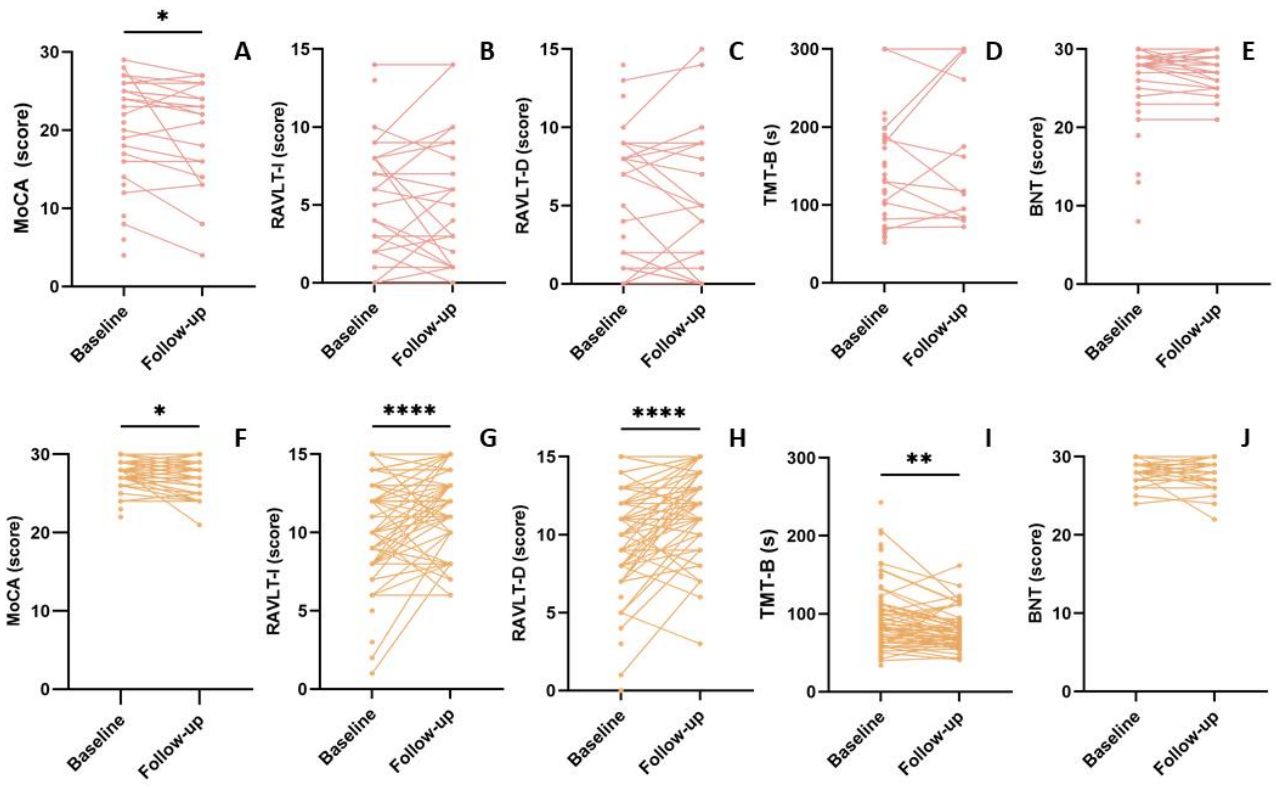
Figure 3. Group-wise comparison of neuropsychological tests at baseline.



Abbreviations: MCI, mild cognitive impairment; AD, Alzheimer's disease; Non-AD, non-AD neurodegenerative diseases; RAVLT-D, Rey auditory verbal learning test, delayed recall; RAVLT-I, Rey auditory verbal learning test, immediate recall; BNT, Boston naming test; MoCA, Montreal cognitive assessment; TMT-B, part B of trail making test; CU, cognitively unimpaired; s, seconds CU, cognitively unimpaired. A +/- indicates Aβ PET positivity and negativity, respectively.

*P<0.05, ** P<0.01, *** P<0.001, ****P<0.0001, derived from Kruskal-Wallis test with *post hoc* Dunn-Bonferroni for multiple comparisons when significant differences were seen across groups.

Figure 4. One-year change in neuropsychological tests in A-E) all CI and F-J) CU.



Abbreviations: MoCA, Montreal cognitive assessment; RAVLT-D/I, Rey auditory verbal learning test, delayed/immediate recall; BNT, boston naming test; TMT-B, part B of trail making test; CI, cognitively impaired; CU, cognitively unimpaired; s, seconds.

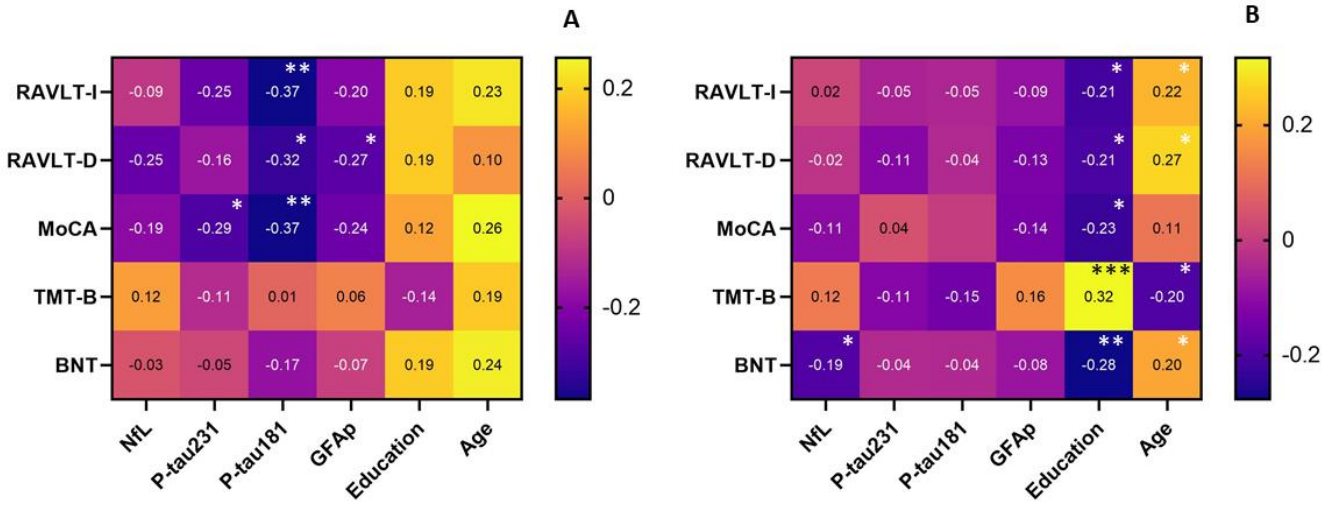
*P<0.05, ** P<0.01, *** P<0.001, ****P<0.0001, derived from paired t-tests.

5.5 Baseline associations between biomarkers and cognition

Furthermore, the associations between plasma biomarker concentrations and cognition were investigated only in the CU, irrespective of Aβ status, and CI with Aβ pathology (Figure 5A and B) to investigate if biomarker levels reflect the degree of cognitive impairment due to AD. As many tests were performed, and no correction for multiple comparisons was performed, the results should be considered exploratory. In CU individuals (Figure 5B), significant associations were seen between cognitive performance, and age as well as years of education

in all cognitive domains (rho = +/- 0.197–0.317, *P* = <0.05–<0.001), except for the MoCA test, where significance was not reached for MoCA score and years of education (*P* = 0.208). In addition, language performance weakly associated with plasma NfL (rho=0.190, *P*<0.05). However, in CI individuals with Aβ pathology, these associations were attenuated. Instead, inverse correlations, reflected by purple in the heat map (Figure 5A) were found between plasma p-tau 181, and scores on RAVLT immediate (rho = -0.367, *P*<0.01) and delayed (rho = -0.323, *P*<0.05) recall. Furthermore, both p-tau231 (rho = -0.287, *P*<0.05) and p-tau 181 (rho = -0.366, *P*<0.01) inversely correlated with global cognition, as indexed with MoCA scores. Plasma GFAp was inversely associated with RAVLT delayed recall (rho = -0.270, *P*<0.01).

Figure 3. Heat map displaying cross-sectional associations between biomarkers and neuropsychological tests in A) Aβ+ CI and B) CU.



Abbreviations: GFAp, glial fibrillary acidic protein; P-tau181, phosphorylated tau 181; P-tau231, phosphorylated tau 231; NfL, neurofilament light; MoCA, Montreal cognitive assessment; RAVLT-D, Rey auditory verbal learning test, delayed recall; RAVLT-I, Rey auditory verbal learning test, immediate recall; BNT, Boston naming test; TMT-B, part B of trail making test; CI, cognitively impaired; CU, cognitively unimpaired; Aβ+, β-amyloid positive.

* *P*<0.05, ** *P*<0.01, ** *P*<0.001. Data in heat map presented as Spearman's correlation coefficient.

5.6 Longitudinal associations between plasma biomarkers and cognition

Furthermore, when assessing the relationship between biomarkers at baseline and change in cognition over a year in the subsample of CU, MCI and AD participants where this data was available (CSF, n = 41-69; plasma, 73-109 depending on test), using multiple linear regression including age, sex and years of education in the models (Supplementary table 1). Similar to the previous section, no corrections for multiple comparisons were made and associations should thus be considered exploratory. Based on inspections of scatterplots (biomarker vs. cognitive test), one obvious outlier was excluded from regression models investigating biomarker effects on MoCA scores (Supplementary figure 1), whereas one outlier was excluded from analyses assessing the predictive value of biomarker levels on TMT-B (Supplementary figure 2), since it was as an obvious outlier in the subset of individuals with CSF and follow-up. No significant associations were seen between biomarker levels and change in cognition at one year.

6 Discussion

In this study, we investigated plasma and CSF biomarkers of AD pathophysiology and their relationship with cognitive subdomains using established neuropsychological tests in a well-characterized research cohort. It was shown that novel plasma and CSF biomarkers reflecting altered tau metabolism reflect AD pathophysiology, with increasing concentrations of plasma and CSF p-tau181 and p-tau231 along the AD continuum but not in non-AD neurodegenerative diseases. In addition, plasma, but not CSF, GFAp demonstrated a similar pattern. No significant group-wise differences were seen for either plasma or CSF NfL were seen. Furthermore, as expected, AD patients performed significantly worse compared with cognitively unimpaired individuals in the cognitive subdomains included in the study; language, memory, executive function/attention and when testing global cognitive functions. However, these differences

were present already in patients at the prodromal stage of AD. When investigating how plasma biomarkers related cross-sectionally with neuropsychological measures, it was shown that worse memory function and global cognition in patients with Aβ pathology and cognitive impairment were associated with higher concentrations of plasma p-tau species. These associations were attenuated in individuals without cognitive impairment, and instead related to age and years of education, which is a well-established finding.[87] However, using multiple regression models, no significant contributions were seen for any of the biomarkers in predicting cognitive worsening at one year.

It is now well-established that p-tau in CSF is elevated in manifest AD,[23] and that clinically meaningful increases are seen already at the prodromal stage.[36,88] However, recent studies have attempted to identify alterations in soluble p-tau concentrations already at the preclinical stage of the disease. A recent study by our group demonstrated that subtle increases in CSF p-tau181 and—even more significantly—p-tau231 can be detected early in the Alzheimer's *continuum*, when only subtle Aβ deposition (below the common threshold for Aβ PET positivity), and that these changes do not occur in other neurodegenerative conditions, albeit having pathological tau depositions, which is the case in primary tauopathies, such as progressive supranuclear palsy, corticobasal degeneration and a share of frontotemporal lobar degeneration. It also seems that these alterations occur before tau is deposited in tangles (as measured with tau PET).[89] The data presented here supports these findings, with a significant increase in CSF p-tau231 being seen already among Aβ+ CU, whereas CSF p-tau181 was only significantly increased at the prodromal stage of AD. Further, p-tau181 was higher in AD dementia compared with Aβ+ CU. However, both biomarkers discriminated between those with clinically manifest AD pathology and non-neurodegenerative diseases. Altogether, the data presented here and previously

suggests that p-tau231 is an earlier biomarker, and p-tau181 is slightly later, possibly implying that the disease-specific tau phosphorylation patterns change along the AD continuum.[85] Furthermore, plasma p-tau 181 and 231 demonstrated similar patterns, albeit with larger overlap. Several recent large studies have demonstrated that meaningful increases also in plasma tau phosphorylated at threonine at amino acids 181, 217 and 231 occur already in the preclinical phase of AD (Aβ+ CU),[56,58,59,90,91] and that soluble tau levels relate to both increasing Alzheimer-type tau pathology and amyloid deposition, but not other neurodegenerative diseases.[56,58,59,90,91] Furthermore, the similar pattern seen for plasma, but not CSF GFAp is peculiar. The previous studies that have been published suggest that this is due to astroglial activation in response to Aβ, and that different routes of clearance could explain the difference between plasma and CSF GFAp. The non-significant differences in NfL across groups in this relatively small study suggests that NfL is not sufficient as a diagnostic biomarker alone in AD, but the majority of clinicians found plasma NfL useful in a questionnaire-based study from Amsterdam, where it (similarly to Sahlgrenska University Hospital), has been introduced into clinical routine, particularly in younger patients with subjective cognitive decline and psychiatric disease.[65]

Thus far, the relationship between specific neuropsychological measures—or cognitive subdomains—and plasma biomarkers are more scarcely evaluated. In this study, we found that among cognitively unimpaired subjects, the only significant associations with cognitive scores were found with age and years of education, which is a well-known factor that influences scores on neuropsychological tests. However, in patients with evidence of Aβ+ pathology and cognitive impairment, associations were seen between plasma p-tau181 and p-tau231 and global cognition. In one of the first studies reporting data on plasma p-tau181, it was found that

higher levels of plasma p-tau181 cross-sectionally in AD and MCI subjects were associated on the delayed drawing attempt of Modified Rey-Benson figures test,[92] being similar to Rey auditory verbal learning test (RAVLT) in the sense that it consists of immediate and delayed recall tasks. However, we extend these findings by showing that also plasma GFAp is associated with worse performance on RAVLT (both immediate and delayed recall) cross-sectionally. In the same study, higher baseline plasma p-tau181 predicted decline in worse delayed figure recall (modified Rey-Benson figures test), verbal function (BNT), and worse global cognitive function (as determined with MMSE) over approximately two years. As mentioned, in this study, we could not see such patterns. There are several possible factors contributing to this. The most obvious explanation is the number of participants in the Aβ+ CI groups in this study (n = 56 vs n = 103). Another possible explanation to why no differences were seen in verbal memory, and possibly also executive function is because of a "floor effect" – it was too difficult for subjects with dementia, implying that the test could not detect further worsening of memory or executive functions in those who were severely impaired already at baseline, and conversely, a roof effect (a test being too easy) for the language test used (see figure 3E). Furthermore, both MCI and AD are slowly progressing conditions, and since there were no significant worsening in most tests for the group of cognitively impaired individuals, it is thus not surprising that these were not predictive in a small set of individuals over a short time-period. The larger longitudinal studies performed thus far such as one in the Alzheimer's diseases neuroimaging initiative (ADNI) (n ~ 700) found that high plasma p-tau 181 entailed large risk of AD dementia in both Aβ+ MCI (hazard ratio (HR) ~ 15) and Aβ+ CU (HR ~ 5) over 84 months as compared with Aβ- CU.[91] Similar findings were seen for general cognition used as a continuous measure. These patterns have been seen also in other studies.[90]

6.1 Strengths and weaknesses

Strengths of this study include the deeply characterized participants, as all included participants had baseline Aβ PET, neuropsychological testing, many novel putative plasma biomarkers of AD and that a subset of participants (including healthy participants) agreed to lumbar puncture, which enabled measurement of corresponding biomarkers in CSF.

Weaknesses include the relatively small number of participants as well as the short follow-up time, which prevented meaningful analysis of the relationship between plasma biomarkers and neuropsychological measures in subgroups cross-sectionally and longitudinally, respectively. The inconsistency in how the groups were assessed, and missing data are caveats which need to be addressed (CU vs CI in one-year cognition, CU+/-, CI+/- in correlations, all groups in cross-sectional biomarkers and neuropsychology, and all participants pooled in the regression models). However, the composition was closely considered in each step. Furthermore, many studies use cognitive composites, both creating global cognitive composites and individual tests in specific to account for test-specific differences in performance, and to reduce the risk of type I errors (false positives) or lessen statistical power due to correction for multiple comparisons. In addition, it is common to construct regression-based age- and education-corrected z-scores based on large healthy populations to determine the compare how subjects perform in relation to what is expected. However, such scores were not available in this study, but the absence of age- and education-differences between groups make group-wise comparisons unlikely to be strongly skewed by such factors.

Nonetheless, we conclude that p-tau231 and p-tau181 both in CSF and plasma as well as plasma GFAp differ significantly between individuals in the Alzheimer's *continuum* and those that are not, and that plasma p-tau181, p-tau231 and GFAp reflect the degree of cognitive impairment

in individuals with objective cognitive decline. In order to draw clearer conclusions about the associations with decline neuropsychological measures, prospective cohort studies with longer follow-up period comparing these plasma biomarker candidates are warranted, both among community-dwelling individuals in population-based studies, and in the specialized memory clinic setting studied here, since the prevalence of cognitive disorders greatly varies between these settings.

Examensarbete 2021, Läkarprogrammet

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7 Populärvetenskaplig sammanfattning

Blodmarkörer för att spegla svårighetsgrad och försämrade hjärnfunktioner över tid vid Alzheimers sjukdom

Alzheimers sjukdom är den vanligaste orsaken till fortskridande kognitiv svikt (påverkan av hjärnans högre funktioner) både i Sverige och världen i stort. Det symtom som kännetecknar sjukdomen är närminnesproblem, men efterhand drabbas även andra av hjärnans funktioner, såsom språk, förmågan att planera och rumsuppfattning. Vi vet idag att de hjärnförändringar som ses vid obduktion av personer med Alzheimers sjukdom är en kombination av klumpar av proteinet β-amyloid, nystan av tau-protein, hjärninflammation samt nervcellsförlust. De senaste decennierna har studier av biomarkörer (mätbara förändringar som speglar normala eller onormala kroppsliga processer) för dessa förändringar i ryggvätska och med avancerad hjärnavbildning tydliggjort att dessa förändringar börjar decennier innan symptom uppstår, och att patienter med dessa förändringar löper högre risk att drabbas av fortskridande kognitiv påverkan än vad som ses vid normalt åldrande. Nya rön från studier som har använt högkänsliga metoder ger en möjlighet att skilja ut de som löper högre risk att drabbas av fortskridande kognitiva problem med ett vanligt blodprov, men det är ännu osäkert vilket av dessa som bäst

skiljer sjuka från friska samt förutsäger vem som försämras över tid. I den här studien jämförde vi två former av modifierat (fosforylerat) tau-protein, en biomarkör för nervcellsskada (neurofilament [NfL]), samt en biomarkör för hjärninflammation (glialt fibrillärt surt protein [GFAp]) som mättes i blodprov på 210 individer, och i ryggvätska på 125. Bland dessa ingick personer med bevarade kognitiva funktioner, personer med mild kognitiv svikt (ett tidigt stadium av hjärnpåverkan), personer med Alzheimerdemens och med andra kognitiva sjukdomar. Utöver detta testades deras högre hjärnfunktioner av en specialiserad psykolog, och efter ett år upprepades dessa tester. Vi såg att de med Alzheimerdemens och mild kognitiv svikt hade högre nivåer av modifierat tau-protein både i blod och ryggvätska jämfört med de kognitivt friska och de med andra kognitiva sjukdomar, medan GFAp bara ökade i blodet. Därefter undersökte vi hur dessa biomarkörer var associerade med högre hjärnfunktioner, och fann att de friska som var äldre och hade lägre utbildning presterade sämre på kognitiva tester, medan detta mönster inte sågs hos de som hade alzheimerförändringar och kognitiv funktionsnedsättning. Istället sågs associationer mellan sämre kognitiv funktion och blodprovsnivåer av framförallt fosforylerat tau och i viss mån också GFAp. Slutligen undersökte vi möjligheten att med blod- och ryggvätskemarkörer förutspå kognitiv försämring över ett års tid, vilket får betraktas som kort tid i sammanhanget, och där sågs inga samband. Sammantaget visar studien att blod- och ryggvätskeprover kan skilja de med tidiga alzheimerförändringar i hjärnan från helt friska och de med andra kognitiva sjukdomar. Dessutom såg vi att nivån av biomarkörer i blod relaterar till grad av kognitiv funktionsnedsättning, men förutser inte förändring på ett års sikt, då detta kan vara en för kort observationstid. Dessa resultat kan ligga till grund för vilka blodtester som bäst speglar de förändringar som sker hos de med Alzheimers sjukdom. För att dra säkrare slutsatser kring vilka

blodprover som bäst förutser kognitiv försämring krävs fler och större jämförande studier med fler individer över längre tid.

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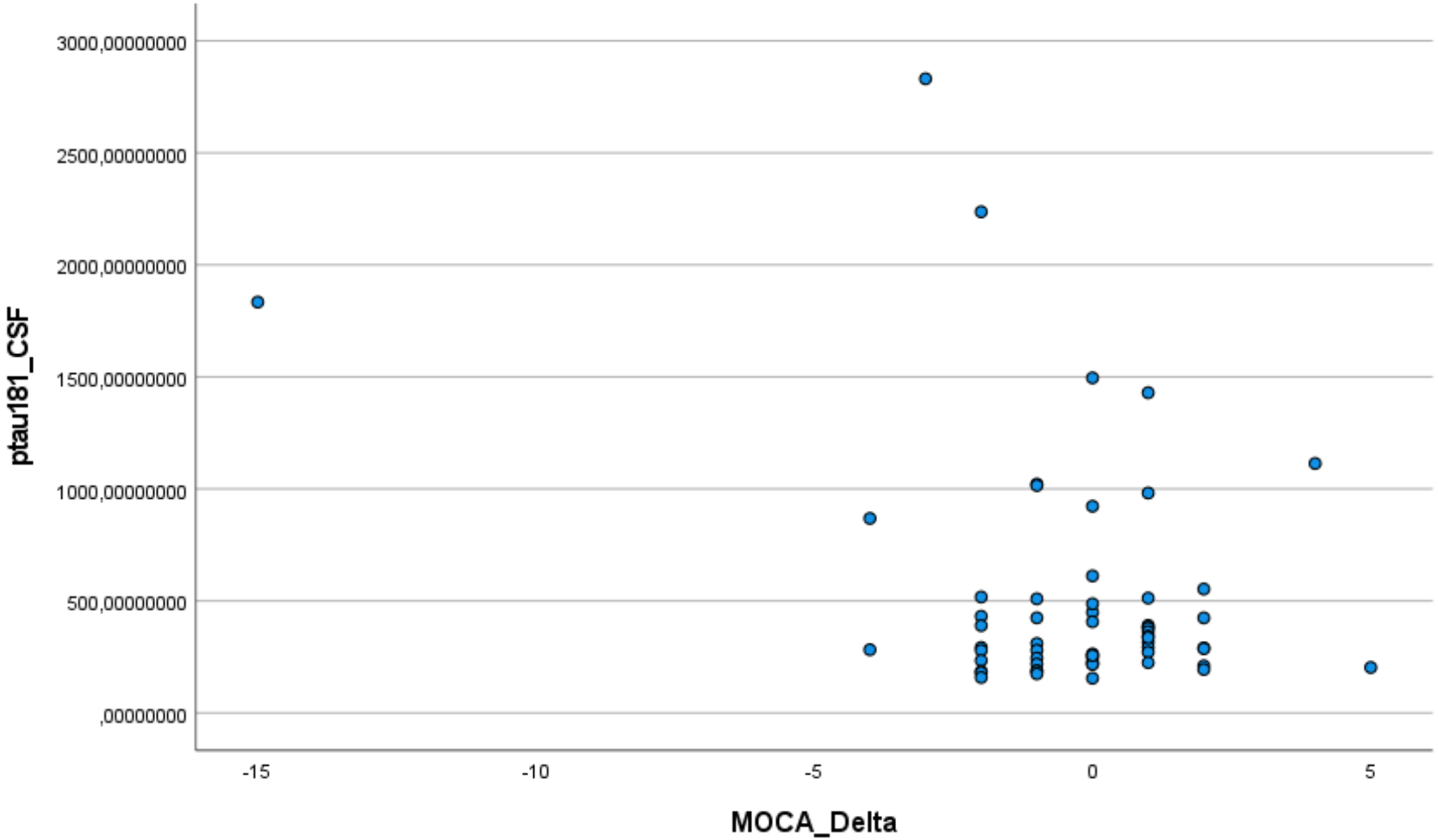
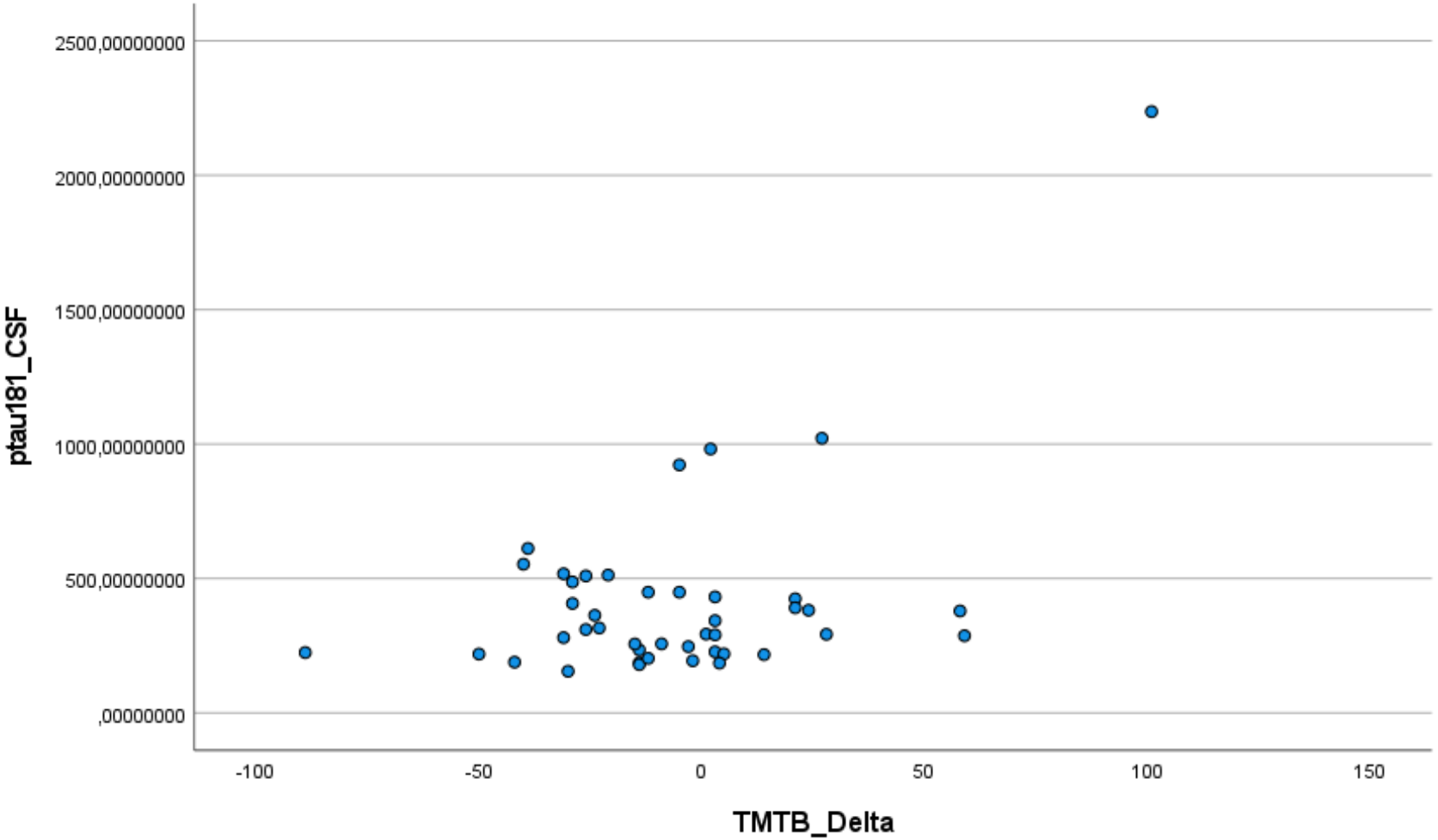
10 Supplementary figures and tables

		Plasma				CSF			
		P-value	β	SE	Participants (n)	P-value	β	SE	Participants (n)
P-tau181	MoCA	0.2	-1.0	0.8	94	0.7	-0.3	0.8	57
	RAVLT-D	0.5	-0.8	1.0	110	0.1	-2.0	1.0	69
	TMT-B	0.9	-2.0	17	73	0.2	26	20	41
	BNT	0.6	0.3	0.5	109	0.4	-0.5	0.6	68
P-tau231	MoCA	0.2	-0.8	0.5	96	0.1	-2.0	1.0	57
	RAVLT-D	0.6	0.5	1.3	110	0.2	-0.03	0.02	69
	TMT-B	0.5	10	15	73	0.1	30	21	41
	BNT	0.4	0.4	0.5	109	0.5	-0.4	0.6	68
GFAp	MoCA	0.2	-1.3	1.0	93	0.7	0.7	2.0	57
	RAVLT-D	0.7	-0.5	1.3	106	0.6	-1.0	2.0	69
	TMT-B	0.4	20	21	70	0.9	-4	29	41
	BNT	0.1	1.0	0.7	105	0.5	-0.7	1.0	68
NfL	MoCA	1.0	-0.03	1.1	90	..	..	..	48
	RAVLT-D	0.9	-0.3	1.4	106	..	..	..	45
	TMT-B	1.0	-0.2	23	69	..	..	..	39
	BNT	0.6	0.4	0.8	105	..	..	..	45

Supplementary table 1. Estimates for biomarker impacts on cognitive outcomes using multiple regression models. Abbreviations: GFAp, glial fibrillary acidic protein; P-tau181, phosphorylated tau 181; P-tau231, phosphorylated tau 231; NfL, neurofilament light; CSF, cerebrospinal fluid; MoCA, Montreal cognitive assessment; RAVLT-D, rey auditory verbal learning test, delayed recall; BNT, boston naming test; TMT-B, part B of trail making test; SE, standard error; β, estimated β coefficient. NfL was excluded from all analyses based on the small sample size (n=40-49).



Supplementary figure 1. Scatterplot identifying outlier, which was excluded from tests with ΔMoCA based on the CSF subsample. A similar pattern was seen also for P-tau231 in CSF. Abbreviations: P-tau181, phosphorylated tau 181; MoCA, Montreal cognitive assessment.



Supplementary figure 2. Rationale behind the exclusion of an outlier from tests with ΔTMT-B based on the CSF subsample. A similar pattern was seen also for p-tau231 in CSF. Abbreviations: P-tau181, phosphorylated tau 181; TMT-B, part B of trail making test.