The impact of tau biomarkers in the A/T/(N) framework of Alzheimer's disease

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Abstract

English abstract:

Alzheimer's disease (AD) dementia is characterized by the accumulation of amyloid-β plaques (A), neurofibrillary tangles (T) and neurodegeneration (N). It is thought to be a combination of pathological processes starting years before the onset of cognitive symptoms, with amyloid-β build up causing neurofibrillary tangles accumulation, in turn triggering neurodegeneration to finally lead up to cognitive symptoms. The clinical diagnosis in living individuals is increasingly supported by clinically validated biomarkers, that are proxies of the aforementioned AD neuropathological changes. Thus, researchers created a biological construct allowing for the *in vivo* identification of AD using available biomarkers: the A/T/(N) framework. It is composed of three groups of biomarkers divided based on the pathology each one measures and allows for disease staging and prediction of pathogenesis and cognitive decline. Even though AD is due to all three hallmarks, tau is considered the pathological process more closely associated with cognition. Our aim was to assess various biomarkers of tau, in plasma, cerebrospinal fluid (CSF) and with positron emission tomography (PET) imaging, and their impact on the A/T/(N) framework of AD.

An increasing number of methods are available to measure tau *in vivo*. Highly sensitive assay for plasma markers of phosphorylated tau (pTau) were recently created. We first provided evidence that plasma pTau181 levels were associated with neurodegeneration cross-sectionally in cognitively unimpaired and impaired individuals. Additionally, it was a predictor of future cortical atrophy in a time-specific manner, based on the individual's cognitive status. We then investigated the degree to which elevated concentrations of different plasma epitopes (here pTau181 and pTau231) deliver similar information and predict positivity status as determined by tau-PET. Tau

status as determined by pTau epitopes and tau-PET was concordant for most individuals, albeit plasma pTau positivity was thought to emerge before tau-PET, for example in cognitively unimpaired amyloid-β positive individuals. Tau statuses was however often accompanied by cognitive impairment, high amyloid-β PET, hippocampal atrophy and CSF pTau181 levels, and higher risk of carrying at least one *APOE*ε4 allele. Finally, we aimed to characterize a novel assay for non-phosphorylated N-terminal tau fragment, named NTA, in a well characterized cohort. We demonstrated that plasma and CSF NTA-tau levels were associated with neurofibrillary tangle accumulation only and not amyloid-β and neurodegeneration. It further predicted future neurofibrillary tangle accumulation and neurodegeneration.

Altogether, we showed that the various tau assessment methods can be used to stage AD pathological progression along the A/T/(N) framework. Plasma pTau levels emerge early in disease progression and might be more closely related to amyloid-β (A). The numerous neurofibrillary tangles (T) markers cannot be used interchangeably as they depict distinct stages of tau pathological progression. Finally, AD-specific tau marker concentrations can be used to predict neurodegeneration (N) years before.

French abstract:

La démence de la maladie d'Alzheimer (MA) se caractérise par l'accumulation de plaques amyloïdes-β (A), d'enchevêtrements neurofibrillaires (T) et de neurodégénérescence (N). Elle serait une combinaison de processus pathologiques commençant des années avant l'apparition des symptômes cognitifs, avec l'accumulation d'amyloïde-β provoquant l'accumulation d'enchevêtrements neurofibrillaires, déclenchant à son tour la neurodégénérescence pour finalement aboutir aux symptômes cognitifs. Le diagnostic clinique chez les personnes vivantes est de plus en plus étayé par des biomarqueurs validés cliniquement, qui sont des substituts des changements neuropathologiques susmentionnés de la MA. Les chercheurs ont donc créé une construction biologique permettant l'identification *in vivo* de la MA à l'aide des biomarqueurs disponibles : le schéma A/T/(N). Il est composé de trois groupes de biomarqueurs divisés en fonction de la pathologie que chacun d'eux mesure et permet de classer la maladie par étapes et de prédire la pathogenèse et le déclin cognitif. Bien que la MA soit due aux trois caractéristiques, la protéine tau est considérée comme le processus pathologique le plus étroitement associé à la cognition. Notre objectif était d'évaluer différents biomarqueurs de la protéine tau, dans le plasma, le liquide céphalorachidien (LCR) et par tomographie par émission de positons (TEP), et leur impact sur le cadre A/T/(N) de la MA.

Un nombre croissant de méthodes sont disponibles pour mesurer la protéine tau *in vivo*. Des tests hautement sensibles pour les marqueurs plasmatiques de la tau phosphorylée (pTau) ont été récemment créés. Nous avons d'abord prouvé que les taux plasmatiques de pTau181 étaient associés à la neurodégénérescence de manière transversale chez des personnes souffrant de troubles cognitifs ou non. De plus, il s'agissait d'un facteur prédictif de l'atrophie corticale future de manière chronologique dans le temps, en fonction de l'état cognitif de l'individu. Nous avons

ensuite étudié dans quelle mesure les concentrations élevées de différents épitopes plasmatiques (ici pTau181 et pTau231) fournissent des informations similaires et permettent de prédire le statut de positivité tel que déterminé par la TEP de tau. Le statut de tau tel que déterminé par les épitopes pTau et la TEP était concordant pour la plupart des individus, bien que la positivité de la pTau plasmatique ait été considérée comme apparaissant avant la TEP, par exemple chez les individus positifs à l'amyloïde-β sans déficience cognitive. Le statut tau était cependant souvent accompagné de troubles cognitifs, d'une élévation de TEP amyloïde-β, d'atrophie de l'hippocampe et de concentrations de pTau181 dans le LCR, ainsi que d'un risque plus élevé de porter au moins un allèle *APOE*ε4. Enfin, nous avons voulu caractériser un nouveau test pour le fragment tau N-terminal non-phosphorylé, appelé NTA, dans une cohorte bien caractérisée. Nous avons démontré que les niveaux de NTA dans le plasma et le LCR étaient associés uniquement à l'accumulation d'enchevêtrements neurofibrillaires et non à l'amyloïde-β et à la neurodégénérescence. Il prédisait en outre l'accumulation future d'enchevêtrements neurofibrillaires et la neurodégénérescence.

Dans l'ensemble, nous avons montré que les différentes méthodes d'évaluation de la protéine tau peuvent être utilisées pour déterminer la progression pathologique de la maladie d'Alzheimer selon le schéma A/T/(N). Les taux plasmatiques de pTau apparaissent tôt dans la progression de la maladie et pourraient être plus étroitement liés à l'amyloïde-β (A). Les nombreux marqueurs des enchevêtrements neurofibrillaires (T) ne peuvent pas être utilisés de manière interchangeable car ils décrivent des étapes distinctes de la progression pathologique de tau. Enfin, les concentrations de marqueurs tau spécifiques de la MA peuvent être utilisées pour prédire la neurodégénérescence (N) des années auparavant.

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Abbreviations

AD	Alzheimer's disease
ADNI	Alzheimer's Disease Neuroimaging Initiative
AIC	Akaike information criterion
ANOVA	analysis of variance
APOE	apolipoprotein E
APP	amyloid precursor protein
AUC	area under the ROC curve
Aβ	amyloid-β
BBB	blood-brain barrier
CDR	clinical dementia rating
CERAD	Consortium to Establish a Registry for AD
CI	cognitively impaired
CSF	cerebrospinal fluid
CU	cognitively unimpaired
CUY	cognitively unimpaired young
DSM	Diagnostic and Statistical Manual of Mental Disorders
FDG	fluorodeoxyglucose
FU	follow-up
FWHM	full width half maximum
GFAP	glial fibrillary acidic protein
GM	grey matter
HRRT	High Resolution Research Tomograph
IWG	International Working Group
LM	linear model
LME	linear mixed effect model
MAO	monoamine oxidase
MAPT	microtubule-associated protein tau
MCI	mild cognitive impairment
MMSE	mini mental state examination
MRI	magnetic resonance imaging
N	Neurodegeneration

NfL	neurofilament light
NFT	neurofibrillary tangles
NIA-AA	National Institute on Aging and Alzheimer's Association
NINCDS-ADRDA	Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association
PET	positron emission tomography
PiB	Pittsburgh Compound B
pTau	phosphorylated tau
RFT	random field theory
ROC	receiver operating characteristics
ROI	region of interest
SD	standard deviation
SUVR	standardized uptake value ratio
T	Tau
TRIAD	Translational Biomarkers in Aging and Dementia
tTau	total tau
VBM	voxel-based morphometry
WM	white matter

Original contribution to knowledge:

The original scientific contributions in this thesis include:

Chapter 2: We measured plasma pTau181 in a large population comprised of cognitively unimpaired and impaired individuals. We first observed a significant difference in plasma pTau181 levels between diagnostic groups at the cross-sectional level. High plasma pTau181 concentration was associated with GM damage in the anterior cingulate and occipital gyrus in the cognitively unimpaired group. In turn, it was associated with GM damage in the temporal lobe and precuneus and WM deterioration in the temporal lobe and corpus callosum in the brain of cognitively impaired individuals. Additionally, longitudinal analyses conducted with data up to four years after baseline revealed that plasma pTau181 changes were not significantly different between cognitively unimpaired and impaired individuals. Baseline levels predicted further GM damage in the temporal lobe and anterior cingulate cortex of the cognitively unimpaired group, 36 months after baseline. On the contrary, it predicted eminent (as soon as 12 months after baseline) degeneration of GM and WM in the brain of cognitively impaired individuals. We observed an incremental pattern of neurodegeneration, starting for GM in the medial temporal and occipital regions, to affect the medial frontal, precuneus, and the whole temporal lobe at 48 months follow-up. The first WM region affected were in the temporal lobe, at 12-month follow-up, to affect all the WM tracts 48 months after baseline. Plasma pTau181 is thus a great marker of present and future neurodegeneration, specific to AD.

Chapter 3: We assessed three markers of tau pathology, plasma pTau231, plasma pTau181 and tau-PET with [^{18}F]MK6240 to evaluate the concordance and discordance in tau status. Most individuals had concordant statuses, however, the presence of discordant groups suggests that

plasma pTau231, pTau181 and tau-PET reflect different stages of tau accumulation. Thus, these tau markers cannot be used interchangeably in the aging and AD spectrum. Nevertheless, positivity to at least one biomarker was often accompanied by a diagnosis of cognitive impairment, higher risk of presenting at least one *APOE*ε4 allele, high amyloid-β PET SUVR, hippocampal atrophy and CSF pTau181 concentration. We observed an incremental pattern, in a sense that individuals that were positive to two biomarkers had an even higher risk of presenting an AD-related pathology or risk factor. Preclinical AD individuals were often positive to plasma biomarkers, but not to tau-PET, suggesting plasma pTau231 and pTau181 might become abnormal before tau-PET, however, longitudinal analyses are needed to confirm such hypothesis. The research project emphasized the importance of plasma assessments, and supports its use in clinical and diagnostic settings, to follow the progression of tau pathology.

Chapter 4: We characterized a novel assay targeting N-terminal tau, NTA-tau, in the plasma and CSF. We observed a significant increase in plasma NTA-tau in individuals at late stages of AD dementia, while CSF NTA-tau seemed to increase earlier on, when participants had abnormal amyloid-β levels as assessed via Aβ-PET. NTA-tau dissociated AD dementia from other types of neurodegenerative condition, including other tauopathies. Even though plasma and CSF NTA-tau correlated with amyloid-β-PET SUVR, tau-PET temporal meta-ROI SUVR and temporal neurodegeneration in ROI-based analyses, tau-PET measures better explained the data. At the voxel-level, we observed that NTA-tau was strongly correlated with tau-PET, and only CSF NTA-tau was slightly associated with amyloid-β-PET. Moreover, longitudinal analyses were carried out with plasma NTA-tau. We showed that plasma NTA-tau only increased in individuals presenting cognitive impairment (MCI or AD) and amyloid-β positivity. Additionally, longitudinal changes

in plasma NTA-tau were associated with longitudinal accumulation of tau-PET in the temporal lobe and the anterior cingulate, as well as neurodegeneration in the temporal lobe. Our manuscript characterized NTA-tau as an assessment tool for tau only, as it did not associate strongly with other hallmarks of AD (i.e. amyloid-β plaques and neurodegeneration).

Contribution of authors

Chapter 2: CT is responsible for the conception and design of the study, acquisition and analysis of data, and drafting a significant portion of the manuscript and figures. ALB, JT, TAP and FZL are responsible for the conception and design of the study, acquisition and analysis of data. PSC, SMM, GB, YTW, JFA, JLR, AS, NJA, TKK are responsible for the acquisition and analysis of the data. MC, KB, HZ, EDVS, PH and SG are responsible for the conception and design of the study. PRN is responsible for the supervision of the project, for the conception and design of the study, and for drafting a significant portion of the manuscript and figures. All authors reviewed and approved the manuscript.

Chapter 3: CT is responsible for the conception and design of the study, acquisition and analysis of data, and drafting a significant portion of the manuscript and figures. JT, PK, ALB, TAP and FZL are responsible for the conception and design of the study, acquisition and analysis of data. NJA, TKK, SS, DLT, JS, NR, NMP, VP, GB, MSK, SSM, YTW, JFA, PCLF, JPFZ are responsible for the acquisition and analysis of the data. MC, EV, KB, HZ and SG are responsible for the conception and design of the study. PRN is responsible for the supervision of the project, for the conception and design of the study, and for drafting a significant portion of the manuscript and figures. All authors reviewed and approved the manuscript.

Chapter 4: CT, JLR and AS are responsible for the conception and design of the study, acquisition and analysis of the data, drafting a significant portion of the manuscript and figures. SS, ALB, NR, LMG, JT are responsible for the conception and design of the study, acquisition and analysis of the data. WSB, JS, FZL, GB, ACM, MC, SSM, TAP are responsible for the acquisition and

analysis of the data. NJA and HZ are responsible for the conception of the study. KB and PRN are responsible for the supervision of the project, for the conception and design of the study, and for drafting a significant portion of the manuscript and figures. All authors reviewed and approved the manuscript.

Original contributions not included in the thesis:

Publications & presentations – first authored

First-authored manuscripts:

Tissot C, Servaes S, Lussier F, Ferrari Souza JP, Ferreira PCL, Bezgin G, Bellaver B, Teixeira Leffa D, Mathotaarachchi S, Chamoun M, Stevenson J, Rahmouni N, Kang MS, Pallen V, Poltronetti NM, Wang YT, Fernandez-Arias J, Benedet AL, Zimmer ER, Soucy JP, Tudorascu DL, Cohen AD, Sharp M, Gauthier S, Massarweh G, Lopresti B, Klunk WE, Baker SL, Villemagne VL, Rosa-Neto M, Pascoal TA. (2022). The association of age-related and off-target retention with longitudinal quantification of [18F]MK6240 tau-PET in target regions.Journal of Nuclear Medicine. https://doi.org/10.2967/jnumed.122.264434

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Publications and presentations – co-authored

Other co-authored manuscripts

Bellaver B, Povala G, Ferreira PCL, Ferrari-Souza JP, Teixeira Leffa D, Lussier FZ, Benedet AL, Ashton NJ, Tissot C, Therriault J, Servaes S, Stevenson J, Rahmouni N, Lopez O, Tudorascu D, Villemagne VL, Gauthier S, Zimmer ER, Zetterberg H, Blennow K, Aizenstein HJ, Klunk WE, Snitz B, Maki P, Thurston R, Cohen A, Ganguli M, Karikari TK, Rosa-Neto P, Pascoal TA. (2023). Astrocyte reactivity influences the association of amyloid-β and tau biomarkers in preclinical Alzheimer's disease.Nature Medicine, Submitted

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Chapter 1: Introduction

1. Alzheimer's disease

1.1 Overview

Alzheimer's disease (AD), which is currently the most common form of dementia, is a neurodegenerative disorder of the brain, leading to memory loss and decline in other cognitive abilities, that interfere with daily life (Alzheimer Association, 2010). The worldwide prevalence of AD is thought to be around 24 million cases, a number predicted to double every 20 years as the population ages (Mayeux & Stern, 2012). AD is currently divided in two main forms, first familial AD, often affecting individuals younger than 65 years of age, and second, sporadic AD, in older individuals. The prevalence of AD varies immensely depending on factors such as age, genetics, level of education, co-morbidities and so on. Currently, AD can only be definitively diagnosed *post-mortem*, at autopsy. However, current research has been focusing on biomarkers of AD that reflect *in vivo* the brain pathophysiological changes causing the disease.

1.2 History of Alzheimer's Disease

In 1906, a German neurologist and psychiatrist, Dr. Alois Alzheimer, discovered the first case of what is currently known as Alzheimer's Disease. Dr. Alzheimer followed a female patient, 50-year-old Auguste D., from 1901 until her death in 1906. He documented the symptomatology and the progression of her symptoms during her illness in an extremely precise manner. She was first brought to the hospital after her husband noticed an untreatable paranoia, sleep disorders, as well as memory and behavioral disturbances (Hippius & Neundörfer, 2003). As the symptoms quickly deteriorated, August D. remained an inpatient in the hospital up to her death. Dr.

Alzheimer was then able to investigate her brain, both morphologically and histologically. He was first able to observe (1) a widespread atrophy of the brain, (2) miliary foci after silver staining, now called amyloid-β plaques and (3) bundles of fibrils, now called neurofibrillary tangles (NFT) (Schachter & Davis, 2000). He observed similar findings in three additional cases, leading to his hypothesis that there is a correlation between the symptoms to the brain changes detected. Dr. Alzheimer's pioneering work, linking clinical symptoms with neuropathological abnormalities, marked the beginning of AD research and provided the fundamental framework for the current models of disease progression (Cipriani et al., 2011; Jack & Holtzman, 2013).

It is in 1908 that Dr. Alzheimer and his collaborator Dr. Emil Kraepelin proposed to call this "peculiar illness of the cerebral cortex": Alzheimer's. Since then, the term "Alzheimer's disease" has developed various meanings, with the goal to better define the disease. Indeed, there are three main categories that are not always in agreement: a clinical entity, a post-mortem pathological entity and finally an *in vivo* pathological model.

In 1984, the first operating criteria for AD dementia was proposed by the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) (McKhann et al., 1984). Other groups have tried to update the criteria proposed, such as the Diagnostic and Statistical Manual of Mental Disorders (DSM) (American Psychiatric Association, 2000), the International Working Group (IWG) (Dubois et al., 2007, 2014), and the National Institute of Aging and the Alzheimer's Association (NIA-AA) (McKhann et al., 2011). With advances in the medical field, definitions have been incorporating novel methods to assess changes in the brain. For example, in 2014 the IWG defined AD as dementia, with the presence of abnormal amyloid-β plaques in the brain. At the time, research had not discovered a method to assess tau *in vivo*, yet, causing it to be left out. Moreover,

as the population ages, various forms of dementia and specifically AD dementia have been appearing. It is important to note that AD can be different from one individual to another, meaning it is critical to include various criteria to define the disease. IWG and DSM were important in incorporating the idea of "atypical AD", which brought challenges in terms of clinical diagnosis. Even though AD remained a clinical entity, markers of amyloid-β plaques were used to ascertain the diagnosis.

Since then, the definition of AD dementia has been updated, and incorporates *in vivo* biomarkers of tau in the diagnosis (McKhann et al., 2011).

1.3 The clinical spectrum of Alzheimer's disease

Dementia is currently defined as the progressive loss of cognitive abilities, that is severe enough to interfere with individual autonomy and normal social functioning. Even though each type of dementia has its own cause and symptoms, they all result from brain cell damage. It is important to note that dementia is a broad term for the decline of cognitive abilities, such as thinking difficulties and memory loss. AD is a disease that affects the brain and causes dementia. It is currently known as the main form of dementia, and is due to the build-up of amyloid-β plaques and NFT, causing symptoms at specific stages of disease progression. It is critical for physicians to assess which disease stage the patient is at, to predict future symptoms and possible courses of treatment. However, each AD case presents a unique set of symptoms, often with varying severity.

As disease progresses, individuals are said to be on the spectrum of the disease, with cognitive and pathophysiological changes being intrinsically connected.

Mild cognitive impairment (MCI) is a term used to describe cognitive decline as compared to age norms, which interfere with activities of daily living but not sufficiently to affect individual

autonomy (Gauthier et al., 2006). Individuals present difficulty in retaining new information, misplacing belongings, as well as some difficulty with problem solving and decision-making. Family and friends are often the first ones to pick up on those changes, that might be also accompanied by personality changes, where a person often chooses to withdraw socially and has difficulty expressing their thoughts. However, MCIs are still able to live independently. It is important to note that MCI is a heterogeneous stage, which does not definitely mean the person is going to progress to AD. Recent research incorporated neuroimaging and cerebrospinal fluid measures to define MCI individuals that are likely on the AD pathway, now termed "MCI due to AD" (Albert et al., 2011). Those individuals present abnormal markers of amyloid-β plaques and NFT accumulation.

The AD dementia stage however is expressed by a highly impacted cognition and physical ability. At this stage, individuals are often unable to communicate, they start relying on others for personal care, and they are not able to function physically. Since the 1980s, the main criterion for AD is memory decline, in association with the deterioration of at least one of the other cognitive functions in absence of other disorders that could justify these said cognitive deficits. The latter, in conjunction with pathological evidence from brain autopsy established a definite diagnosis of AD dementia (McKhann et al., 1984). More recent definition pushes for the recognition of nonamnestic presentations of AD, as well as the importance of defining activities of daily living impairment for the diagnosis of dementia (McKhann et al., 2011). Additionally, the uprising of biomarkers has led to the idea that AD should probably be defined as cognitive deficits, and also abnormality to at least one AD biomarker (i.e. brain atrophy, amyloid-β and/or tau) (Dubois et al., 2007, 2014).

Preclinical AD is a concept that emerged after histopathological observations usually found in the brain of AD patients were done in cognitively unimpaired individuals (Hubbard et al., 1990). However, as present research focuses on *in vivo* biomarkers of the disease, the concept evolved, with specific classifications proposed based on the presence or absence of AD biomarkers in living cognitively unimpaired individuals. The last few years have brought the idea that AD is a developing disease, starting with accumulation of amyloid-β plaques, leading to NFT. Preclinical stages of AD where then considered as cognitively unimpaired with the presence of amyloid-β pathology, with the presence of amyloid-β and neurodegeneration pathologies, and finally, as the presence of amyloid-β, neurodegeneration subtle cognitive decline (Sperling et al., 2011). It was only in 2016 that preclinical AD required the presence of amyloid-β and tau pathologies, however, without the need for neurodegeneration (Dubois et al., 2016).

1.4 Hallmarks and staging of Alzheimer's disease

As mentioned above, AD is characterized by the accumulation of amyloid-β plaques and neurofibrillary tangles, as well as neurodegeneration. Over the last few years, it has been discovered that amyloid-β plaques is the first event happening in the brain, and is necessary but not sufficient to cause AD dementia. Later on, the brain displays accumulation of neurofibrillary tangles, which are necessary to diagnose AD (Hardy & Allsop, 1991; Jack & Holtzman, 2013). Finally, neurodegeneration is a non-AD specific pathology, as it can be seen in various types of brain disorders. However, the topography of the atrophy is specific to the disease (Frisoni et al., 2007).

1.4.1 Amyloid-β plaques

Cleavage of the amyloid precursor protein (APP) by a β- and γ-secretase causes extracellular deposits of amyloid-β aggregates named amyloid-β plaques (Chow et al., 2010). APP has been linked to the regulation of neuronal structure and synaptic function in the healthy brain (Tyan et al., 2012). The APP protein becomes toxic when it is cleaved either in 40 (amyloid-$\beta_{1\text{-}40}$) or 42 (amyloid-$\beta_{1\text{-}42}$) (Selkoe, 1998; Zhang et al., 2011); even though they are close, cleavage at amyloid-$\beta_{1\text{-}42}$ has been shown to cause greater aggregation, thus, neurotoxicity (Nguyen et al., 2016).

Post-mortem studies allowed us to discover a hierarchical accumulation of amyloid-β plaques in the brain. The first studies were conducted by Braak and Braak, where they observed that amyloid-β plaques first accumulated in the basal neocortex (stage A), to then spread to all neocortical regions as well as the hippocampus (stage B), to finally reach the sensorimotor cortex (stage C) (Braak & Braak, 1991). Figure 1 depicts the stage observed by Braak and Braak. Following up on their work, a student of Braak proposed another staging, comprised of five phases. Phase 1 affected only the neocortex, phase 2 the entorhinal and hippocampal cortices, phase 3 the diencephalon nuclei and striatum, phase 4 the brainstem nuclei and finally, at phase 5, the cerebellum (Thal et al., 2002). Figure 2 represents the phases of amyloid-β accumulation as described by Thal *et al*.

Importantly, amyloid-β plaques were observed in individuals exhibiting no cognitive impairment. Participants up to stage 5 were not presenting any sign of cognitive decline (Thal et al., 2002; Vlassenko et al., 2011). This has been interpreted as the fact that the presence of amyloid-β plaques is not sufficient to cause cognitive deficits.

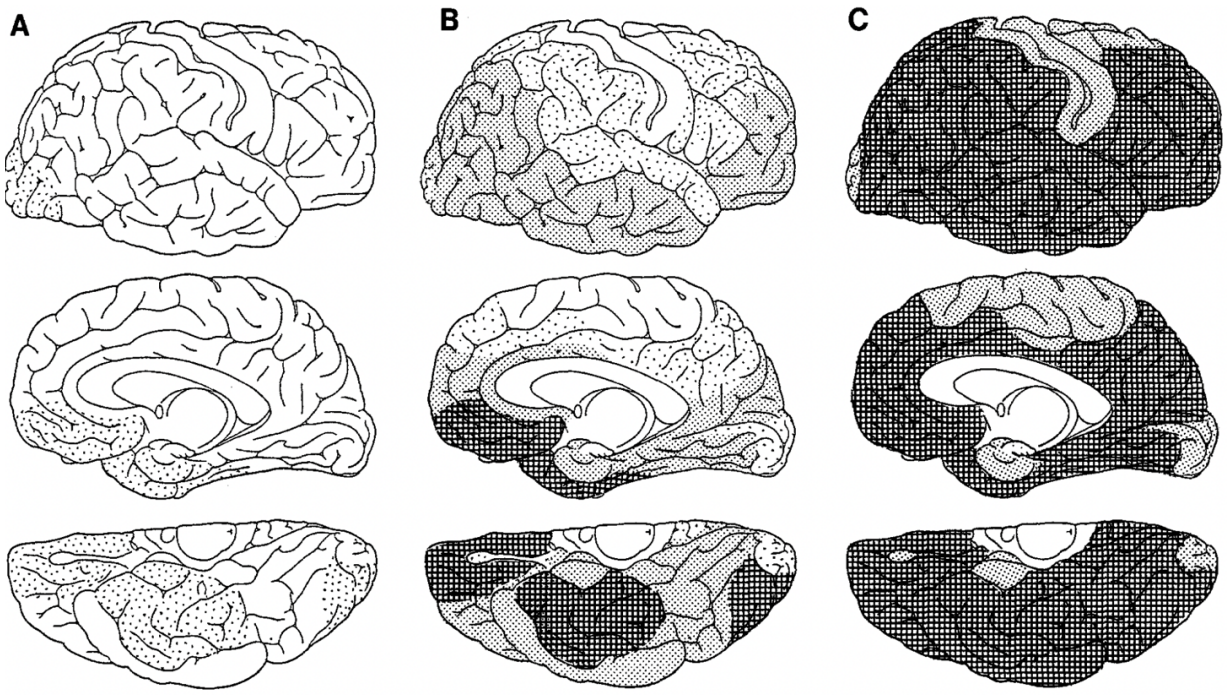

Figure 1: Amyloid-β deposition observed by Braak and Braak (from Braak & Braak, 1991).

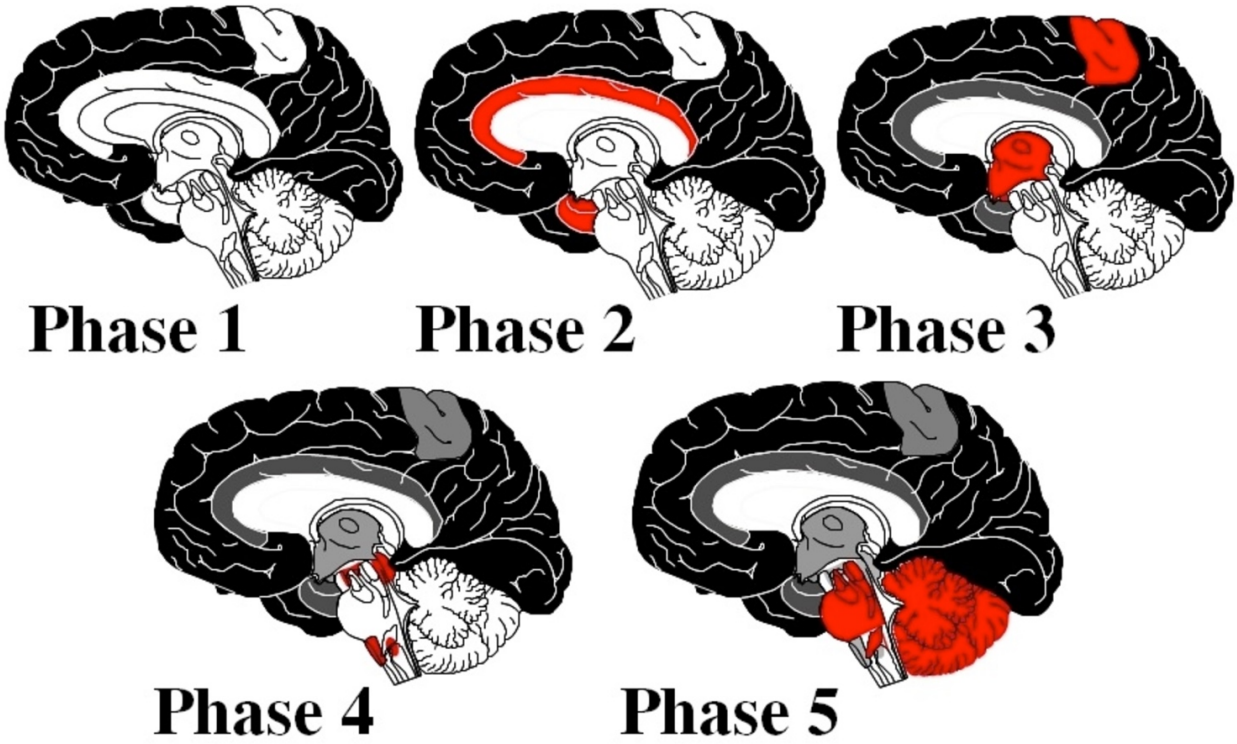

Figure 2: Amyloid-β deposition observed by Thal et al (from Thal et al, 2002).

There is a strong debate in the AD research field in regard to the amyloid-β cascade hypothesis. Based on Hardy *et al*, the cascade hypothesis states that the cleavage of the APP protein (through two pathways) causes amyloid-β aggregates. In turn, amyloid-β plaques trigger the accumulation of NFT in the brain, then initiating cell death (John & Gerald, 1992). Since then, neuroscientists have been trying to model AD, with a high majority supporting this cascade hypothesis (Dubois et al., 2016; Ising et al., 2015; Jack et al., 2016, 2019; Jack & Holtzman, 2013; Long & Holtzman, 2019; McKhann et al., 2011).

On the contrary, another school of thought have been rejecting the amyloid-β cascade hypothesis (Herrup, 2015). This idea is first based on the fact that if an individual presents amyloid-β plaques, they have a higher chance of developing AD. However, it is a slow process and individuals will not certainly be diagnosed with the disease later on (Villemagne et al., 2011). Moreover, NFT accumulation is more closely linked with neurodegeneration (Villemagne et al., 2013), thought to be the main cause of symptom onset. Finally, abnormal tau accumulation can also be observed in other neurodegenerative conditions (V. M. Y. Lee et al., 2001), meaning there are various pathways from which NFT emerge.

Nevertheless, it is important to note that the amyloid cascade hypothesis is often wrongly defined. It does not suggest that amyloid-β plaques are sufficient to cause AD, but is merely based on evidence that cerebral amyloid-β accumulation is the first step towards AD dementia (Hardy & Selkoe, 2002; Long & Holtzman, 2019).

1.4.2 Neurofibrillary tangles

The microtubule-associated protein tau (MAPT) is an essential protein for the for the stabilization of microtubule structure (Witman et al., 1976). The MAPT gene is located on

chromosome 17 and is comprised of 16 exons (Guo et al., 2017). Splicing variants of the gene can be found in the whole body. Six different isoforms can be observed in the brain, that present either three (3R) or four (4R) microtubule-binding domains (Kosik et al., 1989). In the normal and healthy brain, the tau protein is regulated through posttranslational modifications, such as phosphorylation, acetylation and glycolysation (V. M. Y. Lee et al., 1991; Strang et al., 2019). However, in AD, 3R and 4R isoforms undergo hyperphosphorylation, leading to formation of insoluble intracellular aggregates, called paired helical fragments (Ballatore et al., 2007; Vingtdeux et al., 2012). Those paired helical fragments are actually the main constituents of what are named neurofibrillary tangles (Wischik et al., 1988). The accumulation of the toxic intracellular aggregates, together with the loss of soluble tau that is capable of stabilizing microtubules, supposedly leads to abnormal cellular function and neurodegeneration (Iba et al., 2013; V. M. Y. Lee et al., 2001, 2011).

Abnormal tau misfolding, aggregation and accumulation has been linked to multiple diseases: "tauopathies". These include then AD, frontotemporal dementia, progressive supranuclear palsy, among others (Iqbal et al., 2005; V. M. Y. Lee et al., 2001). Genome studies discovered the presence of multiple tau gene mutations in patients suffering from frontotemporal dementia, proving certain MAPT mutations caused abnormal tau, and neurodegenerative disease (Ghetti et al., 2015). These tauopathies are often observed with other pathological abnormalities, such as amyloid-β plaques, α-synuclein or huntingtin (Blum et al., 2015; Jensen et al., 1999). The topographical distribution of tau, together with the second pathological abnormality observed, usually leads to distinct symptomatology and thus, distinct neurodegenerative condition.

In the case of AD, also associated with amyloid-β plaques, the close link between the NFT load and cognitive decline as well we as the presence of neuropsychiatric symptoms has already been

proven (Gomez-Isla et al., 1997; Nelson et al., 2009; Terry et al., 1981; Tissot et al., 2021). Even though the disease is linked to both pathologies, recent research has shown the cognitive decline is mainly due to the accumulation of NFT rather than amyloid-β (Ossenkoppele et al., 2019).

Early on, Braak and Braak observed a hierarchical pattern of NFT accumulation that spans for decades. The extent of NFT is based on the presence, distribution and frequency of NFTs. Braak and Braak divided the system in six stages: stage I/II is thought to affect the transentorhinal cortex, Braak III/IV the limbic regions, and finally, Braak stage V/VI more broadly affects the brain, such as the isocortex and neocortex (Braak & Braak, 1991). More precise analyses were further done separating better the six stages (Braak et al., 2006) (Figure 3). Additionally, there is a Consortium to Establish a Registry for AD (CERAD) scoring also in place: it classifies AD neuropathology in four stages, from negligible to high level (Mirra et al., 1991) (Figure 4). Even though Braak and CERAD staging systems both take into account the localization of NFTs, CERAD focuses more on the density as compared to Braak's.

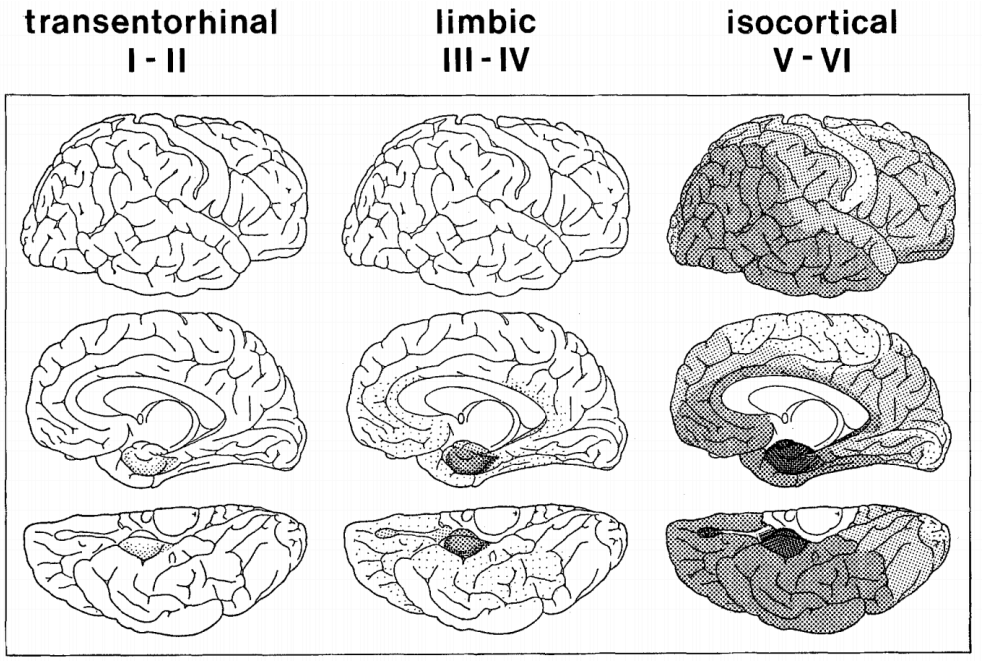

Figure 3: Neurofibrillary tangle deposition observed by Braak and Braak (from Braak & Braak, 1991).

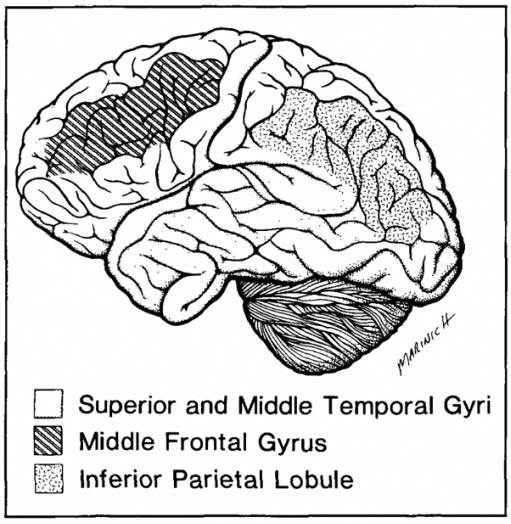

Figure 1. This diagram of the lateral surface of the brain illustrates the areas of neocortex from which recommended neocortical sections are taken.

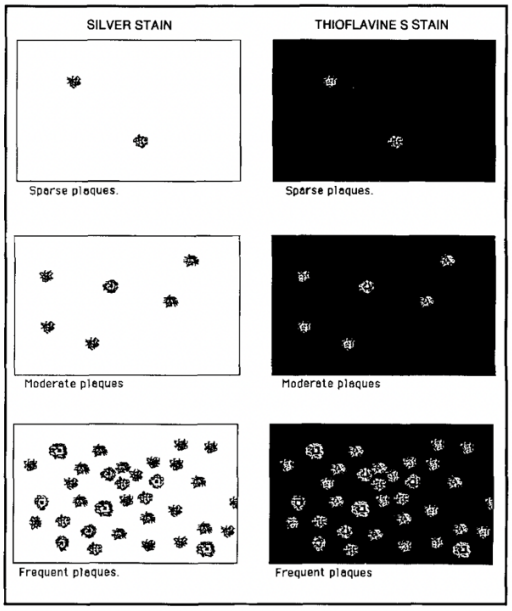

Figure 2. Senile plaques (neuritic) per 100× microscopic field. This cartoon provides a guide to semiquantitative assessment of plaque density per square millimeter.

Figure 4: Neurofibrillary tangle accumulation based on the CERAD staging system (from Mirra et al, 1991).

1.4.3 Neurodegeneration

Neurodegeneration is defined as the pathophysiological processes leading to the loss of grey and white matter, altering the neuronal structure and function (Selkoe, 1991). However, neurodegeneration is not specific to AD, as it has been observed in virtually all brain conditions, as well as in healthy and normal aging (Thal et al., 2004; Wyss-Coray, 2016). Following the accumulation of other AD pathophysiological hallmarks (i.e. amyloid-β plaques and NFT), neurodegeneration is thought to start in the medial temporal, to then affect allocortical and neocortical regions (Crews & Masliah, 2010; Hubbard & Anderson, 1985; Jack et al., 2017). Nevertheless, a system system of neurodegeneration along the course of the AD brain has never been produced.

1.4.4 Other processes involved in Alzheimer's disease

Even though AD is characterized by the three aforementioned pathologies (i.e. amyloid-β, NFT and neurodegeneration), it is thought that other processes are involved in the development of AD. First of all, neuroinflammation has been associated with AD (Heneka et al., 2015). The main discussion revolves around the fact that neuroinflammation could be protective or deleterious, by clearing up the brain (C. Y. D. Lee & Landreth, 2010). Over the last few years, neuropathological studies have revealed the presence of reactive microglia in the AD brain (Akiyama et al., 2000; Holmes, 2013). This is translated as an inflammatory state, which has in turn been associated with amyloid-β plaques and NFT (Bamberger et al., 2003). Our group has recently evidenced through a longitudinal study that microglia activation and tau propagate together along the Braak stages (Pascoal, Benedet, Ashton, et al., 2021).

There is currently a debate in the research field about a possible double peak of neuroinflammation in the course of AD progression. It is believed that the initial peak occurs prior to the development of amyloid-β and tau pathologies, after which inflammation coincides with them until it eventually culminates in a second peak during the later stages of the disease (Fan et al., 2017).

Another process thought to have a link with AD is cerebrovascular disease. Indeed, these two illnesses share common risk factors, such as diabetes mellitus, atrial fibrillation, hypertension, obesity and age (Baglietto-Vargas et al., 2016; Chuang et al., 2016). Studies reported that the presence of vascular risk factors predicted the development of AD, or the conversion from MCI to AD dementia (Blom et al., 2013; Love & Miners, 2016). However, there are possible cofounders when assessing the relationship between cerebrovascular and AD pathologies: the timing of shared risk factors is similar, ischaemic cerebrovascular diseases can lower the threshold for clinical manifestation of AD pathologies, and individuals with history of hypertension or diabetes are often

misdiagnosed as vascular dementia, underestimating the contribution of AD (de Bruijn & Ikram, 2014; Schneider et al., 2004).

Although the links between neuroinflammation, cerebrovascular diseases and AD pathologies are not known, it is critical to keep them in mind when studying the AD continuum.

1.4.5 Risk factors for Alzheimer's disease

The greatest risk factor AD is age, with most cases seen in adults 65 years of age and older. There is 5% of individuals between the ages of 65 and 74 that are diagnosed with AD, as compared to 50% of adults older than 85.

Comorbidities is another strong risk factor in the development of AD. As mentioned previously, cerebrovascular problems have been associated with an increase in amyloid-β plaques and tau tangles, and progression to dementia (Blom et al., 2013; Love & Miners, 2016). Moreover, diabetes, which is due to insulin dysregulation in the body, might lead to an increase in blood levels in the brain, causing harm (Crane et al., 2013).

Genetics are also known to play a significant role in the development of AD pathology. Familial AD is caused by mutations in chromosomes 1, 14 and 21; when one chromosome mutation is inherited, the person will most likely develop AD in their life (Janssen et al., 2003). However, these cases only accounts for less than 10% of AD patients.

Sporadic AD does not seem to be linked to a genetic pattern of inheritance; however, there is a strong correlation with the Apolipoprotein E (ApoE) gene. It carries cholesterol in the blood, and is thus critical for the development, maintenance and repair of the central nervous system (Chernick et al., 2019; Husain et al., 2021). There are three version of the ApoE allele: ε2, ε3, and ε4. The ε2 allele is a protective factor against AD pathology (Mahley & Rall, 2000), as compared

to ε4 being a risk factor (Strittmatter et al., 1993). As individuals carry two alleles for each gene, risk factor was even further increased when an individual carries two ε4 (Corder et al., 1993).

Other lifestyle factors have been associated with higher risk of developing AD, such as low years of education. It is hypothesized that high education levels leads to the formation of synaptic connections in the brain, creating a "synaptic reserve", enabling individuals to compensate for neuronal loss as the disease progresses (Katzman, 1993). Air pollution has been also associated with an increased risk of developing dementia (Moulton & Yang, 2012), and more recently with higher AD pathological load in cognitively impaired individuals (Iaccarino et al., 2021).

It is important to keep in mind that AD is a multifactorial disease, in part explaining the difficulty for researchers to have a definite diagnosis of AD dementia. Throughout this chapter, we have seen evidence on the contribution of various pathological mechanisms in AD onset and progression, showing the pathological complexity and multifaceted toxicity of AD.

1.5 Assessments methods of Alzheimer's disease

1.5.1 Imaging methods

Magnetic resonance imaging (MRI) was first introduced in 1977, creating two or three-dimensional images of the body. MRI imaging is composed of a large magnetic field, and weaker ones. The hydrogen atoms composing the human body alter the magnetic field. While some atoms point towards the patient's head or feet, cancelling each other out, other ones aren't. The MRI machine emits a radio frequency pulse that is specific to hydrogen atoms, causing protons to spin in different directions. Energy is then released when the spinning ceases, which is interpreted by

the MRI scanner. The different tissues in the body react distinctively, leading to an image with shades of grey (Berger, 2002). Images of the brain are thus feasible using an MRI machine.

Positron Emission Tomography (PET) in another type of image obtained through the injection of a radiotracer. The radiotracer is composed of a radioactive part, that is bound to a naturally occurring chemical. Participants are injected, and the radiotracer travels around the body. The compound then binds to a specific target, and when the compound is metabolized, it emits protons. The energy from the protons is then captured by the PET machine, from which an image is obtained. This image reflects the metabolic or biochemical function of the tissues and organs. The first PET tracer was targeting glucose in order to assess the body metabolism. Over the years, radiotracers targeting other proteins have been created.

1.5.2 Biofluid assessments

The most direct measure of brain changes through biofluid assessment is through the cerebrospinal fluid (CSF), which is the clear liquid in which the brain and spinal cord bathe. CSF can be obtained by performing a lumbar puncture. It is critical to provide nourishments, for waste removal and to protect the brain (Spector et al., 2015). It is predominantly secreted from the choroid plexus, and it completely renewed every 5 to 6 hours (Damkier et al., 2013). In brain diseases, the CSF is thought to be more slowly renewed, probably leading to accumulation of toxins in the brain (Sakka et al., 2011). Because the composition of CSF is regulated, abnormal levels of certain proteins can be used for diagnostics (Simon & Iliff, 2016).

Blood tests are also able to reflect changes happening in the body. More specifically, plasma, which is the liquid portion of the blood that remains after red and white blood cells, and platelets are removed. Protein levels can be assessed via basic laboratory work, with current

research trying to focus on more detailed assays for distinct diseases. In the development of a neurodegenerative conditions, the blood-brain barrier, which usually protects the brain from the peripherical system, starts leaking (Zlokovic, 2008). This causes an exchange in products from the brain and the blood, thus, brain proteins can also be assessed in the plasma (Lewczuk et al., 2018).

1.6 Markers of amyloid-β pathology

The most commonly used marker of amyloid-β plaques are CSF levels of amyloid-β. Indeed, early on, researchers observed a decrease in amyloid-β levels in the CSF of AD patients, as the brain accumulation of amyloid-β leads to a decrease in available CSF (Fagan et al., 2006; Rosenmann, 2012; Strozyk et al., 2003). More specifically, amyloid-β 42 peptide is thought to represent the pathology, as compared to amyloid-β 40 peptide. It is currently thought that the ratio between the two peptides gives the most appropriate proxy for amyloid-β load in the brain (Iwatsubo et al., 1994). Lower levels have been detected at early stages of AD, when individuals are not even presenting cognitive decline yet (Racine et al., 2016).

Additionally, PET imaging has allowed to follow the topographical distribution of amyloid-β plaques along the course of AD dementia. The first high-affinity tracer produced for amyloid-β plaques is called [^{11}C]Pittsburgh compound B ([^{11}C]PiB) and shows an increase in cortical uptake in AD participants, as compared to cognitively unimpaired individuals. This corroborates research conducted on post-mortem brain tissues (Ikonomovic et al., 2008; Klunk et al., 2004). The radioactivity of a [^{11}C] leads to a half-life of around 20 minutes, which prevents the chemical to be sent out to other facilities that do not have a radiochemistry facility. However, [^{18}F] have a half-life of 110 minutes, allowing for the product to be produced somewhere and travel to another facility. Other tracers have thus been created, such as [^{18}F]florbetapir, [^{18}F]flobetaben and

[^{18}F]AZD4694 (Cselényi et al., 2012; Newberg et al., 2012; Vandenberghe et al., 2010), and have shown results comparable to [^{11}C]PiB.

Both CSF and PET imaging are now commonly used to assess amyloid-β changes in the brain. However, few studies are reported an inverse correlation between CSF amyloid-β 42 levels and dementia severity (Csernansky et al., 2002), most finding little to no association (Ivanoiu & Sindic, 2005; Mehta et al., 2001). CSF is still critical to assess whether someone is on the AD spectrum, as amyloid-β plaques start accumulating years before the onset of dementia, similar to PET imaging, able to capture pathology accumulation in preclinical stages (Rosenmann, 2012; Vlassenko et al., 2011, 2012) (Figure 5). Interestingly, this supports this idea that amyloid-β accumulation is one of the early stages of disease progression, and amyloid-β alone is not sufficient to cause dementia (Braak & Braak, 1991).

The most critical problem with CSF and PET is that these are costly procedures, and cannot be easily implemented in the clinical care and clinical trial settings. With the development of novel methods for assays, researchers have been focusing on blood-biomarkers of amyloid-β. However, this has yielded heterogeneous results. Candidate biomarkers in the blood were amyloid-β 42 and 40 peptides, which at first were not presenting the expected decrease (Olsson et al., 2016). As assays become more sensitive, a few novel ones have begun to depict changes (de Wolf et al., 2020; Janelidze et al., 2016). Plasma amyloid-β markers are even thought to be able to predict dementia onset (Koyama et al., 2012).

Assays targeting phosphorylated tau (pTau) in the plasma and CSF, which will be discussed after, have been created in the last few years; recent research from our group and others have postulated that pTau markers are more closely associated with amyloid-β rather than tau itself (Mattsson-Carlgren et al., 2021; Therriault, Vermeiren, et al., 2022). Another recent manuscript from our

group presented glial fibrillary acidic protein (GFAP) as a possible marker of amyloid-β (Benedet et al., 2021). Further research is however needed to assess whether current biofluid GFAP or pTau markers, especially in the plasma, could replace amyloid-β markers.

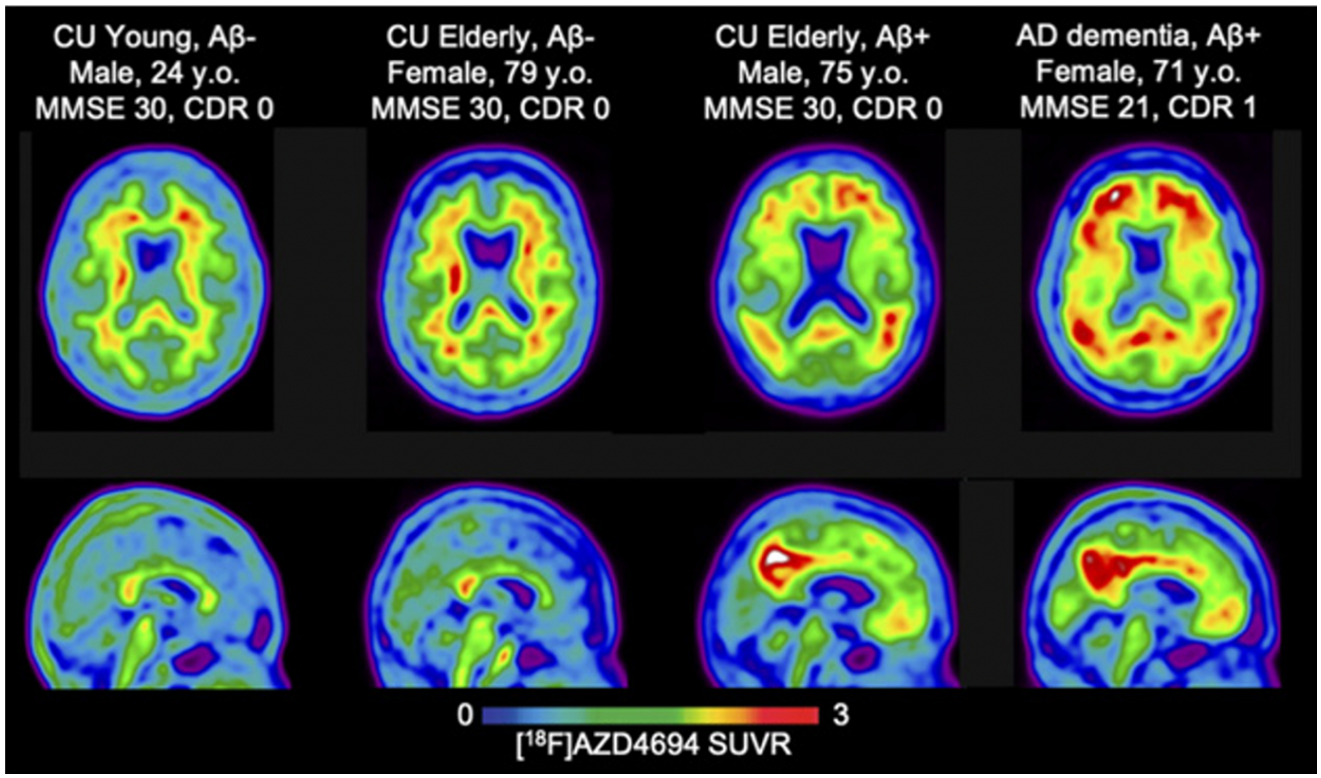

Figure 5: Amyloid-β PET imaging along the aging and Alzheimer's spectrum (from Therriault et al, 2020).

1.7 Markers of neurofibrillary tangle pathology

Neurofibrillary tangles are composed of abnormally phosphorylated tau (i.e. hyperphosphorylated) (Grundke-Iqbal et al., 1986). CSF markers of neurofibrillary tangles have thus focused on total-tau (tTau) and pTau, with assays targeting various epitopes (Rosenmann, 2012). Sites are thought to be phosphorylated in a successive manner, each representing a stage of disease progression (Jack & Holtzman, 2013). The most commonly researched ones are pTau at threonine 181 and 231, and more recently 217 (Hanes et al., 2020; Kohnken et al., 2000; Vanmechelen et al., 2000). There is strong evidence on the association between increased level of

tTau and pTau and cognitive decline, as well as progression to dementia (Ivanoiu & Sindic, 2005; Lin et al., 2009; Tato et al., 1995). At the late stages of the disease, concentrations of pTau and tTau are thought to plateau (Jack et al., 2016).

Similar to amyloid-β pathology, researchers also focused on PET imaging to assess the topographical distribution of neurofibrillary tangles. [^{18}F]AV1451, [^{18}F]THK5117, [^{18}F]THK5317, [^{18}F]THK5351, and [^{11}C]PBB3, grouped together as "first-generation tau-PET tracers", were created and depicted an increase in tracer uptake in the MCI and AD dementia brains *in vivo* (Betthauser et al., 2017, 2019; Chien et al., 2013; Hashimoto et al., 2014). Tau-PET tracers even follow the hierarchical pattern of tau accumulation as assessed by Braak and Braak (Braak & Braak, 1991; Schwarz et al., 2016). Since then, those first-generation tracers have shown critical

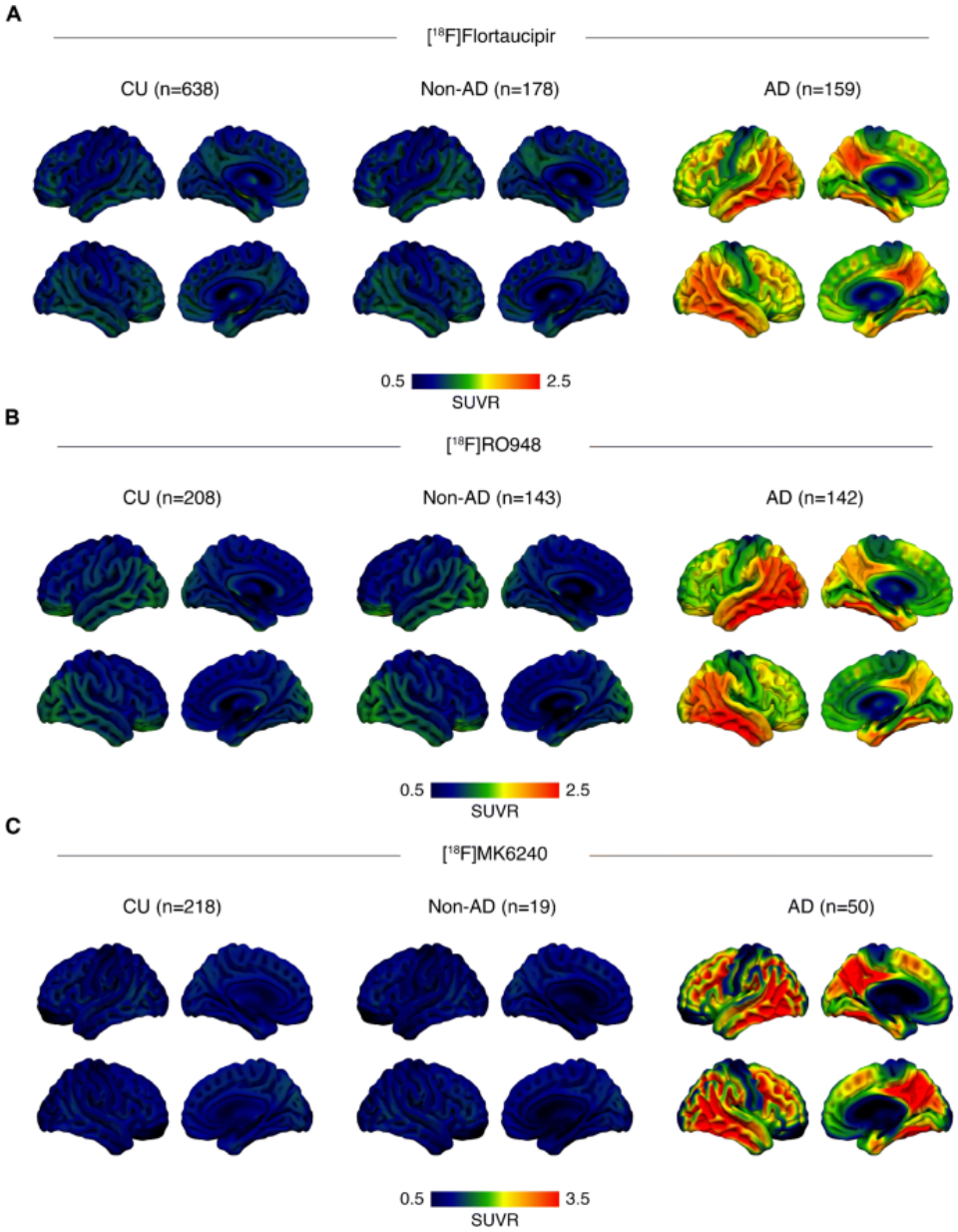

Figure 6: Different tau-PET tracers along the aging and Alzheimer's spectrum (from Leuzy et al, 2021).

disadvantages. Different processes have been linked to changes in tracer uptake: monoamine oxidase (MAO) A for [^{11}C]PBB3, MAO-B for [^{18}F]THK5351 and α-synuclein for [^{18}F]AV1451 (Koga et al., 2017; Ng et al., 2017).

Second-generation tau PET tracers were created taking into account the above-mentioned drawbacks: [^{18}F]MK6240, [^{18}F]RO6958948 and [^{18}F]PI2620 (Kuwabara et al., 2018; Leuzy et al., 2019; Pascoal et al., 2018; Villemagne et al., 2018). They present higher affinity to neurofibrillary tangles as compared to the first-generation ones (Figure 6). [^{18}F]MK6240 seems to be the most sensitive one, presenting the hierarchical Braak staging, and predicting further NFT accumulation along the Braak stages (Pascoal et al., 2020; Pascoal, Benedet, Tudorascu, et al., 2021), with minimal impact from off-target and age-related retention (Tissot, Servaes, et al., 2022) (Figure7). Importantly, PET imaging allowed researchers to link the distribution of pathology across the AD brain with different clinical presentations and neurodegeneration (Ossenkoppele et al., 2016). It also further corroborated the amyloid-β cascade hypothesis, as NFT pathology is almost exclusively observed in individuals with significant amyloid-β burden (Long & Holtzman, 2019).

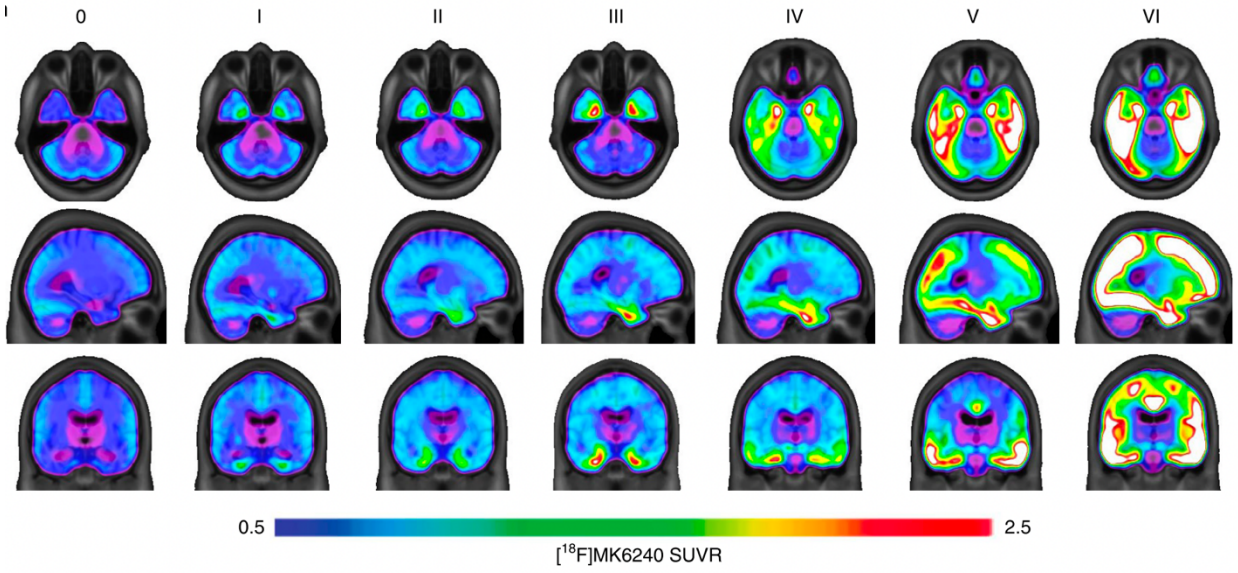

Figure 7: [18F]MK6240 allows to follow Braak staging in Alzheimer's disease (from Therriault et al, 2022).

Over the last few years, a huge part of the research field focused on the assessment of tau in the plasma. Similarly as CSF, plasma pTau 181, 217, and 231 have been the main focus (Barthélemy, Horie, et al., 2020; Mattsson et al., 2016; Zetterberg et al., 2013). Assays created on the Simoa have been proven to be highly specific to AD, sensitive to early changes and follow the AD pathophysiological processes closely (Ashton et al., 2021; Karikari, Pascoal, et al., 2020; Therriault, Pascoal, et al., 2022). The research was previously done on cohort with individuals willing to participate in research. A new window is now opening towards the use of plasma markers in the medical field, more specifically in the diagnostic and clinical trial settings (Hansson, 2021; Parchi et al., 2022). However, as mentioned previously, plasma pTau assays are thought to be more closely linked to amyloid-β rather than tau. Future assays need to focus on the development of assays targeting tau, and tau only.

1.8 Markers of neurodegeneration

Assessment of neurodegeneration in the CSF is done by measuring CSF tTau (Blennow & Zetterberg, 2015; Jack et al., 2016). Indeed, AD brain has shown to have an increase in CSF tTau levels. It is thought that CSF tTau can capture middle to late stages of disease progression (Hampel & Blennow, 2004).

Nevertheless, research has been using MRI to as a proxy of neurodegeneration for years. There is indeed a strong correlation between post-mortem neuronal counts and brain volume measured with MRI (Shimizu et al., 2018). Even though no specific hierarchical staging has been observed in neurodegeneration along the AD spectrum, the first region seemingly impacted in the disease is close to the temporal regions of the brain (Rossor et al., 2002).

Additionally, researchers used glucose metabolism as a proxy of neuronal function. PET imaging done with fluorodeoxyglucose ([^{18}F]FDG) studies showed an age-related hypometabolism, but also AD-related hypometabolism in the brain's default mode network. Indeed, a decrease in [^{18}F]FDG signal was associated with a higher risk of developing dementia later on (Gray et al., 2012; Landau et al., 2012; Therriault et al., 2018; Weissberger et al., 2017). [^{18}F]FDG-PET is currently being used for the clinical diagnosis of AD (Jagust et al., 2007).

As mentioned previously, lumbar punctures, PET and MRI are costly and invasive. The plasma markers researchers focused on are plasma tTau and neurofilament light chain (NfL) (de Wolf et al., 2020; Mattsson et al., 2016). NfL are neuronal cytoplasmic proteins that have been associated with neuronal injury and AD. Plasma tTau has not been showing precise results, as compared to plasma NfL, today considered the best proxy of neuronal injury. Longitudinal studies proved that NfL can be used to predict future neurodegeneration in cognitively unimpaired and impaired individuals, years before (Benedet et al., 2019).

It is important to note that the markers of neurodegeneration discussed here are not AD-specific, but rather tell information about the amount of neuronal injury in the brain. Without visual assessment through MRI or PET, it is impossible to know the disease the person is suffering from. By using disease-specific biofluid markers however, it would be possible to assess the extent of neurodegeneration, and ascertain the diagnosis.

1.9 The modeling of Alzheimer's disease

1.9.1 The use of biomarkers for the progression of Alzheimer's disease

The amyloid-β cascade hypothesis, discussed above, led to Jack and colleagues proposing a model for AD progression using current biomarkers (Jack & Holtzman, 2013). It is thought that

amyloid-β accumulation triggers downstream pathological changes, such as neurofibrillary tangle formation and neurodegeneration, in turn causing cognitive decline. Patients at late stages of the disease do not seem to accumulate amyloid-β plaques anymore, suggesting amyloid-β build-up reaches a plateau. Subsequent disease progression is defined by the severity of tau and neurodegeneration pathologies.

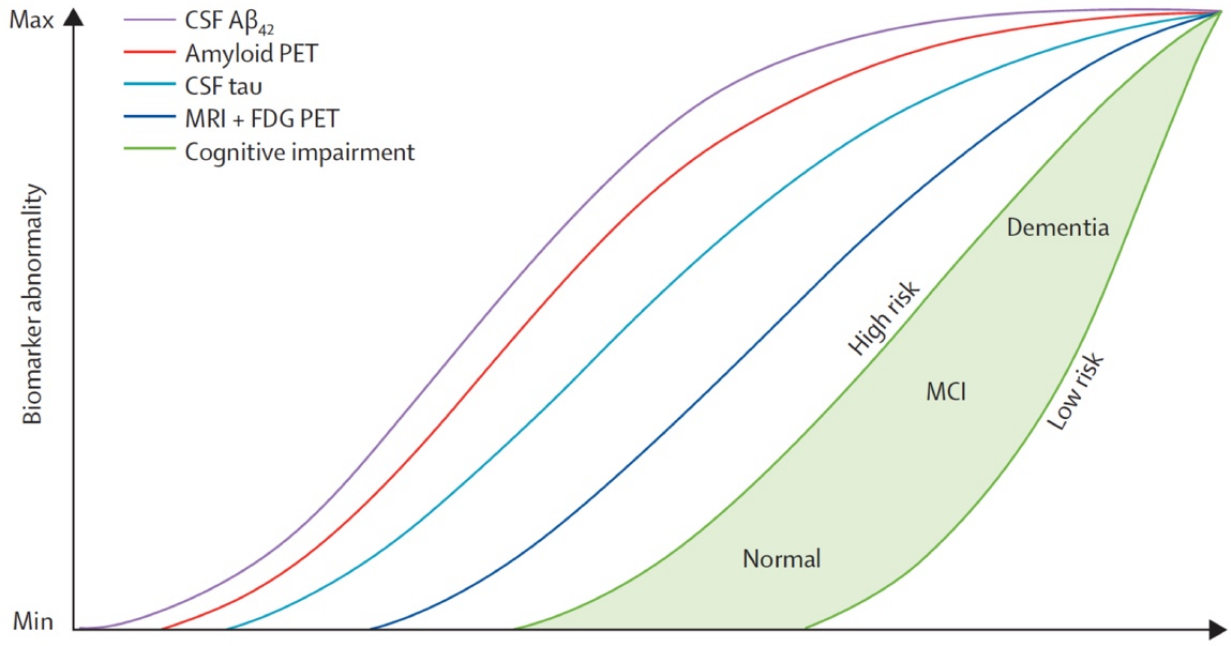

Figure 8: Biomarker modeling of Alzheimer's disease (by Jack et al, 2013).

1.9.2 A/T/(N) framework

As mentioned throughout the introduction, AD is characterized by amyloid-β plaques and neurofibrillary tangles that cause brain degeneration. The clinical diagnosis in living individuals is increasingly supported by various validated biomarkers that are proxies of the AD neuropathological changes. In 2018, the National Institute on Aging and Alzheimer's Association (NIA-AA) established, together, a research framework (Jack et al., 2018). Their goal was to create a biological construct to identify AD *in vivo*, using available biomarkers. In what they named the

A/T/(N) system, there are three groups of biomarkers that are recognized according to the neuropathological processes each one measures. A represents biomarkers of amyloid-β pathology, so as mentioned above, CSF amyloid-β 42 (or amyloid-$\beta_{42/40}$ ratio) and amyloid-β-PET. T represents biomarkers of neurofibrillary tangles or tau, such as CSF pTau181 and tau-PET; a growing body of literature has also been adding other phosphorylation sites as viable markers. Finally, (N) reflects neuronal injury or neurodegeneration, via the assessment of CSF tTau, MRI or [^{18}F]FDG-PET.

Because amyloid-β pathology alone does not cause cognitive decline and neurodegeneration (Vlassenko et al., 2011, 2012), it is thought that A is the earliest detectable marker of AD pathology, thus defined as an "Alzheimer's pathological change". In contrast, AD requires the presence of both A and T. The NIA-AA framework has added the (N) biomarker in parenthesis as, similar to cognitive symptoms, it is not specific to AD neuropathological changes, and therefore can only be used to stage the disease severity, rather than diagnose AD.

With the advances in biofluid assessments, especially in the plasma, researchers have been trying to test whether easily accessible biomarkers can replace brain imaging and CSF methods (Koychev et al., 2021). However, further studies should be conducted on the use of plasma biomarkers in the clinical setting world. For now, methodological differences could lead to distinctive results, thus biasing the diagnosis of AD.

Additionally, the A/T/(N) system was proposed when amyloid-β, tau and neurodegeneration were thought to be the only changes observed in the AD brain. More recent research has discovered the core involvement of neuroinflammation in the development and propagation of AD pathophysiology (Bellaver et al., 2021; Ferrari-Souza et al., 2022; Pascoal, Benedet, Ashton, et al., 2021). The discovery that various pathological processes are also involved

in AD led to the idea that the A/T/(N) system might benefit from adding another marker for either neuroinflammation, or other AD-related change (X). It is possible that in a few years from now, the NIA-AA framework might change into the A/T/(N)/X system.

Moreover, the A/T/(N) system is based on the idea that disease follows a linear trajectory from cognitively unimpaired, to MCI, to AD. Not all patients diagnosed with MCI will progress to AD, meaning other markers of brain pathology (vascular, inflammation and so on) might indicate whether individuals are more likely to develop AD dementia.

However, before any change can be done in the A/T/(N) system, we need to further investigate newly discovered plasma markers, as well as the integration of novel markers.

1.10 Rationale and objectives

The prevalence of AD dementia is increasing around the world, as the population ages. It is thus critical to fully understand all the pathophysiological changes associated with the disease, as well as be able to track those changes over time. Currently, the clinical diagnosis of AD is heterogenous and does not always corroborate AD-related pathophysiological changes (Therriault et al., 2021). This is in part due to the fact that available biomarkers of AD are through CSF and brain imaging (i.e. PET and MRI) assessments. The procedures are invasive and do not allow for an easy implementation in the clinical and clinical trial settings.

Current therapeutic research is focusing on amyloid-β removing drugs (Mintun et al., 2021; Shcherbinin et al., 2022); thus, the assessment of AD pathology *in vivo* and early in the disease process is critical. With established markers of AD, it will also be possible to follow the changes related with drugs.

Among the three AD-related pathologies, tau has been the one more closely associated with cognitive decline, disease severity and progression (Ossenkoppele et al., 2019). Thus, the research field has been focusing on biomarkers of tau pathology, also known to be AD-specific. The implementation of the A/T/(N) framework helped in the assessment of disease progression over time (Jack & Holtzman, 2013). Thus, the overarching goal of this thesis was to investigate the impact of various types of tau biomarkers and how they can assess individuals along the A/T/(N) framework, using multimodal neuroimaging and fluid markers. The overall hypothesis supported by the three papers of this thesis is that the presence and progression of fluid tau species convey information regarding downstream AD pathophysiological events, particularly tau-aggregates and neurodegeneration. The thesis provides insights regarding biofluid tau markers, especially plasma ones, in the A/T/(N) system, which is the most commonly used disease framework.

To determine this, we designed three studies. Novel assays were implemented in the last few years using the Simoa technology. The first study sought to investigate whether a newly discovered plasma pTau181 assay (Karikari, Pascoal, et al., 2020) depicted neurodegeneration in the brain of cognitively unimpaired and impaired individuals. Thanks to the Alzheimer's Disease Neuroimaging Initiative (ADNI) we had longitudinal data available, providing us insights on the association between baseline plasma pTau181 levels and subsequent grey matter loss (**Chapter 2**). Other assays were then created, and seemingly assessed distinct stages of disease progression (Ashton et al., 2021; Karikari, Pascoal, et al., 2020). We thus decided to investigate whether different tau markers could be interchangeably used. For this, we assessed individuals with available plasma pTau181, plasma pTau231 and tau-PET with [^{18}F]MK6240 in the TRIAD cohort, and tested the concordance and discordance of these markers. We associated the

concordant/discordant groups with AD-related markers and risk factors (**Chapter 3**). Nevertheless, those pTau residues have been seen to correlate with both amyloid-β and tau markers (Palmqvist et al., 2019). There is increasing evidence suggesting pTau markers are more closely associated with amyloid-β plaques as compared to tau (Therriault, Vermeiren, et al., 2022). Moreover, pTau residues have been observed to increase early in the disease progression (Jack et al., 2018). Thus, as tau seems to be the marker more closely associated with cognitive problems, biofluid markers that reflect tau pathology only are critical for disease tracking. A novel assay named NTA was created, this time targeting tau species ranging from N-terminal to mid-region; it has been described as AD-specific and presents increased levels in AD individuals (Snellman et al., 2022). In **Chapter 4**, we assessed NTA levels in plasma and CSF in the well-characterized TRIAD cohort, where individuals underwent gold standard imaging measures. The outline of the thesis, added to the A/T/(N) framework of AD can be found in Figure 9.

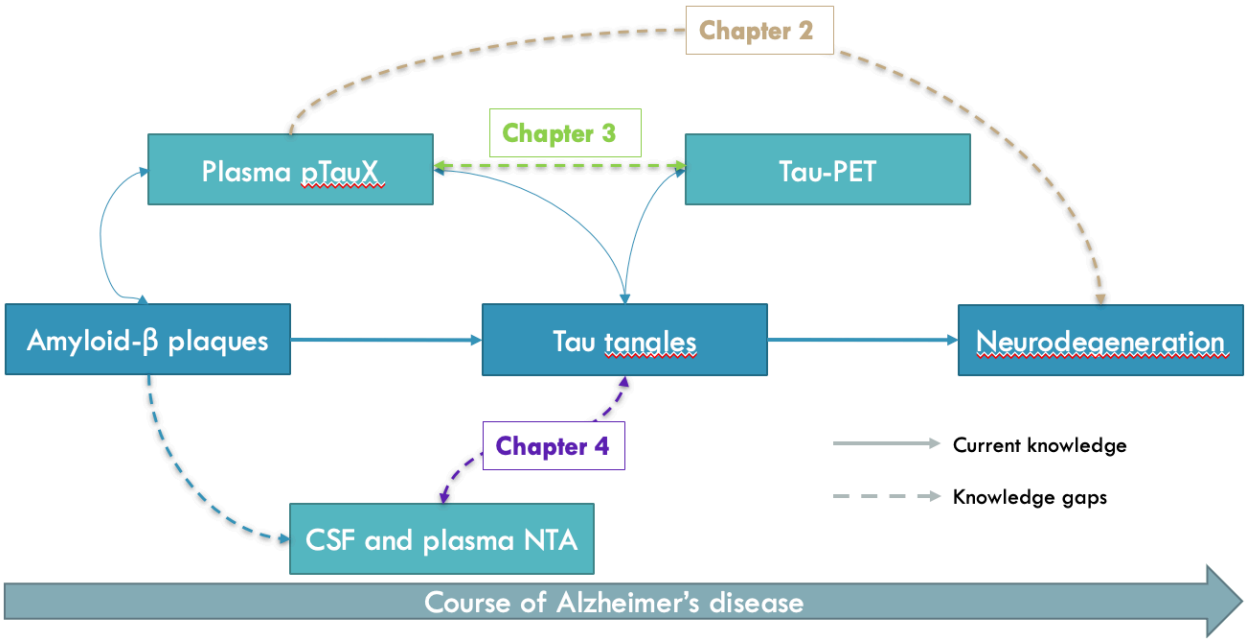

Figure 9: The impact of tau biomarkers in the A/T/(N) system of Alzheimer's disease.

Chapter 2: Plasma pTau181 predicts cortical brain atrophy in aging and Alzheimer's disease.

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*Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf

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2.1 Preface

Over the years, research focused on assays targeting pTau181 in the plasma (Barthélemy et al., 2020; Jack, Wiste, et al., 2018; Yang et al., 2018). Indeed, pTau181 levels observed in the CSF are thought to be one of the markers more closely associated with tau pathology, and thus, cognitive dysfunction (Llibre-Guerra et al., 2019; Vanmechelen et al., 2000). A great advantage of using pTau181 is the specificity it has for AD (Karikari, Benedet, et al., 2020; Thijssen et al., 2020). Recent assays produced using a custom single molecule array (Simoa) machine made them extremely precise and highly sensitive. A novel plasma pTau181 was able to differentiate individuals at distinct stages of the AD *continuum* (Karikari, Benedet, et al., 2020; Karikari, Pascoal, et al., 2020).

Nevertheless, one of the hallmarks of AD is neurodegeneration, which is thought to be the last process, after a significant accumulation of amyloid-β and NFT already occurred (Jack & Holtzman, 2013; Pascoal et al., 2017). However, it is observed in a variety of brain conditions, making biomarkers of neurodegeneration unspecific to AD (Cordato et al., 2005; Davie, 2008; Lee et al., 2001). In Chapter 2, we wondered whether the novel assay for plasma pTau181 was able to assess the extent of neurodegeneration, cross-sectionally and longitudinally in the brain of cognitively unimpaired and impaired individuals.

2.2 Abstract

Background: To investigate the association of plasma pTau181, assessed with a new immunoassay, with neurodegeneration of white matter and grey matter cross-sectionally and longitudinally, in aging and Alzheimer's disease.

Methods: Observational data was obtained from the Alzheimer's Disease Neuroimaging Initiative, in which participants underwent plasma assessment and magnetic resonance imaging. Based on their clinical diagnosis, participants were classified as cognitively unimpaired and cognitively impaired. Linear regressions and linear mixed-effect models were used to test the cross-sectional and longitudinal associations between baseline plasma pTau181 and neurodegeneration using voxel-based morphometry.

Results: We observed a negative correlation at baseline between plasma pTau181 and grey matter volume in cognitively unimpaired individuals. In cognitively impaired individuals, we observed a negative association between plasma pTau181 and both grey and white matter volume. In longitudinal analyses conducted in the cognitively unimpaired group, plasma pTau181 was negatively correlated with grey matter volume, starting 36 months after baseline assessments. Finally, in cognitively impaired individuals, plasma pTau181 concentrations were negatively correlated with both grey and white matter volume as early as 12 months after baseline, and neurodegeneration increased in an incremental manner until 48 months.

Conclusions: Higher levels of plasma pTau181 correlate with neurodegeneration and predict further brain atrophy in aging and Alzheimer's disease. Plasma pTau181 may be useful in predicting AD-related neurodegeneration, comparable to Positron Emission Tomography or cerebrospinal fluid assessment with high specificity for AD neurodegeneration.

Keywords:

Plasma pTau181, neurodegeneration, voxel-based morphometry, Alzheimer's disease.

2.3 Background

Advances in quantification of biofluids made possible the detection of Alzheimer's disease (AD) pathophysiological processes in peripheral plasma. It was recently demonstrated that ultra-sensitive tau phosphorylated at threonine-181 (pTau181) in plasma (Karikari, Pascoal, et al., 2020; Mielke et al., 2018; Tatebe et al., 2017; Yang et al., 2018) provides an inexpensive way to determine the presence of brain neurofibrillary tangles *in vivo*. Recent studies of plasma pTau181 (Benussi et al., 2020; Janelidze et al., 2020; Karikari, Pascoal, et al., 2020; Thijssen et al., 2020) were successful at differentiating AD from other neurodegenerative conditions, and presented a strong correlation with pTau181 concentrations in the cerebrospinal fluid (CSF) (Karikari, Pascoal, et al., 2020). Although the associations between CSF and biomarkers of neurodegeneration have been extensively described, little is known regarding plasma pTau181 and its cross-sectional and longitudinal associations with neurodegeneration of white matter (WM) and grey matter (GM). It was however observed that plasma pTau181 levels correlate with lower grey matter volume in the precuneus and temporal lobe of mild cognitive impairment (MCI) and AD participants (Thijssen et al., 2020). Moreover, the novel method to assess plasma pTau181, which is used in the following analyses, has been shown to predict a one-year cognitive decline, and hippocampal atrophy along the AD spectrum (Karikari, Pascoal, et al., 2020). In the current investigation, we examine whether plasma pTau181 correlates with neurodegeneration assessed via voxel-based morphometry (VBM) cross-sectionally, and longitudinally, over a maximum of a 4-year period. We conducted analyses in cognitively unimpaired (CU) and cognitively impaired (CI) individuals, including MCI and AD participants, who are part of the Alzheimer's disease Neuroimaging Initiative (ADNI). We hypothesize that plasma pTau181 levels are associated with baseline neurodegeneration of WM and GM, as well as predict subsequent atrophy.

2.4 Methods

Study participants:

Data used in the preparation of this article was obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). The ADNI was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early Alzheimer's disease (AD). Data used was downloaded on June 20th, 2020. Each ADNI site received approval from their Ethics Board to conduct the study. Written consent was obtained from all the research participants.

The ADNI inclusion/exclusion criteria are described in detail at www.adni-info.org (accessed June 2020). The individuals underwent MRI scans, an assessment of plasma pTau181, as well as a neuropsychological evaluation. Participants were considered CU when they had a Clinical Dementia Rating (CDR) of 0, MCI when they obtained a CDR of 0.5, while individuals suffering from dementia due to AD were considered such with a CDR of 1 or higher, and met the standard diagnostic criteria for probable AD(McKhann et al., 1984). The CI group was composed of both MCI and AD individuals. Participants who had no objective evidence of cognitive impairment but reported subjective cognitive decline were analyzed together with the CU individuals, as per the National Institute of Aging-Alzheimer's Association(Jack, Bennett, et al., 2018). Baseline diagnosis was used for statistical analyses.

Detailed description of the sample selection can be found in Figure 1. Number of individuals at each timepoint can be found in Table 1.

Imaging analyses:

Pre-processed 1.5T and 3T T1-weighted MRI scans were downloaded from the ADNI database (adni.loni.usc.edu; for pre-processing details, see(Jack et al., 2008)). Anatomical images were segmented into probabilistic grey matter (GM) and white matter (WM) maps using the SPM12 segmentation tool. Each GM and WM probability map was then non-linearly registered (with modulation) to the ADNI template using DARTEL(Ashburner, 2007), and smoothed with a Gaussian kernel of full width half maximum (FWHM) of 8 mm. All images were visually inspected to ensure proper alignment to the ADNI template.

Plasma measurements:

Plasma pTau181 was measured using a clinically validated in-house assay described previously(Karikari, Pascoal, et al., 2020). Plasma pTau181 was measured on Simoa HD-X instruments (Quanterix, Billerica, MA, USA) in April 2020 at the Clinical Neurochemistry Laboratory, University of Gothenburg, Mölndal, Sweden, by scientists blinded to participants' clinical information. Plasma pTau181 data was collected over 47 analytical runs. Assay precision was assessed by measuring three different quality control samples at the start and end of each run, resulting in within-run and between-run coefficients of variation of 3.3%-11.6% and 6.4%-12.7% respectively. Out of 3762 ADNI samples, four were removed due to inadequate volumes. The remaining 3758 all measured above the assay's lower limit of detection (0.25 pg/ml), with only six below the lower limit of quantification (1.0 pg/ml), which were excluded from the study.

Statistical analyses:

R statistical software package (version 4.0.0) was used to perform the nonimaging statistical analyses. Plasma pTau181 results were log-transformed to meet the requirements of parametric statistics. We first conducted t-test (continuous variables) and chi-square tests (categorical variables) for demographics.

We also conducted linear mixed-effect (LME) regression models, using the *lme4* package, in order to compare the progression of plasma pTau181 in each diagnostic group over time. The LME included plasma pTau181 as the dependent variable, and the interaction between time and group as the independent variable. The covariates were sex and age at baseline. To accommodate the correlation arising from multiple measurements on the same participant, we also included a random intercept. The 95% confidence intervals were estimated based on the estimated fitted value across the distribution from 1000 simulations of the model that includes all variations. All tests mentioned previously were two-sided with a statistical significance level of $P < .05$.

For brain imaging, we conducted linear models (LM) and LME at the voxel level using VoxelStats(Mathotaarachchi et al., 2016). We studied the associations between log-transformed plasma pTau181 and GM and WM images. Firstly, we investigated the relationship between log-transformed plasma pTau181 and VBM images cross-sectionally for each diagnostic group (CU and CI) separately, adjusting for sex and age at baseline. Additionally, we investigated the longitudinal associations between baseline plasma pTau181 and VBM images. The LME included VBM images as the dependent variable and the interaction between time and baseline plasma pTau181 as the independent variable. They were adjusted for sex and age at baseline, as well as random intercept. The model was performed in CU and CI individuals separately. Finally, to infer disease progression at each visit, we used the same statistical model using the follow-up visit (time)

as a categorical variable. We used random field theory(Worsley et al., 2004) (RFT) to correct all imaging results for multiple comparisons. Exploratory analyses were also conducted correcting for random slopes.

2.5 Results

Demographics:

Demographic information can be found in Table 2. The sample included a total of 1122 individuals, among which 384 were CU and 738 were CI (539 MCI and 199 AD). The average follow-up time was 22 ± 11.63 months. There was no statistically significant difference between the groups in terms of age. However, there were statistically significant differences in terms of sex, MMSE scores, as well as the plasma pTau181 levels between the CU and CI individuals. CI group was composed predominantly of males, showed lower MMSE scores, and higher plasma pTau181 levels. For the CU group, the mean of plasma pTau181 levels was 15.48 ± 10.02 pg/mL; while for the CI, it was 19.80 ± 10.75 pg/mL (Figure 2 A).

Regional association between plasma pTau181 with WM and GM volume predominate in CI:

Cross-sectional analysis in the CU group revealed that plasma pTau181 was not associated with WM volume. Nevertheless, there was a negative relationship with GM volume. This association was found in the anterior cingulate, and the lateral occipital gyrus (Figure 2 B).

In CI individuals, we discovered strong negative associations between plasma pTau181 and GM as well as WM volumes. Correlations between the blood-based biomarker and GM were observed in the precuneus, the anterior cingulate, and the medial and lateral temporal gyrus. WM and plasma

pTau181 associations were found in the corpus callosum and the temporal lobe (Figure 2 C). R-maps of the cross-sectional analyses can be found in Supplementary Figure 1.

Plasma pTau181 predicts subsequent GM and WM volume decline:

There was no significant difference in the rate of change of plasma pTau181 between CU and CI groups over a period of 48 months ($p = 0.16$) (Figure 3 A). Individual changes can be seen in Supplementary Figure 2. LME models conducted in the CU group showed a negative association between plasma pTau181 and GM volume in the temporal lobe, the precuneus and the anterior cingulate cortex. Similar analyses with WM volume changes did not survive correction for multiple comparisons (Figure 3 B). In the CI individuals, negative associations between plasma pTau181 and GM volume were observed in the precuneus and the frontal cortex, with even stronger associations in the temporal area. Plasma pTau181 and WM volume, showed a negative association in the corpus callosum, and the frontal and temporal lobes (Figure 3 C). When correcting for random slopes, the results were identical.

Negative associations between plasma pTau181 and GM or WM volume spread over time:

Among the CU individuals, negative correlations between plasma pTau181 with GM were observed at the 36-months FU (Figure 4 A) and the 48-month FU (Figure 4 B) in the precuneus, insula, medial frontal, anterior cingulate and finally the temporal lobe. Comparatively, there was no significant negative relationship with WM volume.

In the CI group, areas in which GM volume negatively correlated with plasma pTau181 progressively expanded from 12-48 months FU. As early as 12-month FU, pTau181 and GM

volume correlations were restricted to the medial frontal, precuneus, posterior cingulate and the temporal lobe (Figure 4 C). At 24-months FU, these negative correlations also included the medial occipital cortex (Figure 4 D). From 36 (Figure 4 E) to 48-months FU (Figure 4 F) these associations embraced the whole medial frontal cortex, the precuneus, medial occipital and the temporal lobes. Plasma pTau181 and WM volume associations similarly progressed over time; these correlations were initially confined to the vicinity of the choroidal fissure as well as the temporal horn of the lateral ventricle at 12-months FU (Figure 4 G). Subsequently, they encompassed the lateral periventricular WM at 24-months FU (Figure 4 H), including frontal and occipital lobes at 36-months FU (Figure 4 I) to finally embrace the whole WM 48 months after baseline (Figure 4 J).

2.6 Discussion

In summary we found that plasma pTau181 was associated with GM loss in both CU and CI groups, while its associations with WM loss were observed only in CI cross-sectionally. In CU individuals, plasma pTau181 predicted GM degeneration in AD-related regions starting 36 months after baseline. In CI, plasma pTau181 predicted an incremental degeneration, in both GM and WM, which started in the typical AD-related brain regions, and encompassing the cortex and WM globally four years after the first assessment. The VBM changes seen as early as 12 months after baseline support the hypothesis that plasma pTau181 predicts imminent neurodegeneration.

Cross-sectional analyses suggested that measures of tau phosphorylation with plasma pTau181 informs about GM degeneration among CU individuals. The cross-sectional associations occurred only in areas well known to be affected early in the AD process, such as the cingulate

cortex, in which early amyloid deposition is often observed(Palmqvist et al., 2017). In CU, the anterior cingulate degeneration has been linked to complex attentional deficits, and is known to be impaired along the clinical spectrum of AD(Mesulam et al., 2001). Other studies also presented anterior cingulate atrophy as a predictor of conversion to dementia due to AD in memory-impaired individuals(Killiany et al., 2000), suggesting the region is affected before the onset of cognitive symptoms.

In CI individuals, we observed a strong negative correlation between GM loss and plasma pTau181 in regions commonly affected in AD(Braak & Braak, 1991), such as the medial temporal lobe, the precuneus and the anterior cingulate. The medial temporal region, which encompasses the hippocampus, is well known to be related to early atrophy in AD(Frisoni et al., 2002; Ohnishi et al., 2001). The associations found between plasma pTau 181 and precuneus atrophy also corroborates the finding that this region is often impaired at the early stages of AD(Bailly et al., 2015; Braak & Braak, 1991). Similarly as in the CU individuals, the CI group presented GM degeneration in the anterior cingulate, giving further support to the idea that neurodegeneration in this specific region is associated with tau hyperphosphorylation. Furthermore, we observed associations between plasma pTau181 and WM damage along the temporal lobe as well as the corpus callosum. Temporal WM atrophy has been consistently linked to aging and early AD(Salat et al., 2009). The region is known to connect a network of memory-related areas. The associations between temporal WM and plasma pTau 181 might indicate a vulnerability of these WM tracts to hyperphosphorylation. Finally, the periventricular WM, was also shown to correlate with plasma pTau181 in the CI group. Ventricular dilation and periventricular WM fibers have been presented as a biomarker of neurodegeneration(Teipel et al., 2002). Taken together, the cross-sectional

analyses performed in this study provide evidence that high plasma pTau181 indicates neurodegeneration in brain regions vulnerable to early-AD pathology,

Our longitudinal analyses revealed that plasma pTau181 predicted GM degeneration in various brain regions known to be affected in AD(Braak & Braak, 1991). Particularly, the temporal lobe and posterior cingulate have shown atrophy in people with high plasma pTau181 baseline levels in CU individuals. Atrophy and tau deposition in both regions are well correlated with deficits of memory formation and retrieval in AD(Zhou et al., 2008). Functional alterations of the cingulate cortex imposed by AD pathophysiology has been proved to forecast dementia two years later(Huang et al., 2002). Interestingly, we showed that plasma pTau181 predicts neurodegeneration in those specific regions, making it a possible earlier predictor of upcoming brain atrophy and possibly cognitive changes. By contrast, we did not observe associations between plasma pTau181 and longitudinal WM degeneration in cognitively unimpaired individuals. It is possible that WM is affected later in the disease process as compared to GM. Nevertheless, in CI individuals, we observed a strong negative correlation between plasma pTau181 and both GM and WM degeneration longitudinally. Plasma pTau181 predicted subsequent medial temporal, as well as precuneus, medial frontal and medial occipital degeneration. Those brains regions are known to be included in the Default Mode Network, crucial for cognitive tasks(Buckner et al., 2008). AD pathophysiologies in these brain regions are also associated with memory dysfunction(Hafkemeijer et al., 2012), and are known to accumulate neurofibrillary tangles(Braak & Braak, 1991). It is well described that there is a decrease in the Default Mode Network connectivity along the continuum of normal aging to dementia(Hafkemeijer et al., 2012). The default mode alterations are associated with deficits in

memory retrieval and envisioning the future, among others(Buckner et al., 2008). Regarding WM, plasma pTau181 predicted degeneration more specifically in the temporal and periventricular WM. WM abnormalities is these regions are considered crucial for memory formation and retrieval(Salat et al., 2009), and are also seen as markers of neurodegeneration in AD(Teipel et al., 2002). In WM, tau hyperphosphorylation can potentially come from glia or merely be associated to GM tau(Kovacs et al., 2016). These results indicate that high plasma pTau181 in symptomatic cases harbingers AD-related neurodegeneration.

Further longitudinal analyses conducted at each FU MRI revealed how plasma pTau181 predicted neurodegeneration in both CU and CI groups. Although no effects were observed in the WM of CU individuals, plasma pTau181 predicted cortical neurodegeneration at month 36 after baseline in the temporal GM, the medial frontal cortex, the anterior cingulate and the precuneus. These regions are known to be vulnerable to early AD pathology and important for memory(Braak & Braak, 1991; Frisoni et al., 2002; Killiany et al., 2000). The same analyses with time as an ordinal variable were conducted in the CI group; we observed an incremental degeneration of both WM and GM, resembling Braak staging(Braak & Braak, 1991). At 12-months FU, we observed that plasma pTau181 was related to GM degeneration in the medial temporal, medial frontal and precuneus regions. In the course of the four years post-baseline, we observed that the GM degeneration increased and spread more broadly to the entire cortex. At month 48, the medial temporal cortex, medial frontal cortex and precuneus were significantly associated with plasma pTau181 concentrations. Similarly, the WM deterioration progresses with time. At month 12, alterations were restricted to the temporal lobe, affected early in AD, while at month 48, the frontal WM and the corpus callosum were heavily impaired. In the CI group, plasma pTau181 was able

to predict imminent brain atrophy, which broadened incrementally to affect the entire brain. Taken together, these results suggest that plasma pTau181, in addition to being a cost effective and scalable marker of future tau hyperphosphorylation, incorporates information regarding present and upcoming neurodegeneration.

The current findings between plasma pTau181 and brain atrophy corroborate previous research on CSF and plasma pTau181(Llibre-Guerra et al., 2019; Thijssen et al., 2020). Higher levels of phosphorylated tau predicted neurodegeneration in the medial temporal and periventricular WM among other regions in aging and AD(Gispert et al., 2016; Llibre-Guerra et al., 2019). In CI individuals, plasma pTau181 was correlated with degeneration of GM in the precuneus and temporal lobes(Thijssen et al., 2020). It also predicted atrophy in the hippocampal region along the AD spectrum(Karikari, Pascoal, et al., 2020). Blood-based biomarkers, particularly pTau181, or hyperphosphorylated tau, represent an important step in facilitating disease diagnosis and possibly patient management. Moreover, blood-based biomarkers could serve to enrich a population for clinical trial selection. The last decade has focused on the research of biomarkers useful in AD(O'Bryant et al., 2017), leading to the development of the current assay to assess plasma pTau181. The present study provides evidence of an added value associated with the new immunoassay for plasma pTau181. The higher the plasma pTau181 levels, the higher is the probability of co-existing neurodegeneration and the higher the likelihood of developing brain degeneration in the subsequent years. Indeed, among the CU individuals, it predicted cortical atrophy in AD-related brain regions three years later, while in the CI individuals, it forecasted imminent atrophy of both GM and WM as soon as 12 months later, with a progressive degeneration spreading all over the brain.

Other plasma markers have been studied in AD, such as neurofilament light (NfL) and amyloid-β (Aβ), which are also promising candidates for blood-based biomarkers(Benedet et al., 2019), however, both have shown some disadvantages. Indeed, NfL is a non-specific biomarker, as it has been related to other neurodegenerative conditions(De Marchis et al., 2018; Hansson et al., 2017) and is related to aging. Cerebral amyloidosis can be found in CU individuals(Chételat et al., 2013) as well as a proportion of non-AD dementias. Furthermore, there is a significant production of peripheral Aβ expression(Citron et al., 1994) which may not be related to the AD process. Nevertheless, the current assay for plasma pTau181 is highly specific for AD(Karikari, Pascoal, et al., 2020) with the advantage to inform about coexistent and future neurodegeneration.

Limitations:

The main strength of the study is to provide insights regarding levels of plasma pTau181, using a novel assessing method, and regional brain atrophy in a large cohort of well-characterized individuals. The assay was previously shown to be AD specific, when compared to other neurodegenerative conditions(Karikari, Pascoal, et al., 2020). However, further research is required in larger cohorts with different tauopathies. There are various methodological limitations in the study, such as the fact that not all individuals had follow-up assessments every 12 months along the 4-year period post-baseline. Demographic differences were also present among groups, which were however accounted for in the statistical models. In ADNI(Karikari, Benedet, et al., 2020), a multi-center cohort, and in other single-study cohorts(Karikari, Pascoal, et al., 2020), plasma pTau181 levels have been shown to increase with disease severity. Individuals presenting amyloid positivity had significantly higher levels of plasma pTau181 as compared to CU individuals(Karikari, Benedet, et al., 2020). In our analyses, the CU and CI groups present a

significant difference in plasma pTau181 concentrations, albeit a certain overlap. It is thought to be due to the combination of MCI and AD together in the CI group, and the non-stratification depending on the amyloid status. Apart from these limitations, the new plasma pTau181 immunoassay could be proposed as a simple and scalable way to diagnose AD as it is highly associated with amyloidosis, neurofibrillary tangles and, according to our analysis, predict future brain atrophy in aging and dementia due to AD. To conclude, we provide evidence that this novel test for plasma pTau181 has a strong negative correlation with brain atrophy patterns typical of AD and can also help predict subsequent neurodegeneration in aging and dementia due to AD.

Conclusion

The current study showed, in a large cohort of well characterized individuals, that plasma pTau181 correlates with neurodegeneration at baseline and predicts further brain atrophy. Indeed, CU individuals mainly present GM atrophy, while CI individuals display both GM and WM damage. The incremental patterns of neurodegeneration involve brain regions related to AD pathologies. PET and CSF assessments being more expensive and not readily available, plasma pTau181 may then be a useful, cost-effective and scalable biomarker to predict AD-related neurodegeneration, with high specificity for AD.

List of abbreviations:

AD: Alzheimer's Disease, ADNI: Alzheimer's Disease Neuroimaging Initiative, Aβ: Amyloid-β, CDR: Clinical Dementia Rating, CI: Cognitively Impaired, CSF: CerebroSpinal Fluid, CU: Cognitively Unimpaired, FU: Follow-Up, GM: Grey Matter, LM: Linear Models, LME: Linear Mixed Effects, MCI: Mild Cognitive Impairment, MRI: Magnetic Resonance Imaging, NfL: Neurofilament Light, PET: Positron Emission Tomography, pTau: phosphorylated Tau, RFT: Random Field Theory, VBM: Voxel-Based Morphometry, WM: White Matter

2.7 Declarations:

Ethics approval and consent to participate:

Each ADNI site received approval from their Ethics Board to conduct the study. Written consent was obtained from all the research participants.

Consent for publication:

Not applicable.

Availability of data and material:

All data can be found on adni.loni.usc.edu.

Competing interests:

CT, ALB, JT, TAP, FL, PSC, MC, MS, SM, GB, YTW, JFA, NJA, TKK, EDVS, PH, SG and PR-N have no disclosures to report.

HZ has served at scientific advisory boards for Denali, Roche Diagnostics, Wave, Samumed, Siemens Healthineers, Pinteon Therapeutics and CogRx, has given lectures in symposia sponsored by Fujirebio, Alzecure and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). KB has served as a consultant, at advisory boards, or at data monitoring committees for Abcam, Axon, Biogen, Julius Clinical, Lilly, MagQu, Novartis, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work).

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Author contributions:

CT is responsible for the conception and design of the study, acquisition and analysis of data, and drafting a significant portion of the manuscript and figures. ALB, JT, TAP and FZL are responsible for the conception and design of the study, acquisition and analysis of data. PSC, SMM, GB, YTW, JFA, JLR, AS, NJA, TKK are responsible for the acquisition and analysis of the data. MC, KB, HZ, EDVS, PH and SG are responsible for the conception and design of the study. PRN is responsible for the conception and design of the study, and for drafting a significant portion of the manuscript and figures. All authors reviewed and approved the manuscript.

Acknowledgments

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***2.8 Figures**:*

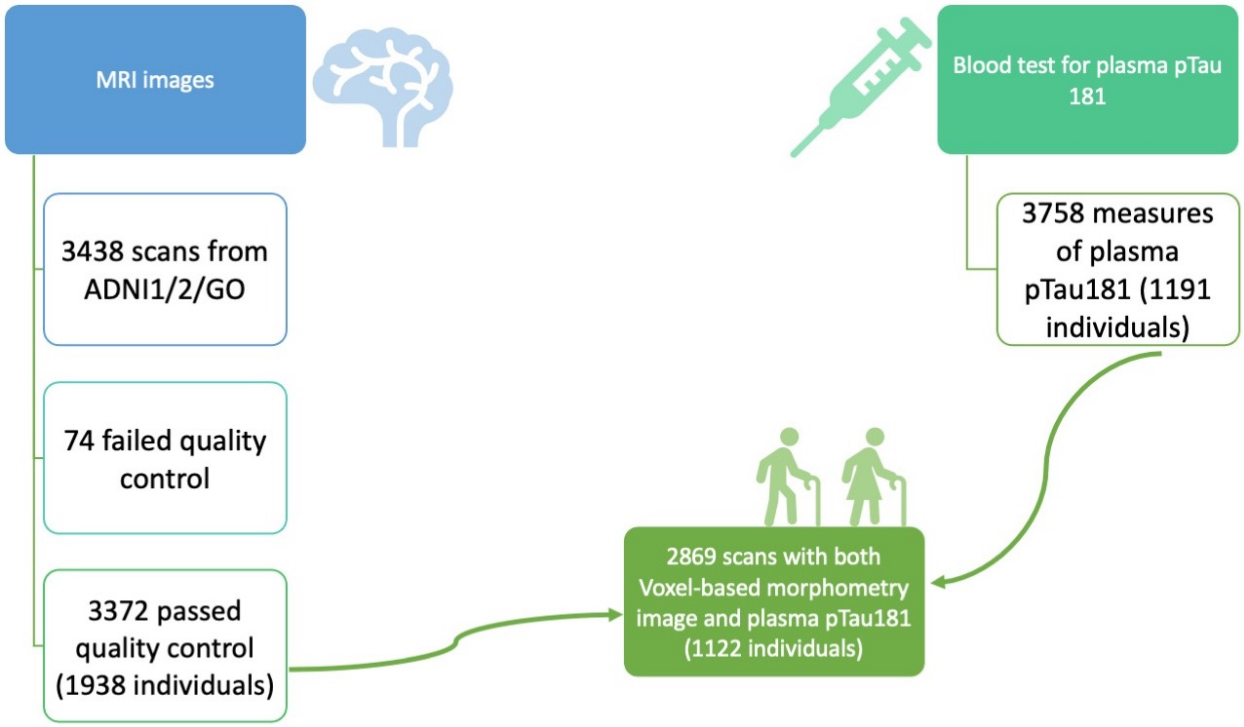


Figure 1: Sample selection from ADNI. 3438 MRI were available from participants, 74 of them failed quality control, ending up to 3364 available scans to study. Similarly, 3758 participants had available results from plasma ptau181. Combined together, we had 2869 available scans and plasma pTau181 results which dates of assessment were within 6 months.

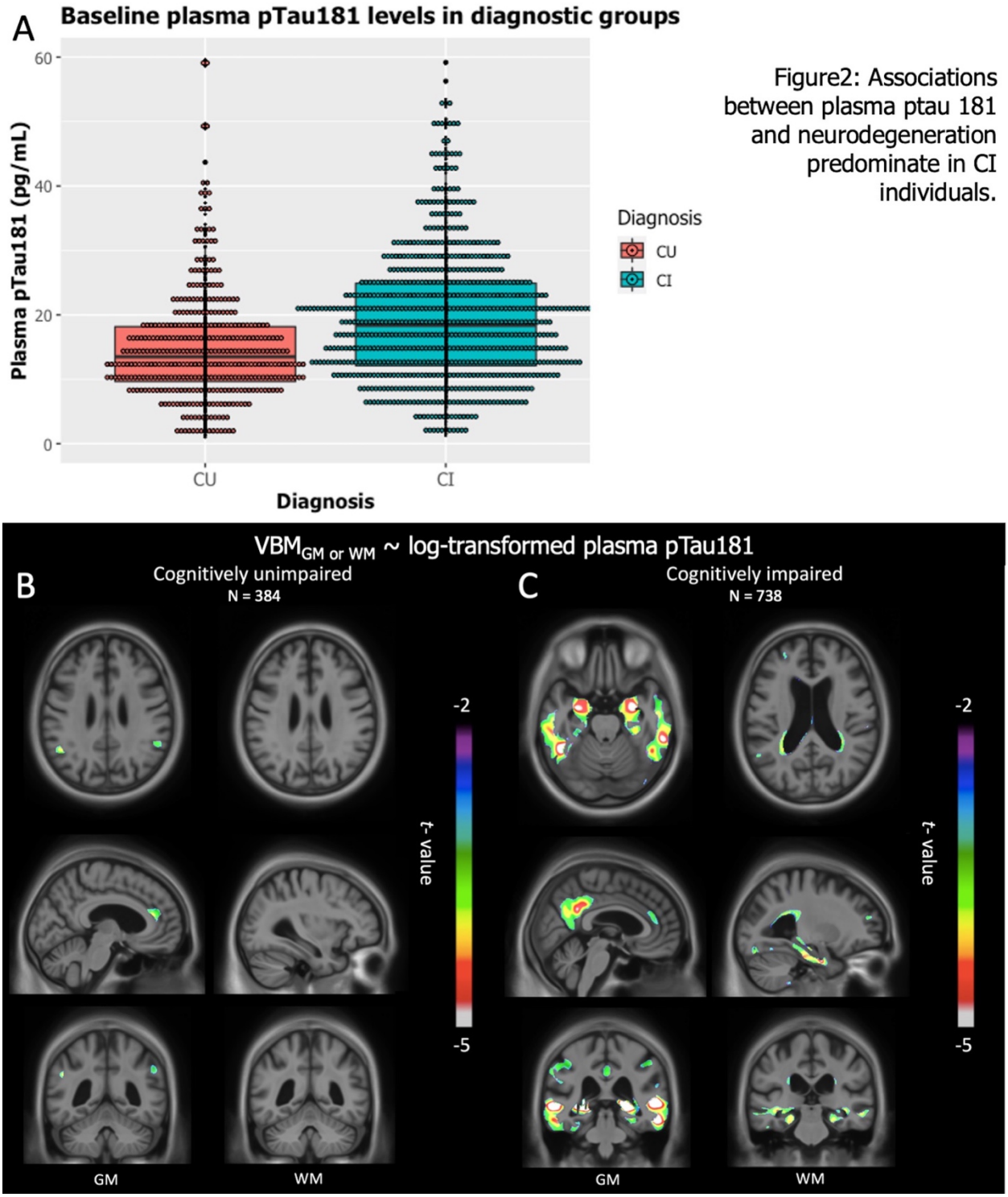


Figure2: Associations between plasma ptau 181 and neurodegeneration predominate in CI individuals.

Figure 2: Associations between plasma tau 181 and neurodegeneration predominate in CI individuals. (A) Mean of log-transformed plasma pTau181 depending on the diagnostic group. (B) CU individuals showed a negative correlation with GM in the anterior cingulate and occipital

gyrus. However, no correlation was found with WM. (C) CI individuals presented a negative correlation with GM in the precuneus, anterior cingulate, medial and lateral temporal gyrus. WM decrease was found in the corpus callosum and the temporal lobe.

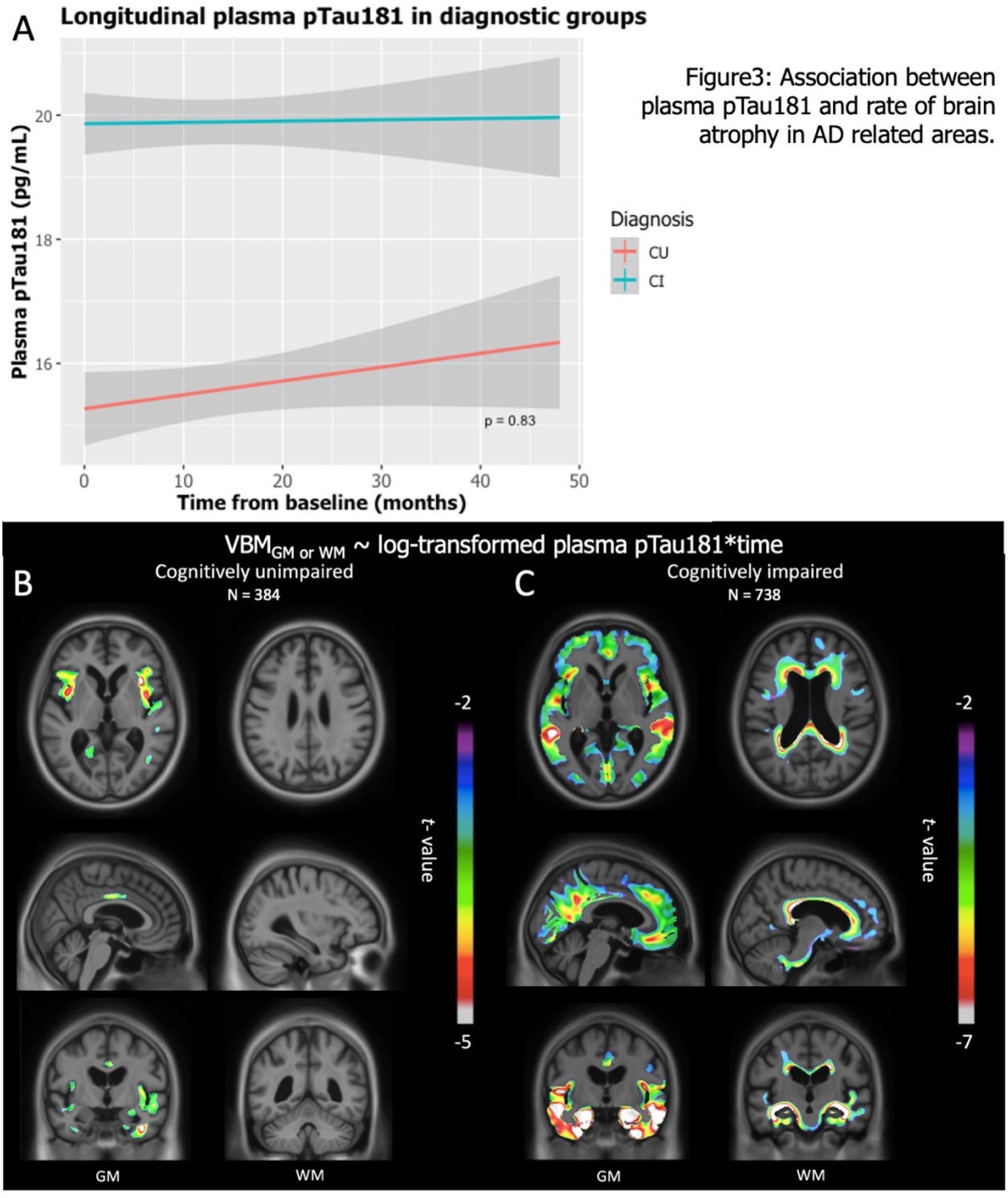


Figure 3: Association between plasma pTau181 and rate of brain atrophy in AD related areas. (A) There is no significant difference between the rates of change of plasma pTau181 between CU and CI individuals. (B) CU individuals presented a negative correlation between plasma pTau181 and

GM in the temporal lobe, precuneus and anterior cingulate cortex. No correlation was found with WM. (C) CI individuals showed a negative correlation with GM in the precuneus, frontal cortex, and the temporal lobe. WM results showed a negative correlation in the corpus callosum, the temporal and the frontal lobes.

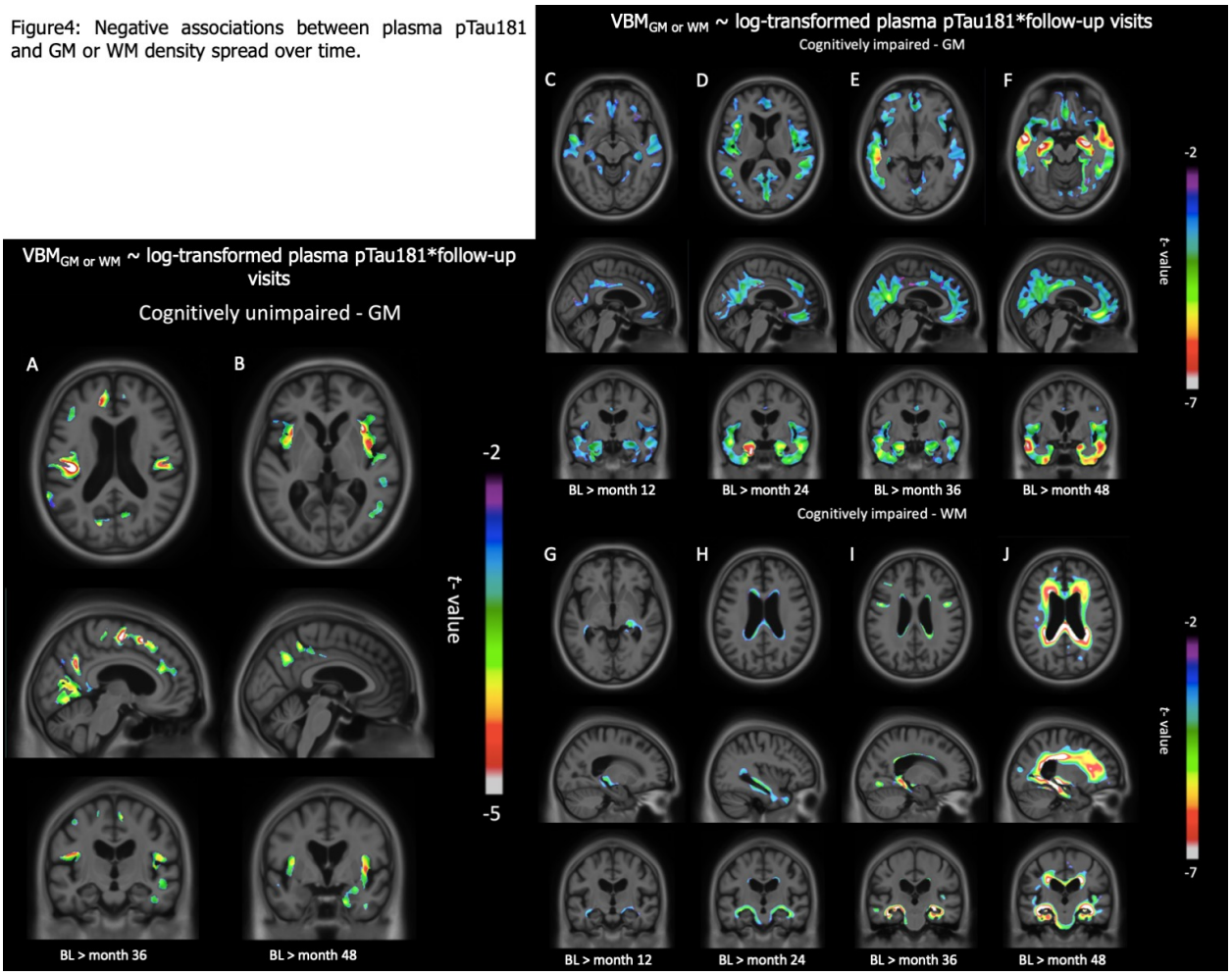


Figure 4: Negative associations between plasma pTau181 and GM or WM volume progressed over time. (A) CU individuals showed GM degeneration 36 months after baseline, in the precuneus, medial frontal, anterior cingulate and temporal lobe. (B) Similar regions were affected 48 months after baseline. (C) The CI group presented GM degeneration 12 months after baseline, in the medial frontal, precuneus, posterior cingulate and temporal lobe. (D) At month 24, similar regions were affected spreading to the medial occipital cortex. (E-F) At month 36 and 48, it spread even further in the medial frontal, temporal and posterior region of the brain. (G) In the CI group, 12 months after baseline, there was a negative correlation between WM and plasma pTau181 in the temporal lobe. (H) At month 24, the started spreading to the corpus callosum. (I) At month 36, the

WM tracts in the frontal and occipital lobe were affected. (J) At month 48, most of the WM in the brain seemed affected.

***2.9 Tables**:*

Table 1: Number of individuals at each timepoint

Time points	**Number of participants**
BL	1122 (384 CU and 738 CI)
V12	786 (238 CU and 548 CI)
V24	605 (240 CU and 365 CI)
V36	177 (51 CU and 126 CI)
V48	179 (73 CU and 106 CI)

Table 2: Participants' characteristics

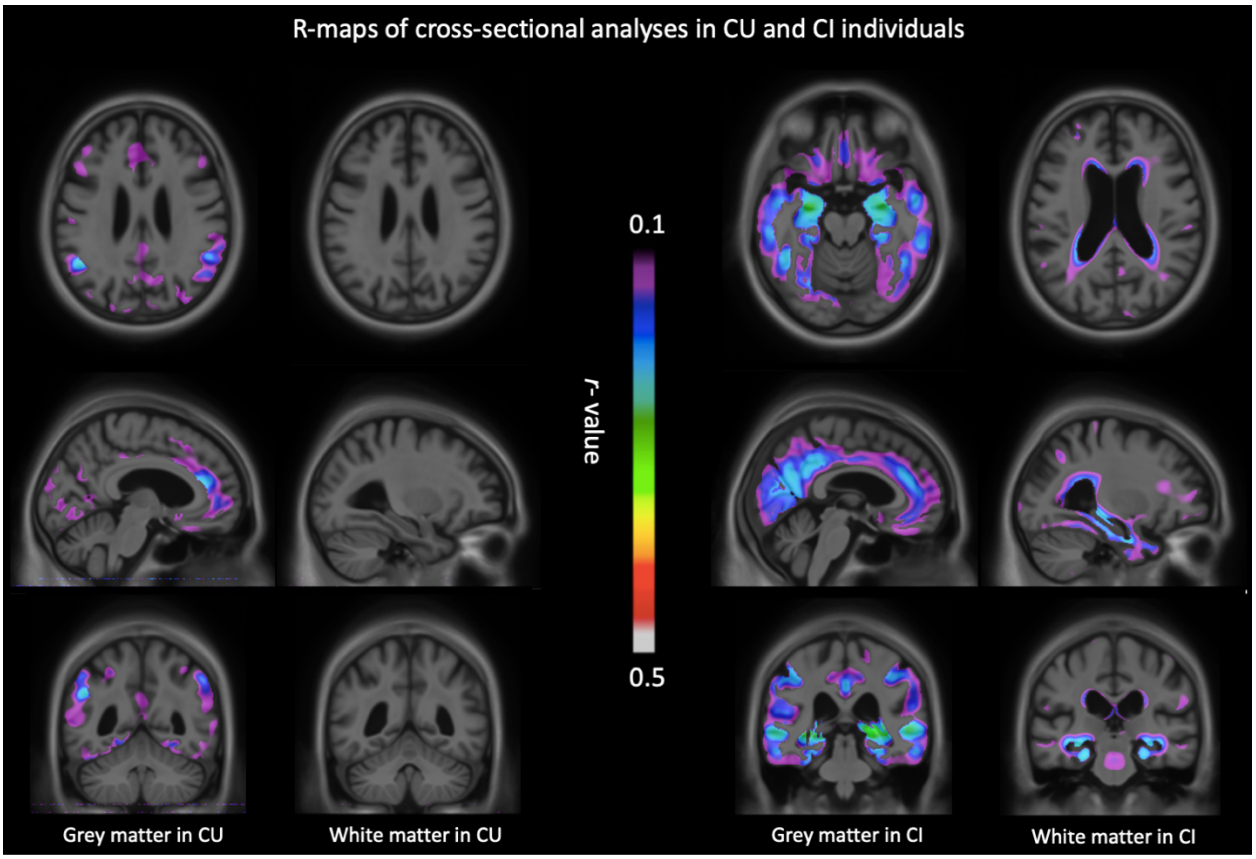
Characteristics	**CU**	**CI**
Number of subjects	384	738
Age (mean, SD) in years	74.40 (6.50)	73.61 (7.94)
Females (n, %)	205 (53%)‡	311 (42%)‡
MMSE score (mean, SD)	29.06 (1.23) †	26.36 (3.68) †
Plasma pTau181 (mean, SD)	15.48 (10.02) †	19.80 (10.75) †

‡Statistical difference between groups (P<0.05).
†Statistical difference between groups (P<0.001).

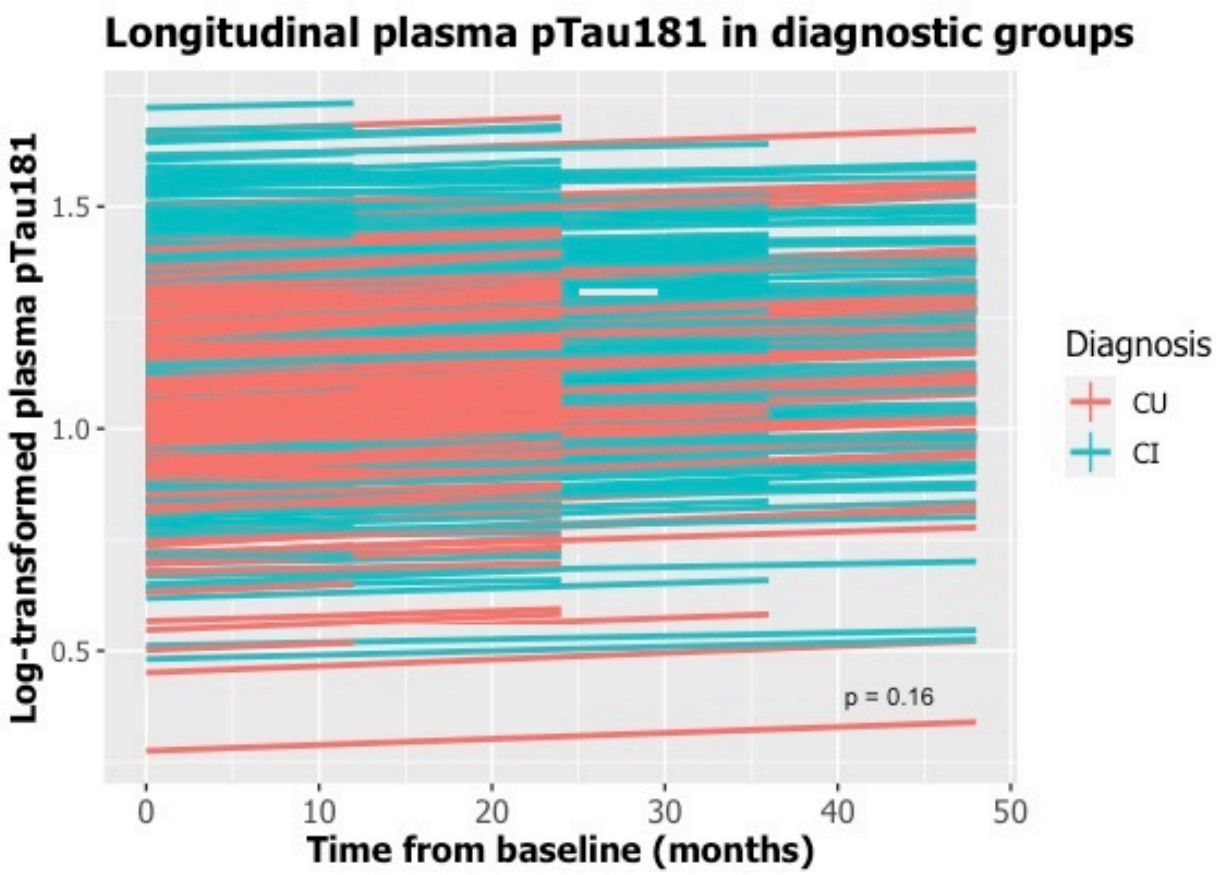
Table 2: Characteristics of participants included in the study. The sample was composed of 384 cognitively unimpaired individuals, and 738 cognitively impaired, among which 539 were diagnosed with mild cognitive impairment and 199 with probable Alzheimer's disease.

2.10 Supplementary material

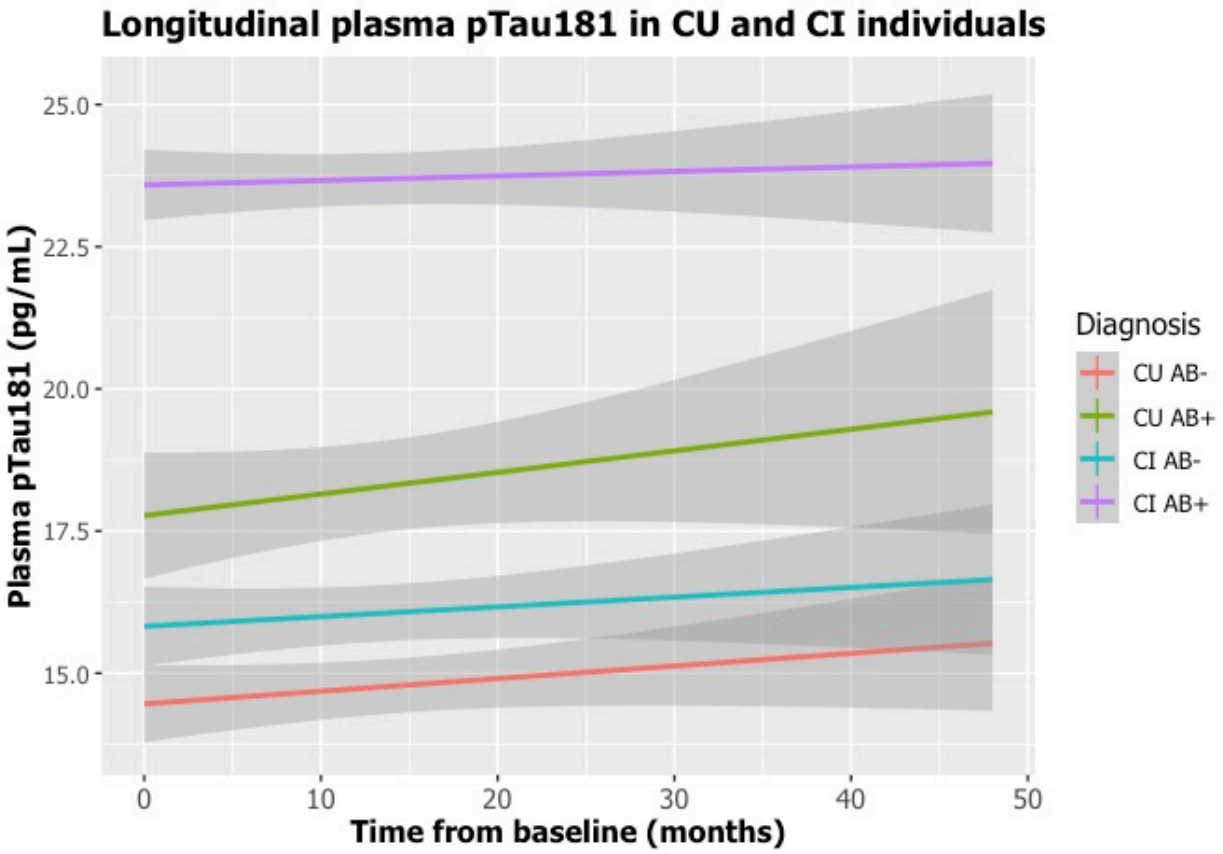
Supplementary figures:



Supplementary Figure 1: Correlations maps (R-maps) of cross-sectional analyses, in both CU and CI groups.



Supplementary Figure 2: Longitudinal changes of plasma pTau181 in each individual.



Supplementary Figure 3: Longitudinal plasma pTau181 changes among cognitively unimpaired and cognitively impaired individuals, stratified by Aβ status.

Supplementary tables:

Characteristics	**CU**	**CI**
Number of subjects	354	685
Age (mean, SD) in years	74.59 (6.61)	73.30 (7.84)
Females (n, %)	188 (53%)‡	290 (42%)‡
MMSE score (mean, SD)	29.06 (1.18) †	27.11 (2.59) †
Plasma pTau181 (mean, SD)	15.35 (9.73) †	19.62 (10.74) †
Aβ status (positive, %)	72 (20%) †	343 (50%) †

‡Statistical difference between groups ($P = 0.001$).
†Statistical difference between groups ($P<0.001$).

Supplementary Table 1: Longitudinal plasma pTau181 changes among cognitively unimpaired and cognitively impaired individuals, stratified by Aβ status.

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Chapter 3: Comparing tau status determined via plasma pTau181, pTau231 and [^{18}F]MK6240 tau-PET.

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3.1 Preface

The A/T/(N) framework has been created to follow the progression of AD-pathophysiological changes (Jack & Holtzman, 2013). Current biomarkers used in the A/T/(N) framework are based on CSF, PET or MRI imaging assessments. However, the methodologies are expensive, invasive, require trained personnel and special equipment, which hampers their use in the clinical setting, and increases drastically the prices of clinical trials. Recent research has been focusing on blood-based biomarkers for AD, which could be easily implemented in the diagnostic and clinical trials settings (Teunissen et al., 2022). CSF, PET and MRI imaging methods are considered better proxies of brain pathology or pathological changes. On the contrary, even though blood markers are highly sensitive, they still cross the blood-brain barrier and are thus more exposed to degradation by proteases, kidney clearance and liver metabolism (Zetterberg & Blennow, 2021).

The second chapter of this thesis allowed us to know plasma markers of tau, more specifically plasma pTau181, is able to track neurodegeneration in the aging and AD brain (Tissot et al., 2021). Importantly, this marker of tau pathology, as well as other newly discovered ones, have shown high specificity for AD (Ashton et al., 2021; Karikari, Pascoal, et al., 2020; Suárez-Calvet et al., 2020). This leads to the idea that plasma markers could be used in the A/T/(N) framework of AD. They could be helpful in knowing the pathological progression of each AD hallmark, in addition to diagnosing the disease.

The advances of biomarker research discussed throughout the thesis have led to the discovery of multiple methods to assess tau. As we try to indicate the best way to diagnose and track the disease progression via the A/T/(N) framework, we need to investigate whether all markers of tau assess the NFT status in a somewhat similar manner. Even though tau pathological progression is a

continuum, it is interesting to categorize individuals as tau positive or tau negative, as it gives insight on the likelihood of someone to develop AD dementia. In Chapter 3, we compared tau statuses as given by plasma pTau (pTau231 and pTau181) and tau-PET. We first indicated whether someone was deemed tau positive based on one of those markers. We then assessed the degree of concordance and discordance among those tau biomarkers' statuses. Thereafter, we evaluated a variety of risk factors and AD hallmark measures, and how they differ based on these categorization groups.

3.2 Abstract:

Background: Tau in Alzheimer's disease (AD) is assessed via cerebrospinal fluid (CSF) and Positron emission tomography (PET). Novel methods to detect phosphorylated tau (pTau) in blood have been recently developed. We aim to investigate agreement of tau status as determined by [^{18}F]MK6240 tau-PET, plasma pTau181 and pTau231.

Methods: We assessed cognitively unimpaired young, cognitively unimpaired, mild cognitive impairment and AD individuals with [^{18}F]MK6240, plasma pTau181, pTau 231, [^{18}F]AZD4694 amyloid-PET and MRI. A subset underwent CSF assessment.

We conducted ROC curves to obtain cut-off values for plasma pTau epitopes. Individuals were categorized as positive or negative in all biomarkers. We then compared the distribution among concordant and discordant groups in relation to diagnosis, Aβ status, *APOE*ε4 status, [^{18}F]AZD4694 global SUVR, hippocampal volume and CSF pTau181.

Findings: The threshold for positivity was 15.085 pg/mL for plasma pTau181 and 17.652 pg/mL for plasma pTau231. Most individuals had concordant statuses, however, 18% of plasma181/PET, 26% of plasma231/PET and 25% of the pTau231/pTau181 were discordant. Positivity to at least one biomarker was often accompanied by diagnosis of cognitive impairment, Aβ positivity, *APOE*ε4 carriership, higher levels of [^{18}F]AZD4694 global SUVR, hippocampal atrophy and CSF pTau181.

Interpretation: Plasma pTau181, pTau231 and [^{18}F]MK6240 seem to reflect different stages of tau progression. Plasma biomarkers can be useful in the context of diagnostic information and clinical trials, to evaluate the disease stage. Moreover, they seem to confidently evaluate tau-PET positivity.

Funding: This study was supported by Weston Brain Institute, Canadian Institute of Health Research and Fonds de Recherche du Québec.

Keywords: tau, plasma, positron emission tomography, Alzheimer's disease.

Research in context:

Evidence before this study:

Assessment of tau *in vivo* is done through positron emission tomography (PET) or cerebrospinal fluid (CSF) assessment. However, those methods are costly and invasive. Research is now focusing on blood-based biomarkers to have an efficient and inexpensive way to assess tau pathology rapidly. Previous work was done on the correlation between plasma phosphorylated-tau (pTau) and tau-PET. However, no study compared the concordance and discordance of tau status, depending on the tau biomarker assessed, either using plasma pTau epitopes or tau-PET. In this study, we compared tau status assessed with plasma pTau231 and pTau181 and [^{18}F]MK6240 tau-PET.

Added value of this study:

The current work demonstrated that most individuals have concordant statuses. This implies assessments of tau seem to confidently evaluate tau presence in the brain of individuals along the Alzheimer's disease spectrum. Additionally, there was an important proportion of individuals showing discrepancy, *i.e.* they were negative to one biomarker but positive to another. This leads to the idea that plasma pTau231, pTau181 and tau-PET reflect different stages of tau progression.

Implications of all available evidence:

Even though statuses using either plasma pTau231, pTau181 or tau-PET did not present perfect concordance, it corroborates a study conducted using CSF demonstrating that biofluid markers are earlier predictors of tau pathology, as compared to tau-PET. Longitudinal analyses are required to assess the disease biomarker trajectories in the plasma. However, our study emphasizes the

importance of plasma assessment, and supports its use in clinical and diagnostic settings to assess tau pathology.

3.3 Introduction:

The core characteristics of Alzheimer's disease (AD) are the accumulation of amyloid-β (Aβ) plaques and phosphorylated tau (pTau) tangles, and plaque-surrounding neurites in the brain, then leading to neurodegeneration(Jack, Bennett, et al., 2018). Positron emission tomography (PET) imaging and cerebrospinal fluid (CSF) assessments are used to detect the presence of AD pathologies *in vivo*. Due to the high cost and perceived invasiveness of these methods, recent research has been focusing on blood-based biomarkers of AD to diagnose and facilitate clinical trial recruitment(Hampel et al., 2018). It was recently demonstrated that ultra-sensitive assays for tau phosphorylated at threonine-181 (pTau181) and threonine-231 (pTau231) in plasma(Ashton et al., 2021; Karikari, Pascoal, et al., 2020; Mielke et al., 2018; Tatebe et al., 2017; Yang et al., 2018) provide an inexpensive way to determine the presence of brain neurofibrillary tangles *in vivo*.

However, recent studies also provided evidence of variability in the biomarker status depending on the method used(Mattsson et al., 2020), which also seems to depend on the clinical stage. CSF Aβ has been suggested to precede Aβ-PET positivity(Palmqvist et al., 2016). Similarly, further evidence supports the idea that CSF pTau181 precedes tau-PET positivity(Meyer et al., 2019). Plasma biomarkers seem to coincide with CSF results more closely than with PET biomarkers(Palmqvist et al., 2019).

Plasma assessments of pTau are promising tools to aid in the diagnosis and clinical management of patients with cognitive impairment, though many questions remain(Thijssen & Rabinovici, 2021). One important question is the degree to which elevated concentrations of different plasma pTau epitopes deliver similar information, and predict tau positivity status as determined by PET.

Here we investigate the concordance and discordance of plasma pTau181, plasma pTau231 and [^{18}F]MK6240 tau-PET biomarker statuses.

3.4 Methods

Study participants and Ethics:

Data was obtained from the TRIAD cohort(Therriault, Benedet, Pascoal, Ashton, et al., 2020), from October 2017 to February 2020. The project was approved by the Douglas Institute Research Ethics Board and written consent was obtained from all participants (Protocols: IUSMD 16-60 and 16-61). 284 individuals (30 cognitively unimpaired young (CUY), 162 cognitively unimpaired (CU), 60 Mild Cognitive Impairment (MCI) and 32 AD) underwent plasma pTau181 and pTau231 assessments, [^{18}F]MK6240 tau-PET, [^{18}F]AZD4694 amyloid-PET, MRI, and a neuropsychological evaluation. Among them, 151 participants were also subjected to CSF pTau181 assessment (22 CUY, 79 CU, 34 MCI and 16 AD). Details on the information gathered from participants can be found here: https://triad.tnl-mcgill.com/. CU individuals are defined as having no cognitive impairment(Jack, Wiste, et al., 2018). Consistent with the biological AD research framework from the National Institute of Aging-Alzheimer's Association(McKhann et al., 2011), participants without a diagnosis of MCI or AD with subjective memory complaints were analyzed with CU individuals. In addition to standard clinical assessments, Mini Mental State Examination (MMSE) and Clinical Dementia Rating (CDR) total scores were used to define MCI operationally as a total MMSE score of 26 or above and a global CDR of 0.5(Petersen, 2004), and dementia due to AD as MMSE lower than 26 and a global CDR above 0.5(McKhann et al., 2011). No participant met the criteria for another neurological or major neuropsychiatric disorder.

PET processing:

PET acquisition and processing of [^{18}F]MK6240 and [^{18}F]AZD4694 can be found elsewhere(Pascoal et al., 2020).

A composite mask including the entorhinal, amygdala, fusiform, inferior and middle temporal cortices was used to calculate [^{18}F]MK6240 temporal meta-ROI SUVR. Those regions are said to capture the changes associated with AD(Meyer et al., 2019; Ossenkoppele et al., 2018). We used a published threshold of 1.24 temporal meta-ROI SUVR(Therriault et al., 2021) to determine tau-PET positivity. In this study, the authors set the threshold by calculating the mean SUVR + 2 standard deviations from the CUY population. A global [^{18}F]AZD4694 SUVR value was estimated by averaging the SUVR from the precuneus, prefrontal, orbitofrontal, parietal, temporal, anterior and posterior cingulate cortices(Jack et al., 2017). The cut-off value for positivity was above a published threshold of 1.55(Therriault, Benedet, Pascoal, Ashton, et al., 2020) global SUVR, used to classify participants as Aβ positive (Aβ+) or Aβ negative (Aβ-). Finally, hippocampal volume was also extracted from MRI images using FreeSurfer.

Biofluid measurements:

All plasma pTau biomarkers were measured using *in-house* Single Molecular Array (Simoa) methods Simoa HD-X instruments (Quanterix, Billerica, MA, USA). Methods were described in the supplementary material, and further detailed elsewhere(Ashton et al., 2021; Karikari, Pascoal, et al., 2020). CSF pTau181 was measured via Lumipulse, at the Clinical Neurochemistry Laboratory, University of Gothenburg, Mölndal, Sweden, by scientists blinded to participants' clinical information.

Statistical analyses:

Receiver Operating Characteristic (ROC) curves analyses were performed to assess the optimal cut-off value for plasma pTau181, and pTau231. CUY were considered as the healthy group, contrasted with AD (Youden Index). We used the CUY as the healthy group as it is known that tau pathology is also related to aging, thus can be observed in CU elderlies(Jagust, 2018; Tissot et al., 2021). CUY were not used in subsequent analyses. Exploratory analyses were also conducted using CU as the healthy group, contrasted with AD. Each individual was categorized as positive or negative in all biomarkers. We obtained four groups: concordant plasma pTau negative / PET negative (Plasma-/PET-), discordant plasma pTau positive / PET negative (Plasma+/PET-), discordant plasma pTau negative / PET positive (Plasma-/PET+) and concordant plasma pTau positive / PET positive (Plasma+/PET+). In the case of plasma pTau231 and pTau181 analyses, the four groups were: concordant negative (pTau231-/pTau181-), discordant plasma pTau231 positive / pTau181 negative (pTau231+/pTau181-), discordant plasma pTau231 negative / pTau181 positive (pTau231-/pTau181+) and concordant positive (pTau231+/pTau181+).

We conducted Spearman correlation analysis between [^{18}F]MK6240 SUVR, plasma pTau181 and pTau231. Using ANOVA and chi-square tests when appropriate, we compared the demographic variables in all groups, and calculated the coefficient of variation.

Further ROC curves were conducted to see how plasma pTau epitopes predicted Aβ-PET positivity.

Role of funders: This work was supported by the Weston Brain Institute, Canadian Institute of Health Research (CIHR), and Fonds de recherche du Québec – Santé. None of the funders had a role in the study design, data collection, data analyses, interpretation or writing of the report.

3.5 Results:

Demographics:

There were significant differences between the diagnostic groups in terms of age, plasma pTau181 and pTau231 levels, temporal meta-ROI SUVR, hippocampal volume, *APOE*ε4 genotype and CSF pTau181. From CU to AD, individuals had higher levels of plasma pTau181, pTau231, CSF pTau181 and temporal meta-ROI [^{18}F]MK6240 SUVR, as well as lower hippocampal volume. Moreover, *APOE*ε4 carriership was more common in individuals with cognitive impairment (either MCI or AD). Similarly, there was a slightly significant difference in the years of education, being higher in CUY as compared to the other diagnostic groups. However, there was no statistically significant difference in terms of sex (Table1). Similar results were observed in the subgroup that underwent CSF assessment (Supplementary Table 1). Moreover, the coefficient of variation (Supplementary Table 2) showed high variation biofluid measures (CSF and plasma), as compared to low variation in imaging (MRI and PET).

Discrepancies between statuses of plasma pTau231, pTau181 and tau-PET:

CUY were only used for the calculation of cut-off values. Using ROC curves (contrasting CUY versus AD – supplementary figure 1), we determined that the cut-off value for positivity for plasma pTau181 was 15.085 pg/mL, and the value for plasma pTau231 was 17.652 pg/mL, using *in vitro* phosphorylated full-length recombinant tau 441 in both cases(Ashton et al., 2021; Karikari,

Pascoal, et al., 2020). When using CU as the healthy group, the cut-off value did not differ for plasma pTau181. Even though the cut-off for plasma pTau231 was higher, it did not impact the results of this study. In the exploratory analyses, we calculated the area under the curve for sensitivity and specificity of plasma pTau231 and pTau181 to evaluate Aβ positivity as assessed via [^{18}F]AZD4694. Analyses revealed that plasma pTau epitopes have acceptable AUC to discriminate between amyloid statuses.

Plasma181/PET demographics can be observed in Table 2, plasma231/PET in Table 3 and finally pTau231/181 in Table 4. In all analyses, we observed significant differences in diagnostic groups regarding plasma pTau181 and pTau231 levels, temporal meta-ROI SUVR, hippocampal volume, *APOE*ε4 presence, and CSF pTau181, while no significant differences in age, sex or years of education.

Significant correlations were observed between [^{18}F]MK6240 SUVR in the temporal meta-ROI and plasma pTau181 ($R = 0.48$, $p < 0.001$[Spearman correlation]) (Figure 1a), and pTau231 ($R = 0.49$, $p < 0.001$ [Spearman correlation]) (Figure 1b), as well as between plasma pTau231 and pTau181 ($R = 0.60$, $p < 0.001$ [Spearman correlation]) (Figure 1c).

For 82% of individuals, the plasma181 and tau-PET assessment methods were in agreement with respect to their tau status. Among the cases where there was a discordance, plasma181+/PET- was observed more frequently. Looking more closely at the plasma181-/PET+ individuals, we observed the majority were cognitively impaired (CI), *i.e.,* MCI or AD. In the plasma231/PET plot, 76% of the individuals were also concordant in terms of their tau status. Additionally, 20% were considered plasma231+/PET-; with a high proportion of CU individuals. The plasma231-/PET+ group was in turn comprised of a high number of CI participants. In 75% of cases, both

plasma pTau231 and pTau181 produced concordant estimates of tau status. Among the discordant results, the proportion of pTau231+/pTau181- was larger than the proportion of pTau231-/pTau181+; the latter was mainly composed of CI individuals.

Demographics in relation to tau statuses:

We first investigated the distribution of diagnostic groups in relation to tau statuses. Plasma181/PET analyses (Figure 2a) revealed that 83% of plasma181-/PET- were CU individuals, while 90% of plasma181+/PET+ were CI. However, we observed that some MCI individuals were considered negative to both tau biomarkers (42% of MCI), and some CU were positive to plasma pTau181 and tau-PET (3% of CU). Among the individuals with tau status discordance, 77% of plasma181+/PET- were CU, while 65% of plasma181-/PET+ were CI. Plasma231/PET analyses showed a similar pattern (Figure 2b) in which 83% of plasma231-/PET- were CU and 86% of plasma231+/PET+ were CI. Nonetheless, in the plasma231-/PET- group, 2% of individuals were AD and 16% MCI, while in the plasma231+/PET+, 14% were CU. Among discordant tau status groups, 82% of plasma231+/PET- individuals were CU with the remaining 16% being MCI and 2% being AD. Finally, 75% of the plasma231-/PET+ group was CI (50% MCI and 25% AD). Plasma pTau231/pTau181 analyses also showed a pattern (Figure 2c) in which 75% obtained concordance in their tau status, and the highest proportion of discrepant individuals was in the pTau231+/pTau181-. In this group, 74% were CU; while they were 56% in pTau231-/pTau181+. When combining diagnosis and Aβ status, we observed that majority of plasma181-/PET- individuals were CU-Aβ- (68%), the remaining being CU-Aβ+ (15%), MCI (Aβ- (11%), Aβ+ (5%)) and AD-Aβ+ (1%) (Figure 2d). Plasma181+/PET+ individuals were mainly composed of CI individuals showing Aβ positivity (2% MCI-Aβ-, 41% MCI-Aβ+, 47% AD-Aβ+). Among the

plasma181/PET groups (Figure 2d), the one with the biggest proportion of CU-Aβ+ individuals was plasma181+/PET-; it is also important to note that the CU-Aβ+ group was often positive for at least one tau biomarker. Finally, cognitive impairment was usually accompanied by tau-PET positivity (plasma181-/PET+). Regarding plasma231/PET statuses, 76% of plasma231-/PET- individuals were CU-Aβ- (Figure2e). One AD-Aβ+ individual was considered plasma231-/PET-. We observed a high proportion of CI-Aβ+ individuals in the plasma231+/PET+ analyses (42% MCI-Aβ+ and 42% AD-Aβ+). Among the individuals with discrepant tau results, CU-Aβ+ were often part of the plasma231+/PET- group. Additionally, CI individuals categorized as plasma231+/PET- were 16% MCI (12% Aβ-, 4% Aβ+) and 2% AD-Aβ+. 75% of the plasma231-/PET+ group were CI individuals (50% MCI-Aβ+, 25% AD-Aβ+). In terms of plasma comparisons, we observed that plasma pTau231-/pTau181- individuals were mainly CU-Aβ- (71%), with some CU-Aβ+ (8%), and a small proportion of MCI (10% Aβ-, 9% Aβ+) and AD-Aβ+ (2%). Conversely, 74% of the pTau231+/pTau181+ participants were categorized as CI (5% MCI-Aβ-, 32% MCI-Aβ+, 38% AD-Aβ+) (Figure2f). In the pTau231+/pTau181- group, we mainly observed Aβ+ individuals (37% CU-Aβ+, 13% MCI-Aβ+). Lastly, the pTau231-/pTau181+ group had a high proportion of CI individuals, showing Aβ positivity (24% MCI-Aβ+ and 18% AD-Aβ+). In all three analyses, when presenting cognitive impairment and/or Aβ positivity, individuals had a tendency to be positive to at least one tau-biomarker. A table summarizing all the percentage of diagnosis and diagnosis combined with Aβ status can be found in the supplementary material (Supplementary table3).

APOE genotype was assessed in a subgroup of 245 individuals. The plasma181/PET analyses showed an incremental relationship in the proportion of *APOE*ε4 carriers, heterozygous or

homozygous (Figure 2g). Indeed, 25% of plasma181-/PET- carried at least one *APOE*ε4 allele, as compared to 60% of plasma181+/PET+. *APOE*ε4 status followed tau-PET positivity more closely than plasma positivity, with 55% of plasma181-/PET+ having at least one *APOE*ε4 allele, and only 25% in the plasma181+/PET- group. Plasma231/PET analyses revealed that plasma231-/PET- had a low proportion (77%) and plasma231+/PET+ had a high proportion (58%) of *APOE*ε4 carriers (Figure 2h). *APOE*ε4 status, in this case too, seemed to correlate with tau-PET positivity closely, with 60% of plasma231-/PET+ and 58% of plasma231+/PET+ being *APOE*ε4 carriers. Finally, in the plasma pTau231/pTau181 analyses, concordant negative individuals were mainly not *APOE*ε4 carriers (74%), while concordant negative were mainly *APOE*ε4 carriers (53%) (Figure2i). The discordant groups had a slightly high proportion of *APOE*ε4 carriers: 36% in pTau231+/pTau181- and 31% in pTau231-/pTau181+.

AD biomarkers in relation to tau statuses:

We first examined the Aβ status distribution in the different tau-assessment groups, based on [^{18}F]AZD4694 SUVR(Therriault, Benedet, Pascoal, Ashton, et al., 2020). We observed that 80% of plasma181-/PET- were Aβ-, while 96% of plasma181+/PET+ were Aβ+ (Figure 3a). Among the cases with a single positive tau biomarker (plasma181+/PET- and plasma181-/PET+), we observed a high percentage of Aβ+ individuals (48% for plasma181+/PET- and 90% for plasma181-/PET+), as compared to the plasma181-/PET- group. Similarly, in the plasma231/PET analyses, we observed a high proportion of Aβ- individuals in the plasma231-/PET- (87%), and Aβ+ individuals in the plasma231+/PET+ (94%) (Figure 3b). Individuals with discrepant tau statuses had a 50% risk of being Aβ+ for plasma231+/PET-, and 94% in the plasma231-/PET+. In both plasma/PET analyses, PET+ individuals had a significantly higher risk of being Aβ+,

independently of the plasma status. Finally, 82% of the pTau231-/pTau181- were also categorized as Aβ- (Figure3c). Comparatively, 88% of the pTau231+/pTau181+ were Aβ+. The groups showing discrepancy in terms of tau statuses had similar results, meaning 54% of the pTau231+/pTau181- and 53% of the pTau231-/pTau181+ had a positive Aβ status.

We compared the [^{18}F]AZD4694 global SUVR levels in each group. Plasma181/PET (Figure 3d) and plasma231/PET (Figure 3e) analyses revealed significant differences in [^{18}F]AZD4694 SUVR among all the groups, except between the plasma-/PET+ and plasma+/PET+ groups. The pTau231/pTau181 analyses revealed that there were significant differences among all groups, except when individuals had discrepant tau results (Figure3f).

We then investigated hippocampal volume results in each group. Plasma181/PET analyses revealed significant differences among all groups, except between the discrepant groups (plasma181+/PET- and plasma181-/PET+) (Figure3g). Similarly, plasma231/PET showed significant differences between groups, except for plasma231+/PET- and plasma231-/PET+ as well as plasma231-/PET+ and plasma231+/PET+ (Figure3h). In the case of pTau231/pTau181, the groups not presenting a statistically significant difference were pTau231-/pTau181- and pTau231+/pTau181- as well as pTau231+/pTau181- and pTau231-/pTau181+ (Figure3i).

Finally, a subgroup of 129 individuals underwent CSF pTau181 assessment, among which 79 CU, 34 MCI and 16 AD. Among the plasma181/PET analyses, we did not obtain significant differences between plasma181-/PET- and plasma181+/PET- as well as plasma181-/PET+ and plasma181+/PET+ (Figure3j). The remaining group comparisons had significant differences. Plasma231/PET revealed significant differences among all groups except between plasma231-/PET+ and plasma231+/PET+ (Figure3k). Lastly, in the pTau231/pTau181 analyses, we discovered statistically significant difference between pTau231-/pTau181- and pTau231-

/pTau181+ as well as pTau231+/pTau181- and pTau231-/pTau181+ (Figure3l). In the remaining group comparisons, we obtained statically significant differences.

3.6 Discussion:

The current study sought to compare the concordance and discordance of plasma pTau181, pTau231 and [^{18}F]MK6240 SUVR positivity in a well-characterized cohort study of aging and AD. In all cases, the rates of concordance were higher than the rates of discordance. The highest rate of concordance was between plasma pTau181 and tau-PET. Discrepant groups differed between the plasma181/PET, plasma231/PET and pTau231/pTau181 statuses, suggesting plasma pTau231, plasma pTau181 and tau-PET abnormality reflect different stages of tau pathology progression. Positivity for one tau biomarker was often accompanied by cognitive impairment, Aβ-PET positivity status, and elevated hippocampal atrophy and CSF pTau181 levels, as well as higher risk of carrying at least one *APOE*ε4 allele.

Previous work on CSF revealed that, among the groups presenting discrepant tau results, CSF pTau abnormality was more common than tau-PET abnormality(Meyer et al., 2019). Moreover, other studies reported that CSF pTau epitopes seemed to appear at different stages of the disease(Barthélemy et al., 2020; Suárez-Calvet et al., 2020). We found a similar pattern for plasma pTau181 and pTau231. Differences observed in the statuses of plasma pTau231, pTau181 and tau-PET suggest distinct stages of tau continuum. In both plasma/PET analyses, the plasma-/PET+ group was the smallest, and individuals often had cognitive impairment. Plasma pTau231 and pTau181 are known to be specific to AD(Ashton et al., 2021; Karikari, Pascoal, et al., 2020), while tau-PET can also be observed in other tauopathies(Leuzy et al., 2019). However, most CI

individuals in the plasma-/PET+ groups were categorized as Aβ+, one of the core characteristics of AD(Jack, Bennett, et al., 2018), suggesting they are also on the AD spectrum. Additionally, plasma+/PET- individuals were mostly cognitively unimpaired individuals, some presenting a positive Aβ status. We observed a higher proportion of discordant individuals in the plasma231/PET analyses, as compared to plasma181/PET. Specifically, there were more plasma231+/PET- individuals, as compared to plasma181+/PET- individuals. Finally, there was some discordance among the plasma epitopes. Plasma pTau231+/pTau181- group was more common as compared to pTau231-/pTau181+. The first group showed Aβ positivity, when the latter individuals usually presented cognitive impairment accompanied by Aβ positivity. When combining both plasma biomarkers, we observed a high rate of pathological as well as cognitive signs of AD. This suggests that even plasma pTau181 and pTau231 reflect different stages of tau continuum, potentially extending species-specific phosphorylation differences in CSF(Barthélemy et al., 2020). Using plasma pTau, our study extends recent CSF biomarker modeling studies which provide evidence that CSF pTau231 abnormality precedes CSF pTau181 abnormality(Ashton et al., n.d.; Suárez-Calvet et al., 2020). This follows the framework in which plasma biomarkers are early detectors of AD pathology(Palmqvist et al., 2019). Tau-PET has been proven effective in providing information regarding the risk of clinical deterioration in the following months(Lu et al., 2021). Having a blood-based assessment giving a strong predictive value of tau-PET status would be critical for both clinical trials and diagnostic settings (Gauthier et al., 2020).

Individuals negative to all tau assessment methods, plasma pTau181, pTau231 and tau-PET were mainly CU-Aβ-, not *APOE*ε4 carriers, with low levels of [^{18}F]AZD4694 global SUVR, hippocampal atrophy and CSF pTau181. Conversely, when individuals were positively concordant in all tau assessment methods, they were often MCI-Aβ+ or AD-Aβ+, with at least one *APOE*ε4

allele, and high levels of [^{18}F]AZD4694 global SUVR, hippocampal atrophy and CSF pTau181. The majority of plasma (231 or 181)+/PET- individuals are either CU-Aβ+ and CI-Aβ+ individuals, which might reflect early stages of the disease. In these cases, tau levels assessed via [^{18}F]MK6240 PET may be below the threshold for positivity. Interestingly, plasma pTau231 positivity, more common than plasma pTau181 positivity, was often observed in individuals categorized as CU-Aβ+.

It has been proposed that plasma pTau181(Karikari, Pascoal, et al., 2020) and pTau231(Ashton et al., 2021) are predictors of AD dementia, and differentiate it from other types of dementia. Conversely, tau-PET is thought to bind to neurofibrillary tangles in the brain. The discordancy may thus result from the difference between the methods, rather than being truly discordant.

Presence of at least one *APOE*ε4 allele is a known risk factor for developing AD(Zhao et al., 2018). Even though research mostly linked it to the presence of Aβ, recent studies focused on its relationship with tau. It has been demonstrated that *APOE*ε4 acts on several mechanisms, including decreasing the clearance of Aβ in the brain, thus leading to higher brain levels of Aβ as well as tau(Therriault, Benedet, Pascoal, Mathotaarachchi, et al., 2020). In both plasma/PET analyses, we observed that *APOE*ε4 presence closely correlated with tau-PET positivity, as having at least one *APOE*ε4 was associated with more than a 50% chance of being tau-PET positive. Concerning the pTau231/pTau181 analyses, the concordant negative and discordant groups had similar results, revealing they had around a 25% risk of having at least one *APOE*ε4. However, more than 50% of the individuals in the pTau231+/pTau181+ group had at least one *APOE*ε4.

We also investigated the relationship between the plasma231 and plasma181/PET and pTau231/pTau181 groups with established AD biomarkers. Plasma biomarkers, in combination with clinical and demographic information, have been proposed to help in the detection of Aβ positivity(Tosun et al., 2021). Our study corroborates this idea, demonstrating that positivity to one tau biomarker correlates with a higher risk of being Aβ positive. Other studies already presented the strong relationship between Aβ and the three biomarkers independently (Ashton et al., 2021; Karikari, Pascoal, et al., 2020). Individuals that obtained a concordant positive tau status for all assessment methods were almost exclusively Aβ positive, while individuals with concordant negative tau were almost exclusively Aβ negative. Accepted biomarker models of AD propose that Aβ accumulation arises before the presence of tau aggregates(Hardy & Selkoe, 2002), and is thus considered an early marker of the disease. The results of both plasma/PET analyses were similar; when individuals were positive to plasma pTau, there was a 50% risk of Aβ positivity, even when obtaining a negative tau-PET. However, when they were tau-PET positive, the risk of being Aβ+ increased dramatically, irrespective of the plasma (231 or 181) status. PTau231/pTau181 analyses revealed that participants had a 50% risk of being Aβ+ when positive to either plasma pTau biomarker. However, combining both results led to an almost certain positive Aβ status. This leads to the hypothesis that tau-PET or the combination of two plasma epitopes, rather than one of pTau231 or pTau181, are great predictors of Aβ status.

When conducting analyses using [^{18}F]AZD4694 global SUVR, we observed that there were no significant differences between the plasma-/PET+ and plasma+/PET+ groups, either using plasma181/PET or plasma231/PET. All other groups had a statistically significant different [^{18}F]AZD4694 SUVR. We noticed a strong variability in the discrepant groups, emphasizing the idea that some individuals might not be on the AD spectrum, while others could be at early disease

stages, with a certain build-up of pathology without cognitive impairment(Chételat et al., 2013; Price & Morris, 1999). Regarding pTau231/181, we observed no significant difference between the discrepant groups. This might be due to the high variability of the pTau levels. We can further hypothesize that combining both biomarkers could be critical in predicting the levels of cortical Aβ, hence be used to predict the advancement of AD pathology(Jack et al., 2010).

We observed that PET status was the best predictor of hippocampal atrophy as all tau-PET+ individuals had low levels of hippocampal volume. It is also important to note that positivity to at least one tau biomarker was related to higher rates of hippocampal atrophy, however, we seemed to obtain similar results when using either pTau231 or 181 combined with tau-PET. Analyses conducted on the comparison between plasma pTau epitopes yet revealed that pTau181 positivity was more closely related to hippocampal atrophy than pTau231. This corroborates the framework in which pTau181 appears at later stages of the disease, when hippocampal atrophy is more prominent(Tissot et al., 2021).

For the established AD biomarker CSF pTau181, rates of concordance and discordance differed widely between analyses. It is important to note that not all participants of the TRIAD cohort underwent a lumbar puncture, lowering the number of individuals in the above results. Plasma biomarkers are thought to closely follow CSF biomarkers in the progress of the disease(Palmqvist et al., 2019). Our study adds to the research framework in which CSF levels of pTau181 are accompanied by abnormal levels of plasma pTau, either 181 or 231, tau-PET, or both, and might reach a plateau at a later disease stage. Again, it seems that the combination of both plasma biomarkers, or tau-PET, was a better predictor of high CSF pTau181 levels.

Importantly, biomarkers assessed in the plasma have crossed the blood-brain barrier (BBB), they are thus at low concentrations as compared to measures in the brain(Snyder et al., 2014). It has been suggested that the BBB is compromised in aging and disease progression(Zipser et al., 2007), leading to an increasing concentration of brain proteins in the plasma as the disease advances. Plasma biomarkers are also known to have a broad coefficient of variation, and may present higher false positive rates, as compared to the more direct assessment of cerebral tau pathology using PET. Plasma assays are a proxy of cortical tau, and do not represent exactly the same components of tau accumulation process as assessed with tau-PET. Moreover, we also focused here on specific phosphorylated sites (i.e. pTau181 and pTau231). Those phosphorylated sites are already thought to appear at different stages of Alzheimer's disease(Barthélemy et al., 2020). Conversely tau-PET assesses neurofibrillary tangles load in the brain(Pascoal et al., 2020), leading to a more direct measure of cortical tau. Because there is this inherent difference, and because similar results were observed when studying the differences between of CSF and tau-PET statuses(Meyer et al., 2019), we do not expect a perfect concordance between tau statuses. Additionally, in our study, ROC curves were conducted based on clinical diagnosis defined through clinical testing. Clinical diagnosis does not perfectly reflect Aβ and tau pathologies at the individual-level. Indeed, recent research showed there is not always a full accordance between the biologically-defined and the clinically-defined AD diagnosis(Therriault et al., 2021).

As new AD therapeutic methods are focusing on Aβ aggregates, we wondered to which degree plasma pTau markers could predict Aβ-PET status. AUC were considered acceptable in discriminating individuals based on Aβ status. As Aβ is known to accumulate years before the onset of clinical symptoms, and appears before tau accumulation(Jack, Bennett, et al., 2018), we expected a strong correlation.

We decided to use here CUY as the reference group to calculate the cut-off values for plasma biomarkers. Brain accumulation of the AD hallmarks is known to be continuous, and CU elderlies tend to show pathology even without cognitive impairment(Jagust, 2018). When using the CU elderlies as the control group, the cut-off was the same for plasma pTau181. For plasma pTau231, we obtained a higher cut-off, however, it did not impact the results observed in this study.

We compared the relationship between tau phospho-forms and as well as their relation to tau-PET status. Even though most individuals had concordant statuses in tau assessment methods, discordant cases were also observed. Analyses comparing plasma231/PET, plasma181/PET and pTau231/pTau181 led to the idea that plasma pTau231, pTau181, and [^{18}F]MK6240 tau-PET reflect distinct aspects of tau accumulation. Our results corroborate a study conducted using CSF pTau epitopes; in autosomal dominant AD, hyperphosphorylation of tau occurred early and exhibited a pattern of site-specific changes at different stages of the disease(Barthélemy et al., 2020; Karikari, Emeršič, et al., 2020; Suárez-Calvet et al., 2020). Longitudinal studies are needed to confirm the ordering of plasma pTau231, pTau181 and tau-PET abnormality. This is potentially useful in clinical trials, in which a plasma test could provide information on the tau pathology stage, rather than using CSF or PET, which are costly and invasive.

The principal strength of the study is the use of a well-characterized cohort of individuals, that underwent gold standard procedures of PET assessment for amyloid-β and tau. Plasma assessments for pTau epitopes also used the most advanced methodologies(Ashton et al., 2021; Karikari, Pascoal, et al., 2020). However, a limitation of our study is the lack of longitudinal measures, which would assess the disease biomarker trajectory. We could also investigate whether

individuals that are positive to one biomarker are more prone to be positive to another one later on, as well as convert to dementia. This could be observed either in individuals that obtained discrepant the plasma/PET results and even between the plasma biomarkers. Moreover, it is important to note that the TRIAD cohort is comprised of a sample of individuals willing to participate in dementia research, thus involving recruitment and sampling biases. Nonetheless, the results show that plasma/PET and pTau231/181 groups correlate well with demographic and clinical information, as well as established biomarkers of AD.

Novel plasma biomarkers and tau-PET measures reflect different stages of tau pathological progression. Even though most measures have concordant statuses, it is thought that plasma biomarkers come at earlier stages of the disease. Positivity to one biomarker is often accompanied by cognitive impairment, presence of Aβ, higher levels of CSF pTau181, as well as higher risk of having at least one *APOE*ε4.

3.7 Declarations

Contributors:

CT: conceptualization, formal analysis, methodology, investigation, writing; JT: conceptualization, methodology, writing, verification of underlying data; PK: conceptualization, validation; ALB: conceptualization, methodology, validation; TKK: validation, investigation, data curation; SS: investigation, data curation; FZL: conceptualization, data curation, verification of underlying data; MC: data curation, project administration; JS: project administration; NR: project administration; NMP: data curation, methodology; VP: data curation, methodology; GB: data curation, software; MSK: software, resources; SSM: software, resources; YTW: data curation; JFA: data curation; PCLF: investigation; JPFS: investigation; EV: methodology; KB: methodology; HZ: methodology; SG: supervision; PRN: writing, supervision. All authors read and approved the manuscript.

Data sharing statement:

All data presented in this study is available upon request to the corresponding author. Data is not publicly available as it contains information that could compromise the privacy of research participants.

Declaration of interests:

Nothing to disclose.

Acknowledgments

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3.8 Figures:

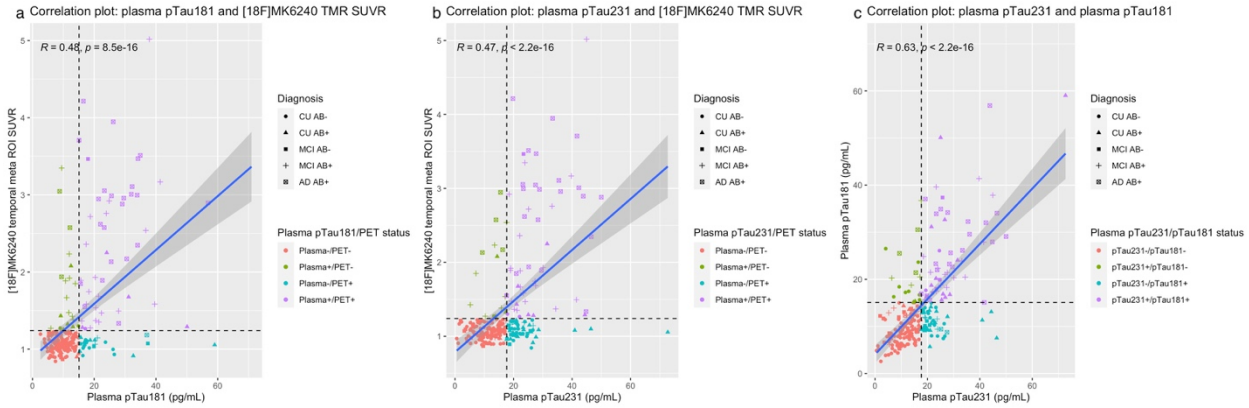


Figure1: Correlation plots (n = 254). a. Correlation between plasma pTau181 and [^{18}F]MK6240 temporal meta-ROI SUVR (R = 0.48, p <0.001), stratified by status and diagnosis. b. Correlation between plasma pTau231 and [^{18}F]MK6240 temporal meta-ROI SUVR (R = 0.49, p <0.001) stratified by status and diagnosis. c. Correlation between plasma pTau231 and pTau181 (R = 0.60, p <0.001) stratified by status and diagnosis.

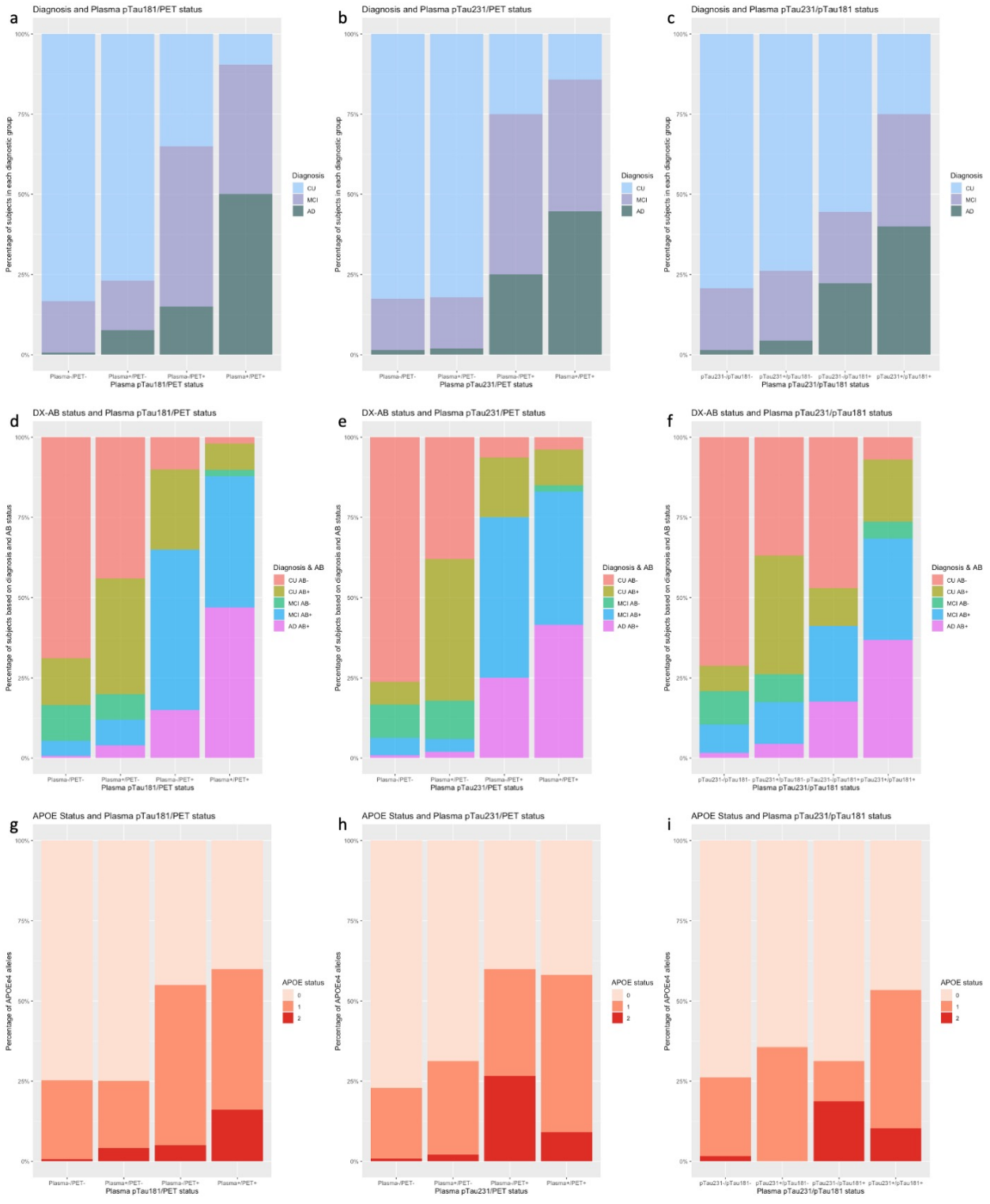


Figure2: Demographic information in relation to plasma/PET statuses (n = 254). a. Diagnosis in plasma181/PET b. Diagnosis in plasma231/PET c. Diagnosis in plasma pTau231/pTau181 d.

Diagnosis and Aβ status in plasma181/PET e. Diagnosis and Aβ status in plasma231/PET f. Diagnosis and Aβ status in plasma pTau231/pTau181 g. *APOE*ε4 status in plasma181/PET and h. *APOE*ε4 status in plasma231/PET. i. *APOE*ε4 status in plasma pTau231/pTau181.

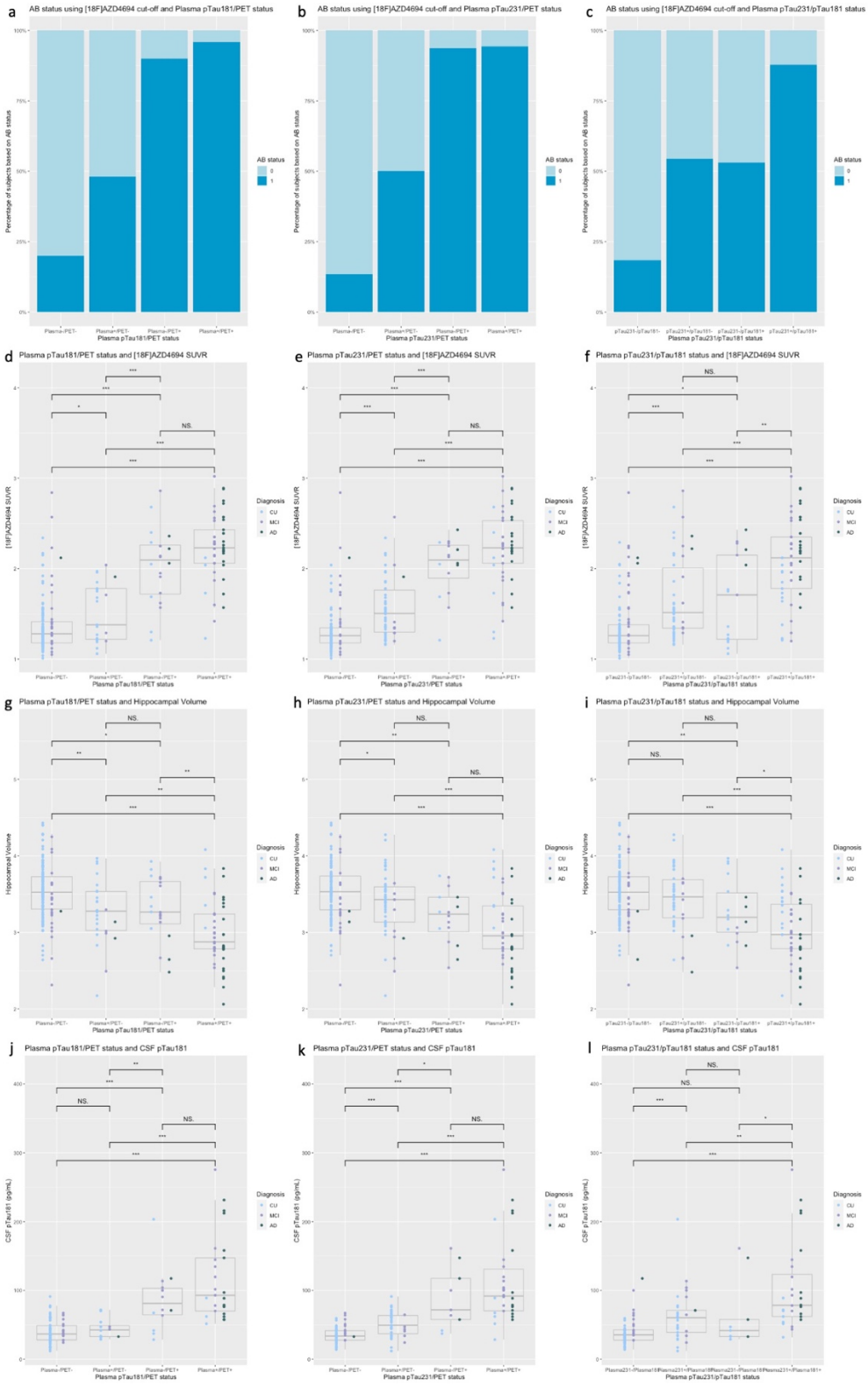


Figure3: Alzheimer's disease biomarkers in relation to plasma/PET statuses (n = 254). a. Aβ status in plasma181/PET b. Aβ status in plasma231/PET c. Aβ status in plasma pTau231/pTau181. d.

[^{18}F]AZD4694 global SUVR in plasma181/PET e. [^{18}F]AZD4694 global SUVR in plasma231/PET f. [^{18}F]AZD4694 global SUVR in plasma pTau231/pTau181 g. Hippocampal volume in plasma181/PET h. Hippocampal volume in plasma231/PET i. Hippocampal volume in plasma pTau231/pTau181 j. CSF pTau181 levels in plasma181/PET k. CSF pTau181 levels in plasma231/PET l. CSF pTau181 levels in plasma pTau231/pTau181.

3.9 Tables:

Table 1: Demographics from the TRIAD cohort

	CUY	**CU**	**MCI**	**AD**	***P* value**
Number of individuals	30	162	60	32	
Age (mean ± sd)	23.0±2.1	69.4±10.3	70.3±9.1	64.9±10.4	<0.001
Sex (Female (%))	19 (63)	102 (63)	27 (45)	16 (50)	0.073
Education (mean ± sd)	17.0±2.2	15.4±3.7	14.8±4.0	13.9±3.4	0.006
Plasma pTau181 pg/mL (mean ± sd)	8.0±3.6	11.3±6.9	16.1±8.6	26.8±12.9	<0.001
Plasma pTau231 pg/mL (mean ± sd)	9.2±5.9	15.4±8.6	18.1±9.5	27.6±11.0	<0.001
Temporal meta-ROI SUVR (mean ± sd)	1.0±0.1	1.1±0.2	1.6±0.8	2.6±0.9	<0.001
Hippocampal Volume (mean±sd)	4.1±0.3	3.5±0.4	3.5±0.4	2.9±0.4	<0.001
APOEε4 (data available) *0 (N (%))* *1 (N (%))* *2 (N (%))*	30 22 (73) 8 (27) 0 (0)	158 116 (73) 40 (25) 2 (1)	58 30 (52) 22 (38) 6 (10)	29 14 (48) 12 (41) 3 (10)	0.002
CSF pTau181 pg/mL Data available *(mean ± sd)*	22 22.4±7.5	79 43.2±25.2	34 76.8±50.2	16 110.6±63.4	<0.001

Table2: Demographics of groups based on Plasma pTau181 and temporal meta-ROI SUVR

	Plasma-/PET-	**Plasma+/PET-**	**Plasma-/PET+**	**Plasma+/PET+**	***P* value**
Number of individuals	156	26	20	52	
Diagnosis *CU* *MCI* *AD*	 130 25 1	 20 4 2	 7 10 3	 5 21 26	<0.001
Age (mean ± sd)	68.9±10.3	73.3±8.6	71.5±5.9	66.4±10.9	0.021
Sex (Female (%))	90 (58)	14 (54)	14 (70)	27 (52)	0.560
Education (mean ± sd)	15.1±4.0	15.6±2.6	14.2±3.1	14.9±3.7	0.615
Plasma pTau181 pg/mL (mean ± sd)	9.3±2.8	24.2±13.7	11.1±2.1	25.9±9.1	<0.001
Plasma pTau231 pg/mL (mean ± sd)	13.6±6.7	20.3±12.8	18.4±8.4	27.9±9.4	<0.001
Temporal meta-ROI SUVR (mean ± sd)	1.1±0.1	1.1±0.8	1.8±0.6	2.4±0.9	<0.001
Hippocampal Volume (mean±sd)	3.5±0.4	3.3±0.4	3.3±0.4	3.0±0.4	<0.001
APOEε4 (data available) *0 (N (%))* *1 (N (%))* *2 (N (%))*	 151 113 (75) 37 (25) 1 (1)	 24 18 (75) 5 (21) 1 (4)	 20 9 (45) 10 (50) 1 (5)	 50 20 (40) 22 (44) 8 (16)	<0.001
CSF pTau181 pg/mL Data available *(mean ± sd)*	 78 40.0±16.3	 12 44.6±14.5	 14 86.4±44.1	 25 117.1±62.1	 <0.001

Table3: Demographics of groups based on Plasma pTau231 and temporal meta-ROI SUVR

	Plasma-/PET-	**Plasma+/PET-**	**Plasma-/PET+**	**Plasma+/PET+**	***P* value**
Number of individuals	132	50	16	56	
Diagnosis					<0.001
CU	109	41	4	8	
MCI	21	8	8	23	
AD	2	1	4	25	
Age (mean ± sd)	68.6±10.6	72.2±8.3	68.9±7.4	67.5±10.7	0.094
Sex (Female (%))	77 (58)	27 (54)	12 (75)	29 (52)	0.389
Education (mean ± sd)	15.5±4.0	14.6±3.4	13.9±3.4	14.9±3.5	0.276
Plasma pTau231 pg/mL (mean ± sd)	11.0±4.1	23.9±8.8	13.4±3.7	28.6±8.7	<0.001
Plasma pTau181 pg/mL (mean ± sd)	10.1±6.6	15.0±9.3	16.6±8.4	23.3±10.3	<0.001
Temporal meta-ROI SUVR	1.1±0.1	1.1±0.1	1.8±0.6	2.3±0.9	<0.001
Hippocampal Volume (mean±sd)	3.5±0.4	3.4±0.4	3.2±0.4	3.0±0.5	<0.001
APOEε4 (data available)	127	48	15	55	<0.001
0	98 (77)	33 (69)	6 (40)	23 (42)	
1	28 (22)	14 (29)	5 (33)	27 (49)	
2	1 (1)	1 (2)	4 (27)	5 (9)	
CSF pTau181 pg/mL					
Data available	59	31	9	30	
(mean ± sd)	35.3±11.4	50.7±18.6	88.6±45.2	111.3±60.5	<0.001

Table4: Demographics of groups based on Plasma pTau231 and Plasma pTau181

	Plasma231-/181-	**Plasma231+/181-**	**Plasma231-/181+**	**Plasma231+/181+**	***P* value**
Number of individuals	130	46	18	60	
Diagnosis					<0.001
CU	103	34	10	15	
MCI	25	10	4	21	
AD	2	2	4	24	
Age (mean ± sd)	68.7±10.3	70.7±8.6	67.8±10.5	68.9±10.8	0.657
Sex (Female (%))	78 (60)	26 (57)	11 (61)	30 (50)	0.614
Education (mean ± sd)	15.3±4.1	14.3±3.4	15.3±3.0	15.1±3.5	0.509
Plasma pTau181 pg/mL (mean ± sd)	9.0±2.8	11.0±2.2	23.7±13.1	25.8±10.1	<0.001
Plasma pTau231 pg/mL (mean ± sd)	11.0±4.1	22.9±6.2	12.9±4.2	29.1±9.9	<0.001
Temporal meta-ROI SUVR	1.1±0.2	1.3±0.5	1.4±0.6	2.1±1.0	<0.001
Hippocampal Volume (mean±sd)	3.5±0.4	3.4±0.4	3.3±0.4	3.0±0.4	<0.001
APOEε4 (data available)	126	45	16	58	<0.001
0	93 (74)	29 (64)	11 (69)	27 (47)	
1	31 (25)	16 (36)	2 (1)	25 (43)	
2	2 (2)	0 (0)	3 (2)	5 (10)	
CSF pTau181 pg/mL					
Data available	59	33	9	28	
(mean ± sd)	39.0±18.4	61.6±35.6	64.8±45.2	102.9±62.7	<0.001

3.10 Supplementary material

Supplementary methods:

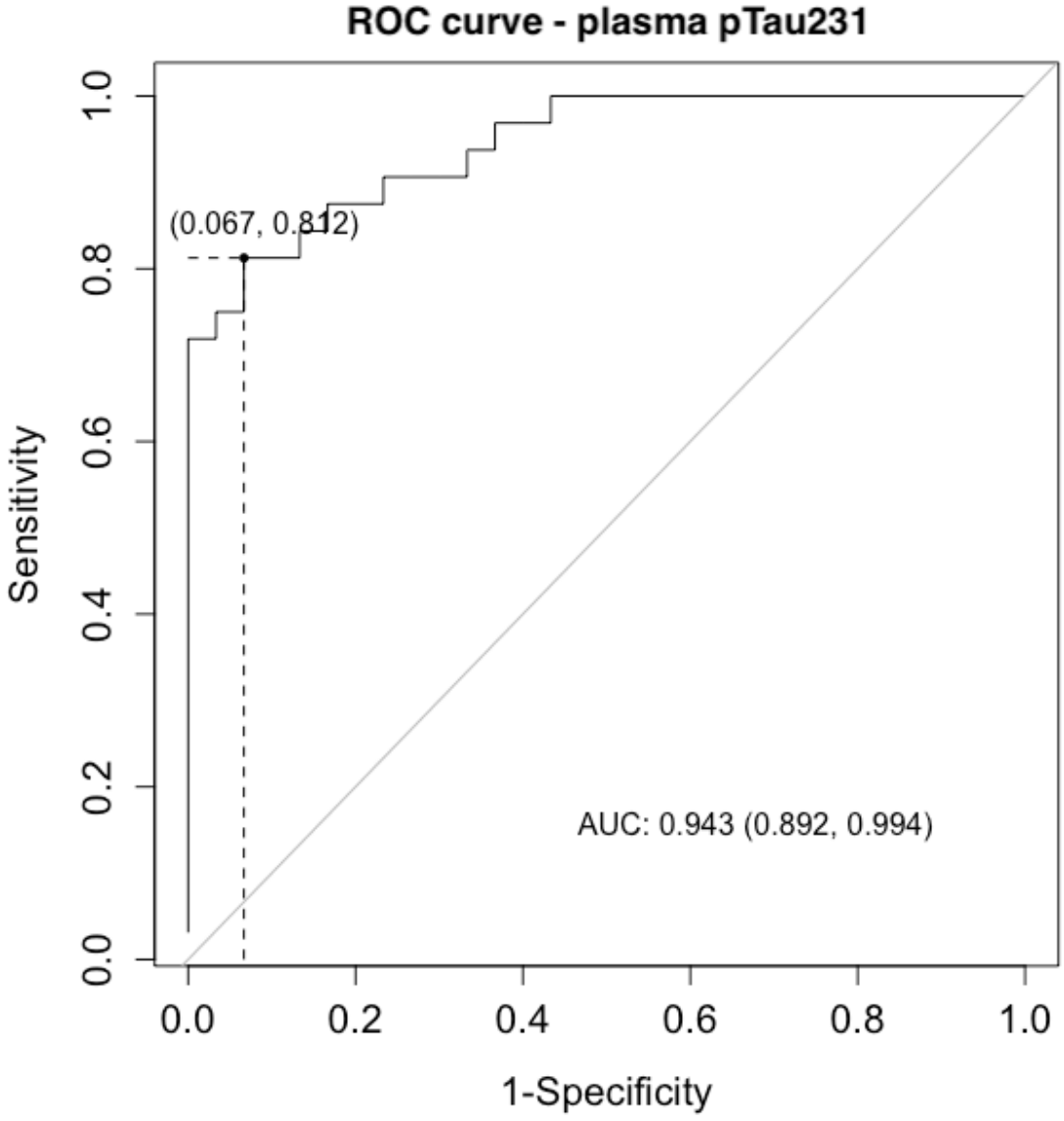
Plasma pTau assays:

Plasma pTau231 and pTau181 were measured using the Simoa HD-1 (Quanterix, Billerica, MA, USA) at the University of Gothenburg (Sweden). For pTau231, the ADx253 monoclonal antibody was used, generated using a synthetic peptide as a KLH-coupled antigen, numbered according to full-length tau-441 phosphorylated at threonine 231. For pTau181, the AT270 mouse monoclonal antibody, that is specific to threonine-181 phosphorylation site, was coupled with magnetic beds, for capture. The detector was anti-tau mouse monoclonal antibody Tau12 (BioLegend, San Diego, CA, USA), which binds to the human tau protein at the N-terminal epitope 6-QEFEVMEDHAGT-18. In both cases, the detection antibody was conjugated to biotin following the manufacturer's recommendations, and the calibrator was full-length recombinant tau-441 phosphorylated *in vitro* by glycogen synthase kinase.

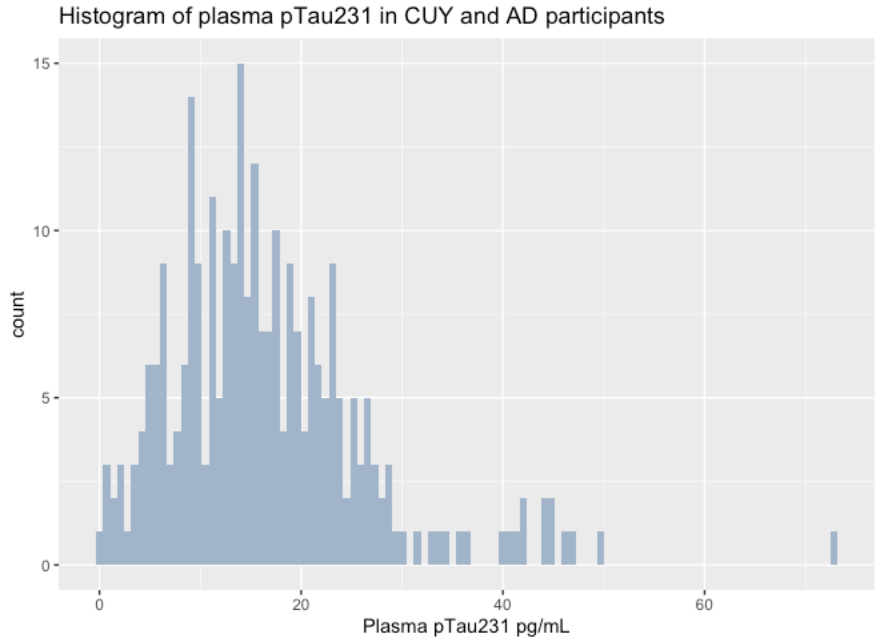
Supplementary figures:

Supplementary Figure 1: ROC curves based on cognitively unimpaired young as the healthy group, versus Alzheimer's disease participants.

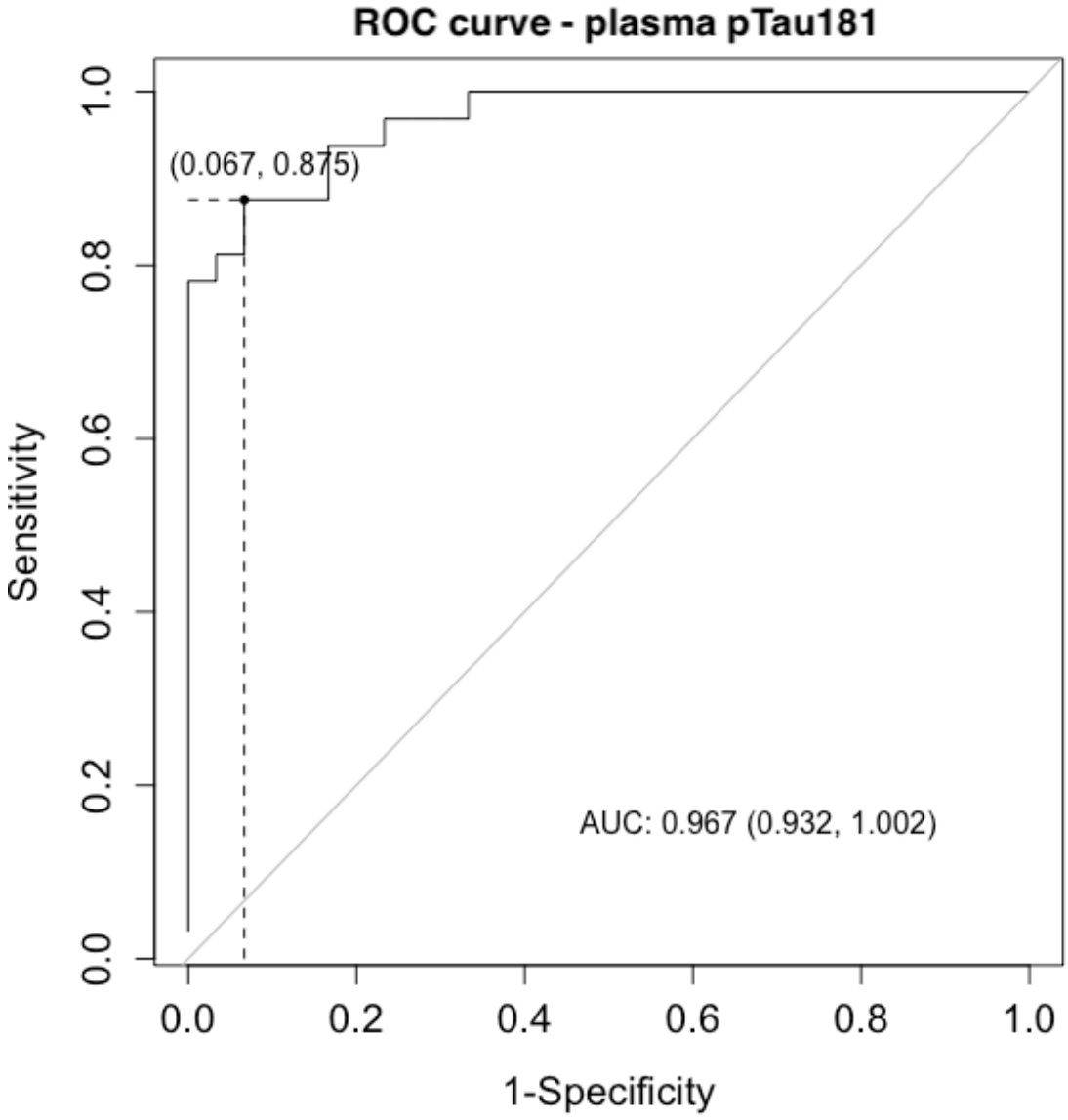
a.1: Plasma pTau231 ROC curve:



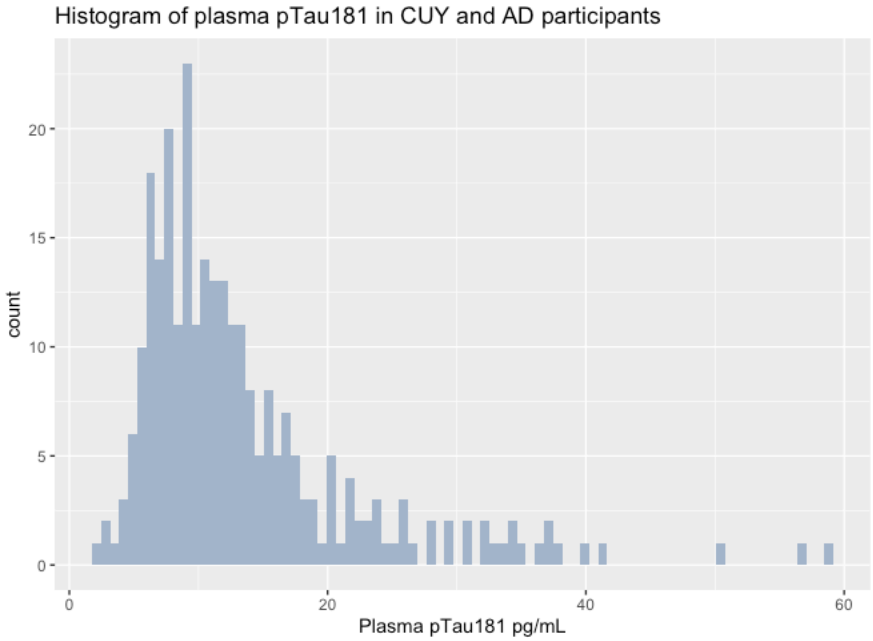
a.2: Histogram of plasma pTau231 levels in CUY and AD individuals



b.1: Plasma pTau181 ROC curve:

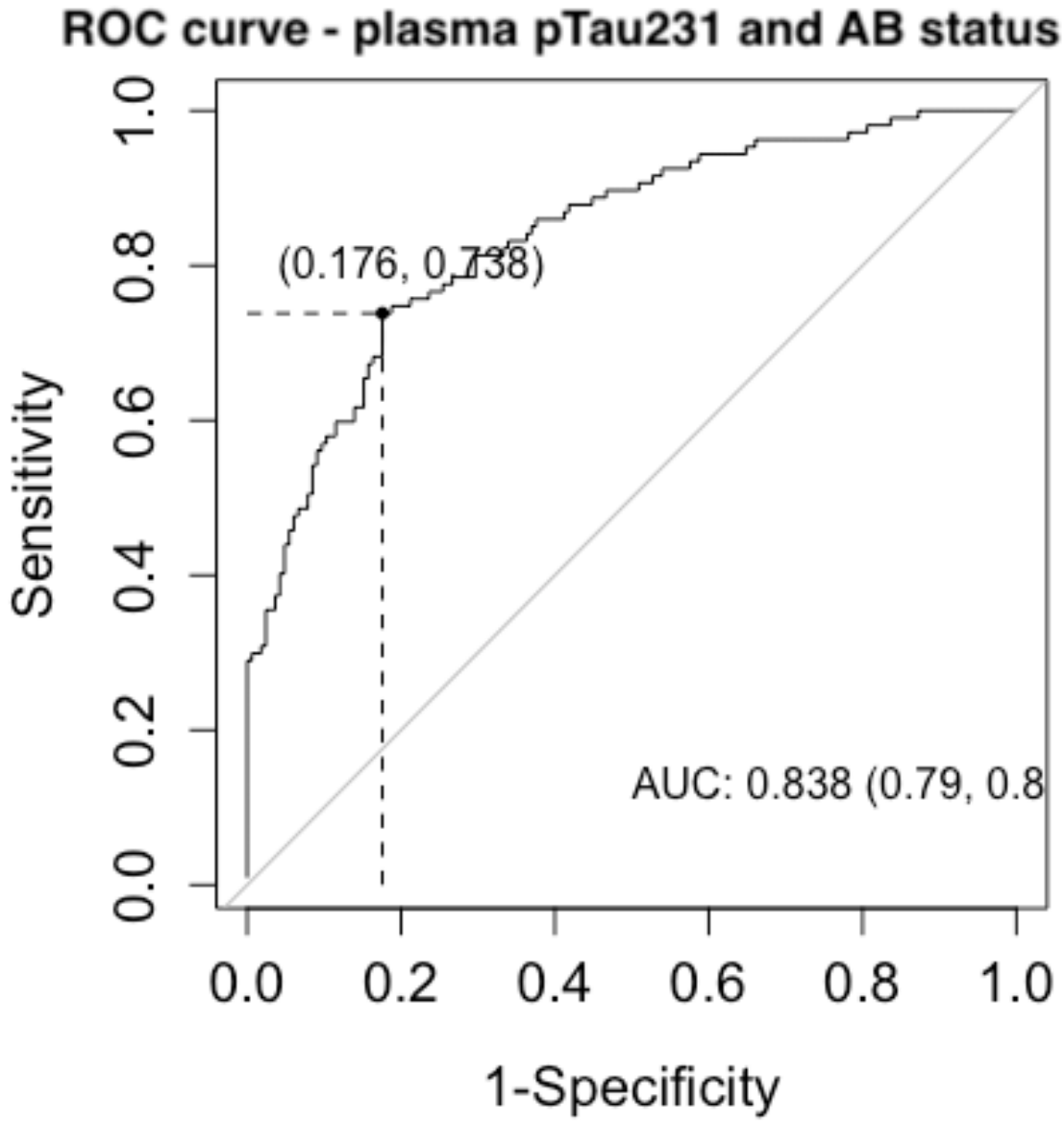


b.2: Histogram of plasma pTau181 levels in CUY and AD individuals

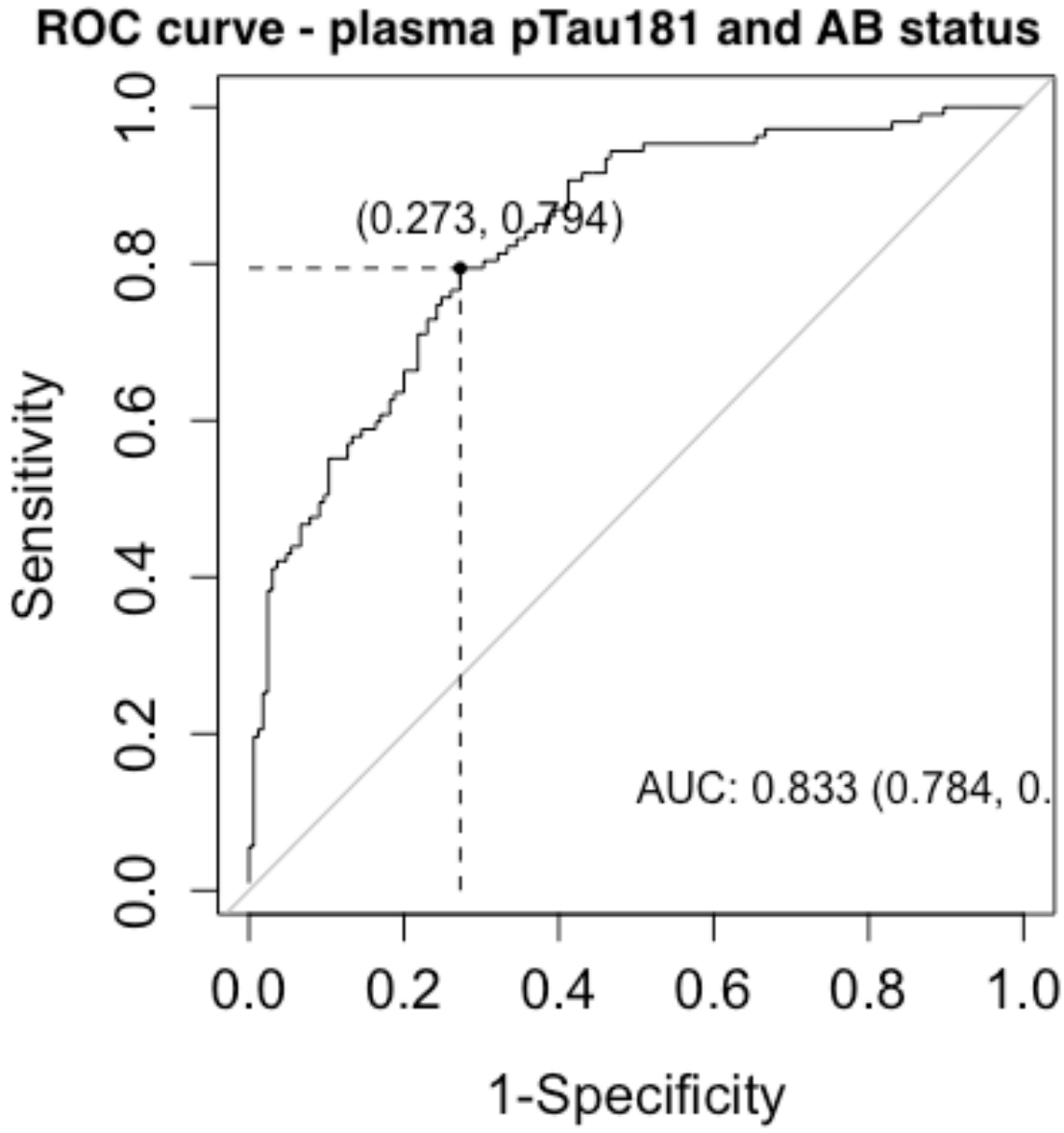


Supplementary Figure 2: ROC curves based on amyloid positive vs amyloid negative individuals, as assessed via [^{18}F]AZD4694 amyloid-PET. Threshold for positivity was 1.55 SUVR.

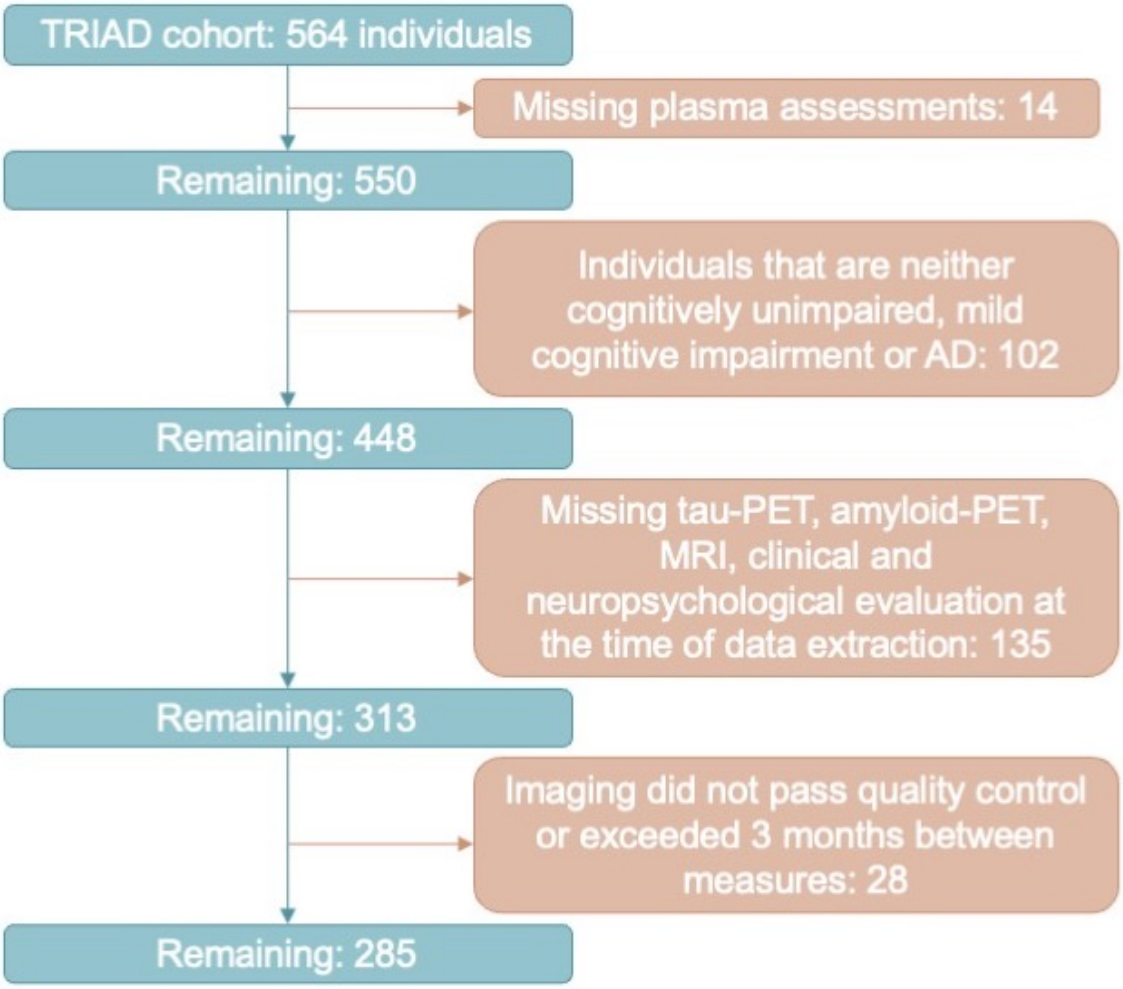
a. Plasma pTau231:



b. Plasma pTau181:



Supplementary Figure 3: Diagram of participants selection



Supplementary tables:

Supplementary Table 1: Demographic information of individuals with cerebrospinal fluid assessment in the TRIAD cohort.

	CU	**MCI**	**AD**	***P* value**
Number of individuals	79	34	16	
Age (mean ± sd)	69.5±9.1	70.5±7.7	63.0±7.8	0.011
Sex (Female (%))	47 (59)	15 (44)	7 (44)	0.228
Education (mean ± sd)	15.0±3.3	14.5±3.6	14.8±3.1	0.795
Plasma pTau181 pg/mL (mean ± sd)	11.8±7.5	14.4±7.7	28.3±16.3	<0.001
Plasma pTau231 pg/mL (mean ± sd)	16.6±10.6	16.9±8.8	27.1±11.4	<0.001
Temporal meta-ROI SUVR (mean ± sd)	1.1±0.2	1.5±0.7	2.7±0.8	<0.001
Hippocampal Volume (mean±sd)	3.5±0.3	3.3±0.4	2.9±0.9	<0.001
CSF pTau181 pg/mL (mean ± sd)	43.2±25.2	76.8±50.2	110.6±63.4	<0.001

Supplementary Table 2: Coefficient of variation for AD pathophysiology measures in the TRIAD cohort.

	CUY	**CU**	**MCI**	**AD**
Number of individuals	30	162	60	32
Plasma pTau181	0.45	0.61	0.53	0.48
Plasma pTau231	0.64	0.56	0.52	0.40
Temporal meta-ROI	0.10	0.18	0.50	0.35
Hippocampal Volume	0.07	0.11	0.11	0.14
CSF pTau181 pg/mL Data available *(mean ± sd)*	 22 0.33	 79 0.58	 34 0.63	 16 0.57

Supplementary Table 3: Table of proportions in biomarker groups.

a. Plasma pTau181 and tau-PET

	Plasma181-/PET-	**Plasma181+/PET-**	**Plasma181-/PET+**	**Plasma181+/PET+**
Number of individuals	156	26	20	52
Diagnosis (%)				
CU	83	77	35	10
MCI	16	15	50	43
AD	1	8	15	47
Diagnosis and Aβ status (%)				
CU Aβ-	68	42	10	2
CU Aβ+	15	35	25	8
MCI Aβ-	11	7	0	2
MCI Aβ+	5	8	50	41
AD Aβ+	1	8	15	47

b. Plasma pTau231 and tau-PET

	Plasma231-/PET-	**Plasma231+/PET-**	**Plasma231-/PET+**	**Plasma231+/PET+**
Number of individuals	132	50	16	56
Diagnosis (%)				
CU	83	82	25	14
MCI	16	16	50	44
AD	2	2	25	42
Diagnosis and Aβ status (%)				
CU Aβ-	76	38	6	4
CU Aβ+	7	44	19	11
MCI Aβ-	10	12	0	2
MCI Aβ+	6	4	50	42
AD Aβ+	2	2	25	42

c. Plasma pTau231 and plasma pTau181

	pTau231-/181-	**pTau231+/181-**	**pTau231-/181+**	**pTau231+/181+**
Number of individuals	130	46	18	60
Diagnosis (%)				
CU	79	74	56	25
MCI	19	22	24	37
AD	2	4	18	38
Diagnosis and Aβ status (%)				
CU Aβ-	71	37	46	6
CU Aβ+	8	37	10	19
MCI Aβ-	10	9	0	5
MCI Aβ+	9	13	24	32
AD Aβ+	2	4	18	38

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Chapter 4: Plasma and CSF concentrations of N-terminal tau fragments associate with *in vivo* neurofibrillary tangle burden.

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4.1 Preface

In Chapter 3, we proved that plasma pTau231, pTau181 and tau-PET reflect different stages of tau pathological progression (Tissot et al., 2022). Since then, other studies were conducted suggesting pTau, in the plasma and in the CSF, emerges in the preclinical phase of AD, when abnormal levels of pathology are only detectable via amyloid-β-PET and CSF-Aβ (Ashton et al., 2021; Jack, Bennett, et al., 2018; Milà-Alomà et al., 2022). Moreover, pTau markers have been more closely associated with amyloid-β-PET rather than tau-PET (Therriault et al., 2022), and also shown to be independently associated with amyloid-β and tau (Mattsson-Carlgren et al., 2021). All these studies propose that pTau might not be the most appropriate marker for "T" in the A/T/(N) system.

Nevertheless, *in vivo* and *postmortem* analyses indicated that NFT load seems more closely associated with cognition and neuropsychiatric symptoms, with higher NFT being related to low cognition and high neuropsychiatric symptoms severity (Gomez-Isla et al., 1997; Nelson et al., 2009; Tissot, Therriault, et al., 2021). It has also been shown to be the best predictor of cognitive decline (Ossenkoppele et al., 2019).

As mentioned throughout the thesis, it would be critical to assess the A/T/(N) system with plasma biofluid markers, are these are more easily implementable in the diagnostic and clinical settings, as compared to CSF, PET and MRI imaging methods. Assessment of tau was first thought to be feasible by quantifying plasma pTau, as it is closely associated with CSF pTau181 (Karikari, Pascoal, et al., 2020). Nevertheless, as pTau levels are associated with two core AD hallmarks, amyloid-β and NFT, it is difficult to know to which extent does NFT accumulation relate to pTau increase. Thus, finding a fluid biomarker capable of quantifying tau, and tau only, is essential. Our collaborators from the University of Gothenburg created a novel assay targeting non-

phosphorylated tau fragments, using the Simoa platform. Their first analyses presented NTA as being highly specific to AD (Snellman et al., 2022). Using gold standard method of pathophysiology assessment available in TRIAD, Chapter 4 aimed at characterizing this novel NTA assay in a cohort of individuals, comprised of cognitively unimpaired, non-AD and individuals on the AD *continuum*. We associated plasma and CSF NTA levels with pathological changes observed in AD, using multimodal imaging, with the goal of assessing which pathology was better explained by NTA. Moreover, we conducted longitudinal analyses determine whether plasma NTA was able to predict further AD pathological changes at follow-up visits.

Our final goal is to use tau biomarkers to assess the A/T/(N) system, which would help in the predicting someone at risk of developing AD dementia, as well as assess the severity of disease progression.

4.2 Preface

Background: Fluid biomarkers capable of specifically tracking tau tangle pathology *in vivo* are highly needed.

Methods: We measured cerebrospinal fluid (CSF) and plasma concentrations of non-phosphorylated N-terminal tau fragments, using a novel immunoassay (NTA) in the TRIAD cohort, consisting of 272 individuals assessed with amyloid (Aβ) PET, tau-PET and MRI imaging and cognitive assessments.

Results: CSF and plasma NTA-tau concentrations were specifically increased in cognitively impaired Aβ-positive groups. CSF and plasma NTA-tau concentrations displayed stronger correlations with tau-PET than with Aβ-PET and MRI, both in global uptake and at the voxel level. Regression models demonstrated that both CSF and plasma NTA-tau is preferentially associated with tau pathology. Moreover, plasma NTA-tau was associated with longitudinal tau-PET accumulation across the aging and AD spectrum.

Discussion: NTA-tau is a biomarker closely associated with *in vivo* tau deposition in AD *continuum* and has potential as a tau tangle biomarker in clinical settings and trials.

Keywords: Plasma, CSF, NTA, Alzheimer's disease, biomarkers, tau

4.3 Introduction

Neuropathological examination is the gold standard for definitive diagnosis of Alzheimer's disease (AD) through post-mortem confirmation of amyloid-β (Aβ) plaques and neurofibrillary tangles (NFTs) (Braak et al., 2006; Braak & Braak, 1991). In 2018, the National Institute on Aging and the Alzheimer's Associations (NIA-AA) Research Framework established AD as a biological construct defined *in vivo* based on cerebrospinal fluid (CSF) and imaging biomarkers, the A/T/(N) framework, where "A" stands for Aβ pathology, "T" for NFT pathology and "N" for neurodegeneration (Jack, Bennett, et al., 2018).

Recently, blood-based biomarkers have shown great promise to identify AD pathophysiological changes (Karikari et al., 2022). Novel assays targeting blood phosphorylated-tau (p-tau) are highly AD specific, however, accumulating evidence suggests that the neuropathological changes inducing increases in soluble p-tau are not explained only by NFT pathology (Karikari, Pascoal, et al., 2020; Milà-Alomà et al., 2022). First, p-tau concentrations increase early during preclinical AD already before tau-PET positivity, and demonstrate an early association with Aβ pathology (Suárez-Calvet et al., 2020). In later symptomatic stages, p-tau presents high association with both Aβ and tau (Ashton et al., 2021, 2022; Karikari, Pascoal, et al., 2020), but is often more closely linked to Aβ as compared to NFT accumulation in the brain measured by PET (Therriault et al., 2022). In addition, plasma p-tau has been found to be increase in amyotrophic lateral sclerosis and linked to spinal cord neuronal loss (Cousins et al., 2022). Thus, it is difficult to determine the specificity of soluble p-tau as a marker of AD-related "T" in the A/T/(N) scheme (Jack, Hampel, et al., 2016). The spatiotemporal accumulation of NFTs in AD correlates more strongly with clinical symptoms and cognitive decline than Aβ plaque depositions, thus, the need for a biomarker capable of specifically tracking tangle pathology remains a priority to the AD field.

Previously, we reported a novel immunoassay targeting non-phosphorylated N-terminal tau fragments in CSF and plasma, referred to as NTA (Snellman et al., 2022), using a Single molecule array (Simoa) platform. Here, we aimed to further investigate the biomarker potential of NTA-tau in a well-characterized cohort including participants across the AD *continuum,* non-AD neurodegenerative diseases and healthy controls. Throughout this paper the abbreviation NTA is used for the novel immunoassay, whereas NTA-tau is used to describe the non-phosphorylated N-terminal tau fragments detected by the NTA assay.

4.4 Methods

Participants:

We included individuals from the TRIAD cohort (McGill University) (Therriault, Benedet, Pascoal, Mathotaarachchi, et al., 2020) (n=272), with data obtained from December 2017 to May 2021. Details of the information gathered from participants can be found elsewhere (https://triad.tnl-mcgill.com/). All participants underwent a full neuropsychological evaluation, magnetic resonance imaging (MRI), [^{18}F]AZD4694 Aβ-PET, [^{18}F]MK6240 tau-PET and plasma NTA assessment within 6 months. A first subset of participants (n=154) had quantification of CSF NTA-tau. A second subset (n=127) had a follow-up visit for plasma, MRI, Aβ-PET, and tau-PET up to three years after baseline (mean follow-up time of 1.86 (SD 0.61) years).

Diagnosis was determined using Mini-Mental State Examination (MMSE), and Clinical Dementia Rating (CDR) scores and the NIA-AA criteria (Jack, Bennett, et al., 2018). Cognitively unimpaired (CU) had no objective impairment, an MMSE score of 26 or more and CDR score of 0 (Jack, Wiste, et al., 2018). Mild cognitive impaired (MCI) individuals had subjective and/or objective cognitive impairment, relatively preserved activities of daily life, an MMSE score of 26 or above

and a CDR score of 0.5 (Petersen, 2004). Diagnosis of AD dementia was assessed with an MMSE score of less than 26 and a CDR of 0.5 or more. Individuals diagnosed with suspected non-AD neurodegenerative diseases were AD biomarker-negative and met clinical criteria for frontotemporal dementia (n=11), progressive supranuclear palsy (n=3), corticobasal degeneration (n=2), Duchenne muscular dystrophy (n=1). Non-AD individuals were categorized by a consensus panel of neurologists based on clinical symptoms. No participant met the criteria for another neurological or major neuropsychiatric disorder.

CSF and plasma biomarker measurements

CSF and plasma NTA-tau levels were quantified using an in-house developed Simoa immunoassay using a Simoa HD-X platform (Quanterix) at the Clinical Neurochemistry Laboratory (Mölndal, Sweden). Development and validation of the NTA assay has been previously described (Snellman et al., 2022). In brief, the NTA assay is comprised by a mouse monoclonal antibody with epitope 159-163aa (HT7, Thermo Scientific) conjugated to paramagnetic beads and used as capture antibody. A mouse monoclonal antibody with epitope 6-18aa (Tau12, BioLegend) is biotinylated and used as detector antibody. Recombinant non-phosphorylated 2N4R tau was used as calibrator (SignalChem).

For CSF NTA, randomized samples were allowed to thaw at room temperature for 45 minutes, vortexed (30s, 2000 rpm), plated and diluted 1:4 using Tau 2.0 assay diluent (Quanterix). An eight-point calibrator curve was run in duplicates. For plasma NTA, randomized samples were allowed to thaw at room temperature for 45 minutes, vortexed (30 seconds, 2000 rpm) and subsequently centrifuged (10 minutes, 4000g). Subsequently, samples were plated and diluted 1:2 using Tau 2.0 assay diluent (Quanterix). An eight-point calibrator curve was run in duplicates. For both CSF and

plasma measurements, two internal quality control (iQC) samples were run in duplicates before and after the analyzed TRIAD samples. The repeatability (CVr%) and intermediate precision (CVRw%) for TRIAD CSF measurements were 5.0% and 9.0%, respectively, and for plasma measurements 6.1% and 8.5%, respectively (Supplementary Table1). Eight out of 531 samples (1.5%) were under the calculated limit of detection (0.032 pg/ml).

Image processing

Detailed description concerning acquisition and processing of Aβ-PET and tau-PET and MRI can be found in the supplementary material. Global Aβ standardized uptake value ratio (SUVR) was determined using an average of Aβ-PET SUVR in the precuneus, prefrontal, orbitofrontal, parietal, temporal, anterior and posterior cingulate cortices. Individuals were categorized as Aβ- or Aβ+ based on a threshold of 1.55 SUVR (Therriault, Benedet, Pascoal, Ashton, et al., 2020). *In vivo* classification of PET-based Braak stages was done following Pascoal *et al*. (Pascoal et al., 2020), where cut-offs were assessed as 2.5 standard deviation above the mean of CU young adults. Temporal meta-ROI SUVR of tau-PET was acquired from a composite mask including the entorhinal, amygdala, fusiform, inferior and middle temporal cortices, which capture changes associated with AD (Meyer et al., 2019; Ossenkoppele et al., 2018). Finally, neurodegeneration was assessed in an AD-signature mask comprised of the entorhinal, inferior temporal, middle temporal cortices, and fusiform gyrus (Jack et al., 2017).

Statistical analyses

Non-imaging statistical analyses were performed on R statistical software (version 4.0.0). ANOVA tests were conducted for continuous variables and Fisher tests for categorical variables

for demographic information. ANOVA with Tukey's multiple comparison test compared plasma and CSF NTA-tau concentrations across diagnostic groups. Non-AD cases were only included in statistical analyses when comparing diagnostic groups. MCI Aβ- were kept when studying the aging and the AD *continuum* due to their symptomatology as their Aβ levels were close to the threshold value for Aβ positivity. Spearman's rank correlations (R) assessed the one between NTA-tau concentrations and AD pathophysiological hallmarks. Additionally, smoothing splines were generated (GraphPad Prism v8.4.0) to assess plasma and CSF NTA-tau changes along Braak stages. Linear regression models, adjusted for age and sex, tested the effect of different predictors: neocortical Aβ-PET (A), temporal meta-ROI tau-PET (T), temporal neurodegeneration (N), Aβ-PET and tau-PET (A+T), and all predictors (A+T+N) on plasma and CSF NTA. To compare these nested models, we used the Akaike information criterion (AIC) and the adjusted coefficient of determination (R^2), as measures of how well the model fits the data and of how much of the outcome variability is explained by the model. ΔAIC was calculated as the difference between two AIC values for a given biomarker, and the best model was defined as the simplest model presenting the lowest AIC value. Linear mixed models assessed changes in plasma NTA-tau concentrations over time. The model included plasma NTA as the dependent variable and the interaction between time and diagnostic group as the independent variable. The models' covariates were age at baseline, sex and random intercept.

Voxel-wise analyses were performed on VoxelStats, a statistical toolbox implemented in Matlab (Mathotaarachchi et al., 2016). Linear models assessed the cross-sectional relationship between NTA-tau concentrations and Aβ-PET, tau-PET and VBM images, correcting for age, sex, and diagnosis. Linear mixed models had brain imaging (either Aβ-PET, tau-PET or VBM) as the dependent variable, and the interaction between time and plasma NTA as the independent variable.

Other predictors were age at baseline, sex, and diagnosis, and the mixed models were fitted with random intercepts on the participant level. We further corrected images for multiple comparisons using random field theory (RFT) correction (Worsley et al., 2004).

4.5 Results

Demographics

We included 272 individuals categorized as CU Aβ-, CU Aβ+, MCI Aβ-, MCI Aβ+, AD Aβ+ and non-AD neurodegenerative condition. We observed no significant between-group differences in sex or years of education. However, there was a significant difference in terms of age, with the non-AD neurodegenerative condition group being younger than the other groups (Table1). Demographic information on the CSF and longitudinal subsamples can be found in the Supplementary Table2 and Supplementary Table3, respectively.

NTA-tau concentrations across diagnostic groups

In plasma, NTA-tau concentrations were significantly higher ($p < 0.001$) in AD Aβ+ individuals as compared to all other diagnostic groups (CU Aβ-, CU Aβ+, MCI Aβ-, MCI Aβ+ and non-AD neurodegenerative conditions) (Figure1A). NTA-tau concentrations in CSF were increased across all cognitively impaired (i.e., MCI and AD) Aβ+ groups. First, NTA-tau was significantly increased in AD Aβ+ individuals compared to all other groups (CU Aβ-, CU Aβ+, MCI Aβ- and non-AD dementia, *p-value*<0.001 for all), except MCI Aβ+. Moreover, MCI Aβ+ individuals had higher CSF NTA-tau levels as compared to CU Aβ- (*p-value*<0.001), CU Aβ+ (*p-value*<0.05), MCI Aβ- (*p-value*<0.05) and non-AD neurodegenerative conditions (*p-value*<0.001) (Figure1B).

NTA-tau concentrations associate with global measures of neuroimaging markers of A/T/(N)

Plasma NTA-tau concentrations (n=254) correlated positively with Aβ-PET SUVR (R=0.34, *p-value*<0.001) and tau-PET SUVR (R=0.47, *p-value*<0.001), and negatively with temporal VBM (R=-0.31, *p-value*<0.001) (Figure2A). Among the regression models with plasma NTA-tau as outcome, the highest adjusted R^2 value included the combination of Aβ, tau and neurodegeneration (A+T+N: R^2=0.335), the second highest was tau only (T: R^2=0.329), and the third one included Aβ and tau (A+T: R^2=0.326). However, AICs of tau only and A+T+N model were the same (AIC=19.07); thus, the simplest model – that is tau only – was considered as the best-fitting model (Figure 2A). In Aβ-PET-positive individuals (n=129), Aβ-PET and temporal VBM correlations were similar, whereas tau-PET R^2 improved compared to the whole sample (R=0.64, *p-value*<0.001) (Supplementary Figure1A).

CSF NTA-tau measures (n=141) showed similar results to those of plasma, correlating with Aβ-PET SUVR (R=0.48, *p-value*<0.001), tau-PET SUVR (R=0.53, *p-value*<0.001), and temporal VBM (R=-0.27, *p-value*=0.006) (Figure2B). Among the regression models with CSF NTA-tau as outcome, the tau only model had the highest R^2 (T: R^2=0.483), followed by A+T+N (R^2=0.482), and the combination of Aβ and tau (A+T: R^2=0.4780). The smallest AIC was for the tau only model (T: AIC=1426), significantly lower than A+T+N (ΔAIC=2.78) and A+T (ΔAIC=11.5) (Figure 2B). In Aβ-PET-positive individuals (n=70), CSF NTA-tau concentrations did not correlate with Aβ-PET SUVR (R=0.17, *p*-value=0.15). On the other hand, the correlation of CSF NTA-tau with tau-PET SUVR (R=0.57, *p-value*<0.001) and temporal VBM (R=-0.32, *p*-value=0.0079) slightly improved (Supplementary Figure1B).

Finally, we modeled the cross-sectional, group-level trajectories of plasma and CSF NTA-tau concentrations as a function of Braak stages defined by tau-PET. Plasma NTA-tau began

increasing at Braak stage III, until Braak stage VI. Contrarily, CSF NTA-tau started increasing at Braak stage I and continued until Braak stage VI (Figure2C). In Aβ-PET-positive individuals, plasma NTA-tau behaved similarly. Nevertheless, CSF NTA-tau trajectory was slightly different; it also started to increase at Braak I but reached a plateau already at Braak IV (Supplementary Figure1C).

Voxel-wise association between NTA-tau concentrations with neuroimaging markers of A/T/(N)

We then conducted voxel-wise analyses between plasma and CSF NTA-tau concentrations and Aβ-PET, tau-PET and VBM, correcting for age, sex and diagnosis. Plasma NTA-tau was more strongly associated with tau-PET signal in the precuneus, temporal and medial frontal lobes, while no results survived RFT correction for Aβ-PET and VBM (Figure3A). CSF NTA-tau concentrations associated with Aβ-PET signal in the medial frontal and hippocampus. It strongly associated with tau-PET signal throughout the medial cortex, and temporal lobes. Associations with VBM were not significant after RFT correction (Figure3B).

In Aβ+ individuals (Supplementary Figure2) significant associations with tau-PET were observed for both plasma and CSF NTA-tau in similar regions. No results survived RFT correction for Aβ-PET. NTA-tau concentrations were also negatively associated with VBM, in the superior temporal and occipital lobe for plasma NTA-tau, and in the inferior temporal lobe for CSF NTA-tau.

Longitudinal changes in plasma NTA-tau concentrations

We observed a longitudinal increase in plasma NTA-tau concentrations in participants classified as AD Aβ+, and MCI Aβ+ at baseline (*p-value*<0.001 and *p-value*<0.01 respectively), whereas no changes were seen in the other groups (Figure4A). Voxel-wise linear mixed model assessed the association between longitudinal changes in plasma NTA-tau and each imaging modality. We

observed no association with Aβ-PET, however, we found a significant positive associations with tau-PET, especially in the medial frontal, precuneus and temporal lobes. Changes in plasma NTA-tau concentrations were also associated with decrease in VBM in the medial temporal lobe (Figure4B).

4.6 Discussion

In this study, we report the first comprehensive characterization of the novel NTA immunoassay in both plasma and CSF, using a cohort comprised of individuals across the AD *continuum* as well as non-AD cases, all of which were characterized through imaging biomarkers. Our results support that (i) NTA-tau is a specific biomarker of AD, (ii) NTA-tau concentration is more closely associated with tau-PET than with Aβ-PET or neurodegeneration (indexed by VBM) and (iii) plasma NTA-tau is associated with longitudinal tau-PET accumulation throughout the cortex, as well as neurodegeneration in medial temporal areas.

In the last few years, there has been a successful development of various immunoassays capable of measuring brain-derived biomarkers in blood and CSF (Ashton et al., 2021; Karikari, Pascoal, et al., 2020; Suárez-Calvet et al., 2020). Among them, p-tau, specifically p-tau181, p-tau217 and p-tau231, have proven to be especially promising (Ashton et al., 2021; Karikari, Pascoal, et al., 2020; Mattsson-Carlgren et al., 2021). Among dementia disorders, p-tau species are the most specific for AD pathology and start to increase early during preclinical stages, when only subtle changes in CSF Aβ are detectable and prior to tau NFT pathology being severe enough to be visualized on tau-PET (Suárez-Calvet et al., 2020). On the other hand, quantification of tau fragments in blood irrespective of isoform and phosphorylation state, traditionally referred to as t-tau, has rendered mixed results. For example, blood t-tau assays have proven meaningful in acute

neurological conditions, such as traumatic brain injury, and in chronic neurological diseases characterized by intense neurodegeneration such as Creutzfeldt-Jakob disease (Rubenstein et al., 2017; Zerr et al., 2021). However, in AD, t-tau assays show large overlaps between diagnostic groups, raising uncertainty around their potential clinical utility (Mattsson et al., 2016; Zetterberg et al., 2013), possibly due to the presence of peripherally produced "t-tau" species. Recently, a t-tau assay referred to as NT1 and targeting tau species ranging from N-terminal to mid-region, showed promising results in blood (Chen et al., 2019; Chhatwal et al., 2020; Mengel et al., 2020). This assay was shown to be AD specific and predicted cognitive decline and neurodegeneration (Chhatwal et al., 2020; Mengel et al., 2020). Based on these findings, and given the success of several N-terminal directed p-tau immunoassays in identifying early AD pathophysiological changes, the same N-terminal targeted strategy was used when developing NTA (Snellman et al., 2022). In a previous study, CSF NTA-tau was increased in MCI Aβ+ and AD Aβ+ compared to AD biomarker-negative neurological controls, MCI Aβ- and non-AD Aβ- individuals (Snellman et al., 2022), and similar findings were also observed here. Interestingly, our results suggest that plasma and CSF NTA show different emergences along the AD continuum. CSF NTA-tau concentrations increase during preclinical AD, while plasma NTA-tau is increased in AD Aβ+ cases. This difference may be explained by the fact that NTA-tau concentrations are approximately 100-fold lower in plasma than in CSF (Snellman et al., 2022), and tau biomarkers generally perform better and show higher fold changes when measured in CSF (Palmqvist et al., 2019), since tau protein in plasma is more exposed to degradation by proteases, kidney clearance and liver metabolism. Altogether, this might prevent the NTA assay from successfully detecting subtle early alterations in plasma levels of N-terminal tau fragments, which are however detectable in CSF.

Despite p-tau being currently categorized as a tangle marker in the A/T/(N) framework, accumulating evidence suggests p-tau concentrations in CSF and blood rise in response to Aβ pathology (He et al., 2018; Zhang et al., 2020). Various studies support the idea that increased tau phosphorylation is an early event in the Aβ cascade (Choi et al., 2014; Kaeser et al., 2022; Mattsson-Carlgren et al., 2021; Sato et al., 2018). A recent study showed that p-tau abnormality is one of the first events related to AD pathogenesis, and is more closely associated with Aβ pathology, rather than NFT accumulation (Therriault et al., 2022). Altogether, these studies bring to light that p-tau measures in CSF and plasma need to be used cautiously in the A/T/(N) system, as they might not exclusively reflect "T". NTA-tau, however, seems to be more closely associated with tau accumulation as compared to other AD pathophysiological processes. Both plasma and CSF NTA-tau concentrations correlated with Aβ-PET accumulation and temporal neurodegeneration, but only at global, not voxel-level analysis. NTA-tau levels on the other hand were associated with tau-PET in both voxel-wise and ROI-based analyses. Our comparison of nested regression models showed that temporal meta-ROI measures of tau-PET better explained plasma and CSF NTA-tau concentrations, with the tau only model often presenting as the most parsimonious one. Importantly, NTA-tau changes followed the hierarchical Braak staging system (Braak & Braak, 1991). CSF NTA-tau increases after tau-PET positivity is detected in Braak stage I (transentorhinal), while plasma NTA-tau concentrations increase at a later stage, starting Braak III (amygdala, parahippocampal gyrus, fusiform gyrus, and lingual gyrus). This further corroborates the idea that CSF and plasma NTA-tau indicate different stages of NFT progression, with CSF NTA increasing first.

We then repeated the same analyses in the Aβ-positive individuals only, and comparison of goodness-of-fit metrics enforced the idea that NTA-tau concentrations are more strongly

associated with tau-PET rather than other AD hallmark. However, in this subgroup, cross-sectional ROI-based and voxel-wise analyses revealed also an association between NTA-tau concentrations and neurodegeneration. As individuals presenting Aβ positivity are more advanced in disease progression, they are expected to display more neurodegeneration.

Additionally, a subset of individuals had follow-up measures of plasma NTA-tau, tau-PET, Aβ-PET and MRI. First, we observed that only cognitively impaired Aβ+ individuals showed a significant increase in plasma NTA-tau concentrations over time. This suggests a potential novel ability for plasma NTA-tau to track late disease progression, since commonly studied AD plasma biomarkers such as p-tau usually start to increase at preclinical AD, but reach a plateau at advanced AD stages (Karikari, Benedet, et al., 2020). Most notably, plasma NTA-tau predicted tau-PET accumulation in middle to late Braak regions (Braak & Braak, 1991). Comparatively, plasma p-tau markers have been related to longitudinal accumulation of Aβ, NFT and neurodegeneration in broader brain regions (Lantero Rodriguez et al., 2020; Milà-Alomà et al., 2022; Tissot, Benedet, et al., 2021). This finding corroborates the idea that plasma NTA-tau is a predictor of mid- to late-stage tau accumulation. Plasma NTA-tau also predicted neurodegeneration in medial temporal lobe, related to memory problems observed in AD dementia (Frisoni et al., 2002). Among key AD pathophysiological changes, neurodegeneration has been observed as the latest stages (Jack & Holtzman, 2013). Following the progression of AD pathophysiological changes, we would expect plasma NTA-tau concentrations to rise before neurodegeneration, when NFT accumulation is high enough to induce neuronal damage.

Since AD is characterized by the accumulation of both Aβ and tau pathologies, and current fluid p-tau markers seem more closely related to Aβ, there is an urgent need for fluid biomarkers that can specifically track tau pathology. Because of their close association with Aβ and tau

pathologies, it is difficult to determine to which extent NFT deposition ultimately contributes to soluble p-tau signal, and their strong association with Aβ is also supported by the reduction of p-tau after Aβ removal (Shcherbinin et al., 2022). Thus, NTA-tau measurements could potentially be useful in clinical settings, providing a cost-effective tool capable of tracking tau pathology *in vivo,* and in clinical trials, as an inclusion/exclusion criterion (Mintun et al., 2021), or to potentially monitor the downstream effects of anti-Aβ drugs. This is especially important as N-terminal tau is thought to be closely linked with presynaptic toxicity, which is currently exploited therapeutically (Amadoro et al., 2020; Zhou et al., 2017). Moreover, plasma NTA could be an easily implementable tool to detect individuals at middle to late stages of AD, as well as individuals at risk of accumulating further tau. Our results suggests NTA-tau would be a suitable fluid marker for the "T" category of the A/T/(N) system (Jack, Bennett, et al., 2016, 2018). However, further studies in different cohorts in combination with other AD markers are still required.

This study has both some strengths and limitations. A strength is that the TRIAD cohort is comprised of participants across the AD *continuum* and with other neurodegenerative diseases, extensively characterized using multiple state-of-the-art biomarkers. Moreover, this cohort includes follow-up blood and imaging collection, allowing for longitudinal analysis. Additionally, matching plasma and CSF samples were available. Taken altogether, this enabled a very detailed characterization of the novel NTA assay, shedding light on the underlying pathophysiological mechanisms that induce abnormal increase of NTA-tau in plasma and CSF. Limitations include that CSF was not available for all subjects, which limited our ability to conduct certain analyses, *e.g.,* longitudinal analysis using CSF NTA. Secondly, despite the consistency of our findings in plasma and CSF, having a replication cohort would have further strengthened our results. Additionally, it would have been interesting to investigate the concordance between CSF and

plasma NTA measurements with post-mortem Braak staging. Finally, TRIAD is composed of individuals willing to participate in research focused on dementia, thus creating sampling and self-selection biases.

To conclude, our study provides evidence that NTA-tau differentiates individuals in distinct diagnostic groups across aging and AD *continuum* and is a biomarker more closely associated with NFT accumulation in AD, rather than Aβ and neurodegeneration. Moreover, plasma NTA-tau is a predictor of tau-PET progression in middle to late Braak stage regions.

4.7 Declarations

Acknowledgements

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Conflicts of interest

HZ has served at scientific advisory boards and/or as a consultant for Abbvie, Acumen, Alector, Alzinova, ALZPath, Annexon, Apellis, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, Optoceutics, Passage Bio, Pinteon Therapeutics, Prothena, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen, and Roche, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work).

4.8 Figures

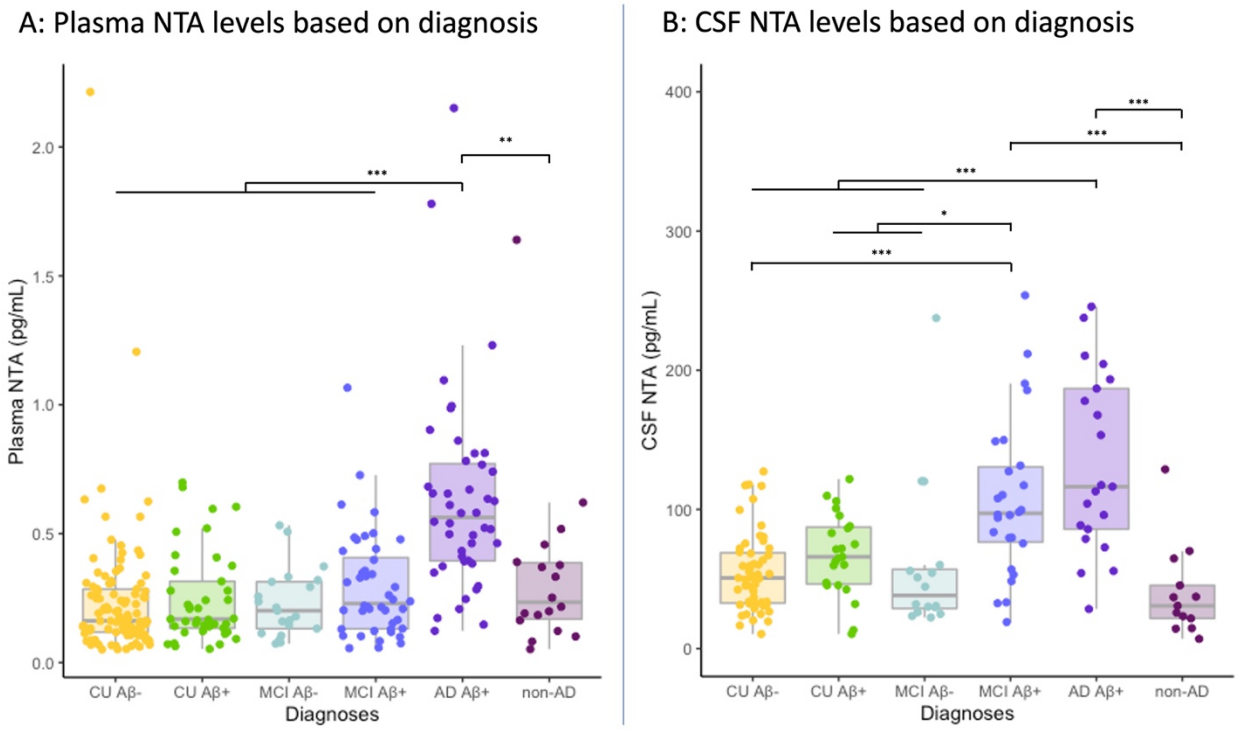


Figure 1: Plasma (A) and CSF (B) NTA-tau concentrations stratified by diagnosis and beta-amyloid (Aβ) positivity.

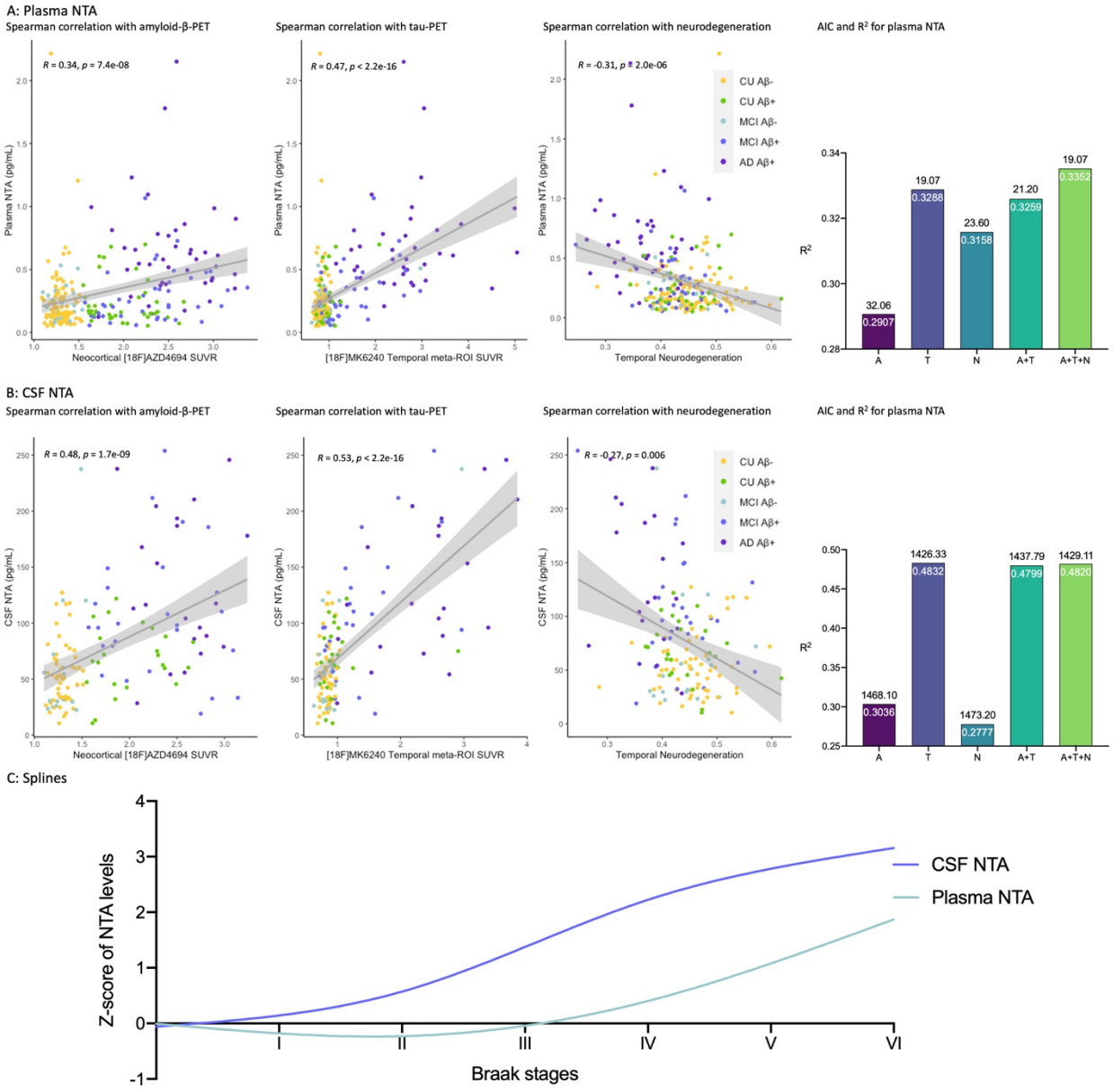


Figure 2: Associations of NTA-tau concentration with Alzheimer's disease hallmarks. Correlation of plasma (A) and CSF (B) NTA-tau concentration with Aβ-PET, tau-PET, and neurodegeneration estimated by voxel-based morphometry (VBM). Corresponding AIC and R^2 AIC and R^2 values for each predictor were obtained from linear regression models correcting for age, sex, and diagnosis. (C) Smoothing splines present the changes in plasma and CSF NTA-tau concentrations along the *in vivo* Braak staging system assessed with [^{18}F]MK6240.

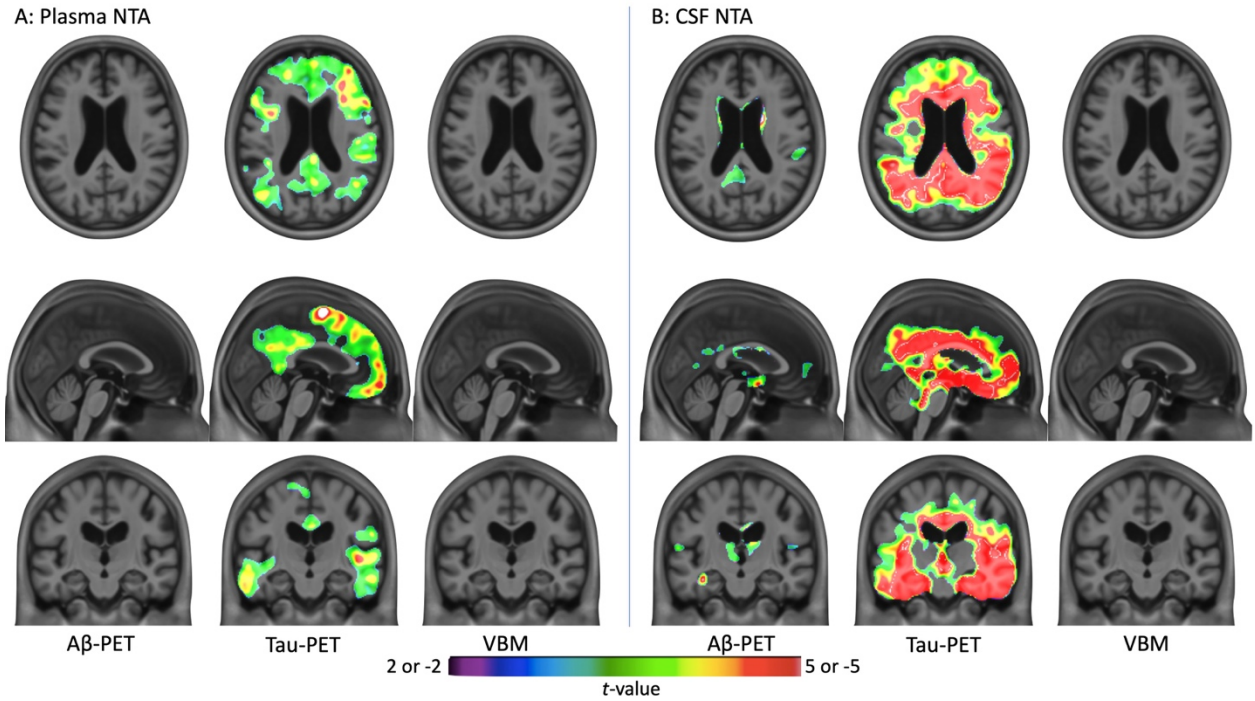


Figure 3: Voxel-wise associations between plasma (A) and CSF (B) NTA-tau concentrations and Aβ-PET, tau-PET and neurodegeneration assessed by voxel-based morphometry (VBM).

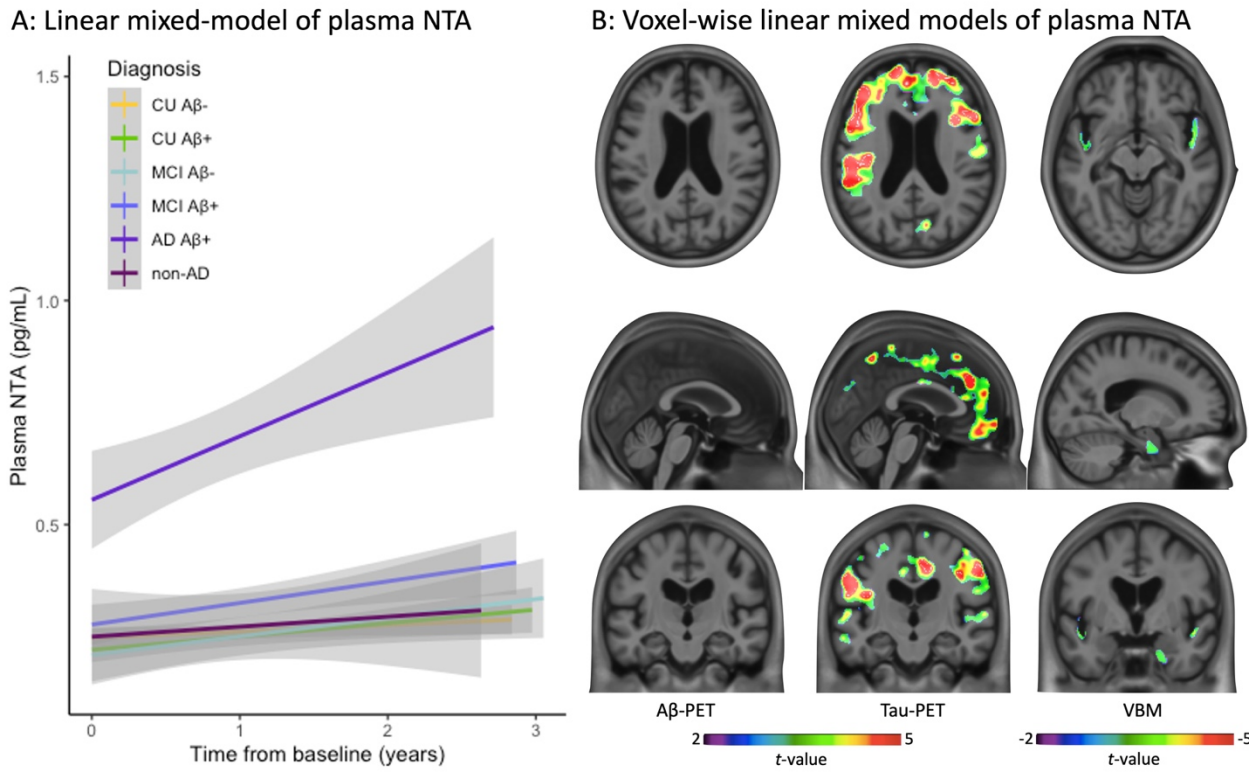


Figure 4: Longitudinal changes in plasma NTA-tau concentrations. (A) Longitudinal changes in plasma NTA levels based on diagnosis. (B) Linear mixed models presenting the association between longitudinal changes in plasma NTA-tau concentrations and in Aβ-PET, tau-PET and neurodegeneration assessed by voxel-based morphometry (VBM).

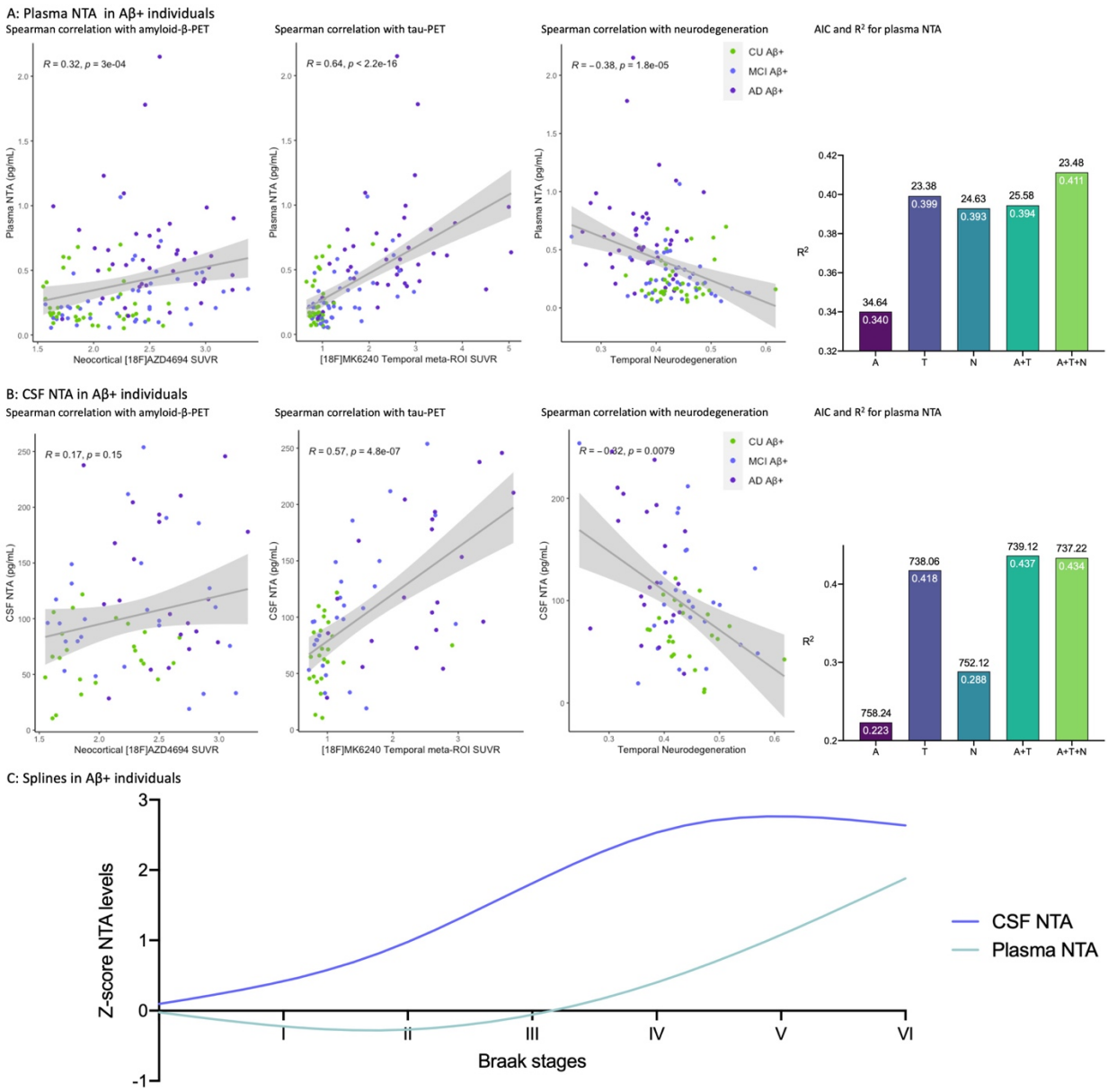
4.9 Tables

Table 1: Demographics

	CU Aβ- (N=104)	CU Aβ+ (N=41)	MCI Aβ- (N=21)	MCI Aβ+ (N=44)	AD Aβ+ (N=44)	non-AD (N=18)	P-value
Age (mean (SD))	69.71 (9.0)	71.34 (10.3)	72.60 (5.2)	71.08 (5.5)	68.13 (8.6)	61.36 (8.6)	<0.001
Sex							0.197
Female	62 (59.6%)	29 (70.7%)	8 (38.1%)	26 (59.1%)	27 (61.4%)	13 (72.2%)	
Male	42 (40.4%)	12 (29.3%)	13 (61.9%)	18 (40.9%)	17 (38.6%)	5 (27.8%)	
Years of education (mean (SD))	15.54 (3.8)	14.66 (3.7)	15.00 (4.5)	15.50 (3.6)	14.62 (3.3)	13.83 (3.2)	0.314
Aβ-PET neocortical SUVR (mean (SD))	1.29 (0.1)	2.01 (0.3)	1.31 (0.1)	2.30 (0.5)	2.53 (0.4)	1.19 (0.1)	<0.001
Tau-PET temporal meta-ROI SUVR (mean (SD))	0.84 (0.1)	0.94 (0.2)	0.94 (0.5)	1.38 (0.6)	2.42 (1.0)	0.81 (0.1)	<0.001
Plasma NTA (pg/mL) (mean (SD))	0.24 (0.3)	0.25 (0.2)	0.23 (0.1)	0.29 (0.2)	0.63 (0.4)	0.35 (0.4)	<0.001

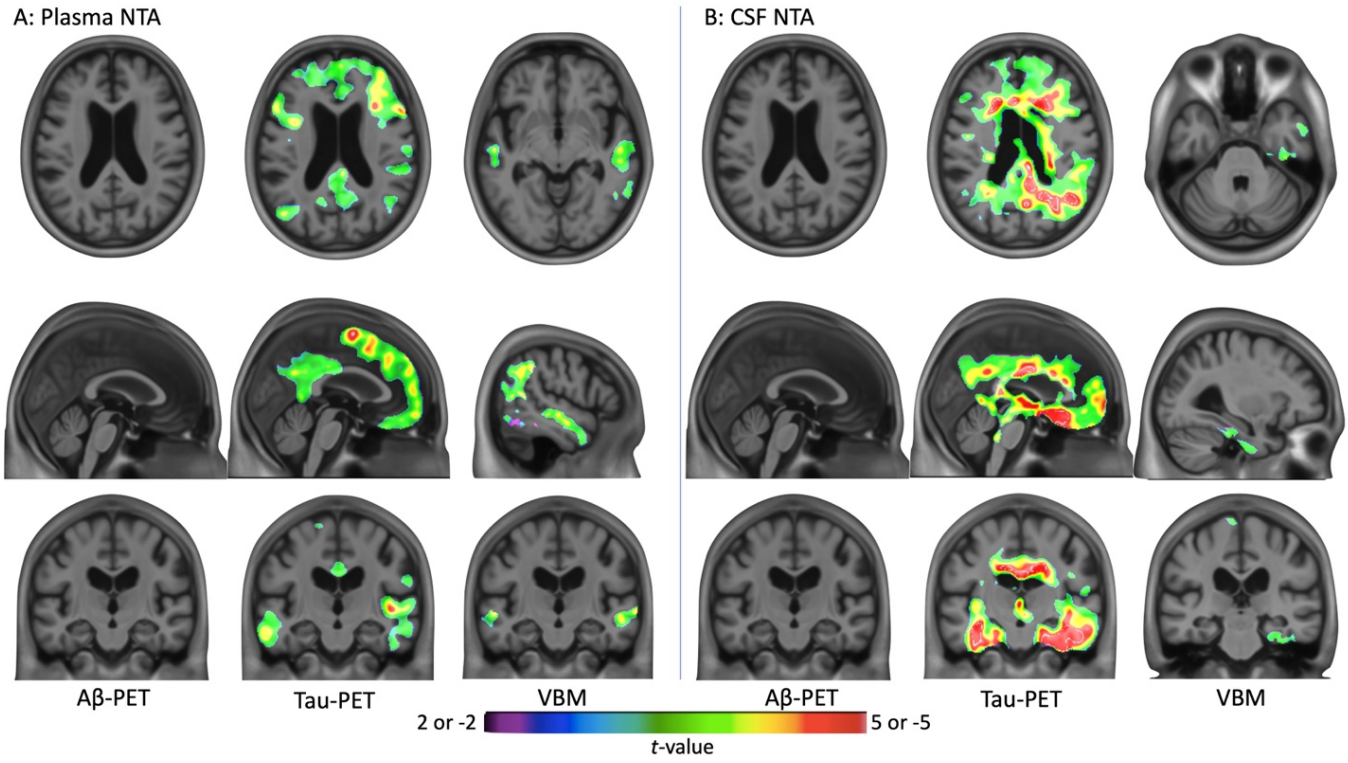
4.10 Supplementary material

Supplementary figures



Supplementary Figure 1: Associations of NTA-tau concentration with AD hallmarks in Aβ positive individuals. Correlation of plasma (A) and CSF (B) NTA-tau concentration with Aβ-PET, tau-PET, and neurodegeneration estimated by voxel-based morphometry (VBM), with corresponding AIC and R^2 values. AIC and R^2 values for each predictor were obtained from linear regression models correcting for age, sex, and diagnosis. C: Smoothing splines showing changes

in plasma and CSF NTA-tau concentrations along the *in vivo* Braak staging system assessed with [^{18}F]MK6240.



Supplementary Figure 2: Voxel-wise associations of **(A)** plasma and **(B)** CSF NTA-tau concentrations with Aβ-PET, tau-PET and neurodegenerations assessed by voxel-based morphometry (VBM).

Supplementary tables

Supplementary Table 1: Repeatability and intermediate precision of the NTA assay in TRIAD cohort, both in CSF and plasma.

NTA	iQC	Concentration (pg/ml)	Repeatability (CVr) %	Intermediate precision (CVRw) %
CSF	LowQC	2.4	4.2	10.4
	HighQC	75	5.8	7.5
	Mean		5.0	9.0
Plasma	LowQC	0.2	5.6	8.4
	HighQC	2.4	6.6	8.6
	Mean		6.1	8.5

Abbreviations: iQC, internal quality control

Supplementary Table 2: Demographic information and biomarker concentrations of the CSF subsample (n = 154).

	CU Aβ- (N=55)	CU Aβ+ (N=23)	MCI Aβ- (N=16)	MCI Aβ+ (N=26)	AD Aβ+ (N=21)	non-AD (N=13)	P-value
Age (mean (SD))	70.50 (7.2)	69.90 (9.7)	72.17 (5.7)	70.77 (5.7)	65.78 (8.0)	63.76 (7.6)	0.00398
Sex							0.777
Female	29 (52.7%)	15 (65.2%)	7 (43.8%)	16 (61.5%)	12 (57.1%)	8 (61.5%)	
Male	26 (47.3%)	8 (34.8%)	9 (56.3%)	10 (38.5%)	9 (42.9%)	5 (38.5%)	
Years of education (mean (SD))	15.58 (3.4)	14.65 (3.5)	15.44 (5.0)	15.54 (2.7)	14.62 (3.0)	13.23 (2.8)	0.205
Aβ-PET neocortical SUVR (mean (SD))	1.30 (0.1)	2.01 (0.4)	1.31 (0.1)	2.27 (0.5)	2.54 (0.4)	1.21 (0.1)	<0.001
Tau-PET temporal meta-ROI SUVR (mean (SD))	0.83 (0.1)	1.01 (0.4)	0.97 (0.5)	1.35 (0.6)	2.40 (0.8)	0.79 (0.1)	<0.001
CSF NTA (pg/mL) (mean (SD))	54.75 (27.9)	67.80 (29.2)	60.21 (56.4)	106.95 (57.1)	132.81 (64.0)	40.07 (32.6)	<0.001
Plasma NTA (pg/mL) (mean (SD))	0.24 (0.2)	0.27 (0.2)	0.23 (0.1)	0.31 (0.2)	0.63 (0.4)	0.41 (0.4)	<0.001

Supplementary Table 3: Demographic information of the longitudinal sample (n = 127).

	CU Aβ- (N=50)	CU Aβ+ (N=22)	MCI Aβ- (N=8)	MCI Aβ+ (N=28)	AD Aβ+ (N=16)	non-AD (N=3)	P-value
Age (mean (SD))	70.37 (7.0)	72.37 (8.7)	70.08 (5.8)	71.96 (5.2)	69.10 (9.2)	66.90 (5.3)	0.244
Sex							0.152
Female	29 (58.0%)	16 (72.7%)	4 (50.0%)	14 (50.0%)	14 (87.5%)	2 (66.7%)	
Male	21 (42.0%)	6 (27.3%)	4 (50.0%)	14 (50.0%)	2 (12.5%)	1 (33.3%)	
Years of education (mean (SD))	15.90 (3.9)	15.09 (2.9)	14.25 (5.5)	15.57 (3.1)	15.28 (2.5)	12.67 (4.0)	0.681

Supplementary methods

Imaging methods:

MRI data was obtained at the Montreal Neurological Institute, using a 3T Siemens Magnetom with a standard head coil. A volumetric magnetization prepared rapid gradient echo (MPRAGE) MRI (TR: 2300 ms, TE: 2.96ms) sequence was employed to obtain a high-resolution T1-weighted anatomical image of the entire brain. An optimized voxel-based morphometry (VBM) protocol (Good et al., 2001) was followed for the preprocessing of T1-weighted MR images. Voxel values were modulated by multiplying them by the Jacobian determinants derived from the spatial normalization step, and images were smoothed with a 8mm isotropic Gaussian kernel.

PET scans were obtained with a Siemens High Resolution Research Tomograph (HRRT). [^{18}F]MK6240 images were acquired 90-110 minutes post-injection, and used the inferior cerebellar grey (CG) as the reference region (Pascoal et al., 2018). [^{18}F]AZD4694 images were obtained 40-70 minutes post-injection, and used the CG as the reference region (Cselényi et al., 2012)**.** For attenuation correction, each PET scan was followed by a 6-minute transmission scan conducted with a rotating ^{137}Cs point source. Moreover, the images were corrected for dead time, decay and random and scattered coincidences.

Using an in-house pipeline, T1-weighted images were further corrected for non-uniformity and field-distortion. PET images were automatically registered to the T1-weighted image space, and the T1-weighted images linearly and non-linearly registered to the ADNI template space. Afterwards, we performed a PET non-linear registration using the linear and non-linear transformations from the T1-weighted image to the ADNI space and the PET to T1-weighted image registration. Finally, the PET images were spatially smoothed to achieve a final resolution

of 8mm full-width at half maximum. All images underwent quality control to ensure alignment was adequate.

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Chapter 5: Discussion

The overarching goal of the thesis is to depict the impact of tau biomarkers on the A/T/(N) framework of AD dementia. **Chapter 1** allowed us to have a comprehensive perspective of the current research in AD. In **Chapter 2**, we used a novel assay for plasma pTau181 and evaluated its predictive value for current and future neurodegeneration, in individuals not presenting cognitive dysfunction and in individuals diagnosed with cognitive impairment. In **Chapter 3**, we compared multiple tau assessment methods and noticed they were associated with distinct stages of tau pathological progression; some biomarkers emerging at early stages of the disease when pathophysiological changes are only due to amyloid-β plaque accumulation. Finally, **Chapter 4** assessed a novel biofluid marker of tau, which, as compared to other current biofluid markers, seem to be only related to tau and no other AD pathophysiology. In **Chapter 5** we thus discuss how tau biomarkers, often specific to AD dementia, are able to track pathophysiological changes that occur in the disease, related to amyloid-β plaque and NFT accumulation, as well as neurodegeneration.

Research around the world has revealed an increasing prevalence of AD. Currently known as the most common form of dementia, it is one of the leading causes of death in individuals aged 65 and older (Alzheimer Association, 2010). As population ages, the number of "oldest old", meaning elderlies 85 and more, will affect the number of people living with AD. In turn, this will cause a dramatic increase in the number of individuals becoming caregivers, which have been shown to present emotional distress and increased financial burden (Papastavrou et al., 2007). The

goal to limit the symptoms of AD is not only to help individuals living with dementia, but also their caregivers and families.

As more individuals develop AD dementia, it is critical to have a definition of the disease, as well as an easier diagnosis of it. Today, AD dementia is diagnosed through cognitive testing, and a definite diagnosis can only be done after *post-mortem* assessment of amyloid-β and NFT accumulation. It is however important to note that a high number of individuals have concordant living clinical and *post-mortem* diagnoses (Grandal Leiros et al., 2018).

Over the last years, biomarkers of dementia, and especially of AD dementia have been created, leading to the NIA-AA research framework. AD dementia is a *continuum* and staging the disease would be of great help in the diagnostic and clinical trial settings. Most importantly, biomarkers are based on *in vivo* measures assessed via CSF and imaging (MRI and/or PET). In this case, the diagnosis is not based on symptomatology and rather on underlying pathologic processes.

AD dementia has been related to accumulation of amyloid-β plaques and NFT, and neurodegeneration (Braak & Braak, 1991). Furthermore, the amyloid-β cascade hypothesis led to the idea that AD dementia starts with accumulation of amyloid-β years before the onset of symptoms, which leads to NFT, and finally to neurodegeneration (John & Gerald, 1992). Various studies corroborated this idea, documenting the existence of a preclinical phase of AD, that can be assessed using *in vivo* biomarkers (Dubois et al., 2016; Palmqvist et al., 2016; Sperling et al., 2011). This is especially critical for physicians to predict future cognitive decline and neuropsychiatric symptoms, as well as in clinical trials as inclusion criteria.

Following the above findings, the NIA-AA research framework developed the A/T/(N) scheme for AD. It classifies individuals based on biomarker measures of amyloid-β (A), NFT (T) and

neurodegeneration (N) biomarkers (Jack et al., 2018). The system was created using imaging and CSF markers, but also to be flexible, in a sense that new groups and new biomarkers could be added when becoming available. Most importantly, it is accomplished using continuous measures, respecting the pathological processes of AD dementia that progress over time.

Measures of pTau181 in the CSF have been accepted as a clinical tool to assess pathology load (Niemantsverdriet et al., 2017). In recent years, researchers have been trying to assess pTau181 levels in the blood. One assay, created at the University of Gothenburg has shown to be highly sensitive and specific to AD (Karikari, Pascoal, et al., 2020). Using a diverse cohort of individuals with follow-up up to four years after baseline, we assessed the cross-sectional and longitudinal associations between plasma pTau181 and neurodegeneration. In **Chapter 2** we first depicted cross-sectional differences between cognitively unimpaired and impaired individuals. While grey matter decrease was only observed in small regions of the cognitively unimpaired group, we found both grey matter and white matter decrease in cognitively impaired individuals. Moreover, plasma pTau181 presented itself as a great tool to predict longitudinal changes. Indeed, it was able to predict future grey matter degeneration 36-months after baseline in the cognitively unimpaired group. Nevertheless, it predicted eminent (i.e. as soon as 12-months after baseline) grey matter and white matter degeneration in cognitively impaired individuals.

Other blood-based biomarkers have been related to neurodegeneration and seen as promising candidates, such as neurofilament light chain (NfL) (Benedet et al., 2019). Nevertheless, NfL is not an AD-specific marker, as levels are also increasing in normal aging and other neurodegenerative conditions (Hansson et al., 2017). In this case, plasma pTau181 is only increased across the AD spectrum, and is also able to reflect stages of disease progression.

Interestingly, plasma pTau181 levels have displayed significant differences based on the amyloid-β status (Karikari, Benedet, et al., 2020; Karikari, Pascoal, et al., 2020). However, stratification was not possible in this sample size, due to low number of individuals undergoing amyloid-β PET or CSF amyloid-β assessments. It would be interesting to know the prediction power of plasma pTau181 in combination with assessments of CSF or PET amyloid-β.

In addition to plasma pTau181, other proxies of tau load were discovered. For example, plasma pTau231, thought to be an early marker of AD pathology progression (Ashton et al., 2021). As mentioned previously, the A/T/(N) system is based on CSF and imaging measures. However, these are invasive and expensive assessment methods, and usually require special settings, which hampers their use around the world. Thus, plasma markers would be of great advantage to create an easier diagnosis of AD based on clinically validated markers. In **Chapter 3** we compared tau statuses given based on three distinct tau measurement methods, plasma pTau231, pTau181 and tau-PET ([^{18}F]MK6240). We then examined measures of AD pathophysiologies, comprised in the A/T/(N) system, and risk factors of AD based on the tau status. The analyses revealed that plasma and imaging measures reflect distinct stages of tau pathological progression, and cannot be used interchangeably. However, when an individual obtained a positive status in one of the biomarkers, it was most often associated with cognitive impairment (either MCI or AD), positive amyloid-β-PET status, higher risk of carrying at least one *APOE*ε4 allele, high hippocampal atrophy and high levels of CSF pTau181. Incorporating plasma markers in the A/T/(N) system would probably allow for a more detailed staging of the disease. Most importantly, it is thought that distinct phosphorylation sites emerge at different stages of the disease of disease progression (Barthélemy, Li, et al., 2020; Suárez-Calvet et al., 2020), meaning measuring the amount of pTau in a specific

site would allow for an *in vivo* staging. A recent study conducted in our group further corroborated this idea. Braak staging was assessed via tau-PET with [^{18}F]MK6240 (Pascoal et al., 2020), allowing for a biomarker modeling of the disease. Changes in CSF and plasma pTau species revealed the differential evolution of each one: CSF markers were observed to increase at earlier stages and showed a more drastic increase, as compared to plasma pTau species, presenting a slower increase. However, pTau epitopes were increasing in the same order in CSF and plasma (Therriault, Pascoal, et al., 2022).

A study conducted using CSF pTau measures and tau-PET revealed that positivity to one CSF measure predicted future tau-PET positivity (Meyer et al., 2019). This study lacked longitudinal analyses, which would have revealed if individuals presenting plasma biomarker positivity at baseline would have predicted a transition to positivity of the other plasma marker – and which one was increasing first – as well as tau-PET.

Over the years, plasma pTau markers were revealed to be related to amyloid-β plaques and NFT (Ashton et al., 2021; Karikari, Pascoal, et al., 2020). They were also indicated to emerge before tau-PET, when only abnormal levels of amyloid-β are detected (Palmqvist et al., 2016; Suárez-Calvet et al., 2020). A recent study has even shown both plasma and CSF pTau levels are more closely associated with amyloid-β-PET rather than tau-PET (Therriault, Vermeiren, et al., 2022). We can thus question to which extent does pTau reflect increases in amyloid-β rather than NFT pathology. As cognitive and neuropsychiatric deficits are more closely related to NFT pathology as compared to amyloid-β (Gomez-Isla et al., 1997; Nelson et al., 2009; Terry et al., 1981; Tissot et al., 2021), there is a critical need for a biofluid marker capable of tracking NFT pathology only. Researchers focused on non-phosphorylated tau markers, t-tau, except these were

not specific to AD, as they presented a large overlap with other acute neurological conditions (Mattsson et al., 2016; Zetterberg et al., 2013). More recently, the NT1 assay targeted tau species ranging from N-terminal to mid-region was revealed to be promising in the blood (Chen et al., 2019; Chhatwal et al., 2020; Mengel et al., 2020). Thus, in collaboration with the University of Gothenburg, we created a novel assay called NTA-tau, using the same N-terminal targeted strategy (Snellman et al., 2022). In **Chapter 4**, we characterized this novel assay in the TRIAD cohort (Therriault et al., 2020), where individuals undergo gold standard imaging procedures. NTA-tau levels in plasma and CSF correlated more strongly with tau-PET rather than amyloid-β-PET measures. Most importantly, we observed that NTA-tau was AD-specific, and even able to track tau pathological progression across Braak stages. Longitudinal analyses were conducted with plasma NTA-tau only, as we did not have enough power to conduct them in the CSF. We found that plasma NTA-tau was associated with longitudinal tau-PET accumulation as well as neurodegeneration.

Taken together, the studies indicated that markers of tau pathology can help in the prediction of A/T/(N) status, as well as to follow the progression along the disease *continuum*. More specifically, markers of "A" are currently known as CSF and PET measures of amyloid-β. This research, in conjunction with other ones, have revealed that pTau assays might be reflecting amyloid-β pathology better than tau (Milà-Alomà et al., 2022; Therriault, Vermeiren, et al., 2022). This is particularly important as current plasma markers of amyloid-β have rendered mixed results (Janelidze et al., 2016). First, they are not AD-specific and might not be sensitive enough to capture the changes observed through CSF and imaging analyses. Current pTau assays are able to assess early changes in amyloid-β and tau pathology. We can hypothesize that present pTau measures

can be used to define “A”. Nevertheless, it is important to note that pTau epitopes cannot be used interchangeably, as they assess distinct stages of disease progression (Suárez-Calvet et al., 2020; Therriault, Pascoal, et al., 2022; Tissot, Therriault, et al., 2022). Longitudinal analyses will be required to assess which epitopes emerge first, and are more or less closely linked to either AD-related pathophysiology.

Markers of “T”, presently CSF and PET measures, were the first plasma markers investigated performing well in the discrimination of AD from non-AD cases (Zetterberg et al., 2013). In the last decade, highly sensitive assays were created, especially using the Simoa platform. Tau can be observed in a variety of diseases called tauopathies (V. M. Y. Lee et al., 2001), thus existing total tau assays were not specific to AD. Conversely, researchers realized that pTau assays were AD-specific. By focusing on the N-terminal to mid-region parts of the tau protein, we were able to create an assay differentiating AD from other neurodegeneration conditions, which was not associated with amyloid-β and neurodegeneration (i.e. the other AD hallmarks). Most importantly, it was able to track tau pathological progression across PET-defined Braak stages (Braak & Braak, 1991; Pascoal et al., 2020), as well as predict future cognitive decline (Snellman et al., 2022) and tau accumulation. This research was the first one measuring NTA-tau in a large cohort of individuals; we would need to replicate the findings in another one before adding NTA-tau in the A/T/(N) framework of AD.

Finally, “N” is known to be a non-disease specific change, as it was observed in normal aging and various brain conditions (Thal et al., 2004). The current biomarkers for neurodegeneration in the A/T/(N) system are based on CSF, PET or MRI imaging. In the last few years, researchers created markers supposedly reflecting AD-specific neurodegeneration, especially when using MRI imaging (Jack et al., 2017). Nevertheless, biofluid markers have been shown to increase in normal

aging, acute and chronic neurodegenerative conditions (Hansson et al., 2017), rendering their use in the A/T/(N) system complicated. Even though NfL was promising in reflecting AD pathophysiological changes (Benedet et al., 2019), another biomarker of AD was always necessary to assure a participant was considered on the AD *continuum*. The tau biomarkers used in the present thesis were, first of all, AD specific, and secondly, able to reflect existing and/or future neurodegeneration. Plasma pTau181 could predict neurodegeneration in both cognitively unimpaired and impaired group and NTA-tau was able to predict eminent degeneration across the aging and AD spectrum.

An important aspect of the A/T/(N) system is to predict future cognitive decline and AD pathophysiological changes. We hereby showed that tau markers were able to reflect the current biomarker status of individuals, as well as predict future changes in a timely manner. By including information regarding their cognitive status, we were able to predict whether someone was subject to neurodegeneration eminently or a few years later using plasma pTau181. NTA-tau predicted eminent tau accumulation as well as neurodegeneration. Even though it has been tested in a limited number of cohorts, its strong association with tau-PET, suggests NTA-tau could be of great use to evaluate tau changes *in vivo* through a simple blood test.

Yet, the assays used in this thesis are novel, not commercially available nor spread out around the world. Even though they always present AD-specificity and similar results among study groups, various manuscripts have indicated different cut-off values for positivity depending on the cohort used (Karikari, Benedet, et al., 2020; Tissot, Therriault, et al., 2022). Before their implementation in the clinical and clinical trial settings, more research is needed to identify what are the factors that would cause such changes and variations in the cut-off values. Furthermore, plasma markers

are presently being investigated as surrogate markers of AD-related pathology, to study if they could replace CSF and/or imaging measures. In the clinical trial setting, researchers could use plasma markers specific to AD, first as inclusion and/or exclusion criteria, and then to assess disease progression when exploring the effects of disease-modifying therapies (Mintun et al., 2021; Shcherbinin et al., 2022). Plasma pTau181 for example was already assessed as a surrogate marker of disease progression and has shown great results in decreasing the cost of clinical trials (Ferreira et al., 2022). However, these are still markers of early-stages pathological changes, thus we could wonder whether using later AD-specific biomarkers renders better results.

In the clinic, there is still a long way before making those assays available to everyone. The inherent problem is the inability to determine brain region-specific changes, when visually assessing brain damage gives information on the type of brain condition the patient is suffering from. Biomarkers are currently being tested to be specific for one particular disease. When an individual comes into the clinic with dementia symptoms, researchers will thus have to determine which biomarker or array of biomarkers to test in order to define the diagnosis. There is still some discussion as to how we can use current plasma markers in the clinic. Instead of using them to determine if someone has a specific diagnosis, it could be used to exclude possible ones. Analyses are currently being conducted to use plasma markers' negative predictive value rather than their positive predictive value.

Future studies also need to investigate the ethnic, racial and sex differences tau markers can have, and how it could change their use in the A/T/(N) system. There are indeed significant differences in the prevalence of AD based on sex and race (Alzheimer Association, 2010). For now, little research has been conducted on the sex differences in plasma markers, albeit, a few that

suggested that the clinical interpretation could be impacted by it (Tsiknia et al., 2022). Furthermore, race and ethnic differences were clearly observed using plasma and CSF measures; for example African Americans present lower CSF pTau181 and t-tau levels for a similar cognitive impairment (Hajjar et al., 2022; Morris et al., 2019). In order to implement the use of tau biomarkers in the A/T/(N) system worldwide, we would first need to fully understand the sex and race differences and account for them in the framework.

Further research is required to investigate gender differences in AD. While genetic factors related to dementia have been identified, gender is also likely to play a significant role (Podcasy & Epperson, 2016). For instance, studies have linked lower education levels with a higher risk of cognitive impairment (Katzman, 1993; Stern et al., 1994), and historically, women have had less access to education than men. However, this is still an emerging area of research that requires emphasis from the research community.

The thesis focused on the impact of tau biomarkers in the A/T/(N) framework of AD. With research finding novel assessment methods, we will be able to include them in the A/T/(N) framework, and evaluate possible differences among all of them. As seen in **Chapter 3** and in other manuscripts, we expect that distinct biomarkers of tau emerge at different stages of disease progression. By using a combination of tau markers, rather than only one, we might be able to stage the AD *continuum* and better predict progression to dementia. Taking together the latest research and the present analyses, we observed that plasma pTau markers are early markers of AD pathogenesis, and even though they were first created as markers of tau pathology, they might be more appropriate to define "A". We further showed that "T" can be quantified via numerous tau biomarkers that cannot be used interchangeably, nevertheless, each one provides information on

the disease stage. Finally, “N”, that is observed in various brain conditions, can be predicted years before using tau biomarkers; by using AD-specific markers it also allows to ascertain the neurodegeneration observed is due to this disease and not another.

It is important to note that the knowledge regarding AD onset and progression increases every year. It is currently thought that neuroinflammation, cerebrovascular problems and other factors might affect the disease. We presently focused on tau to explain A/T/(N) as it follows more closely the cognitive symptoms as compared to the other pathologies. Nevertheless, we must keep in mind that other biomarkers, related to the different factors associated with the disease, might be better to track the progression. For example, neuroinflammation has been closely related with amyloid-β and tau pathologies; GFAP predicts amyloid-β and YKL-40 predicts tau accumulation (Benedet et al., 2021; Ferrari-Souza et al., 2022). Incorporating biomarkers of neuroinflammation, or any other factor affecting the disease, in the A/T/(N) system might help further stage AD pathogenesis.

6. Conclusion

To conclude, this thesis investigated currently available tau biomarkers and their impact of the A/T/(N) framework. We hereby proved that biofluid and imaging markers of tau can help in the assessment of a person's stage along the A/T/(N) framework of AD. The presence, as well as the progression of fluid tau species convey information on the downstream AD pathophysiological events, such as tau aggregates and neurodegeneration (Figure 1). Importantly, these biomarkers are specific to the disease, while amyloid-β and neurodegeneration can be observed in various brain disorders. Thus, it allows for the assessment of amyloid-β and neurodegeneration, in addition to tau, specifically associated with the disease. The A/T/(N) framework is applied to better understand and better predict the onset and progression of AD dementia. Amyloid-β accumulation occurs years before clinical symptoms, and, alone, it is not sufficient to cause the disease. However, markers of amyloid-β plaques give insight on the risk of progression to dementia. Additionally, neurodegeneration markers are not specific to AD, thus cannot be used to identify the disease but can help in staging it. Altogether, tau biofluid markers have currently shown to be AD-specific, while imaging markers can be observed in different tauopathies. On the one hand, biofluid assessments are specific for a disease and usually more easily performed around the world; on the other hand, they do not depict the topographical information that PET tracers present, often allowing for a more precise diagnosis.

We first observed that plasma pTau can be used to predict present and future cortical atrophy in a disease-stage-specific manner. We hereby tested only plasma pTau181 but it is expected that all plasma pTau epitopes behave in a similar way: they emerge when abnormal levels of amyloid-β can be detected and can help in staging disease progression. Secondly, we depicted differences in tau statuses when using different methods of NFT assessment. This suggests they

each represent distinct stages of tau progression, with plasma and CSF positivity preceding tau-PET positivity. Finally, we seem to have found a marker of tau pathology that is only associated with NFT accumulation and no other AD hallmark. Interestingly, plasma pTau181 – and possibly other pTau epitopes – and NTA-tau were able to predict neurodegeneration in a timely manner.

The results suggest that tau markers are specific to a disease stage and can be used to track the progression of AD pathologies, along the A/T/(N) framework.

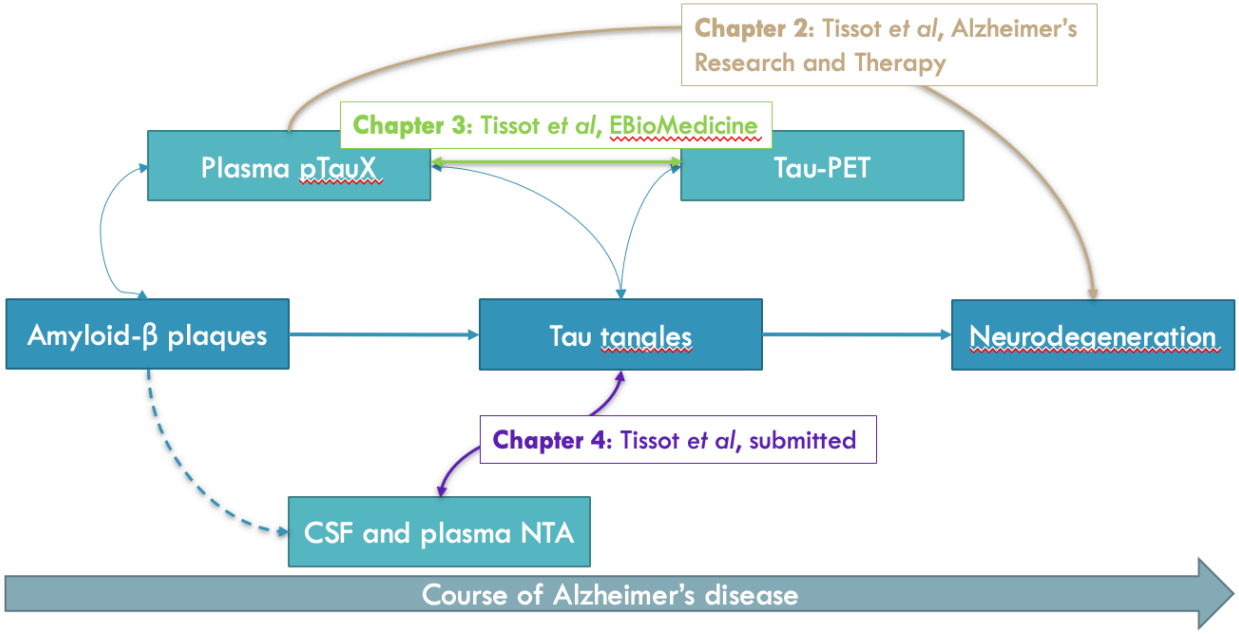

Figure 10: Rationale of the thesis.

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