Blood-Based Biomarkers of Alzheimer's disease: Bringing plasma p-Tau to the Clinic

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Bringing Plasma p-Tau to the Clinic

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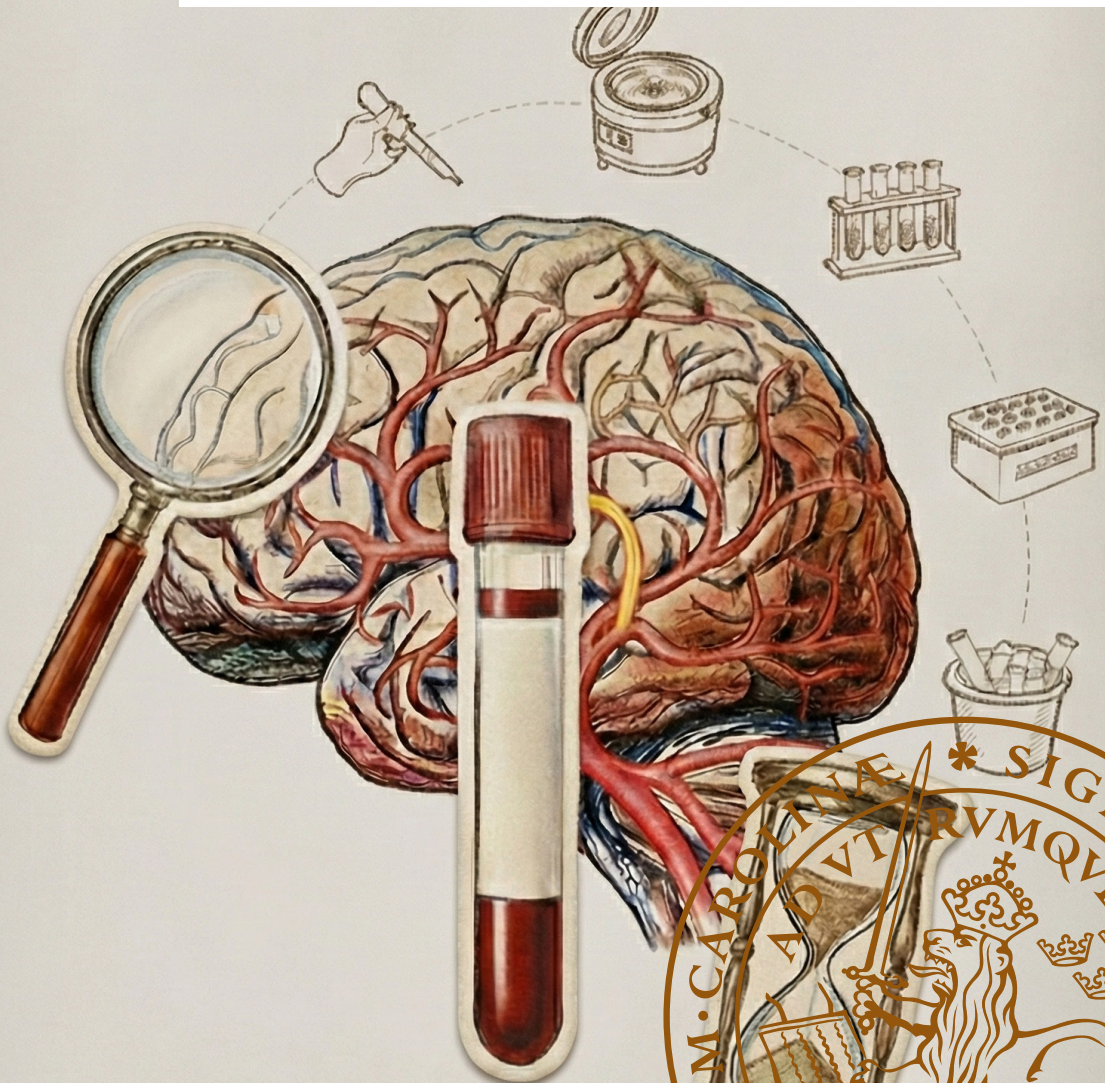

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About the author

Divya Bali holds a Master's degree in Biotechnology from Lund University, Sweden. In 2021, she began her PhD studies in the field of neurology. In her PhD thesis, she focused on investigating the diagnostic and prognostic utility of various blood-based biomarkers of Alzheimer's disease, particularly p-Tau, as well as the impact of pre-analytical factors on the concentration and performance of p-Tau217. This work contributes to supporting the implementation of plasma p-Tau217 for the diagnosis and prognosis of Alzheimer's disease, as well as for monitoring disease progression in both routine clinical practice and treatment trials. In addition to her research, Divya has served as a Membership Officer at Lunds Doktorandkår (LDK) and has held leadership roles as Head of Events and Vice-Chair of the Medical Doctoral Student Union (MDR) at Lund University.

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Bringing Plasma p-Tau to the Clinic

Divya Bali

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Abstract: Alzheimer's disease, the leading cause of dementia, is neuropathologically defined by the deposition of amyloid-beta (Aβ) plaques and neurofibrillary tau tangles. Cerebrospinal fluid (CSF) and imaging biomarkers can reliably detect underlying Aβ and tau pathologies. However, their widespread use is impractical in routine clinical practice globally. Therefore, more accessible and inexpensive tools, such as blood-based biomarker (BBM) tests, are needed. Advances in sensitive immunoassays and mass spectrometry (MS-based) techniques, have made it possible to measure various phosphorylated tau (p-Tau) species in blood. Recent evidence indicates that blood p-Tau may be a highly useful biomarker of Alzheimer's disease related pathologies.

The aim of this thesis was to facilitate the integration of plasma p-Tau as a biomarker of Alzheimer's disease in routine clinical practice and treatment trials. This was studied using the well-characterized, longitudinal, prospective Swedish BioFINDER-1 study, as well as an independent cohort of patients with mild cognitive impairment (MCI) and the neuropathology Banner cohort.

In paper I, we found that the optimal sample handling conditions for measuring plasma p-Tau217 on the Mesoscale Discovery (MSD) platform involved thawing samples at room temperature followed by centrifugation prior to analytical assessment.

In paper II, on assessing the performance of various p-Tau variants and assays for predicting abnormal Aβ status and progression to Alzheimer's disease dementia, we demonstrated that MS-based p-Tau217 had the best performance. Several p-Tau217 immunoassays showed high accuracy even though it was lower than the accuracy of MS p-Tau217.

In paper III, on assessing the performance of ALZpath p-Tau217 assay against the neuropathological outcomes, we found that it performed similarly to Lilly p-Tau181 assay. However, its associations with core measures of Alzheimer's disease pathology were significantly weaker than those of p-Tau217$_{\text{Lilly}}$.

In paper IV, we found that normalizing longitudinal CSF biomarkers to a reference protein and specifically to Aβ40 strengthened their associations with longitudinal measures of disease progression such as cognitive decline and brain atrophy. This improvement did not apply to plasma biomarkers. In addition, we found that plasma p-Tau217 and CSF p-Tau205/Aβ40 in combination with NfL could serve as surrogate markers for monitoring treatment responses.

Together, our findings support the implementation of plasma p-Tau217 for diagnosis and prognosis of Alzheimer's disease, as well as for monitoring of disease progression in both routine clinical practice and treatment trials.

Key words: Alzheimer's disease, plasma p-Tau217, fluid biomarkers, amyloid-beta, tau

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Cover by Gemini AI tool (concept by Divya Bali)

This cover illustrates the scientific journey of translating complex biological signals from the brain into measurable data through the medium of blood, specifically focusing on neurodegenerative conditions like Alzheimer's disease. The central brain, detailed with its intricate vasculature, emphasizes the critical neurovascular link, while the blood collection tube acts as a diagnostic "window" into the organ. The magnifying glass signifies the rigorous scrutiny required to detect biomarkers within complex biological noise. The hourglass symbolizes the critical dimension of time, highlighting both the progressive nature of Alzheimer's and the urgency of early diagnosis ("Time is Brain"). Finally, the surrounding arc of laboratory icons represents the methodological rigor of the scientific process from sample to analysis.

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Dedicated to my parents

“She believed she could, so she did”- R.S.Grey

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Original papers and manuscripts included in the thesis

I. **Bali, D.,** Hansson, O., & Janelidze, S. (2024). Effects of certain pre-analytical factors on the performance of plasma phospho-tau217. Alzheimers Research & Therapy, 16(1), 31. doi.org/10.1186/s13195-024-01391-1

II. Janelidze, S., **Bali, D.,** Ashton, N. J., Barthelemy, N. R., Vanbrabant, J., Stoops, E., Vanmechelen, E., He, Y., Dolado, A. O., Triana-Baltzer, G., Pontecorvo, M. J., Zetterberg, H., Kolb, H., Vandijck, M., Blennow, K., Bateman, R. J., & Hansson, O. (2023). Head-to-head comparison of 10 plasma phospho-tau assays in prodromal Alzheimer's disease. Brain, 146(4), 1592-1601. doi.org/10.1093/brain/awac333

III. **Bali, D.,** Salvado, G., Beach, T. G., Serrano, G. E., Atri, A., Reiman, E. M., Jeromin, A., Hansson, O., & Janelidze, S. (2025). Comparison of plasma ALZpath p-Tau217 with Lilly p-Tau217 and p-Tau181 in a neuropathological cohort. Acta Neuropathol Commun, 13(1), 144. doi.org/10.1186/s40478-025-02064-2

IV. **Bali, D.,** Salvado, G., Ashton, N. J., Palmqvist, S., Rodriguez, L. J., Stromud, E., Carlgren, M. N., Janelidze, S., Hansson, O. Tracking Alzheimer's disease progression using longitudinal CSF and plasma biomarkers and their ratios to Aβ40. *Manuscript.*

Author's contribution to the papers

I. Divya contributed to the study design with other authors. She performed the data collection independently, as well as carried out all the statistical analyses and interpreted the outcomes with some input from coauthors. She wrote and revised the manuscript with input from coauthors.

II. Divya helped to design some parts of the study and independently collected data with input from the shared first author. This study also comprised of other biomarker measurements, which were conducted in collaboration with other laboratories. She actively performed and cross-checked all statistical analyses. She contributed to the results interpretation and manuscript writing.

III. Divya contributed to the study design together with the main supervisor. She collected data independently. Neuropathological assessments and plasma p-Tau217 measurements using ALZpath immunoassay were conducted with our research partner. She drafted and revised the manuscript.

IV. Divya, along with the main supervisor, contributed to the design of the study and independently conducted data collection. Other biomarker measurements were also included in this study, which were conducted in collaboration with our collaborators. She performed the statistical analysis, interpreted the outcomes and drafted the manuscript with some input from the main supervisor.

Papers not included in the thesis

I. Salvado, G., Horie, K., Barthelemy, N. R., Schindler, S. E., Janelidze, S., Dolado, A. O., **Bali, D.,** Stomrud, E., Mattsson-Carlgren, N., Palmqvist, S., Vogel, J. W., Bateman, R. J., Ossenkoppele, R., & Hansson, O. (2025). Alzheimer's Imaging Consortium. Alzheimers Dement, 21 Suppl 8(Suppl 8), e110831. https://doi.org/10.1002/alz70862_110831

II. Lantero-Rodriguez, J., Janelidze, S., Palmqvist, S., Braun-Wohlfahrt, L. S., Vavra, J., **Bali, D.**, Dolado, A. O., Mattsson-Carlgren, N., Stomrud, E., Zetterberg, H., Blennow, K., Hansson, O., Montoliu-Gaya, L., & Salvado, G. (2025). P-Tau205 Is a Biomarker Linked to Tau-Pet Abnormality: A Cross-Sectional and Longitudinal Study. medRxiv.

III. Kjaergaard, D., Simonsen, A. H., Stomrud, E., **Bali, D.**, Janelidze, S., Hansson, O., Waldemar, G., & Nielsen, T. R. (2025). Combining plasma p-tau217 and cross-cultural cognitive tests to identify Alzheimer's Disease in an ethnically diverse mixed memory clinic cohort. J Neurol Sci, 478, 123739. doi.org/10.1016/j.jns.2025.123739

IV. Hellquist, E., Janelidze, S., Weidung, B., Franzon, K., Giedraitis, V., Ingelsson, M., **Bali, D.,** Velickaite, V., Zetterberg, H., Hansson, O., & Kilander, L. (2025). Plasma p-tau(217) correlates strongly with cerebrospinal fluid Abeta(42) and increases over a ten-year period in amyloid-positive, non-demented very old men. Journal of Alzheimers Disease, 13872877251390387. doi.org/10.1177/13872877251390387

V. Kumar, A., Pichet Binette, A., **Bali, D.,** Janelidze, S., Stomrud, E., Palmqvist, S., Vogel, J. W., Hansson, O., & Mattsson-Carlgren, N. (2025). Cell-weighted polygenic risk scores are associated with beta-amyloid and tau biomarkers in Alzheimer's disease. Brain Commun, 7(5), fcaf353. doi.org/10.1093/braincomms/fcaf353

VI. Salvado, G., Janelidze, S., **Bali, D**., Dolado, A. O., Therriault, J., Brum, W. S., Pichet Binette, A., Stomrud, E., Mattsson-Carlgren, N., Palmqvist, S., Coomans, E. M., Teunissen, C. E., van der Flier, W. M., Rahmouni, N., Benzinger, T. L. S., Gispert, J. D., Blennow, K., Dore, V., Feizpour, A., Groups, P.-A. S. (2025). Plasma Phosphorylated Tau 217 to Identify Preclinical Alzheimer Disease. Jama Neurology.

VII. Kjaergaard, D., Nielsen, T. R., Stomrud, E., Palmqvist, S., **Bali, D**., Dolado, A. O., Janelidze, S., Hansson, O., Waldemar, G., & Simonsen, A. H. (2025). Influence of ethnicity on the diagnostic accuracy of plasma p-tau217 for the identification of Alzheimer's disease: A consecutive mixed memory clinic cohort study. Alzheimers Dement, 21(6), e70315.

VIII. Janelidze, S., Ashton, N. J., Orduna Dolado, A., Nordstrom, U., **Bali, D.,** Forsberg, K. M. E., Keskin, I., Mastrangelo, A., Vacchiano, V., Liguori, R., Blennow, K., Zetterberg, H., Mattsson-Carlgren, N., Gonzalez-Ortiz, F., Parchi, P., Andersen, P. M., & Hansson, O. (2025). A comparison of p-tau assays for the specificity to detect tau changes in Alzheimer's disease. Alzheimers Dement, 21(4), e70208. doi.org/10.1002/alz.70208

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XI. Warmenhoven, N., Salvado, G., Janelidze, S., Mattsson-Carlgren, N., **Bali, D.**, Orduna Dolado, A., Kolb, H., Triana-Baltzer, G., Barthelemy, N. R., Schindler, S. E., Aschenbrenner, A. J., Raji, C. A., Benzinger, T. L. S., Morris, J. C., Ibanez, L., Timsina, J., Cruchaga, C., Bateman, R. J., Ashton, N.,…Hansson, O. (2025). A comprehensive head-to-head comparison of key plasma phosphorylated tau 217 biomarker tests. Brain, 148(2), 416-431. doi.org/10.1093/brain/awae346

XII. Pereira, J. B., Kumar, A., Hall, S., Palmqvist, S., Stomrud, E., **Bali, D.,** Parchi, P., Mattsson-Carlgren, N., Janelidze, S., & Hansson, O. (2023). DOPA decarboxylase is an emerging biomarker for Parkinsonian disorders including preclinical Lewy body disease. Nat Aging, 3(10), 1201-1209. doi.org/10.1038/s43587-023-00478-y

XIII. Groot, C., Cicognola, C., **Bali, D.**, Triana-Baltzer, G., Dage, J. L., Pontecorvo, M. J., Kolb, H. C., Ossenkoppele, R., Janelidze, S., & Hansson, O. (2022). Diagnostic and prognostic performance to detect Alzheimer's disease and clinical progression of a novel assay for plasma p-tau217. Alzheimers Research & Therapy, 14(1), 67. doi.org/10.1186/s13195-022-01005-8

XIV. Pichet Binette, A., Palmqvist, S., **Bali, D**., Farrar, G., Buckley, C. J., Wolk, D. A., Zetterberg, H., Blennow, K., Janelidze, S., & Hansson, O. (2022). Combining plasma phospho-tau and accessible measures to evaluate progression to Alzheimer's dementia in mild cognitive impairment patients. Alzheimers Research & Therapy, 14(1), 46. doi.org/10.1186/s13195-022-00990-0

Abstract

Alzheimer's disease, the leading cause of dementia, is neuropathologically defined by the deposition of amyloid-beta (Aβ) plaques and neurofibrillary tau tangles. Cerebrospinal fluid (CSF) and imaging biomarkers can reliably detect underlying Aβ and tau pathologies. However, their widespread use is impractical in routine clinical practice globally. Therefore, more accessible and inexpensive tools, such as blood-based biomarker (BBM) tests, are needed. Advances in sensitive immunoassays and mass spectrometry (MS-based) techniques, have made it possible to measure various phosphorylated tau (p-Tau) species in blood. Recent evidence indicates that blood p-Tau may be a highly useful biomarker of Alzheimer's disease related pathologies.

The aim of this thesis was to facilitate the integration of plasma p-Tau as a biomarker of Alzheimer's disease in routine clinical practice and treatment trials. This was studied using the well-characterized, longitudinal, prospective Swedish BioFINDER-1 study, as well as an independent cohort of patients with mild cognitive impairment (MCI) and the neuropathology Banner cohort.

In paper I, we found that the optimal sample handling conditions for measuring plasma p-Tau217 on the Mesoscale Discovery (MSD) platform involved thawing samples at room temperature followed by centrifugation prior to analytical assessment.

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In paper IV, we found that normalizing longitudinal CSF biomarkers to a reference protein and specifically to Aβ40 strengthened their associations with longitudinal measures of disease progression such as cognitive decline and brain atrophy. This improvement did not apply to plasma biomarkers. In addition, we found that plasma

p-Tau217 and CSF p-Tau205/Aβ40 in combination with NfL could serve as surrogate markers for monitoring treatment responses.

Together, our findings support the implementation of plasma p-Tau217 for diagnosis and prognosis of Alzheimer's disease, as well as for monitoring of disease progression in both routine clinical practice and treatment trials.

Popular scientific summary

Dementia is a condition that affects the brain and causes a gradual decline in an individual's cognitive abilities. Although some cognitive decline is expected as people get older, dementia is not part of normal aging. Globally, around 55 million individuals are living with dementia, and this figure is expected to grow as the population ages. There are several forms of dementia, and Alzheimer's disease is the most common. In an Alzheimer's disease brain, there is an abnormal accumulation of proteins, called amyloid-beta and tau. These proteins can be detected and measured using cerebrospinal fluid (CSF) and brain imaging biomarkers. A biomarker is defined as a measurable indicator that reflects what is happening inside a person's body. It may be a molecule, protein, gene, or enzyme present in blood, tissues or other body fluids. CSF and brain imaging biomarkers have been successfully used in research settings and in specialized memory clinics in some countries to diagnose Alzheimer's disease. However, these methods are costly, invasive and require specialized staff and facilities, making them difficult to be implemented widely. Therefore, the need of the hour is simpler, less costly and minimally invasive test, such as, "blood tests".

Recent advancements in laboratory techniques have made it possible to detect and measure various Alzheimer's disease related protein forms in blood, including phosphorylated tau (p-Tau). Existing evidence have shown that blood p-Tau, particularly p-Tau217, could be a highly useful biomarker for detecting brain changes related to Alzheimer's disease.

The goal of this thesis was to support the implementation of plasma p-Tau as a biomarker for Alzheimer's disease in everyday clinical use and treatment trials. This work was addressed using three very well-characterized, independent cohorts.

It is known that the way plasma samples are handled before biomarker measurement can influence biomarker levels, making comparisons across studies challenging. Therefore, to minimize the effects of these sample handling factors, known as pre-analytical factors, it is essential to establish a standardised protocol. In paper I, we investigated the effects of certain pre-analytical sample handling factors and proposed certain sample handling conditions that ensure the best performance of plasma p-Tau217 on the Mesoscale platform.

Over the years, several laboratory assays have been developed to measure several p-Tau forms. Different studies have reported different results regarding the

performance of these p-Tau species, partly due to differences in assays performance and cohort characteristics. To find a high performing assay suitable for clinical practice and drug trials, it is necessary to conduct side-by-side comparisons within the same participants. Therefore, in paper II, using 10 assays we performed a side-by-side comparison of three main p-Taus i.e. plasma p-Tau217, p-Tau181 and p-Tau231 and we found that among all the laboratory methods, mass spectrometry-based method for measuring p-Tau217 in plasma was the best at identifying individuals with Alzheimer's disease and predicting those who would progress to Alzheimer's disease over time. Furthermore, some immunoassays also demonstrated high accuracy for both outcomes. However, although, mass-spectrometry based methods are highly accurate, they may not be as suitable as immunoassays for routine clinical practice, due to a more complex handling and measurement procedures.

Whenever a new assay is developed, its performance is best validated against neuropathological outcomes (amyloid and tau pathology), which are consider the gold standard. ALZpath, developed a commercial immunoassay for measuring p-Tau217 in blood. Some studies have reported its high clinical diagnostic accuracy for identifying individuals with Alzheimer's disease, but none had determined its performance in the sample with neuropathology confirmed diagnosis. Therefore, in paper III, we assessed the performance of p-Tau217$_{ALZpath}$ against the neuropathological measures of brain amyloid and tau pathology in comparison with established p-Tau biomarkers, Lilly p-Tau217 and p-Tau181. We found that the performance of p-Tau217$_{ALZpath}$ was comparable to p-Tau181$_{Lilly}$, however p-Tau217$_{ALZpath}$ did not perform as well as p-Tau217$_{Lilly}$.

Blood and CSF markers have shown great promise for identifying individuals with Alzheimer's disease and predicting Alzheimer's disease progression from a single sample at baseline. However, their ability to monitor Alzheimer's disease progression, meaning tracking changes in these fluid markers over time to downstream effects of the disease such as cognitive decline or atrophy remains understudied. Therefore, in paper IV, we examined both plasma and CSF biomarkers to identify the best biomarker or combination of biomarkers that could help track Alzheimer's disease related changes. Recent studies have also shown that adjusting biomarkers to a reference protein can improve their performance by reducing the biological differences that exists in the biomarker levels between individuals. In this study, we found that adjusting CSF but not plasma biomarkers to a reference protein such as Aβ40 improved their associations with longitudinal measures of cognition and brain atrophy. Additionally, we found that plasma p-Tau217 and CSF p-Tau205/Aβ40 in combination with NfL could potentially be used as surrogate markers for tracking disease advancement.

In summary, we first established the best pre-analytical conditions to ensure optimal performance of p-Tau217 assay on the Mesoscale platform. We also report that mass-spectrometry assays and certain immunoassays demonstrate sufficiently high

accuracy in detecting Alzheimer's disease and predicting who will progress to Alzheimer's disease dementia over time. Our data indicate that it is very important to validate the results of novel ALZpath p-Tau217 immunoassays against the gold standard neuropathological outcomes. Finally, we found that plasma p-Tau217 and CSF p-Tau205/Aβ40 along with NfL could be useful for tracking disease progression.

Populärvetenskaplig sammanfattning

Summary in Swedish

Demens är ett tillstånd som påverkar hjärnan och orsakar en gradvis försämring av en persons kognitiva förmågor. Demens är inte en del av det normala åldrandet även om en viss kognitiv försämring är förväntad när människor blir äldre. Globalt lever cirka 55 miljoner personer med demens och detta antal förväntas öka i takt med en åldrande befolkning. Det finns flera former av demens där Alzheimers sjukdom är den vanligaste. I hjärnan hos en person med Alzheimers sjukdom finns en onormal ansamling av proteiner, nämligen amyloid-beta och tau. Dessa proteiner kan upptäckas och mätas med hjälp av prover på cerebrospinalvätska (CSV) samt biomarkörer från hjärnavbildning. En biomarkör definieras som en mätbar indikator som återspeglar vad som händer i en persons kropp. Det kan vara en molekyl, ett protein, en gen eller ett enzym som finns i blod, vävnader eller andra kroppsvätskor. CSV- och hjärnavbildningsbiomarkörer har framgångsrikt använts för att diagnostisera Alzheimers sjukdom inom forskningsmiljöer och på specialiserade minneskliniker i vissa länder. Dessa metoder är dock kostsamma, invasiva och kräver specialiserad personal och utrustning, vilket gör det svårt att införa dem i stor skala. Därför behövs enklare, billigare och minimalt invasiva tester, nämligen ”blodtester”.

Nya framsteg inom laboratorieteknik har möjliggjort upptäckt och mätning av olika proteiner i blodet som är kopplade till Alzheimers sjukdom, till exempel fosforylerat tau (p-tau). Befintlig evidens har visat att p-tau i blod, särskilt p-tau217, kan vara en mycket användbar biomarkör för att identifiera hjärnförändringar relaterade till Alzheimers sjukdom.

Målet med denna avhandling var att bidra till införandet av plasma p-tau som en biomarkör för Alzheimers sjukdom i klinisk vardag och i behandlingsstudier. Detta arbete genomfördes med hjälp av tre välkarakteriserade och oberoende kohorter.

Det är sedan tidigare känt att hur plasmaprover hanteras före biomarkörmätning kan påverka biomarkörnivåerna, vilket försvårar jämförelser mellan olika studier. Därför är det viktigt att fastställa ett standardiserat protokoll för att minimera effekterna av dessa provhanteringsfaktorer, så kallade pre-analytiska faktorer. I artikel I undersökte vi effekterna av vissa preanalytiska hanteringsfaktorer och föreslog provhanteringsförhållanden som säkerställer optimal prestanda för plasma p-tau217 på en Mesoscale-plattform.

Under åren har flera laboratorieanalyser utvecklats för att mäta flera olika p-tau-former. Olika studier har rapporterat varierande resultat gällande prestandan hos dessa p-tau-varianter, delvis på grund av skillnader i analysprestanda och kohortegenskaper. För att identifiera en högpresterande analys som är lämplig för klinisk användning och läkemedelsprövningar krävs jämförelser inom samma

deltagare. Därför genomförde vi i artikel II en direkt jämförelse av tre huvudsakliga p-tau-varianter (p-tau217, p-tau181, p-tau231) med hjälp av tio olika laboratorieanalyser. Här fann vi att, bland alla laboratoriemetoder, en masspektrometribaserad mätning av p-tau217 i plasma var bäst på att identifiera individer med Alzheimers sjukdom och på att förutsäga vilka som skulle utveckla Alzheimers sjukdom över tid. Dessutom visade vissa immunoanalyser hög noggrannhet för båda utfallen. Trots den höga noggrannheten är masspektrometribaserade metoder dock mindre lämpade för rutinmässig klinisk användning än immunoanalyser, eftersom de kräver mer komplex hantering och mer avancerade mätprocedurer.

När en ny laboratorieanalys utvecklas så är det bäst att validera dess prestanda mot neuropatologiska utfall (amyloid- och taupatologi), vilket betraktas som guldstandard. ALZpath Inc. har utvecklat en kommersiell immunoanalys för att mäta p-tau217 i CSV och blod. Vissa studier har rapporterat hög diagnostisk noggrannhet för denna analys vid identifieringen av individer med Alzheimers sjukdom, men ingen studie har tidigare jämfört dess prestanda i ett urval med personer som också har neuropatologiskt bekräftade diagnoser. Därför utvärderade vi i artikel III prestandan av p-tau217ALZpath i förhållande till neuropatologiska mått på amyloid- och taupatologi, i jämförelse med etablerade p-tau-biomarkörer såsom Lilly p-tau217 och p-tau181. Vi fann att prestandan för p-tau217ALZpath var jämförbar med p-tau181Lilly, men att p-tau217ALZpath inte uppvisade lika bra prestanda som p-tau217Lilly.

Biomarkörer i blod och CSV har visat stor potential för att identifiera personer med Alzheimers sjukdom samt för att förutsäga sjukdomsprogression baserat på ett enskilt prov vid baseline. Däremot är deras förmåga att över tid följa sjukdomens utveckling – det vill säga att relatera förändringar i biomarkörnivåer till sjukdomens konsekvenser såsom kognitiv försämring eller hjärnatrofi – fortfarande otillräckligt studerad. I artikel IV undersökte vi både plasma- och CSV-biomarkörer för att identifiera den biomarkör, eller kombination av biomarkörer, som bäst lämpar sig för att följa Alzheimersrelaterade förändringar över tid. Nya studier har också visat att justering av biomarkörer mot ett referensprotein ett protein som inte varierar mycket mellan olika individer kan förbättra deras prestanda genom att minska biologiska variationer mellan personer. I denna studie fann vi att när vi normaliserade CSV-biomarkörer mot ett referensprotein som till exempel Aβ40 så förbättrades deras samband med longitudinella mått på kognition och hjärnatrofi, vilket inte var fallet med plasmabiomarkörer. Dessutom såg vi att plasma p-tau217 och CSV p-tau205/Aβ40, i kombination med NfL, potentiellt kan användas som surrogatmarkörer för att följa sjukdomsutvecklingen.

Sammanfattningsvis fastställde vi först de bästa pre-analytiska förhållandena för att säkerställa optimal prestanda för analysen av p-tau217 på Mesoscale-plattformen. Vi rapporterar också att masspektrometribaserade analyser och vissa immunoanalyser uppvisar tillräckligt hög noggrannhet för att upptäcka Alzheimers

sjukdom och förutsäga vilka som kommer att utveckla Alzheimersdemens över tid. Vår data understryker vikten av att validera resultat från nya ALZpath p-tau217-analyser mot neuropatologiska guldstandardmått. Slutligen fann vi att plasma p-tau217 och CSV p-tau205/Aβ40, tillsammans med NfL, kan vara användbara för att följa sjukdomsprogression.

विज्ञान का सरल सारांश

Summary in Hindi

डिमेंशिया एक ऐसी स्थिति है जो मस्तिष्क को प्रभावित करती है और किसी व्यक्ति की संज्ञानात्मक क्षमताओं में धीरे-धीरे कमी का कारण बनती है। हालांकि उम्र बढ़ने के साथ कुछ संज्ञानात्मक गिरावट सामान्य है, लेकिन डिमेंशिया सामान्य बुढ़ापे का हिस्सा नहीं है। विश्वभर में लगभग 5.5 करोड़ लोग डिमेंशिया के साथ रह रहे हैं, और जैसे-जैसे जनसंख्या उम्रदराज़ होगी, यह संख्या और बढ़ने की संभावना है। डिमेंशिया के कई प्रकार होते हैं, जिनमें अल्ज़ाइमर रोग सबसे आम है। अल्ज़ाइमर रोग से प्रभावित मस्तिष्क में दो प्रोटीन , अमाइलॉयड-बीटा और टाउ , असामान्य रूप से जमा हो जाते हैं। इन प्रोटीनों का पता लगाना और उन्हें मापना मस्तिष्कमेरु द्रव (सीएसएफ) और मस्तिष्क इमेजिंग बायोमार्कर की मदद से संभव है। एक बायोमार्कर को शरीर के भीतर क्या चल रहा है, इसका एक मापने योग्य संकेतक माना जाता है। यह किसी व्यक्ति के रक्त, ऊतकों या अन्य शारीरिक द्रवों में उपस्थित कोई अणु, प्रोटीन, जीन या एंज़ाइम हो सकता है। सीएसएफ और मस्तिष्क इमेजिंग बायोमार्कर को शोध कार्यों और कुछ देशों में विशेषीर्कत मेमोरी क्लीनिकों में अल्ज़ाइमर रोग के निदान के लिए सफलतापूर्वक उपयोग किया गया है। हालांकि, ये विधियाँ महंगी, आक्रामक हैं और इनके लिए विशेष-trained स्टाफ तथा सुविधाओं की आवश्यकता होती है, जिससे इन्हें बड़े पैमाने पर लागू करना कठिन हो जाता है। इसलिए, वर्तमान समय की आवश्यकता है सरल, कम खर्चीले और न्यूनतम आक्रामक परीक्षणों की जैसे कि "रक्त परीक्षण"।

हाल के वर्षों में प्रयोगशाला तकनीकों में हुई प्रगति के कारण रक्त में अल्ज़ाइमर रोग से संबंधित विभिन्न प्रोटीन रूपों, जैसे फॉस्फोराइलेटेड टाउ (p-Tau), का पता लगाना और मापना संभव हुआ है। उपलब्ध प्रमाण यह दर्शाते हैं कि रक्त में p-Tau, विशेषकर p-Tau217, अल्ज़ाइमर रोग से संबंधित मस्तिष्कीय परिवर्तनों की पहचान के लिए एक अत्यंत उपयोगी बायोमार्कर हो सकता है।

इस शोध प्रबंध (थीसिस) का लक्ष्य था अल्ज़ाइमर रोग के लिए प्लाज़्मा p-Tau को एक बायोमार्कर के रूप में नियमित नैदानिक उपयोग और उपचार-आधारित अध्ययनों में लागू करने में सहायता करना। यह कार्य तीन अत्यंत सुव्यवस्थित और स्वतंत्र कोहॉर्ट्स का उपयोग करके किया गया।

यह अच्छी तरह से ज्ञात है कि बायोमार्करों को मापने से पहले प्लाज़्मा नमूनों को किस प्रकार संभाला जाता है, इससे उनके स्तर प्रभावित हो सकते हैं, जिससे विभिन्न अध्ययनों के परिणामों की तुलना

करना चुनौतीपूर्ण हो जाता है। इसलिए, इन सैम्पल-हैंडलिंग कारकों जिन्हें प्री-एनालिटिकल फ़ैक्टर कहा जाता है के प्रभाव को कम करने के लिए मानकीकृत प्रोटोकॉल स्थापित करना आवश्यक है। पेपर I में, हमने कुछ प्री-एनालिटिकल सैम्पल-हैंडलिंग कारकों के प्रभावों की जांच की और मेसॉस्केल प्लेटफॉर्म पर प्लाज़्मा p-Tau217 के सर्वश्रेष्ठ प्रदर्शन को सुनिश्चित करने वाली नमूना-हैंडलिंग स्थितियों का प्रस्ताव किया।

पिछले कई वर्षों में विभिन्न p-Tau रूपों को मापने के लिए कई प्रयोगशाला जांच विकसित की गई हैं। लेकिन अलग-अलग अध्ययनों में इन p-Tau रूपों के प्रदर्शन के संबंध में भिन्न-भिन्न परिणाम सामने आए हैं, संभवतः जांचों के प्रदर्शन और कोहॉर्ट विशेषताओं में अंतर के कारण। नैदानिक अभ्यास और दवा परीक्षणों के लिए एक उच्च-प्रदर्शन करने वाली जांच खोजने के लिए आवश्यक है कि एक ही प्रतिभागियों के भीतर इन जांचों की सीधी तुलना की जाए। इसलिए, पेपर II में हमने 10 जांचों का उपयोग करके तीन प्रमुख p-Tau रूपों; p-Tau217, p-Tau181 और p-Tau231 का सीधा तुलनात्मक विश्लेषण किया। हमने पाया कि प्लाज़्मा p-Tau217 को मापने के लिए मास स्पेक्ट्रोमेट्री आधारित विधि अल्ज़ाइमर रोग की पहचान करने और यह अनुमान लगाने में कि कौन व्यक्ति समय के साथ रोग की ओर बढ़ेगा, सबसे अधिक सटीक थी। इसके अलावा, कुछ इम्यूनोअस्से ने भी दोनों परिणामों के लिए उच्च सटीकता दिखाई। हालांकि, यह ध्यान देने योग्य है कि यद्यपि मास स्पेक्ट्रोमेट्री विधियाँ अत्यंत सटीक हैं, उनकी जटिल प्रक्रियाओं के कारण वे नियमित नैदानिक उपयोग के लिए इम्यूनोअस्से जितनी उपयुक्त नहीं हो सकतीं।

जब भी कोई नई जांच विकसित की जाती है, उसकी सत्यता का सर्वोत्तम आकलन न्यूरोपैथोलॉजिकल परिणामों (अमाइलॉयड और टाउ पैथोलॉजी) के विरुद्ध किया जाता है, जिन्हें स्वर्ण मानक माना जाता है। ALZpath ने रक्त में p-Tau217 मापने के लिए एक वाणिज्यिक इम्यूनोअस्से विकसित किया है। कुछ अध्ययनों ने अल्ज़ाइमर रोग के निदान में इसकी उच्च सटीकता की रिपोर्ट की है, लेकिन अब तक इसके प्रदर्शन का मूल्यांकन उन व्यक्तियों में नहीं किया गया था जिनमें न्यूरोपैथोलॉजी-प्रमाणित निदान उपलब्ध था। इसलिए, पेपर III में हमने p-Tau217ALZpath के प्रदर्शन की तुलना स्थापित p-Tau बायोमार्करों (Lilly p-Tau217 और p-Tau181) से करते हुए, मस्तिष्क अमाइलॉयड और टाउ पैथोलॉजी के न्यूरोपैथोलॉजिकल मापों के विरुद्ध इसका आकलन किया। हमने पाया कि p-Tau217ALZpath का प्रदर्शन p-Tau181Lilly के तुलनीय था, लेकिन यह p-Tau217Lilly जितना अच्छा प्रदर्शन नहीं कर पाया।

रक्त और सीएसएफ मार्करों ने अल्ज़ाइमर रोग की पहचान करने और एकल बेसलाइन नमूने के आधार पर रोग की प्रगति की भविष्यवाणी करने में बेहतरीन क्षमता दिखाई है। हालांकि, समय के साथ इन मार्करों में होने वाले परिवर्तनों का संज्ञानात्मक गिरावट या मस्तिष्क क्षय जैसे रोग के डाउनस्ट्रीम प्रभावों से संबंध स्थापित करने की उनकी क्षमता पर अभी तक पर्याप्त अध्ययन नहीं हुआ है। इसलिए, पेपर IV में, हमने प्लाज़्मा और सीएसएफ दोनों बायोमार्करों का विश्लेषण किया ताकि यह पता लगाया जा सके कि कौन-सा बायोमार्कर या बायोमार्करों का संयोजन अल्ज़ाइमर रोग से संबंधित परिवर्तनों को सबसे अच्छी तरह ट्रैक कर सकता है। हालिया अध्ययनों में यह भी दर्शाया गया है कि बायोमार्करों को Aβ40 जैसे एक संदर्भ प्रोटीन के अनुपात में समायोजित करने से व्यक्तियों के बीच मौजूद जैविक अंतर कम हो सकते हैं, जिससे उनका प्रदर्शन बेहतर हो सकता है। इस अध्ययन में, हमने पाया कि सीएसएफ बायोमार्करों को एक संदर्भ प्रोटीन के अनुसार समायोजित करने से लेकिन प्लाज़्मा मार्करों में नहीं संज्ञानात्मक और मस्तिष्क क्षय के दीर्घकालिक मापों के साथ उनके संबंध बेहतर हुए। इसके अतिरिक्त, हमने पाया कि प्लाज़्मा p-Tau217 तथा सीएसएफ p-Tau205/Aβ40, NfL के साथ मिलकर रोग प्रगति को ट्रैक करने के लिए संभावित रूप से उपयोगी हो सकते हैं।

सारांश में, हमने पहले मेसॉस्केल प्लेटफॉर्म पर p-Tau217 जांच के सर्वोत्तम प्रदर्शन को सुनिश्चित करने के लिए उचित प्री-एनालिटिकल स्थितियों की स्थापना की। हमने यह भी पाया कि मास स्पेक्ट्रोमेट्री अस्से और कुछ इम्यूनोअस्से अल्ज़ाइमर रोग की पहचान करने और यह अनुमान लगाने में कि कौन-सा व्यक्ति समय के साथ अल्ज़ाइमर रोग डिमेंशिया की ओर बढ़ेगा, उच्च सटीकता प्रदान करते हैं। हमारे डाटा यह इंगित करते हैं कि नवीन ALZpath p-Tau217 इम्यूनोअस्से के परिणामों को स्वर्ण मानक न्यूरोपैथोलॉजिकल परिणामों के विरुद्ध सत्यापित करना अत्यंत महत्वपूर्ण है। अंततः, हमने पाया कि प्लाज़्मा p-Tau217 और सीएसएफ p-Tau205/Aβ40, NfL के साथ मिलकर रोग प्रगति को ट्रैक करने में सहायक हो सकते हैं।

List of abbreviations

Aβ	Amyloid-beta
Aβ-PET	Amyloid positron emission tomography
AD	Alzheimer's disease
ADD	Alzheimer's disease dementia
ADAD	Autosomal dominant Alzheimer's disease
ADLs	Activities of daily living
ADNC	Alzheimer's disease neuropathologic change
APP	Amyloid precursor protein
ARIA	Amyloid-related imaging abnormalities
AUC	Area under the curve
A+	Amyloid positive
A-	Amyloid negative
BBMs	Blood-based biomarkers
CI	Confidence Interval
CERAD	Consortium to Establish a Registry for Alzheimer's disease
Cond	Condition
CSF	Cerebrospinal fluid
CU	Cognitively unimpaired
EOAD	Early-onset Alzheimer's disease
FDA	Food and Drug Administration
GFAP	Glial Fibrillary Acidic Protein
IWG	International working group
LATE	Late-onset Alzheimer's disease
MCI	Mild cognitive impairment

MRI	Magnetic resonance imaging
MS	Mass spectrometry
MTBR	Microtubule binding region
MRI	Magnetic positron imaging
NA	Not applicable
NC	Non-centrifugation
NFTs	Neurofibrillary tangles
NfL	Neurofilament light
NTA-tau	N-terminal fragment of total Tau
PET	Positron emission tomography
p-Tau	Phosphorylated Tau
PHFs	Paired helical filaments
ROC	Receiver operating characteristic curve
ROI	Region of Interest
RT	Room temperature
SUVR	Standardized uptake value ratio
SCD	Subjective cognitive decline
t-Tau	Total Tau
tau-PET	Tau positron emission tomography

Introduction

Alzheimer's disease

Dementia is an umbrella term which is commonly defined by a loss of cognitive functions (ability to think, remember and reason) to an extent that severely interferes with an individual's day to day activities (1, 2). According to the World Health Organization, more than 55 million individuals are currently living with dementia, and about ten million new cases are diagnosed each year (3). Alzheimer's disease is one of the leading causes of dementia and accounts for up to 60-80% of dementia cases (4). The global cost of treating Alzheimer's disease and other dementias was estimated to be around $384 billion in 2025, and as the population ages, this cost is projected to rise to around $1 trillion by 2050 (5). Thus, Alzheimer's disease poses a massive economic burden on the healthcare system, and solutions for it are an urgent need for the whole society.

Alzheimer's disease is a neurodegenerative disorder, which typically begins with subtle memory problems (forgetfulness). As the disease advances, the memory problems worsen, followed by difficulties in other cognitive functions including language, learning, motor planning, executive function, attention and visuospatial abilities (6, 7). This represents a "typical" clinical presentation of Alzheimer's disease. However, there are less common, "atypical", clinical manifestations which do not usually begin with memory problems; instead they first affect visual, language, executive, behavioral, or motor functions (8).

Alzheimer's disease can be categorized into early-onset Alzheimer's disease (EOAD) and late-onset Alzheimer's disease (LOAD) (9). EOAD and LOAD primarily differ by the age of onset of Alzheimer's disease symptoms. Symptom onset in LOAD usually occurs between 60-65 years of age. The onset of EOAD typically occurs between 30 years and 60-65 years of age. EOAD is typically linked to autosomal dominant Alzheimer's disease (ADAD), which is characterized by rare genetic mutations such as Amyloid Precursor Protein (APP), presenilin 1 or presenilin 2 (10-12). However, EOAD can also be sporadic, that is demonstrating no clear pattern of inheritance, and is commonly influenced by genetic risk factors (*Apolipoprotein E(APOE)* ε4 allele*),* age, or environmental exposures (13).

Hallmarks of Alzheimer's disease

Historically, Alzheimer's disease was coined after the German psychiatrist and neuropathologist, Alois Alzheimer. In 1907, while performing a histopathological examination of the brain of his patient, Auguste Deter, who passed away in a mental asylum in Frankfurt, he identified abnormal alterations inside and outside neurons. Dr. Alzheimer described these as cortical miliary foci and intracellular neurofibrillary alterations (14). These abnormal changes, today recognized as amyloid plaques and neurofibrillary tangles (NFTs), are the hallmarks of Alzheimer's disease (Figure 1).

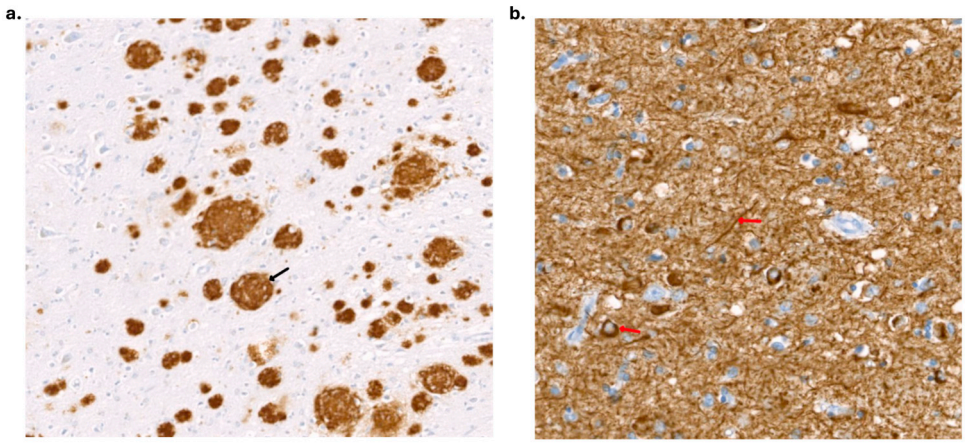

Figure 1. The illustration represents the main hallmarks of Alzheimer's disease.
Neuropathological sections from the brain of an Alzheimer's disease patient. a) The balck arrow represents plaque containing predominantely Aβ42; b) The upper red arrow represents neuropil thread, the lower red arrow represents NFTs. *The image was obtained from the Alzforum.*

Amyloid pathology

Amyloid plaques are primarily composed of extracellular deposits of peptides called amyloid-beta (Aβ). Several forms of Aβ deposits exist, among which dense-core plaques and diffuse plaques are the most common forms found in Alzheimer's disease patients (15). Dense core plaques are primarily composed of tightly packed core of fibrillar Aβ, with a more loosely organized Aβ in the vicinity and are mostly found in advance stages of the disease (15-18). However, not all dense core plaques are alike (15). A subset of dense core plaques are neuritic plaques, i.e., they have a centre core of Aβ and are additionally surrounded by damaged nerve cell processes (neurites) (15). These damaged neuronal processes are known as dystrophic neurites (15). Dystrophic neurites often contain either abnormal tau or APP, or both (19). Some of the dystrophic neurites found in the proximity of neuritic plaques are comprised of neuron-specific neurofilament proteins (15, 20, 21), which provide structural support to axons (15, 22). This suggests that cytoskeletal alterations might

contribute to neurodegenerative mechanisms. Activated microglia cells and reactive astroglia, the key drivers of neuroinflammation, are often found surrounding the neuritic plaques (15, 17, 23-25). Likewise, synaptic loss has been commonly reported in the vicinity of these plaques (15, 17, 25, 26). Diffuse plaques lack a well-defined core and are more diffuse in appearance (hence the name "diffuse") and are predominantly found in the early stages of Alzheimer's disease (15).

Aβ is produced by proteolytic cleavage of APP (27). APP undergoes processing via two distinct pathways: the non-amyloidogenic and amyloidogenic (16, 27) (Figure 2). In the non-amyloidogenic pathway, APP is cleaved by α-secretase, within the Aβ domain preventing the formation of Aβ (16, 28). This cleavage leads to the formation of a soluble monomeric peptide (sAPPα) (27). On the contrary, in the amyloidogenic pathway, APP is sequentially cleaved by β- and γ- secretase (presenilin 1 and presenilin 2 are catalytic subunit of γ-secretase complex) (29), resulting in the formation of Aβ peptides (mainly Aβ40 and Aβ42) (27). The longer form (Aβ42) is more prone to aggregation.

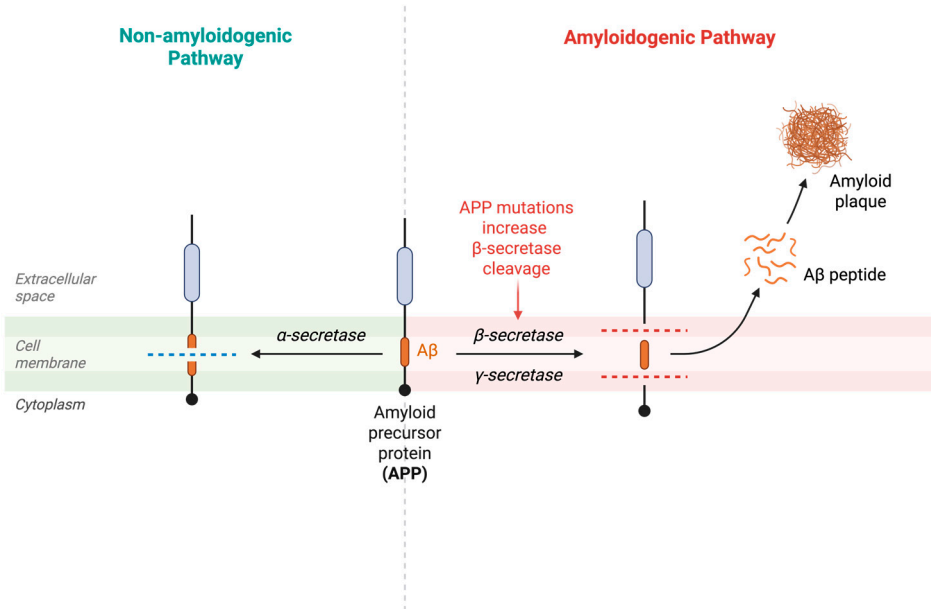

Figure 2. The illustration demonstrates the cleavage pathway of the transmembrane protein APP. APP is cleaved via two pathways: the non-amyloidogenic (left) and amyloidogenic (right). In the non-amyloidogenic pathway, APP is cleaved by α secretase within the Aβ domain. Whereas, in the amyloidogenic pathway, APP is sequentially cleaved by β- and γ- secretase resulting in the formation of Aβ peptides, which ultimately results in the formation of Aβ plaques. *Figure created with Biorender.*

In a healthy human brain, soluble monomeric Aβ peptides are generated continuously (28). However, in the brain of an Alzheimer's disease patient, due to an imbalance between the production and clearance of Aβ peptides, the monomeric peptides first aggregate into oligomers, followed by protofibrils and finally into insoluble fibrils, a key component of Aβ plaques (27).

Tau pathology

Intracellular NFTs are predominantly made up of filaments of misfolded tau protein (30, 31). They develop through distinct stages, and exhibit varying morphological and biochemical characteristics (32). NFTs first appear as pre-tangles and subsequently develop into mature tangles, which ultimately disrupt the nucleus of the neuron, resulting in neuronal death. Finally, the remains of mature tangles after cell death, known as ghost tangles, are released extracellularly (32). Furthermore, lesions of tau, known as neuropil threads (abnormal thread-like structures containing filamentous tau) are also found in the Alzheimer's disease brain (33).

The microtubule associated protein Tau, encoded by the MAPT gene, is present in various neuronal compartments (axons, cell bodies, dendrites and synapses) (34-38). The MAPT gene undergoes alternative splicing (exon 2, 3, and 10), resulting in 6 different isoforms of Tau (Figure 3) containing 0, 1, or 2 inserts in the N-terminal domain and three repeats (3R) or four repeats (4R) in the microtubule binding domain: 0N3R, 1N3R, 2N3R, 0N4R, 1N4R, and 2N4R isoforms (36). Equal numbers of 3R and 4R tau are found in the adult brain (39) but different levels are found across primary and secondary tauopathies. A mix of 4R and 3R tau is particularly present in paired helical filaments (PHFs) in Alzheimer's disease (6).

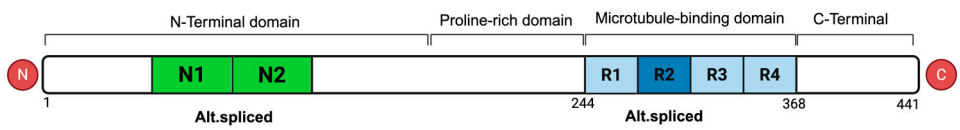

Figure 3. The illustration of different domains of Tau protein.
Tau is comprised of 4 major domains, i.e. N-terminal domain, proline rich domain, microtubule binding domain and C-terminal domain. The alternate splicing of Tau protein (at exon 2, 3 and 10) results in the formation of 6 different Tau isoforms. *The figure was adapted from Bali et al. (2025) (40), and modified using Biorender.*

Under normal physiological conditions, tau undergoes phosphorylation and plays a key role in microtubule stabilization, promoting axonal transport within neurons (35, 41). In the Alzheimer's disease brain, tau undergoes abnormal hyperphosphorylation (42, 43), resulting in its detachment from microtubules (44) and the formation of misfolded tau proteins within neurons (6, 30, 31). These misfolded tau proteins further aggregate into PHFs, which in later stages form NFTs and neuropil threads (42, 43, 45) (Figure 4).

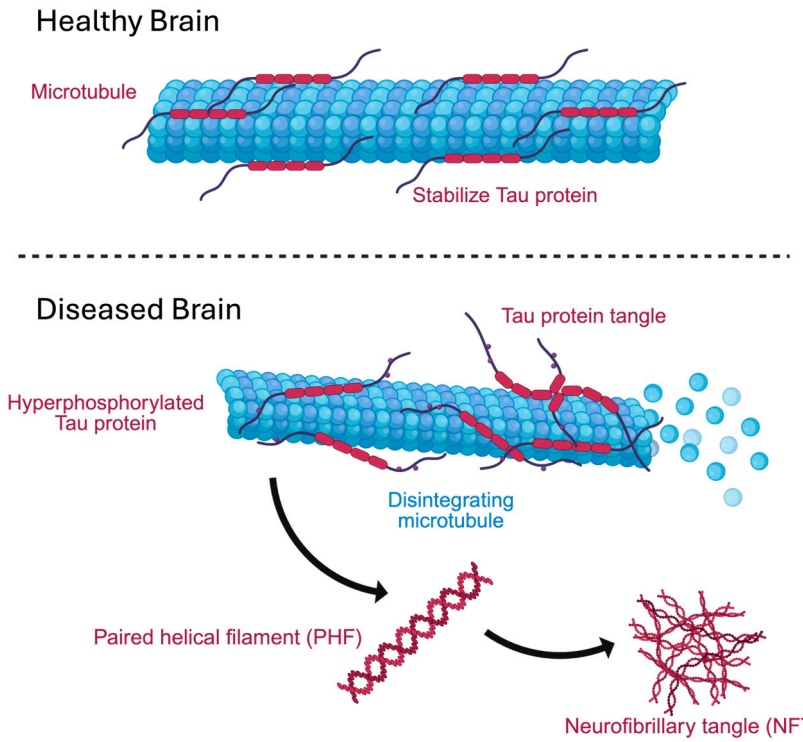

Figure 4. The illustration represents the role of tau in the healthy and diseased brain.
In healthy brain, tau plays a key role in stabilizing the microtubule (panel above). In diseased brain, tau undergoes abnormal hyperphosphorylation and detaches from the microtubule (panel below). This results in the formation of misfolded tau proteins, which aggregates into PHFs and eventually results into the formation of NFTs. *Created using Biorender.*

The amyloid cascade hypothesis has long dominated the field of Alzheimer's disease, describes temporal evolution of pathological processes in Alzheimer's disease. According to this hypothesis, abnormal accumulation of Aβ plaques trigger a series of pathological events, including hyperphosphorylation of tau, formation of PHFs, development of NFTs, neuroinflammation, and finally neurodegeneration, leading to the appearance of clinical symptoms (46).

The progression to Alzheimer's Disease

Progression of the neuropathologic burden

Aβ plaques deposition tends to follow a stereotypical spatiotemporal pattern (47). The earliest Aβ deposits are found in the frontal, parietal, temporal or occipital neocortex (Phase 1), followed by spreading to the entorhinal region, CA1 and insular cortex (Phase 2), further progressing to subcortical regions (Phase 3) and eventually affecting the midbrain (Phase 4). Lastly, Aβ can be identified in the brainstem and cerebellum (Phase 5). This staging pattern which describes the anatomical progression of Aβ deposition, was introduced by Dr. Dietmar Thal (a neuropathologist) and is therefore known as Thal staging (47) (Figure 5a).

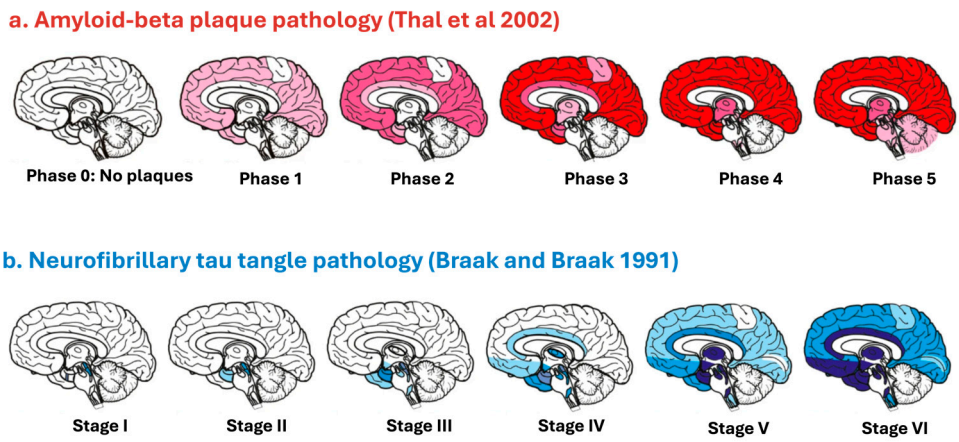

Figure 5. The image represents the spreading of amyloid (a) and tau (b) pathology. *The image was obtained with permission from Springer Nature (48) and was modified using Biorender.*

The Consortium to Establish a registry for Alzheimer's disease (CERAD) score (49), is another semi-quantitative neuropathological scoring system designed to estimate the density of neuritic plaques in neocortical regions for the diagnosis of Alzheimer's disease. Based on the neuropathological evaluation of neuritic plaques density, rather on its location as with the Thal method, the Alzheimer's disease diagnostic categories can be divided into 1) no Alzheimer's disease (no neuritic plaques), 2) possible Alzheimer's disease (sparse neuritic plaques), and 3) probable (moderate neuritic plaques) or definite Alzheimer's disease (frequent neuritic plaques). According to this scoring system, a diagnosis of Alzheimer's disease requires the presence of moderate or frequent neuritic plaques in one or more neocortical regions. Furthermore, in case of probable Alzheimer's disease, a patient must exhibit a decline in memory without a history or evidence of other illness that

could account for mental impairment. Instead of measuring the anatomical progression of amyloid pathology, the CERAD score reflects the coexistence of amyloid and phosphorylated tau in plaques (48).

Similar to Thal staging, the accumulation of NFTs in Alzheimer's disease also follows a typical spatial temporal pattern (50). The NFTs first appear in the transentorhinal cortex and then progress to the entorhinal cortex (Stage I and II). As the disease advances, NFTs further spread to hippocampus (Stage III and IV) and finally reach the neocortical regions (Stage V and VI). This staging pattern, describing the progression and anatomical distribution of NFTs is known as Braak staging, named after Heiko Braak and Eva Braak (50) (Figure 5b).

A global score was developed by the National Institute on Aging and the Alzheimer's Association (NIA-AA) (51). In this scoring system, A represents the Aβ phases ranging from A0 (no Aβ plaques) to A3 (Thal phase 4-5), B represents the Braak NFT stages ranging from B0 (no NFT tangles) to B3 (Braak stage V or VI) and C represents the CERAD score ranging from C0 (no neuritic plaques) to C3 (frequent neuritic plaques). Using this ABC score, the Alzheimer's disease neuropathologic changes (ADNC) can be categorized into four levels: 1) None 2) Low 3) Intermediate 4) High.

Neurodegeneration in Alzheimer's disease is tightly linked to the spread of tau pathology. Neuronal loss and brain atrophy first occur in the entorhinal cortex, followed by the hippocampus, amygdala and other medial temporal regions (52, 53). *Neurodegeneration and Neuroinflammation is outside the scope of this thesis and will not be discussed in further detail.*

Clinical Progression

It is well recognized that the Alzheimer's disease progression follows a continuum both biologically and clinically. However, three distinct clinical stages are used to classify individuals in the clinical settings and in research studies: cognitively unimpaired (CU), mild cognitive impairment (MCI) and dementia (54). In CU individuals, cognitive function is objectively within the expected range for age and sex norms. This assessment is made based on clinical evaluation and/or cognitive test results. Individuals who report subjective cognitive decline (SCD) and/or exhibit a slight decline during repeated cognitive testing are considered CU. In MCI, cognitive performance is objectively altered. Evidence of a drop in cognitive abilities is present. An individual with MCI can perform daily activities independently but may require support with complex activities of daily life. Finally, in the dementia stage, significant cognitive dysfunction affecting several domains and/or neurobehavioral symptoms are observed. These impairments have a substantial impact on activities of daily life, and the individual can no longer function independently, requiring assistance with the daily tasks. Alzheimer's

disease dementia can be further subdivided into mild, moderate, and severe stages that vary in the extent of functional impairment.

Recently a clinical staging of Alzheimer's disease, which applies to individuals who have the pathological changes (measured using biomarkers) associated with the disease, has been proposed in a paper by the Alzheimer's Association working group (55). The six clinical stages include "stage 1: asymptomatic, biomarker evidence only; stage 2: transitional decline, mild detectable change, but minimal impact on daily function; stage 3: cognitive impairment with early functional impact; stage 4: dementia with mild functional impairment; stage 5: dementia with moderate functional impairment; and stage 6: dementia with severe functional impairment."(55).

Treatments for Alzheimer's disease

For a long time, Alzheimer's disease was treated primarily with symptomatic therapies using medications such as acetylcholinesterase inhibitors (56) (donepezil, rivastigmine and galantamine) and the N-methyl-D-aspartate (NMDA) receptor antagonist memantine (57). These medications were primarily focused on mitigating symptoms in patients with mild to moderate Alzheimer's disease and have no effect on the underlying disease pathology or its progression. Cholinesterase inhibitors provide relief by increasing the levels of acetylcholine, a neurotransmitter that is decreased in Alzheimer's disease patients due to neuronal loss (56). In contrast, memantine acts by inhibiting the effects of glutamate, a neurotransmitter found in excess in Alzheimer's disease, which can promote neuronal damage (57).

Based on the amyloid cascade hypothesis previously discussed, most clinical trials conducted in Alzheimer's disease have targeted Aβ pathology. For a long time, therapeutic approaches designed to reduce or clear Aβ plaques failed for various reasons. For example, in some cases, the efficacy of anti-Aβ drugs was suboptimal (58). In others, there was misclassification of participants (substantial number of participants did not have Aβ pathology in the brain). Furthermore, severe side effects were seen for some drugs. AN1792, the first active vaccine, caused meningoencephalitis (59); monoclonal antibody, bapineuzumab, which was designed to neutralize the Aβ species, led to amyloid-related imaging abnormalities (ARIA) at high doses (60, 61).

In 2021, an anti-amyloid treatment (monoclonal antibody), aducanumab, was the first to demonstrate a clear effect on removing amyloid from the brains of Alzheimer's disease patients and received approval from the U.S. Food and Drug Administration (FDA) under the accelerated approval pathway. However, this drug was discontinued due to inconsistent evidence of clinical efficacy (i.e., effects on cognition) (62, 63). Later, two effective anti-amyloid treatments, donanemab (64)

and lecanemab (65), have shown consistent positive results on reducing cognitive decline, which led to their traditional approval by the FDA. They have also been approved by the European Medicines Agency (EMA), Japan, and Chinese regulatory authorities and in some other parts of the world. These drugs have been shown to significantly reduce Aβ plaques and slow cognitive decline by around 30%. Both, donanemab and lecanemab target fibrillar Aβ aggregates. However, lecanemab targets soluble Aβ protofibrils (66), whereas donanemab targets the insoluble pyroglutamate-modified N-terminal truncated form of Aβ found exclusively in mature plaques. (67). Their binding promotes plaque removal via microglial mediated phagocytosis (68-70). Both treatments are associated with ARIA, requiring magnetic resonance imaging (MRI) monitoring. Importantly, for patients to receive these drugs, they must demonstrate an adequate amount of Aβ pathology in the brain.

Biomarkers of Alzheimer's disease

Importance of biomarkers

Earlier, Alzheimer's disease was diagnosed primarily based on clinical evaluation. Patients with neurodegenerative diseases are often first managed in primary care and then referred to specialised dementia or memory clinics depending on the severity of their clinical symptoms, although the specific protocols can vary by countries. It was reported that about 20-30% (71) of individuals with a clinical diagnosis of Alzheimer's disease are misdiagnosed, with even higher rates in primary care settings (71). Such misdiagnosis can lead to suboptimal patient treatment and care, as well as unnecessary medical visits and costly medical examinations. Furthermore, this was also one of the reasons why some previous clinical trials may have failed, as they included participants who were misdiagnosed with Alzheimer's disease. This situation has drastically improved with the implementation of cerebrospinal fluid (CSF) (72) and neuroimaging biomarkers (73) that can detect amyloid and tau pathology in patients with Alzheimer's disease.

A biomarker is defined as an objective, measurable, and quantifiable indicator, of biological or pathogenic process or responses to therapeutic intervention (74, 75). It can be a molecule, gene, protein, enzyme, or physiological indicator present in tissues, blood, or other body fluids (74, 75).

CSF and imaging biomarkers of Aβ and tau

CSF biomarkers

CSF, a colourless liquid in contact with the brain, is found in the ventricles and the subarachnoid space surrounding the brain and spinal cord (76). Some of this fluid flows down the spinal cord toward the lumbar region. CSF is routinely obtained via

lumbar puncture, a procedure in which a small volume of CSF is extracted using a needle inserted into the lumbar vertebra (Figure 6) (77). Biochemical changes occurring in the brain can be mirrored in the CSF. CSF protein concentrations can be measured using various analytical methods, including immunoassay and mass spectrometry (MS) (78).

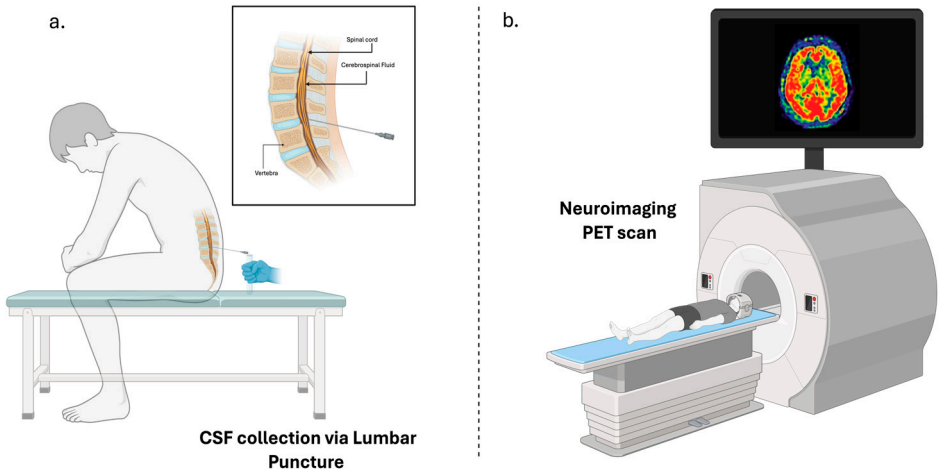

Figure 6. The figure represents a) the CSF collection procedure using lumbar puncture, and b) the patient undergoing a positron emission tomography (PET) scan. Abbreviations: PET, positron emission tomography. *The image was created using Biorender.*

In 1992-1993, CSF Aβ (79) and CSF total Tau (t-Tau) (80) were identified using immunoassays. In 1995, two studies demonstrated that CSF Aβ42 and tau levels were reduced in Alzheimer's disease patients compared to controls (81, 82). Since then, core CSF biomarkers including Aβ42 or the Aβ42/Aβ40 ratio as a measure of Aβ pathology and p-Tau as a measure of tau pathology have been studied extensively.

Aβ

Several Aβ peptides species are secreted into the CSF and blood, with Aβ40 being the most abundant, while Aβ42 concentrations are usually lower (83, 84). Studies have shown a roughly 50% reduction in CSF levels of Aβ42 in patients with Alzheimer's disease compared to CU older individuals (85), as Aβ42 tends to aggregate into amyloid plaques, reducing its secretion in the CSF (86) (Figure 7). Notably, the CSF Aβ42/Aβ40 ratio demonstrates higher diagnostic ability to identify Alzheimer's disease instead of CSF Aβ42 alone (87, 88). This is suspected to be related to the fact that CSF Aβ40 reflects total Aβ production and using the ratio accounts for the inter-individual differences in overall protein production and

clearance. This implies that an actual decrease in Aβ42 can be detected more accurately in individuals who produce more Aβ in general, whereas slightly low CSF Aβ42 levels in those who produce less Aβ overall would not be misinterpreted as suggestive of brain amyloidosis (78, 89). Another reason for superior performance of CSF Aβ42/Aβ40 could be that the pre-analytical handling of CSF samples (i.e., how samples are treated prior to analysis) has the same impact on both Aβ isoforms (90, 91).

P-Tau

In Alzheimer's disease, abnormal tau undergoes phosphorylation at multiple epitopes (amino acids 181, 217, 231, 205), primarily localised in the proline rich domain of the tau protein [99]. Several soluble p-Tau fractions are secreted and can be measured in CSF (Figure 7). Among p-Tau isoforms, p-Tau181 is the most abundant (92, 93) and was historically the first biomarker to discriminate Alzheimer's disease from controls with high accuracy. CSF p-Tau181 and p-Tau217 are elevated in Alzheimer's disease patients and can distinguish Alzheimer's disease from other neurodegenerative diseases with high accuracy (71, 78, 94). However, CSF p-Tau217 shows stronger correlations with tau tangle load and severity of the disease than CSF p-Tau181 (95), demonstrates superior performance in identifying Alzheimer's disease pathology and dementia, as well as in distinguishing Alzheimer's disease from other neurodegenerative disorders (93, 95-101). Levels of CSF p-Tau181 and p-Tau217 increase during preclinical Alzheimer's disease, before the emergence of insoluble tau aggregates in the brain (101, 102) indicating that these biomarkers start to change in response to abnormal accumulation of Aβ plaques. Interestingly, CSF p-Tau217 is more strongly associated with Aβ pathology in Alzheimer's disease than p-Tau181 (103). Furthermore, in comparison to CSF p-Tau217, CSF p-Tau217/tau217 (phosphorylated/non-phosphorylated tau) demonstrated even stronger associations with Aβ pathology indicating that similar to Aβ42/Aβ40, the CSF p-Tau217/tau217 ratio might mitigate the effects of inter-individual differences not related with Alzheimer's disease (103).

CSF levels of another p-Tau species, p-Tau231, also rise very early in the Alzheimer's disease continuum, serving as an early indicator of emerging Aβ pathology (104). A neuropathological study showed that p-Tau231 is primarily found in pre-tangles, an earlier event in Alzheimer's disease progression, whereas p-Tau181 is primarily found in mature NFTs (a later event) (105). CSF p-Tau231 has demonstrated higher specificity than CSF p-Tau181 for distinguishing Alzheimer's disease patients from healthy controls (106).

In contrast, CSF p-Tau205 has emerged as a more specific marker of Alzheimer's disease tau-aggregate pathology showing stronger correlations with tau-PET than Aβ-PET (103, 107). CSF p-Tau205 levels rise markedly in Aβ-PET and tau-PET positive (A+T+) individuals compared to A+T- and A-T- participants but this increase occurs after elevations in p-Tau217 and p-Tau181 levels. (107). In ADAD,

CSF p-Tau205 had the strongest correlation with brain atrophy and hypermetabolism compared to other p-Tau isoforms (108).

Lastly, a new tau species containing microtubule binding region of tau protein (MTBR-tau) has been found in CSF. MTBR-tau is a central element of insoluble tau aggregates (109-113). Recently, a specific MTBR-tau species, MTBR-tau243, has been shown to closely associate with tau tangle pathology and cognitive measures (114).

Imaging biomarkers

PET imaging allows the study of the amount and spatial distribution of the Aβ and tau pathologies and neurodegeneration in the brain of living people. There are currently three Aβ-PET ligands, (18F)florbetapir, (18F)flutemetamol, (18F)florbetaben), that have been approved and have been commonly used for the diagnostic evaluation of Alzheimer's disease (71, 115). These ligands detect insoluble Aβ plaques with high precision and have been validated against neuropathology (116-118). In MCI individuals, Aβ-PET can help identify individuals who are at a higher risk of developing Alzheimer's disease dementia (ADD) (119), while in CU individuals, abnormal Aβ-PET is associated with future cognitive decline (120). Conversely, negative Aβ-PET could rule out that an individual with cognitive impairment might have Alzheimer's disease.

The first tau-PET tracer, (18F)flortaucipir, which measures insoluble filamentous deposits (insoluble tau fibrils) was developed more recently (121). While several other tau-PET tracers have become available since then (including (18F)MK6240, (18F)RO948 and (18F)PI-2620) (121, 122), only (18F)flortaucipir has been so far approved by the FDA for use in clinical practice for diagnosis of Alzheimer's disease (123). Notably, tau-PET demonstrates higher diagnostic precision in comparison to Aβ-PET and CSF Aβ42/Aβ40 when differentiating ADD from other neurodegenerative diseases (124, 125). Furthermore, tau-PET has been found to be strongly linked to early clinical symptoms compared to Aβ-PET (126). *The imaging biomarkers remain outside the scope of this thesis and will not be discussed in further detail.*

Despite their proven diagnostic ability, CSF and imaging biomarkers have several limitations that restrict their widespread implementation (71, 78). CSF sampling via lumbar puncture is invasive and may cause mild adverse events; PET exposes individuals to radiation and requires complex facilities; while both can only be performed by specialised staff. Moreover, these biomarkers are expensive and demands substantial financial investment. Consequently, considerable effort has been devoted to develop blood-based biomarkers (BBMs), which can measure pathological processes associated with Alzheimer's disease with high precision and accuracy. Since their development few years ago, BBMs have revolutionised the

field of Alzheimer's disease. They are minimally invasive, cost-effective and easily accessible.

Blood-based biomarkers of Aβ and tau

Aβ

Initially, it was challenging to measure plasma Aβ42/Aβ40, with studies reporting inconsistent findings (127). But in 2016, a study using an ultrasensitive method (immunoassay) to quantify plasma Aβ42/Aβ40 (128) demonstrated moderate accuracy in predicting abnormal Aβ-PET. With advancements in technology, several MS-based methods (129-131) and immunoassays (132-134) were developed showing very promising results. In comparison to most immunoassays, MS-based methods clearly demonstrate higher accuracy in detecting cerebral Aβ pathology (132-134). Nevertheless, the utility of plasma Aβ42/Aβ40 is limited, as individuals with abnormal Aβ status demonstrate only 10-20% decrease in plasma Aβ42/Aβ40 levels (in comparison CSF Aβ42/Aβ40 is decreased by 50%) (Figure 7). One possible explanation for this discrepancy is that plasma Aβ42/Aβ40 levels are also influenced by Aβ produced outside the brain (129, 131-133).

P-Tau

Over the years, various highly sensitive assays have been developed that could measure p-Tau species (Figure 7) in blood such as p-Tau181 (135-138), p-Tau217 (139, 140), and p-Tau231 (141) with high accuracy and precision. They have also been found to be significantly correlated with their corresponding CSF isoforms (139, 141, 142). Some studies have shown that plasma p-Tau217 performed significantly better at detecting Alzheimer's disease pathology (using Aβ-PET, tau-PET, CSF Aβ42/Aβ40) and predicting future advancement to ADD (143-145). Neuropathological studies demonstrated significant correlation between plasma p-Tau217 and Alzheimer's disease autopsy confirmed cases (146, 147). Plasma p-Tau217 exhibits high accuracy in distinguishing preclinical Alzheimer's disease from controls and outperforms p-Tau181 in discriminating Alzheimer's disease from other neurodegenerative diseases (101, 138, 139, 148-150). Higher fold change in plasma p-Tau217 levels have been reported between Alzheimer's disease individuals and controls compared to plasma p-Tau181 (140, 151, 152). Longitudinal plasma p-Tau217 levels have also been found to be significantly associated with cognitive decline and brain atrophy in preclinical Alzheimer's disease (153). MS-derived plasma %p-Tau217 (phosphorylated/non-phosphorylated tau) demonstrates equivalent diagnostic performance to FDA approved CSF tests for determining Alzheimer's disease pathology (154). Plasma p-Tau217, p-Tau181 and p-Tau231 have been found to be more strongly associated with Aβ pathology (Aβ-PET) than tau pathology (tau-PET) and their levels have been found to be increased early and prior to detection of tau aggregation by PET (101, 136, 144, 155-159). Levels of plasma p-Tau231 might increase earlier than

plasma p-Tau217 and p-Tau181 along the Alzheimer's disease continuum (141, 148, 160). Another p-Tau variant, p-Tau212, have shown similar diagnostic accuracy as p-Tau217 for both amyloid and tau pathology but outperformed p-Tau231 and p-Tau181 (161). Elevated plasma p-Tau212 has also been reported in individuals with Down Syndrome (162). Recently, more tangle associated biomarkers have also been identified. Plasma p-Tau205 has been found to be more closely associated with tau than Aβ pathology (measured by Aβ-PET and tau-PET) (163). Furthermore, emerging eMTBR-tau 243 in plasma showcased tighter association with tau tangle pathology (measured by tau-PET) and cognitive performance (164).

Since the first p-Tau assays were developed, various new assays for different p-Tau variants have been established that use different tau-specific antibodies, detection systems and analytical procedures. It was not clear how these assays compared in terms of performance because different studies used different assays and cohorts, making direct comparison impossible.

Biomarkers of neurodegeneration and neuroinflammation

One of the most established fluid biomarkers of neurodegeneration is neurofilament light (NfL). NfL plays a key role in providing structural support, promoting growth and facilitating signal transmission in axons (165). Increases in CSF and plasma NfL levels occur as a result of its release from injured axons (Figure 7). Elevated levels of NfL have been found not only in Alzheimer's disease but also in other neurodegenerative diseases, for example amyotrophic lateral sclerosis, frontotemporal dementia and atypical parkinsonian disorders and vascular pathologies (71, 166, 167). Thus, NfL serves as an unspecific proxy for neurodegeneration and brain atrophy (78).

t-Tau is also considered a biomarker of neurodegeneration because its CSF and plasma levels are increased in Creutzfeldt-Jacob disease (168), head trauma, brain stroke (169, 170), anoxia and peripheral nerve disorders (171, 172) However, existing evidence suggests t-Tau levels may also reflect Alzheimer's disease-related tau pathology. For example, strong correlations between CSF t-Tau and p-Tau are seen in Alzheimer's disease. Moreover, CSF and plasma t-Tau concentrations have been found to increase early in ADAD (173). Finally, N-terminal fragments of t-Tau (NTA-tau) measured in CSF and plasma using a novel NTA assay were found to closely associate with tau tangle pathology (174).

Neuroinflammation and specifically astrocytic activation are characteristic features of Alzheimer's disease. Glial Fibrillary Acidic protein (GFAP) is a component of cytoskeleton involved in reactive remodelling of astrocytes. GFAP can be detected in both CSF and plasma and has been used as a marker of astrogliosis (Figure 7). Increased plasma GFAP levels (and to a less extent GFAP levels in CSF) (175) have been found in individuals with Aβ pathology (176) and are predictive of future

cognitive decline and conversion to ADD in MCI and CU individuals (177, 178). However, changes in plasma GFAP are not specific for Alzheimer's disease and its levels are also elevated in other neurodegenerative disorder such as frontotemporal dementia (179). Other fluid markers of inflammatory response exist, such as sTREM2 in relation to microglial activation, *but they are outside the scope of this thesis*.

Structural MRI is commonly used to assess cortical and subcortical brain atrophy as indicators of neurodegeneration (180, 181). Other neuroimaging markers of neurodegeneration (e.g., fluorodeoxyglucose-positron emission tomography) as well as inflammatory responses and glial activation (e.g., translocator protein positron emission tomography (TSPO-PET)), *are outside the scope of this thesis.*

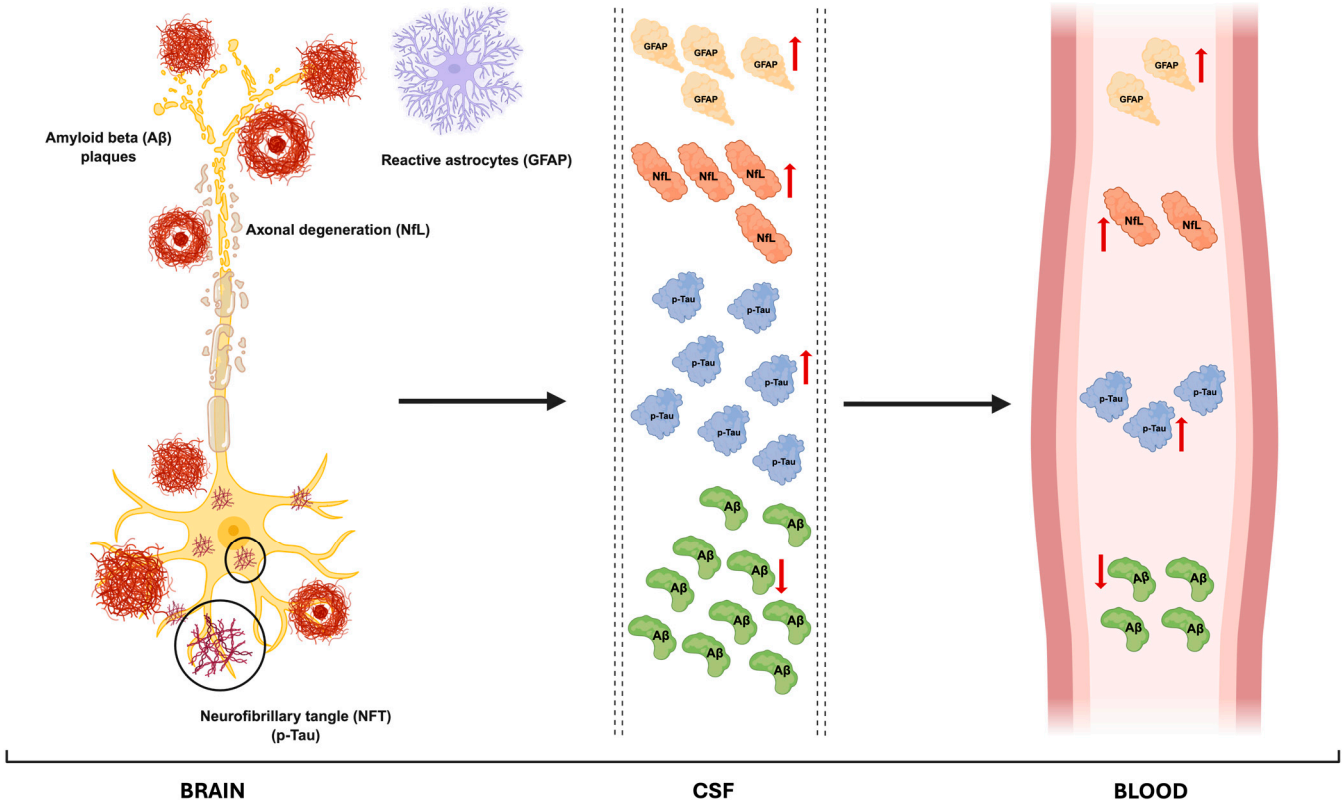

Figure 7. Schematic representation of pathological mechanisms and their related fluid biomarkers that can be reliably measured in the CSF and blood of individuals with Alzhiemer's disease. The biomarkers shown are: Aβ, p-Tau, NfL and GFAP. The red arrow indicates their relative levels in each biofluid. Figure adapted from (182) and modified using Biorender.

Pre-analytical sample handling affects fluid biomarkers

Pre-analytical factors refer to sample handling conditions that could potentially influence the concentrations of fluid biomarkers (CSF and plasma) prior to analytical assessment (Figure 8).

Previous studies have revealed that pre-analytical factors such as tube material, aliquot tube volume, storage time, repeated freeze-thaw cycles, additives and centrifugation, have a significant impact on the core CSF Alzheimer's disease biomarker (Aβ42, p-Tau, t-Tau) concentrations (183-198). The effects of these pre-analytical factors were pronounced for CSF Aβ42, followed by CSF t-Tau and lastly CSF p-Tau (185), accounting for a large part of the total variability found in biomarker concentrations (184, 185, 199). Therefore, a consensus emerged that harmonizing the pre-analytical sample handling could substantially reduce variability in biomarker concentrations, improve biomarker diagnostic accuracy, minimize rates of patient misclassification and thereby allow comparison of biomarker results across various laboratories and ultimately promote the inclusion of CSF biomarker testing in both research and routine clinical practice (185). As a result, in 2021, a simple and standardized pre-analytical protocol for CSF collection and handling was established (200).

Figure 8. The image highlights some pre-analytical factors related to plasma and CSF biomarkers.

Some studies have investigated the effects of pre-analytical factors on plasma biomarker concentrations, albeit not as extensively as for CSF biomarkers. These studies revealed that levels of plasma Aβ40, Aβ42 and t-Tau were affected by delayed centrifugation and storage temperature, tube types, freeze thaw cycles, tube transfer, and anticoagulants (201-206), whereas, only levels of plasma Aβ40 and Aβ42 were found to be affected by delayed processing after the blood drawn prior to centrifugation (201). Using the ratio of plasma Aβ40/Aβ42 instead of individual plasma Aβ40 and Aβ42, only partially mitigated the pre-analytical effects (202, 203, 207). Plasma p-Tau181 levels were found to be affected by freeze-thaw cycles, tube types and storage temperature (202-205, 207). However, not many studies have examined the effect of pre-analytical factors on plasma p-Tau217 concentrations. These studies have demonstrated that plasma p-Tau217 levels exhibit high robustness, showing no significant influence from pre-analytical conditions such as delays in blood processing, storage temperature and time, anticoagulants, tube transfer, tube types and remain stable up to four freeze-thaw cycles (202, 206, 208).

Biological diagnosis and staging of Alzheimer's disease

The Alzheimer's Association criteria for diagnosis and staging of Alzheimer's disease

In 2024, the Alzheimer's Association Workgroup revised the 2018 diagnosis and staging criteria of Alzheimer's disease, taking into account recent progress in the BBMs, regulatory approval of the first disease-modifying therapies and better insights into distinction and similarity between imaging, CSF and BBMs (55). A unified biological (based on biomarkers) and clinical staging of Alzheimer's disease was outlined.

Biological diagnosis

The biomarkers were categorized into three broad categories: 1) core biomarkers of Alzheimer's disease neuropathologic change (ADNPC); 2) biomarkers of pathological processes common for Alzheimer's disease and other neurodegenerative disorders; and 3) biomarkers of co-pathologies that often co-occur with Alzheimer's disease. These categories are further divided (Table 1) as:

- Category 1, comprised of Core 1 and Core 2 biomarkers. Core 1 biomarkers are further divided into A (Aβ proteinopathy) and T_1 (phosphorylated and secreted Alzheimer's disease tau). Core 2 biomarkers comprise the T_2 (Alzheimer's disease tau proteinopathy) category.

- Category 2, includes N (neurodegeneration) and I (inflammatory markers) categories.

- Category 3, consists of V (vascular brain injury) and S (alpha synucleinopathy) categories.

Table 1: Categorization of fluid analyte and imaging biomarkers. ***This table is reproduced from Jack et al 20204 (55)***

Abbreviations: αSyn-SAA, alpha-synuclein seed amplification assay; CT, computed tomography; FDG, fluorodeoxyglucose; WMH, white matter hyperintensity

Biomarker category	CSF or plasma analytes	Imaging
Core Biomarkers		
Core 1		
A: (Aβ proteinopathy)	Aβ42	Amyloid PET
T_1: (phosphorylated and secreted AD tau)	p-Tau217, p-Tau181, p-Tau231	
Core 2		
T_2: (AD tau proteinopathy)	MTBR-Tau243, other phosphorylated tau forms (eg: p-Tau205), non-phosphorylated mid-region tau fragments	Tau PET
Biomarkers of non-specific process involved in Alzheimer's disease pathophysiology		
N: (injury, dysfunction, or degeneration of neuropil)	NfL	Anatomic MRI, FDG-PET
I: (Inflammation) Astrocytic activation	GFAP	
Biomarkers of non-Alzheimer's disease pathology		
V: vascular brain injury		Infarction on MRI, or CT,WMH
S: α-synuclein	αSyn-SAA	

According to the proposed criteria, abnormalities in Core 1 biomarkers (Aβ PET, CSF Aβ42/Aβ40, CSF p-Tau181/Aβ42, CSF t-Tau/Aβ or accurate plasma assays and in particular p-Tau217) (Table 2) can be used to diagnose Alzheimer's disease in asymptomatic and symptomatic people. However, diagnostic testing is not currently recommended for CU individuals because there are no approved treatments for this population. Core 2 biomarkers (MTBR-Tau243, p-Tau205, tau-PET, non-phosphorylated mid-region tau fragments) (Table 2) should not be used as a standalone diagnostic test for Alzheimer's disease. Instead, Core 2 biomarkers combined with Core 1 biomarkers can be used for identifying the biological stages of disease and to inform about: a) the possibility that the symptoms are linked to Alzheimer's disease; b) the rate of disease progression in symptomatic patients; and c) likelihood of short-term progression in asymptomatic individuals (55).

Table 2: Intended use for imaging, CSF and plasma biomarker assays. ***This table is reproduced from Jack et al 20204 (55)***

Abbreviations: αSyn-SAA, alpha-synuclein seed amplification assay; CT, computed tomography; FDG, fluorodeoxyglucose; WMH, white matter hyperintensity

Intended use	CSF	Plasma	Imaging
Diagnosis			
A: (Aβ proteinopathy)	_	_	Amyloid PET
T_1: (phosphorylated and secreted Alzheimer's disease tau)	_	p-Tau217	_
Hybrid ratios	p-Tau181/Aβ42, t-tau/Aβ42, Aβ40/Aβ42	% p-Tau217	_
Staging, prognosis, as an indicator of biological treatment effect			
A: (Aβ proteinopathy)	_	_	Amyloid PET
T_1: (phosphorylated and secreted Alzheimer's disease tau)	_	p-Tau217	_
Hybrid ratios	p-Tau181/Aβ42, t-tau/Aβ42, Aβ40/Aβ42	% p-Tau217	_
T_2: (Alzheimer's disease tau proteinopathy)	MTBR-Tau243, other phosphorylated tau forms (eg: p-Tau205), non-phosphorylated mid-region tau fragments	MTBR-Tau243, other p-Tau forms (eg, p-Tau205)	TauPET
N: (injury, dysfunction, or degeneration of neuropil)	NfL	NfL	Anatomic MRI, FDG-PET
I: (Inflammation) Astrocytic activation	GFAP	GFAP	_
Identification of copathology			
N: (injury, dysfunction, or degeneration of neuropil)	NfL	NfL	Anatomic MRI, FDG-PET
V: vascular brain injury	_	_	Infarction on MRI, or CT,WMH
S: α-synuclein	αSyn-SAA	_	

Staging

The biological staging of Alzheimer's disease is based solely on core biomarkers. This staging implies that an individual progresses in a sequence, from initial stages to advanced stages. A four-stage scheme is proposed. According to this scheme, changing biomarkers correspond to the following stages: Stage A (early); Stage B (initial); Stage C (intermediate); and Stage D (advanced).

The staging is done employing Aβ-PET and tau-PET or with the combination of Core 1 fluid biomarkers and tau-PET (Table 3) (55). Abnormal Aβ-PET or Core 1 biomarkers indicate stage A or higher, whereas tau-PET distinguishes stages A-D from one another, reflecting the spread of tau pathology from the medial temporal lobe into neocortical regions and the amount of neocortical tau-PET uptake: stage A, negative tau-PET ($A+T_2-$); stage B, tau-PET uptake limited to the medial temporal region ($A+ T_{2MTL}+$); stage C, moderate tau-PET uptake in a neocortical region of interest (ROI) ($A+ T_{2MOD}+$); stage D, high tau-PET uptake in the same neocortical area ($A+ T_{2HIGH}+$). CSF/plasma biomarker-based staging may be done in the future when there is enough evidence to support such an approach.

Table 3: Biological Staging. ***This table is reproduced from Jack et al 20204 (55)***

	Intial-stage biomarkers (A)	**Early-stage biomarkers (B)**	**Intermediate-stage biomarkers (C)**	**Advanced-stage biomarkers (D)**
PET	Amyloid PET	Tau PET medial temporal region	Tau PET moderate neocortical uptake	Tau PET high neocortical uptake
	A+ T_2 -	A+ T_{2MTL}+	A+ T_{2MOD}+	A+ T_{2HIGH}+
Core 1 fluid	CSF Aβ40/Aβ42, p-Tau181/Aβ42, t-tau/Aβ42 and accurate Core 1 plasma assays can establish that an individual is in biological stage A or higher, but cannot discriminate between PET stages A-D at present			

The biological as well as clinical staging described above are integrated into a single staging scheme (Table 4) reflecting the link between biological stages and progression of clinical symptoms. The highlighted diagonal (Table 4) represents the expected Alzheimer's disease progression trajectory. But this may be modified by the presence of comorbid pathologies and resilience.

Table 4: Integrated biological and clinical staging. ***This table is reproduced from Jack et al 2024 (55)***

	Stage 0	**Clinical Stage 1**	**Clinical Stage 2**	**Clinical Stage 3**	**Clinical Stage 4**
Initial biological stage (A)	X	1A	2A	3A	4-6 A
Initial biological stage (B)	X	1B	2B	3B	4-6 B
Initial biological stage (C)	X	1C	2C	3C	4-6 C
Initial biological stage (D)	X	1D	2D	3D	4-6 D

International working group (IWG) recommendations for clinical-biological diagnosis of Alzheimer's disease

Diagnostic criteria for Alzheimer's disease proposed by the International Working Group (IWG) share several similarities with the Alzheimer's Association criteria, such as both groups agree that: a) abnormal biomarker levels must be present for an Alzheimer's disease diagnosis; b) in clinical settings, individuals who do not show any symptoms of Alzheimer's disease should not undergo biomarker testing; c) assessing an individual's risk is essential; d) Alzheimer's disease often does not occur alone and is frequently accompanied by co-pathologies that contribute to cognitive impairment; e) Alzheimer's disease related changes begin many years before symptoms appear.

However, one major difference between the two criteria lies in how they classify individuals who show no symptoms but have abnormal Alzheimer's disease biomarker levels. The IWG states that asymptomatic individuals with abnormal biomarkers should not be labelled as having Alzheimer's disease; rather, they must be considered "at risk" of developing the disease. In contrast, the Alzheimer's Association workgroup views Alzheimer's disease as a biological process and considers individuals who have abnormal biomarker levels, even if they do not yet show symptoms, as having Alzheimer's disease pathology.

Aims of the thesis

The overall aim of this thesis was to aid the implementation of plasma p-Tau as a biomarker of Alzheimer's disease in clinical practice and drug trials. Specifically, we aimed to:

- Develop standardized pre-analytical sample handling procedures to ensure optimal performance of plasma p-Tau217 which is considered one of the best plasma p-Tau biomarkers for Alzheimer's disease **(paper I).**
- Compare the performance of p-Tau217 and other p-Tau variants measured using different analytical approaches in their ability to detect abnormal Aβ status and to predict progression to ADD **(paper II).**
- Evaluate the performance of a novel ALZpath p-Tau217 immunoassay in comparison to established p-Tau217 and p-Tau181 immunoassays in a neuropathological cohort **(paper III).**
- Determine the best performing CSF and plasma p-Tau biomarkers that could be utilised (in combination with other promising biomarkers) for monitoring Alzheimer's disease progression **(paper IV).**

Methods

This section will provide a brief overview of the methods. For detailed description concerning methodology and sensitivity analysis please refer to the individual papers in the appendix of this thesis.

Study cohorts

The studies included in this thesis primarily utilized data from three cohorts. Each study has a distinct aim, design and is based on different data modalities. **Paper I and IV** used data from Swedish BioFINDER-1 cohort, **Paper II** utilized data from MCI cohort and **Paper III** used data from the Arizona Study of Aging and Neurodegenerative Disorders cohort.

Swedish BioFINDER- 1

Swedish BioFINDER-1 (NCT01208675, https://biofinder.se/one/) study was started in 2009, with the aim to understand the underlying causes of different neurodegenerative diseases including Alzheimer's disease. This cohort is comprised of more than 1600 participants who were recruited at the memory clinics at Skåne University Hospital and Ängelholm hospital, Sweden. This is a longitudinal study with a follow-up of up to 10 years including primarily CU individuals and patients with MCI and dementia. Study participants underwent an extensive cognitive, neurological and psychiatric assessment, CSF and blood collection and MRI.

MCI cohort

MCI cohort included individuals with a baseline diagnosis of MCI who were recruited at the Memory clinic, Skåne University Hospital, Malmö, Sweden. All the participants went through an extensive cognitive, neurological and psychiatric assessment and were followed up for about 4.9 years. Petersen criteria were fulfilled by every participant who was diagnosed as MCI at baseline. During follow up, participants who eventually developed ADD had fulfilled the dementia criteria by

DSM-IIIR and probable Alzheimer's disease criteria by NINCDS-ADRDA and had abnormal CSF Aβ42/Aβ40 ratio.

Arizona Study of Aging and Neurodegenerative disorders cohort

In 1987, at Banner Sun Health Research Institute, a program known as The Brain and Body Donation Program (BBDP), was started with the main aim of collecting donated brains and currently has a collection of approximately more than1600 brains. The BBDP program is now described as the Arizona Study of Aging and Neurodegenerative disorders (AZSAND). The participants included in this cohort are mainly from the retirement communities based in the urban areas of Phoenix, Arizona, with a clinical diagnosis of CU, MCI, Alzheimer's disease or non-Alzheimer's disease dementia. All participants included in this cohort undergo an extensive medical check-up (including blood sampling), neurological, neuropsychological and movement disorder assessments and after death their brains are analyzed by highly experienced and medically licensed pathologists. *Arizona Study of Aging and Neurodegenerative disorders (AZSAND) is referred to as the Banner neuropathology cohort in the thesis.*

Clinical studies

Ethical Approval

Study I, II and IV in this thesis were approved by the Ethics Committee at Lund University, Sweden and specifically Study III which was conducted in collaboration with Arizona Banner Institute was approved by the Institutional Review Board of the Brain and Body Donation Program, Arizona, United States. Written consent was acquired from all the participants or their legal responsible person.

Study Design

Paper I

In paper I, we included 50 Aβ+ and 50 Aβ- participants from the Swedish BioFINDER-1 cohort. The impact of pre-analytical conditions on the concentration and performance of plasma p-Tau217 was examined. Plasma samples were either thawed at room temperature (RT) or on ice. For each of the thawing method, we compared samples that were centrifuged and those that were not centrifuged, resulting in four conditions: (1) thawed at RT, not centrifuged (2) thawed at RT,

centrifuged (3) thawed on ice, not centrifuged (4) thawed on ice, centrifuged. Additionally, three freeze-thaw cycles were also tested.

Paper II

A total of 135 participants, who were diagnosed as MCI at baseline were included. All these participants were followed for up to 4.9 years. Participants were categorized into MCI-ADD if they developed ADD during follow-up (n=45) and non-progressors (MCI-stable cases and those who progressed to non-ADD, n=90). Non-progressors were further divided into A+ (n=26) and A- (n=64) based on the CSF Aβ40/Aβ42 ratio. Plasma and CSF samples were analyzed for several p-Tau species that were measured with different analytical methods (immunoassays and MS). We primarily focused on evaluating the ability of different p-Tau biomarkers to accurately detect abnormal Aβ status and predict future advancement to ADD. Furthermore, we also performed sensitivity analyses in a subcohort of participants, who had data available for all p-Tau species included in the study.

Paper III

From the Banner neuropathology cohort, we included 72 participants who all had antemortem plasma samples and underwent postmortem neuropathological assessment. Study participants were divided into two groups based on the ADNC scale, i.e. none/low ADNC (n=30) and intermediate/high ADNC (n=42). We evaluated the associations of different plasma biomarkers with density scores of amyloid plaque and neurofibrillary changes (Braak, CERAD) as well as with ADNC. The clinical diagnosis of participants ranged from CU, MCI, Alzheimer's disease to non-Alzheimer's disease dementia.

Paper IV

We included 850 participants from the Swedish BioFINDER-1 cohort who were followed for up to 8 years. The participants were diagnosed as either CU (n=675) or MCI (n=175) at baseline. Data on various plasma and CSF biomarkers, cognitive assessments and neuroimaging (MRI) was available. In this study, we assessed the associations between the longitudinal changes in plasma and CSF biomarkers (with and without normalization to reference proteins) and longitudinal changes in cognition and brain atrophy. Furthermore, we identified the most parsimonious CSF and plasma biomarker demonstrating strongest associations with change in cognitive decline and atrophy.

CSF and blood sample collection

CSF

In paper I, II and IV, CSF samples were collected from all the participants via lumbar puncture and centrifuged for 10 minutes at 2000g, 4°C. After centrifugation, CSF samples were aliquoted and stored at -80°C until further analysis.

Plasma

In paper I, II and IV, blood samples were collected in ethylenediaminetetraacetic acid (EDTA) tubes and were centrifuged for 10 minutes at 2000g, 4°C. After centrifugation, plasma was extracted, transferred to another 50 ml tube, mixed and aliquoted and stored at -80°C until further analysis.

In paper III, blood samples were collected at the Arizona Banner Health Institute and one of the antemortem plasma aliquots was shipped to our laboratory at Lund University, Sweden for analysis.

CSF and blood samples – Biochemical analysis

Paper I

Immunoassay developed by Lilly laboratories on mesoscale discovery (MSD) platform was used to measure plasma and CSF p-Tau217. CSF Aβ40/Aβ42 was measured using a fully automated Elecsys immunoassay developed by Roche Diagnostics.

Paper II

Several assays based on different platforms were used to measure p-Tau species (p-Tau217, p-Tau181 and p-Tau231). Information about different assays is summarised in Table 5 below:

Table 5: Description of various p-Tau assays

p-Tau species	Plasma	CSF	Name of the platform/ assay	Name of the Institute/ company
p-Tau217			MSD immunoassay	Eli Lilly
			MS-based assay	Washington University
			Simoa immunoassay	Janssen Research and Development
p-Tau181			MSD immunoassay	Eli Lilly
			MS-based assay	Washington University
			Simoa immunoassay	Gothenburg University
			Simoa immunoassay	ADx Neuroscience
			Lumipulse immunoassay	Fujirebio

			Splex immunoassay	MSD
p-Tau231			Inhouse Simoa immunoassay	Gothenburg University
			Splex immunoassay	MSD

Paper III

Plasma p-Tau217 was measured using ALZpath immunoassay based on the SIMOA platform in Phoenix, Arizona. Lilly MSD immunoassays were used to measure plasma p-Tau217 and p-Tau181 at Lund University.

Paper IV

Several CSF and plasma biomarkers were measured using different immunoassays. Overview of all the biomarkers is described below:

- CSF and plasma p-Tau217: MSD immunoassay (Lilly)
- CSF p-Tau181, t-Tau, Aβ40, Aβ42: Elecsys immunoassay (Roche)
- CSF NfL, GFAP: NeuroToolKit assay (Roche)
- CSF p-Tau205: Simoa immunoassay (Quanterix)
- Plasma Aβ40, Aβ42, p-Tau181, GFAP, NFL: Prototype immunoassays (Roche)
- Plasma p-Tau212, NTA-Tau: Simoa immunoassay (Quanterix)

Postmortem methods

Neuropathological assessment

In paper III, the data obtained from the histopathological scoring was utilised. The scoring was done by a single neuropathologist at Arizona Banner institute (Dr. Thomas G. Beach). CERAD template was used to calculate the total plaque density score, and the same template was used for calculating the neurofibrillary density score. Braak staging for spatial distribution of neurofibrillary changes, CERAD classification of neuritic plaque density scores and Thal staging of Aβ plaque distribution were used as neuropathology measures. Global ADNC was calculated based on the Braak, CERAD and Thal scales (209). *For a detailed description of histopathological scoring please refer to paper III.*

Imaging

As a part of Paper IV, cortical thickness was used as a measure of brain atrophy. For this, first a 3T MRI scanner was utilized to obtain T1-weighted MRI scans.

FreeSurfer 6.0 was employed for the generation of structural segmentations and for the calculation of the cortical thickness. Cortical thickness was measured in Alzheimer's disease signature regions (temporal meta-ROI) (210). *For a detailed description of MRI acquisition and processing refer to paper IV.*

Cognitive testing

In paper IV, two measures evaluating cognitive functions were used: (1) the Mini-Mental State Examination (MMSE) and (2) the modified Preclinical Alzheimer's Cognitive Composite (mPACC). MMSE is a measure of one's cognitive abilities, evaluated on a scale of 0 to 30 points. The higher the score, the better the cognitive performance and vice versa. However, this test did not demonstrate high sensitivity (211). Hence, a more sensitive cognitive measure, mPACC score, was also used. This score measures an individual's global cognition. *A detailed description about the calculation of the mPACC score can be found in paper IV.*

Statistical analysis

In paper I, all statistical analyses were performed using SPSS software, whereas for papers II-IV, R studio was used.

Paper I

For determining the correlations between CSF biomarkers (p-Tau217 and Aβ42/Aβ40) and plasma p-Tau217, the Spearman's correlation was utilized. To measure how well plasma p-Tau217 could distinguish abnormal from normal CSF Aβ42/Aβ40 and p-Tau217 status, receiver operating characteristic (ROC) was used. The influence of pre-analytical factors on the concentrations of plasma p-Tau217 was determined with a two-way repeated measures ANOVA.

Paper II

Group differences in plasma p-Tau biomarker levels were examined with univariate general linear models (adjusted for age, sex) and additionally for duration of follow-up when comparing MCI who advanced to ADD to those who did not. To assess the correlation between various CSF and plasma biomarkers, the Spearman's correlation was used. Differences between correlation coefficients were measured using bootstrapping. ROC curve was utilised to assess the diagnostic performance of CSF biomarkers. The areas under the curve (AUC) between two curves were compared using the DeLong test.

Paper III

The Spearman's test was used to examine the cross-correlation between different plasma biomarkers (p-Tau217$_{ALZpath}$, p-Tau217$_{Lilly}$ and p-Tau181$_{Lilly}$). To assess the associations of plasma biomarkers with Aβ plaque and neurofibrillary density scores, partial Spearman's correlation was used (adjusted for age, sex and time between blood drawn to death). Similar sets of analyses were performed with models also including neurofibrillary density score as a covariate when assessing the associations between biomarkers and Aβ plaque score, and vice versa. The significant differences between correlation coefficients were assessed using bootstrapping. Kruskal-Wallis and Mann-Whitney tests were used to compare biomarker levels between different pathological groups. ROC curve analysis was utilised to assess diagnostic performance of various plasma biomarkers. To compare the AUC between two curves, the DeLong test was used.

Paper IV

Linear mixed-effect models were used to assess the associations between longitudinal changes in biomarkers (CSF/plasma) and longitudinal changes in cognitive decline (measured using MMSE and mPACC) and brain atrophy (measured using cortical thickness). In the independent models, CSF/plasma slopes were included as predictors and longitudinal cognition and atrophy as outcomes. The slopes were calculated using linear mixed-effect models, with time as the only predictor. Age and sex, and their interactions with time, were included as covariates in each model. Education and its interaction with time were also included as covariates in the cognition models. We determined differences in beta estimates using bootstrapping to compare the strength of associations between biomarkers or their respective ratios with cognitive decline or brain atrophy. Finally, we identified the most parsimonious CSF and plasma biomarker models showcasing the strongest associations with longitudinal cognition and brain atrophy.

Summary of key results

In the following section, the main results of each paper are described. For full details on the methodology and additional analyses please refer to the individual papers in the appendix of this thesis.

Paper I

Plasma biomarkers have revolutionized the Alzheimer's field. However, there are still substantial uncertainties regarding the influence of pre-analytical conditions on their levels and performance. Therefore, the impact of pre-analytical factors on plasma p-Tau217 (one of the top-performing plasma Alzheimer's disease biomarkers) was assessed in this study.

We tested how conditions such as thawing temperature, centrifugation and freeze-thaw cycles could impact concentration of p-Tau217, and also its ability to distinguish abnormal CSF Aβ42/Aβ40 and/or CSF p-Tau217 status. To this end, we quantified plasma p-Tau217, CSF p-Tau217 and CSF Aβ42/Aβ40 in individuals with (Aβ+, n=50) and without (Aβ-, n=50) evidence of brain amyloid pathology, from the Swedish BioFINDER-1 cohort. Plasma samples were either thawed at room temperature (RT) or on ice. For each thawing method, we compared samples that were centrifuged and samples that were not, resulting in four conditions: (1) thawed at RT, not centrifuged; (2) thawed at RT, centrifuged; (3) thawed on ice, not centrifuged; (4) thawed on ice, centrifuged. Additionally, three freeze-thaw cycles were tested.

For all four conditions, there were significant correlations between plasma p-Tau217 and the CSF biomarkers (i.e., p-Tau217 and Aβ42/Aβ40) in the whole cohort and between plasma p-Tau217 and CSF p-Tau217 in the Aβ+ group (Table 6). Surprisingly, only samples that were centrifuged exhibited significant correlations between plasma p-Tau217 and CSF Aβ42/Aβ40 in Aβ+, and between plasma p-Tau217 and CSF p-Tau217 in Aβ-. Plasma p-Tau217 separated with high precision people who had abnormal levels of CSF p-Tau217 or CSF Aβ42/Aβ40 from those with normal levels of these biomarkers (Figure 9). Of note, its discriminative accuracy was somewhat lower for samples thawed at RT and not

centrifuged, as reflected by the numerically lower AUC in comparison to other conditions (Figure 9).

Table 6: Spearman's correlations between plasma p-Tau217 and/or CSF Aβ42/40 / CSF p-Tau217
Data displayed as Spearman's correlation coefficients (p-value, adjusted/unadjusted). Abbreviations: C, centrifugation; Cond, condition; CSF, cerebrospinal fluid; NC, non-centrifugation; RT, room temperature (212).

Plasma p-Tau217	**CSF Aβ42/Aβ40** R (p-value adjusted/unadjusted)	**CSF p-Tau217** R (p-value adjusted/unadjusted)
All (n = 100)		
Cond 1: thaw at RT, NC	**-0.515 (<0.001 / <0.001)**	**0.614 (<0.001 / <0.001)**
Cond 2: thaw at RT, C	**-0.636 (<0.001 / <0.001)**	**0.713 (<0.001 / <0.001)**
Cond 3: thaw on ice, NC	**-0.607 (<0.001 / <0.001)**	**0.666 (<0.001 / <0.001)**
Cond 4: thaw on ice, C	**-0.652 (<0.001 / <0.001)**	**0.717 (<0.001 / <0.001)**
Aβ+ (n=50)		
Cond 1: thaw at RT, NC	-0.215 (0.172 / 0.133)	**0.506 (<0.001 / <0.001)**
Cond 2: thaw at RT, C	**-0.394 (0.010 / 0.005)**	**0.579 (<0.001 / <0.001)**
Cond 3: thaw on ice, NC	-0.284 0.079 / 0.046)	**0.511 (<0.001 / <0.001)**
Cond 4: thaw on ice, C	**-0.406 (0.007 / 0.003)**	**0.550 (<0.001 / <0.001)**
Aβ- (n=50)		
Cond 1: thaw at RT, NC	0.230 (0.162 / 0.108)	0.190 (0.202 / 0.186)
Cond 2: thaw at RT, C	0.073 (0.615 / 0.615)	**0.394 (0.007 / 0.005)**
Cond 3: thaw on ice, NC	0.210 (0.172 / 0.143)	0.184 (0.202 / 0.202)
Cond 4: thaw on ice, C	0.105 (0.511 / 0.468)	**0.334 (0.022 / 0.018)**

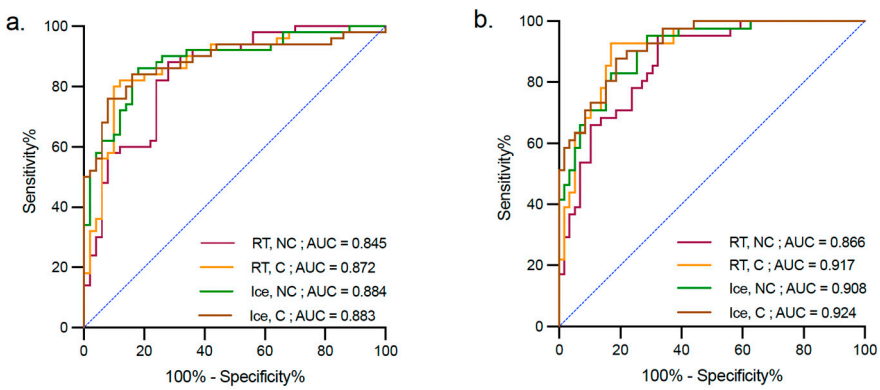

Figure 9. Discriminative accuracy of plasma p-Tau217
Identifying individuals with abnormal a) CSF Aβ42/Aβ40 status and b) CSF p-Tau217 status. Abbreviations: C, centrifugation; Cond, condition; CSF, cerebrospinal fluid; NC, non-centrifugation; RT, room temperature. *Figure reprinted from (212).*

Furthermore, we found that there was a significant interaction between Aβ status and sample handling conditions on plasma p-Tau217 levels, meaning that the difference in p-Tau217 levels between sample handling conditions were not the

same for Aβ+ and Aβ- individuals. Specifically, higher p-Tau217 concentrations were found in non-centrifuged samples than in centrifuged samples. Finally, plasma p-Tau217 levels did not differ significantly between samples subjected to one, two, or three freeze-thaw cycles (Figure 10).

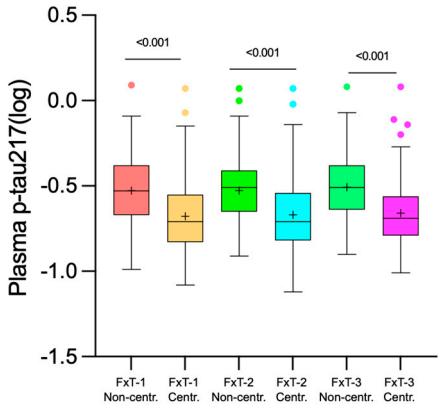

Figure 10. Plasma p-Tau217 levels across different conditions

Cond1, Plasma samples underwent one freeze-thaw cycle without centrifugation, Cond 2, centrifuged prior to analysis , Cond 3, underwent two freeze thaw cycles without centrifugation, Cond 4, centrifuged before the analysis, Cond 5, underwent three freeze thaw cycles without centrifugation, Cond 6, centrifuged prior to analysis. Abbreviations: C, centrifugation; FxT, freeze-thaw cycle; NC, non-centrifugation. *Figure reprinted from (212).*

Based on the results of this paper, we proposed that plasma samples should be thawed at RT and centrifuged before their analysis with the MSD p-Tau217 assay.

Paper II

Plasma contains multiple p-Tau forms, and over the years a variety of analytical approaches, including immunoassays and MS-based methods have been developed to quantify these species. Despite these developments, comparing the relative performance of different assays or different p-Tau forms has been challenging, mainly due to heterogeneous assay platforms, cohorts, and sample-handling protocols. Hence, in this study we compared, head-to-head, the performance of the three main p-Tau variants (p-Tau181, p-Tau217 and p-Tau231) measured using either MS-based methods or various immunoassays. The study comprised 135 individuals recruited at the memory clinic with a clinical diagnosis of MCI at baseline, and with a follow-up duration of up to 4.9 years. CSF Aβ status and future progression to ADD were our primary outcomes.

First, we examined how well different plasma p-Tau species could discriminate individuals with and without abnormal Aβ status at baseline (Table 7). MS-based p-Tau217^{WashU} assay showed the strongest diagnostic accuracy, compared to other p-Tau biomarkers. Among immunoassays, p-Tau217^{Lilly} exhibited the highest AUC but was not significantly higher than p-Tau217^{Janss}, p-Tau181^{ADx} (both immunoassays), or p-Tau181^{WashU} (MS). The remaining immunoassays demonstrated comparatively inferior performance. The largest difference in plasma biomarker levels between A+ MCI and A- MCI was seen for p-Tau217^{WashU} gradually decreasing for p-Tau217^{Janss}, p-Tau217^{Lilly} and p-Tau181^{ADx} (Figure 11).

Table 7: Associations between plasma p-Tau variants and CSF Aβ42/Aβ40
Data obtained from the ROC curve analysis. CSF Aβ42/Aβ40 was used to classify MCI participants as amyloid positive/A+ or amyloid negative/A-. Abbreviations: AUC, area under the curve; CI, confidence interval; NA, not applicable (213).

Plasma p-Tau	AUC (95% CI)	P-value versus p-Tau217^{WashU}	P-value versus p-Tau217^{Lilly}
p-Tau217^{WashU}	0.947 (0.907-0.987)	NA	0.015
p-Tau217^{Lilly}	0.886 (0.827-0.944)	0.015	NA
p-Tau217^{Janss}	0.858 (0.795-0.920)	0.004	0.38
p-Tau181^{ADx}	0.841 (0.768-0.913)	<0.001	0.24
p-Tau181^{WashU}	0.835 (0.765-0.906)	<0.001	0.20
p-Tau231^{UGOT}	0.784 (0.703-0.864)	<0.001	0.029
p-Tau181^{Lilly}	0.759 (0.676-0.841)	<0.001	<0.001
p-Tau181^{UGOT*}	0.743 (0.652-0.833)	<0.001	0.005
p-Tau181^{Fuji}	0.694 (0.604-0.784)	<0.001	<0.001
p-Tau181^{Splex*}	0.642 (0.533-0.751)	<0.001	<0.001

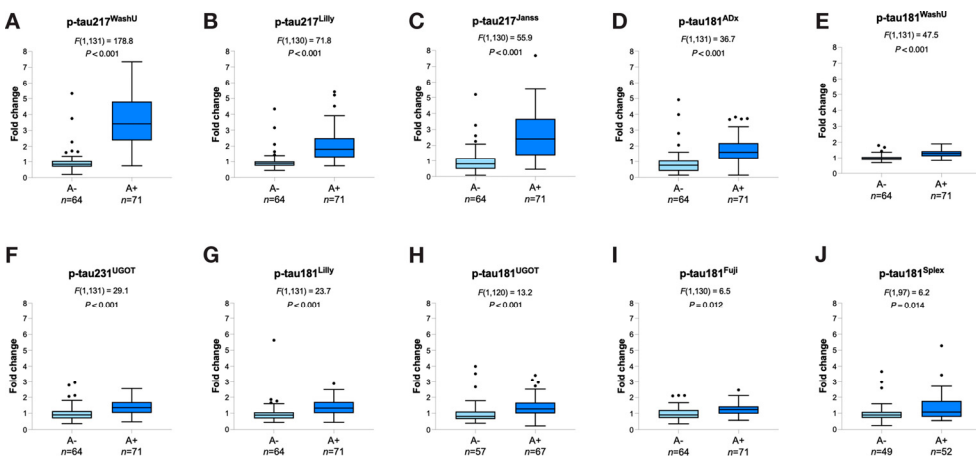

Figure 11. p-Tau biomarkers levels in A+ and A- MCI.
Results displayed as fold change from the A- MCI group average. Univariate general linear models were used for calculating F-values and p-values (adjusted for age and sex). Each box represents interquartile range, the horizontal lines are medians and the outliers and wishkers were illustrated using the Tukey method. *Figure reproduced from (213).*

Next, we tested the ability of the different p-Tau species to predict clinical progression to ADD (Table 8). Here again p-Tau217^{washU} showed the top performance, with an AUC significantly greater than those of the other biomarkers, followed by p-Tau217^{Lilly}, p-Tau217^{Janss}, p-Tau181^{ADx} (all immunoassays) and p-Tau181^{WashU} (MS) all of which demonstrated comparable performance.

Table 8: Plasma p-Tau biomarkers associations with future progression to ADD
Data obtained from the ROC curve analysis. Abbreviations: AUC, area under the curve; CI, confidence interval; NA, not applicable (213).

Plasma p-Tau	AUC (95% CI)	P-value versus p-Tau217^{WashU}	P-value versus p-Tau217^{Lilly}
p-Tau217^{WashU}	0.932 (0.891-0.974)	NA	0.027
p-Tau217^{Lilly}	0.889 (0.833-0.946)	0.027	NA
p-Tau217^{Janss}	0.872 (0.814-0.931)	0.027	0.53
p-Tau181^{ADx}	0.846 (0.777-0.916)	0.007	0.16
p-Tau181^{WashU}	0.835 (0.764-0.906)	0.001	0.09
p-Tau181^{Lilly}	0.813 (0.734-0.892)	0.002	0.013
p-Tau231^{UGOT}	0.777 (0.699-0.856)	<0.001	0.009
p-Tau181^{UGOT}	0.775 (0.692-0.858)	<0.001	0.014
p-Tau181^{Fuji}	0.735 (0.649-0.821)	<0.001	0.002
p-Tau181^{Splex}	0.688 (0.579-0.796)	<0.001	<0.001

Significant elevations in the three p-Tau217 biomarkers (p-Tau217^{Lilly}, p-Tau217^{WashU}, p-Tau217^{Janss}) and the best performing p-Tau181 biomarker (p-Tau181^{WashU} and p-Tau181^{ADx}) levels were observed in both A+ non-progressors and MCI-ADD in comparison to A- non-progressors (Figure 12). Levels of the three p-Tau217 biomarkers were also higher in MCI-ADD than in A+ non-progressors, whereas neither p-Tau181 nor p-Tau231 levels differ significantly between these groups.

The largest fold increase in both A+ non progressors and MCI-ADD groups in comparison to A- non-progressors was found for p-Tau217^{WashU}. Amongst the immunoassays, p-Tau217^{Lilly}, p-Tau181^{ADx} and p-Tau217^{Janss} demonstrated greater fold increases in MCI-ADD in comparison to A+ non progressors.

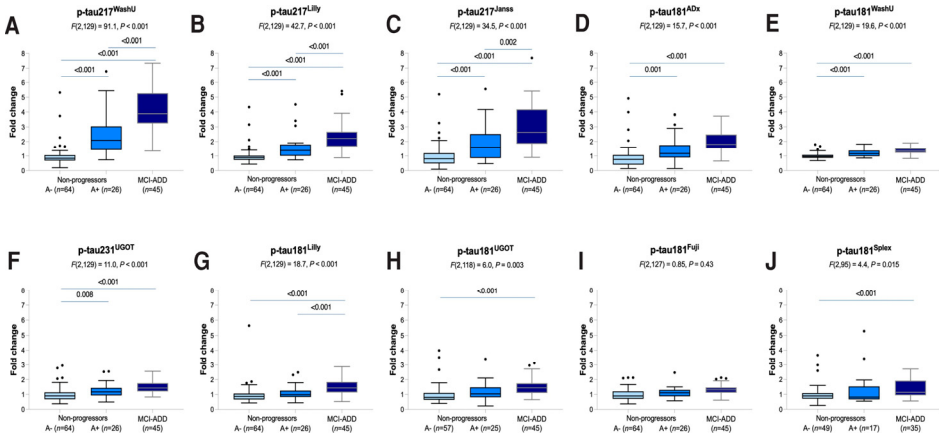

Figure 12. p-Tau biomarkers levels in individuals who advanced to ADD during follow-up as well as in A+ and A- non progressors.

Data displayed as fold change from the A- MCI group average. Univariate general linear-models were used for calculating F-values and p-values (adjusted for age, sex, and follow up time). Each box represents interquartile range, the horizontal lines are medians and the outliers and wishkers were illustrated using the Tukey method. *Figure reproduced from (213).*

Finally, p-Tau217^{WashU}, followed by p-Tau217^{Lilly} showed the top correlations between CSF and plasma values (Figure 13).

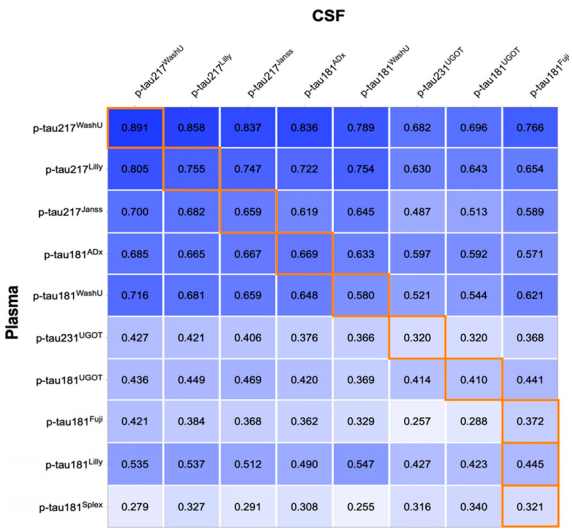

Figure 13. Spearman-based associations between CSF and plasma p-Tau biomarkers. Figure reproduced from (213).

Evidence from our study points to the superiority of MS-based assay and sufficiently high precision of several immunoassays to identify Aβ positivity and predict conversion from MCI to ADD.

Paper III

ALZpath developed a commercial immunoassay for measuring plasma p-Tau217, available worldwide for research use. Several reports demonstrated its high clinical diagnostic accuracy for Alzheimer's disease. In this study, we, for the first time, assessed ALZpath p-Tau217 performance against the gold standard neuropathological outcomes in comparison to plasma Lilly p-Tau217 and Lilly p-Tau181. We included 72 participants from the Banner neuropathology cohort. The clinical diagnosis of participants ranged from CU to MCI, Alzheimer's disease and non-Alzheimer's disease dementia.

First, we found significant associations between the three plasma biomarkers (p-Tau217$_{ALZpath}$, p-Tau217$_{Lilly}$ and p-Tau181$_{Lilly}$) and Aβ plaque density scores and neurofibrillary density scores (Table 9, Figure 14). Still, in both analyses, correlations with the outcome measures were significantly higher for p-Tau217$_{Lilly}$ compared to p-Tau217$_{ALZpath}$. When adjusting for neurofibrillary density scores, the significant correlations between the plasma biomarkers and Aβ density scores still held true. However, only p-Tau217$_{Lilly}$ was associated with neurofibrillary density scores when adjusting for all the covariates and Aβ plaque density scores (Table 5).

Table 9: Associations between different plasma biomarkers and neuropatholgical outcomes
Results obtained from partial Spearman's correlation (ρ). Bold values indicate significant associations. Significant differences between the correlation coefficients were assessed using bootstrapping (40).

	Models adjusted for covariates (age, sex and time blood-death)			**Models adjusted for covariates and neurofibrillary density scores**		
Plaques	ρ	p-value	p-value	ρ	p-value	p-value
p-Tau217$_{ALZpath}$	0.58	**<0.001**	Reference	0.53	**<0.001**	Reference
p-Tau181$_{Lilly}$	0.65	**<0.001**	0.328	0.59	**<0.001**	0.491
p-Tau217$_{Lilly}$	0.78	**<0.001**	**0.012**	0.73	**<0.001**	**0.015**
	Models adjusted for covariates (age, sex and time blood-death)			**Models adjusted for covariates and amyloid plaque density scores**		
Neurofibrillary changes	ρ	p-value	p-value	ρ	p-value	p-value
p-Tau217$_{ALZpath}$	0.26	**0.031**	Reference	0.03	0.82	Reference
p-Tau181$_{Lilly}$	0.37	**0.002**	0.225	0.15	0.33	0.29
p-Tau217$_{Lilly}$	0.51	**<0.001**	**0.004**	0.32	0.022	**0.003**

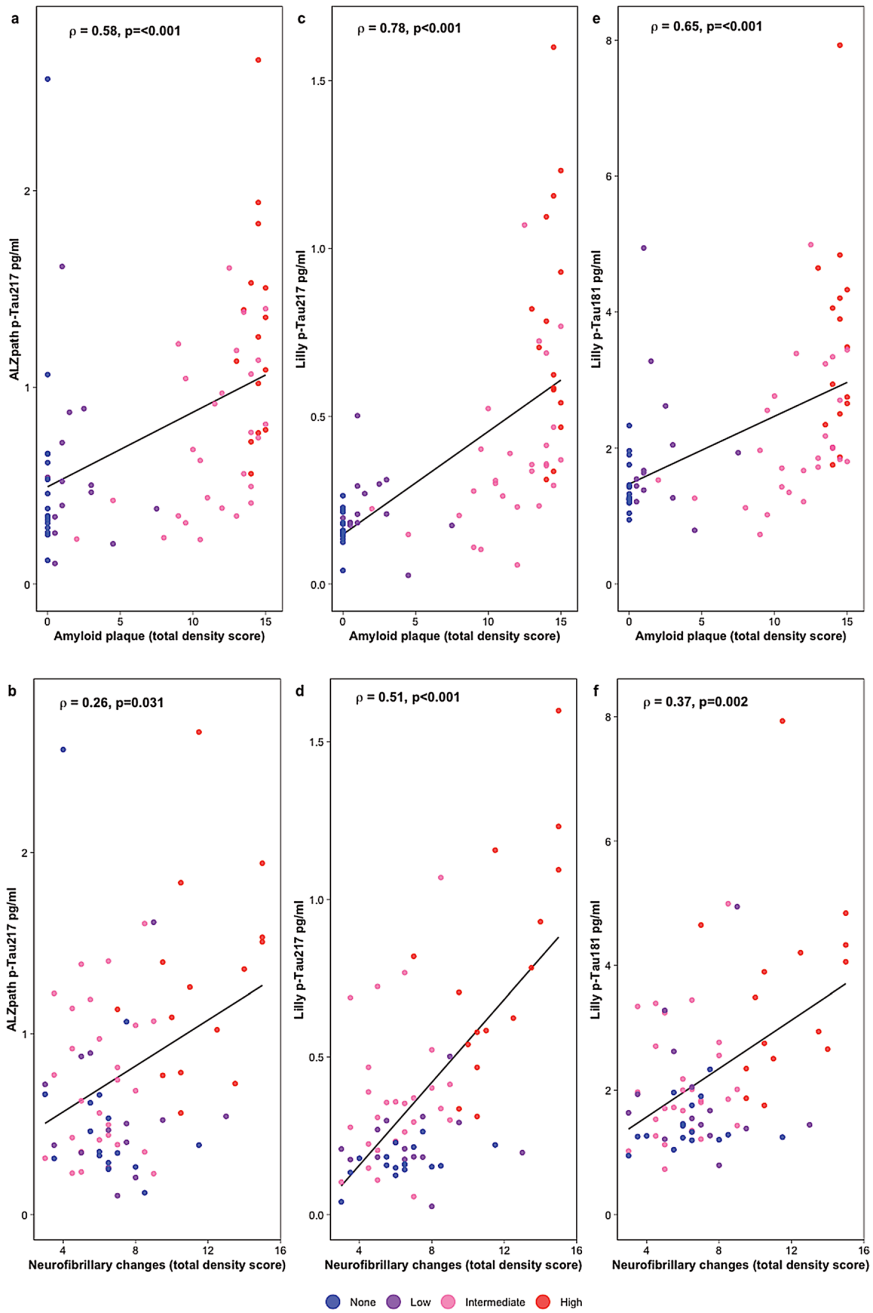

Figure 14. Associations of p-Tau biomarkers with Aβ plaque and neurofibrillary density scores. Data shown as partial Spearman correlation coefficients (ρ) and p-values. *Figure reproduced from (40).*

Next, all the plasma biomarkers accurately predicted ADNC classification, with p-Tau217$_{Lilly}$ again demonstrating significantly higher AUC compared to p-Tau217$_{ALZpath}$. Furthermore, the results were very similar when performing similar set of analysis for Braak staging and CERAD classification; that is p-Tau217$_{Lilly}$ demonstrated superior performance compared to p-Tau217$_{ALZpath}$.

Table 10: Predicting classification based on neuropatholgical scales.
Data obtained from ROC curve analysis for predicting (a) ADNC, (b) Braak and (c) CERAD classification. Significant differences between the AUCs of two ROC curves were assessed using The DeLong test. Abbreviations: ADNC, Alzheimer's disease neuropathologic change; CI, confidence interval; CERAD, The Consortium to Establish a Registry for Alzheimer´s Disease (40).

	AUC	95% CI.	p-value comp.
ADNC			
p-Tau217$_{ALZpath}$	0.75	(0.63,0.87)	Reference
p-Tau181$_{Lilly}$	0.76	(0.65,0.88)	0.72
p-Tau217$_{Lilly}$	0.87	(0.78,0.96)	**0.021**
BRAAK			
p-Tau217$_{ALZpath}$	0.74	(0.61,0.87)	Reference
p-Tau181$_{Lilly}$	0.75	(0.61,0.88)	0.88
p-Tau217$_{Lilly}$	0.82	(0.70,0.94)	**0.021**
CERAD			
p-Tau217$_{ALZpath}$	0.78	(0.66,0.89)	Reference
p-Tau181$_{Lilly}$	0.79	(0.68,0.90)	0.85
p-Tau217$_{Lilly}$	0.89	(0.80,0.97)	**0.024**

In summary, the performance of p-Tau217$_{ALZpath}$ and p-Tau181$_{Lilly}$ was comparable; however, p-Tau217$_{Lilly}$ demonstrated significantly higher associations with core measures of Alzheimer's disease pathology in comparison to p-Tau217$_{ALZpath}$.

Paper IV

It is at present largely unknown whether CSF and plasma Alzheimer's disease biomarkers can be used to track disease progression. In this study, we analyzed several plasma (p-Tau217, p-Tau181, p-Tau212, Aβ42, Aβ40, NTA-tau, GFAP, NfL) and CSF (p-Tau217, p-Tau181, p-Tau205, Aβ42, Aβ40, t-Tau, NfL and GFAP) biomarkers in a cohort of 850 participants from the Swedish BioFINDER-1 study who were followed for up to 8 years. We assessed the associations between longitudinal changes in various CSF and plasma biomarkers, both with and without normalization to Aβ40, Aβ42 or t-Tau and longitudinal cognitive decline (measured using MMSE and mPACC) and brain atrophy (measured using cortical thickness). The associations were tested in the whole cohort as well as in Aβ+ participants only.

First, we examined the associations between changes in longitudinal CSF biomarker levels with changes in longitudinal cognition and atrophy. In the whole cohort, we found significantly stronger associations with longitudinal cognitive decline and brain atrophy for CSF p-Tau205/Aβ40, p-Tau217/Aβ40 and p-Tau181/Aβ40 compared with p-Tau205, p-Tau217 and p-Tau181, respectively, and with their ratios to CSF Aβ42 and t-Tau (Figure 15). In Aβ+ participants, slopes of p-Tau205/Aβ40, p-Tau217/Aβ40 and p-Tau181/Aβ40 demonstrated significant associations with longitudinal cognition and atrophy.

Next, we identified the most parsimonious CSF biomarker model demonstrating the best associations of cognitive decline and atrophy. Such a model for longitudinal MMSE and mPACC in the whole cohort as well as for longitudinal brain atrophy in both the whole cohort and the Aβ+ group included p-Tau205/Aβ40 slopes and NfL slopes, (Table 11). A model comprising only p-Tau205/Aβ40 best predicted longitudinal cognitive decline in Aβ+ participants.

We then performed similar sets of analyses for plasma biomarkers. Here, contrary to CSF, none of the plasma biomarker ratios demonstrated significantly stronger associations with the three longitudinal outcomes (MMSE, mPACC and cortical thickness), in comparison to longitudinal plasma measures of p-Tau217, p-Tau181, p-Tau212, NfL and GFAP (Figure 16). All non-normalized biomarker slopes demonstrated significant associations with accelerated cognitive decline and atrophy in the whole cohort. In the Aβ+, slopes of p-Tau217, p-Tau212, NfL and GFAP exhibited significant associations with longitudinal cognitive decline and atrophy. p-Tau181 slope was found to be significantly associated with cognition but not with brain atrophy.

In plasma, a model including p-Tau217 slope demonstrated the best associations with MMSE and mPACC changes in the whole cohort and Aβ+ participants, and with brain atrophy in the Aβ+ group (Table 12). However, in the whole cohort, the most parsimonious model for longitudinal brain atrophy combined slopes of plasma p-Tau217 and NfL.

Finally, we found associations of plasma p-Tau217 with all three longitudinal outcomes were not significantly different from those of CSF p-Tau205/Aβ40 and p-Tau217/Aβ40.

In conclusion, normalization to Aβ40 enhances the strength of associations between longitudinal CSF biomarkers, but not plasma biomarkers, and longitudinal cognitive decline and atrophy. The most parsimonious CSF and plasma models for associations with longitudinal cognition and brain atrophy included slopes of CSF p-Tau205/Aβ40 and plasma p-Tau217, respectively, either alone or in combination with NfL slopes.

CSF Biomarkers

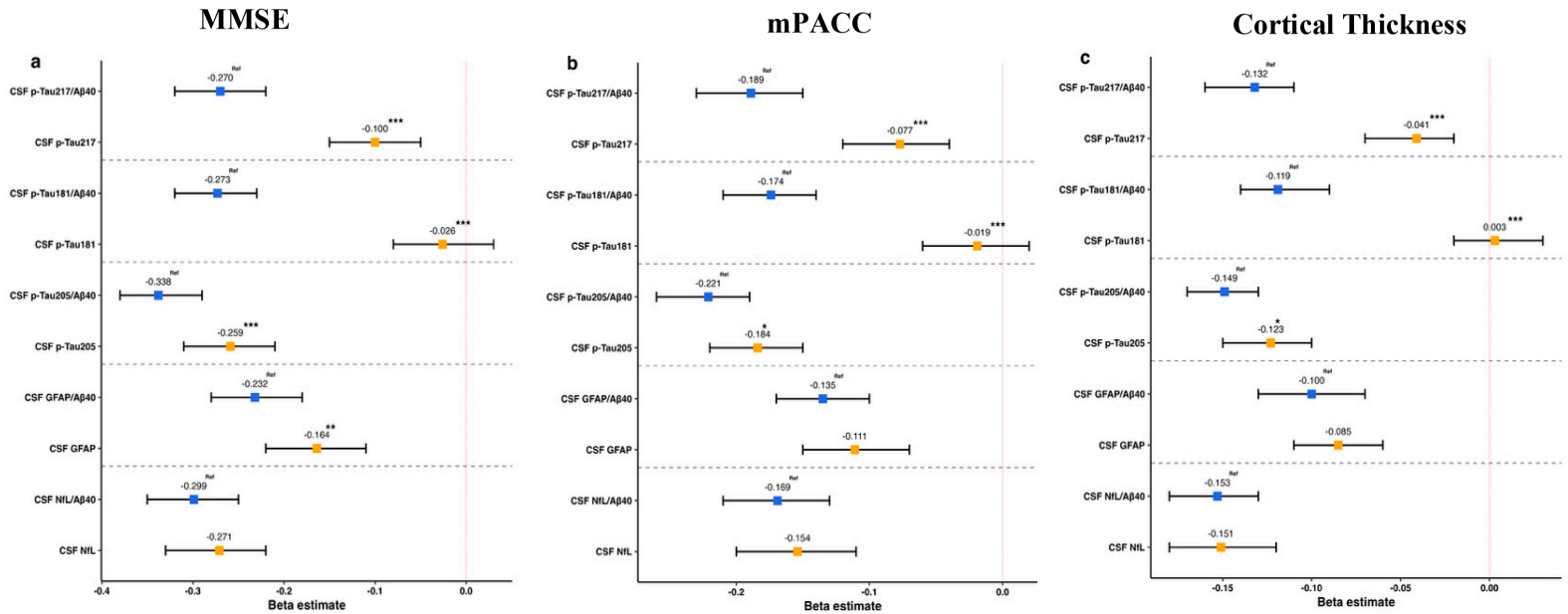

Figure 15. The figure demonstrate longitudinal change in individual CSF biomarkers and longitudinal changes in cognitive decline and brain atrophy in the whole cohort.

In this forest plot, each segment is divided by a dash line, which comprised of a beta eastimate of individual CSF biomarker normalized to Aβ40 (Reference) (blue square) followed by the beta estimate of individual CSF biomarker (orange square). The beta estimate are derived from the linear-mixed effect models. Bootstrapping approach was used to estimate the difference in beta estimates with respect to the reference biomarker slope. Abbreviations: mPACC, modified Preclinical Alzheimer's Cognitive Composite; MMSE, Mini-Mental State Examination.

Plasma Biomarkers

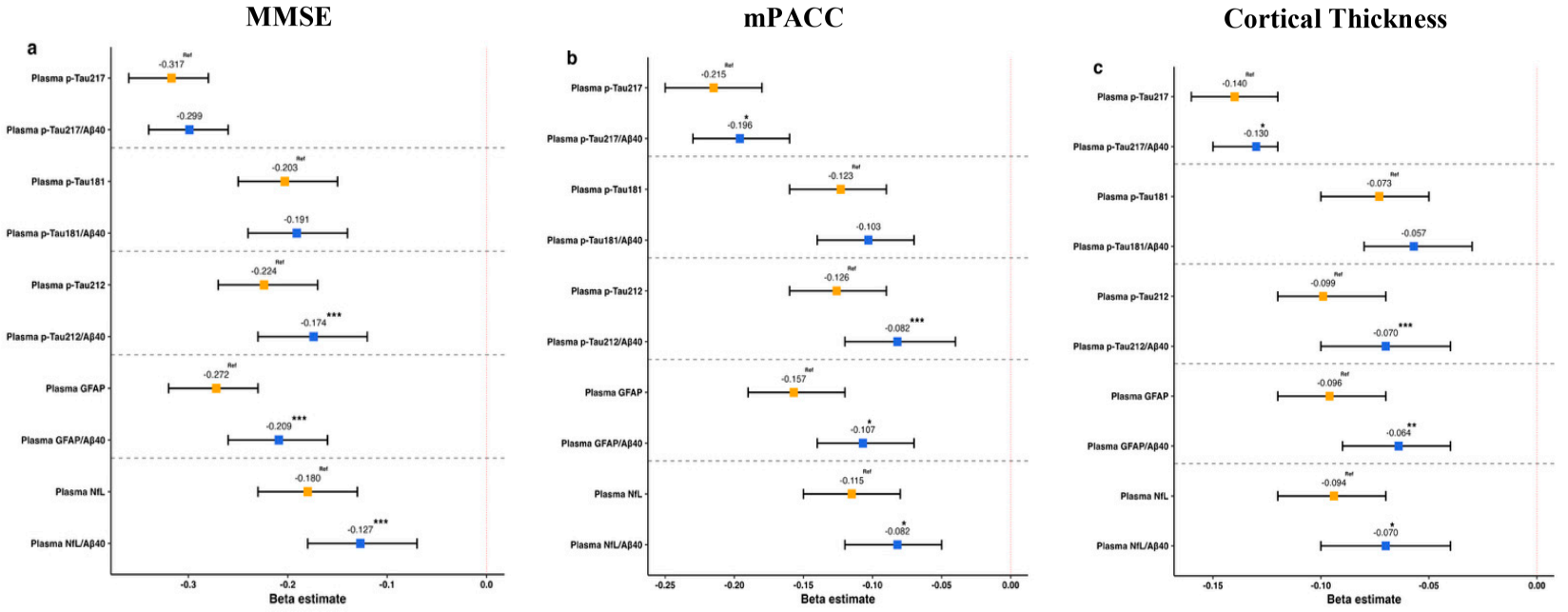

Figure 16. The figure demonstrate longitudinal change in individual plasma biomarkers and longitudinal changes in cognitive decline and brain atrophy in the whole cohort.

In this forest plot, each segment is divided by a dash line, which comprised of a beta eastimate of individual plasma biomarker (Reference) (orange square) followed by the beta estimate of individual plasma biomarker normalized to Aβ40 (blue square). The beta estimate are derived from the linear-mixed effect models. Bootstrapping approach was used to estimate the difference in beta estimates with respect to the reference biomarker slope. Abbreviations:mPACC, modified Preclinical Alzheimer's Cognitive Composite; MMSE, Mini-Mental State Examination.

Table 11: Most parsimonious CSF models for associations with longitudinal cognition and brain atrophy.

The most parsimonious models were derived using the dedrge function from the MuMIn package in R software. Abbreviations: mPACC, modified Preclinical Alzheimer's Cognitive Composite; MMSE, Mini-Mental State Examination.

Outcome	CSF Biomarkers	AIC	R^2	β Estimate (CI)	P-value
Whole Cohort					
MMSE change	Slope CSF p-Tau205/Aβ40*time + Slope CSF NFL*time	2971	0.40	-0.351 (-0.395, - 0.308)	**<0.001**
				-0.081 (-0.127, -0.035)	**0.001**
mPACC change	Slope CSF p-Tau205/Aβ40*time + Slope CSF NFL*time	2436	0.40	-0.224 (-0.259, -0.189)	**<0.001**
				-0.072 (-0.108, -0.036)	**<0.001**
Cortical Thickness change	Slope CSF p-Tau205/Aβ40*time + Slope CSF NFL*time	1761	0.32	-0.139 (-0.16, -0.118)	**<0.001**
				-0.100 (-0.122, -0.078)	**<0.001**
Aβ+					
MMSE change	Slope CSF p-Tau205/Aβ40*time	1115	0.38	-0.341 (-0.407, -0.275)	**<0.001**
mPACC change	Slope CSF p-Tau205/Aβ40*time	976	0.31	-0.206 (-0.265, -0.148)	**<0.001**
Cortical Thickness change	Slope CSF p-Tau205/Aβ40*time + Slope CSF NFL*time	699	0.31	-0.117 (-0.154, -0.081)	**<0.001**
				-0.088 (-0.125, -0.05)	**<0.001**

Table 12: Most parsimonious plasma models for associations with longitudinal cognition and brain atrophy.

The most parsimonious models were derived using the dedrge function from the MuMIn package in R software. Abbreviations: mPACC, modified Preclinical Alzheimer's Cognitive Composite; MMSE, Mini-Mental State Examination.

Outcome	Plasma Biomarkers	AIC	R^2	β Estimate (CI)	P-value
Whole Cohort					
MMSE change	Slope Plasma p-Tau217*time	2779	0.29	-0.283 (-0.326, -0.241)	**<0.001**
mPACC change	Slope Plasma p-Tau217*time	2248	0.27	-0.196 (-0.229, -0.163)	**<0.001**
Cortical Thickness change	Slope Plasma p-Tau217*time + Slope Plasma NFL*time	1735	0.24	-0.120 (-0.144, -0.096)	**<0.001**
				-0.045 (-0.07, -0.019)	**0.001**
Aβ+					
MMSE change	Slope Plasma p-Tau217*time	869	0.33	-0.274 (-0.348, -0.201)	**<0.001**
mPACC change	Slope Plasma p-Tau217*time	718	0.34	-0.222 (-0.278, -0.166)	**<0.001**
Cortical Thickness change	Slope Plasma p-Tau217*time	594	0.27	-0.147 (-0.183, -0.112)	**<0.001**

Discussion and future perspectives

The work presented in this thesis focused on supporting the implementation of plasma p-Tau as a biomarker of Alzheimer's disease in clinical practice and clinical trials. In this regard, in **paper I,** we investigated the effects of certain pre-analytical sample handling factors on the concentrations and performance of plasma p-Tau217. In **paper II** we compared the performance of p-Tau species (p-Tau217, p-Tau181 and p-Tau231) quantified using different analytical approaches such as MS-based methods (the gold standard for protein identification) and immunoassays to evaluate their ability to detect Aβ positivity and predict progression to ADD. In **paper III,** the performance of a commercially available immunoassay manufactured by ALZpath Inc for measuring p-Tau217 in blood was assessed in comparison with Lilly immunoassays (Lilly p-Tau217 and Lilly p-Tau181) in a neuropathological study population. Finally, in **paper IV,** we identified the best candidate CSF and plasma biomarkers that could be utilized for monitoring Alzheimer's disease progression and further examined whether normalizing longitudinal CSF/plasma biomarkers to reference proteins strengthened their associations with changes in longitudinal clinical outcomes (such as cognition and brain atrophy).

A significant amount of variability in the levels of Alzheimer's disease biomarkers observed across laboratories is due to differences in pre-analytical sample handling procedures (200). Therefore, it is of utmost importance to establish unified protocols that would allow the standardization of biomarker measurements. To this end, in **paper I**, we examined the impact of certain pre-analytical factors on the concentration and performance of plasma p-Tau217. Interestingly we found that even though p-Tau217 concentration was higher in non-centrifuged compared with centrifuged samples, centrifugation improved correlations of plasma p-Tau217 (measured using the Lilly immunoassay) with CSF p-Tau217 and Aβ42/Aβ40. Although the underlying mechanism remains unclear, it is likely that centrifugation reduces the non-specific signal in the assay. We did not observe any significant effects of thawing samples at RT or on ice nor of up to three freeze-thaw cycles on the performance of plasma p-Tau217. Our results regarding freeze-thaw cycles are consistent with prior research (202). Based on these data, we recommended centrifugation of plasma samples to achieve optimal performance of the Lilly p-Tau217 assay. Additionally, although we did not find any significant differences between thawing samples at RT or on ice, we still recommended thawing plasma

samples at RT since it is quicker and more feasible in clinical chemistry laboratories. The protocol we proposed was specific for p-Tau217 measured on the Mesoscale platform. Future studies are needed to comprehensively address the effects of pre-analytical sample handling factors on all the p-Tau assays (measured using different analytical methods) that would be potentially implemented for the diagnostic work-up of Alzheimer's disease (205, 214). Because different p-Tau assays are based on different antibodies, analytical platforms and detection systems, they may differ in their susceptibility to pre-analytical sample handling.

While most previous studies have evaluated the effects of various pre-analytical factors on plasma p-Tau217 concentration (202-205, 207), in our study, for the first time, we assessed the performance of plasma p-Tau217, which is highly relevant for its application in clinical practice and drug trials. Thus, our study provided a template for future investigations determining the influence of pre-analytical sample handling factors across different Alzheimer's disease plasma biomarkers.

Following early work demonstrating the high diagnostic accuracy of plasma p-Tau181 and p-Tau217, numerous assays were developed to measure different p-Tau variants (p-Tau217, p-Tau181, p-Tau231) (97, 137, 140, 145, 215-217). However, studies have reported inconsistent findings about the performance of different p-Tau species which could be due to differences in assay performance as well as pre-analytical factors and cohort characteristics. Therefore, in **paper II** we measured p-Tau217, p-Tau181 and p-Tau231 using 10 different assays in the same samples from patients with MCI which allowed direct head-to-head comparison of the p-tau biomarkers. The results of this study indicated that p-Tau217 measured using a MS-based assay performed exceptionally well in predicting Aβ pathology and progression to ADD, outperforming the top immunoassays, p-Tau217^{Lilly}, p-Tau217^{Janss}, p-Tau217^{ADx}, which all showed high accuracy. A likely explanation is that MS-based methods offer very high analytical accuracy and can reliably measure low-abundance proteins in complex, protein-dense matrices (218). We also showed that when both p-Tau217 and p-Tau181 were quantified using the same MS-based methods, p-Tau217 outperformed p-Tau181, likely because, p-Tau217 is a more dynamic biomarker with much lower levels in non-Alzheimer's individuals. Taken together these findings highlighted substantial variability in performance across different p-Tau variants and assays, even when measured in the same samples, suggesting that while some assays are suitable for implementation, others require further optimization.

Since our study, additional head-to-head comparisons have confirmed that p-Tau217 measured using MS-based methods outperforms immunoassays not only in predicting Aβ pathology and advancement to ADD (supporting our results) but also in detecting tau pathology (219-221). These findings were important for the regulatory approval of p-Tau217 (in ratio with Aβ42) for Alzheimer's disease diagnosis.

Because p-Tau variants begin to change in response to Aβ pathology, abnormal plasma (or CSF) p-Tau levels do not necessarily reflect tau pathology. Therefore, future studies should aim at developing biomarkers whose levels alter specifically in response to abnormal tau aggregation in the brain. Such biomarkers are needed for effective diagnostic workflow, as they would be more closely linked to clinical symptoms onset. Recently, one such candidate was identified. Studies have shown that a specific MTBR-tau species, MTBR-tau243, is more strongly associated with tau pathology than other tau variants (114, 164).

Plasma p-Tau217 is already being used in clinical trials for participant screening and since 2022 the Alzheimer's Association recommended cautious use of BBMs in memory clinics (222). With the escalating interest in p-Tau217, multiple assays have been developed. For example, ALZpath Inc has developed a commercially available immunoassay for measuring p-Tau217 in plasma. Several studies have demonstrated its high diagnostic accuracy in clinical cohorts. However, for assessing the usefulness of novel tests, validation against neuropathological outcomes, which is considered the gold standard, is crucial. In **paper III**, we evaluated the performance of this assay in a neuropathological cohort and found that p-Tau217$_{\text{ALZpath}}$ and p-Tau181$_{\text{Lilly}}$ demonstrated similar associations with core measures of Alzheimer's disease pathology. However, these associations were significantly weaker for p-Tau217$_{\text{ALZpath}}$ compared with p-Tau217$_{\text{Lilly}}$. Consistent with our findings, p-Tau217$_{\text{Lilly}}$ also demonstrated stronger associations with tau-PET than p-Tau217$_{\text{ALZpath}}$ in the Swedish BioFINDER-2 cohort, even though both biomarkers showed similar associations with Aβ-PET (219). Still, other studies have reported comparable performance of p-Tau217$_{\text{ALZpath}}$ to p-Tau217$_{\text{Lilly,}}$ p-Tau217$_{\text{Lumipulse}}$ and p-Tau217$_{\text{+ Janssen}}$, when determining Aβ- and tau-PET positivity as well as distinguishing Alzheimer's disease from other neurodegenerative disorders (219, 223, 224). The discrepancies between our results and previously published findings could be due to the high mean age of the participants in our study. Of note, the size of our cohort was relatively small, and thus future work is needed to validate our results in larger independent neuropathology cohorts.

BBMs and CSF biomarkers have shown great promise for diagnosis and prognostication of Alzheimer's disease. However, their ability to monitor disease progression remains insufficiently explored. Thus, in **paper IV**, we conducted a simultaneous analysis of longitudinal CSF (p-Tau217, p-Tau181, p-Tau205, Aβ42/Aβ40, total-tau, GFAP, NfL) and plasma (p-Tau217, p-Tau181, p-Tau212, Aβ42/Aβ40, NTA-Tau, GFAP, NfL) biomarkers to identify the best biomarkers or their combinations for tracking longitudinal changes in cognition and brain atrophy. Furthermore, given emerging evidence that normalization to reference proteins improves biomarker performance (225), we also examined CSF and plasma biomarkers normalized to reference proteins such as Aβ40, Aβ42 and t-Tau. We found that normalizing CSF biomarkers (particularly CSF p-Tau205, p-Tau217, p-Tau181) to Aβ40 strengthened their association with longitudinal cognitive decline

and brain atrophy, whereas the correspondent plasma biomarkers showed no such improvement. These results indicate that normalizing CSF biomarkers to Aβ40 helps mitigate not only inter-individual (as shown in previous studies) but also intra-individual variability (which arises due to changes in CSF production and clearance). In contrast, plasma Aβ40 levels are influenced by peripheral production, making it a less robust reference protein. Further plasma proteomics research is needed to identify an effective reference protein for plasma biomarkers. Once the optimal reference proteins and ratios are established for both plasma and CSF biomarkers, their performance for monitoring disease progression should be further assessed.

Among all tested biomarkers, longitudinal plasma p-Tau217 and CSF p-Tau205/Aβ40 in combination with NfL showed the strongest association with our outcome measures of disease progression. Interestingly, the associations with longitudinal changes in cognition and brain atrophy were comparable for plasma p-Tau217, CSF Tau205/Aβ40 and CSF p-Tau217/Aβ40 ratio suggesting that plasma p-Tau217 is logistically a more suitable marker to track disease progression. However, future longitudinal studies should be conducted to evaluate the potential of more specific markers of tau pathology, e.g. MTBR-tau243, as well as FDA cleared Lumipulse plasma p-Tau217.

Concluding remarks

The main findings of this thesis are:

- The optimal pre-analytical sample handling conditions for plasma p-Tau217 quantified on an MSD platform involve thawing plasma samples at RT followed by centrifugation, prior to analytical assessment
- MS-based methods performed exceptionally well and better than immunoassays when identifying individuals with abnormal Aβ status as well as those who progressed to ADD. Some immunoassays exhibited high and consistent accuracy across both outcomes.
- The performance of a commercially available immunoassay ALZpath p-Tau217 against the gold standard neuropathological assessments is similar to Lilly p-Tau181. However, the associations between core measures of AD pathology and ALZpath p-Tau217 were significantly lower compared to Lilly p-Tau217.
- Normalization of longitudinal CSF biomarkers to Aβ40 strengthened their associations with longitudinal clinical outcomes (cognitive decline and brain atrophy). However, no effect on normalization was seen for longitudinal plasma biomarkers. Changes in CSF p-Tau205/Aβ40 and plasma p-Tau217 potentially in combination with NfL, may serve as a surrogate marker for monitoring some elements of disease progression during clinical practice and treatment trials.

Declaration of use of Artificial Intelligence (AI)

While writing this thesis, AI language model such as ChatGPT and Gemini were used strictly for checking grammar and improving writing style. No new text was generated using these tools. The author takes full responsibility for the content of the “kappa”/comprehensive summary of this thesis.

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