Investigating amyloid precursor protein and tau in human platelets as potential blood biomarkers in individuals at risk of Alzheimer's disease

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Abstract

Background: While detecting neocortical amyloid-beta (NAL) by positron emission tomography (PET) offers a unique opportunity to recognise individuals at potential risk of Alzheimer's disease (AD), its uneconomical and invasive nature calls for a more accessible and cost-effective biomarker for greater clinical utility. Platelets are rich with Amyloid Precursor Protein (APP), the parent protein for amyloid-beta, with literature reporting significant alterations in platelet APP isoform ratio (APPr), APP processing secretases ADAM10 (a disintegrin and metalloproteinase) and BACE1 (beta-secretase 1), and tau in AD compared to normal individuals. Therefore, this study aims to explore whether these platelet proteins of interest are associated with NAL prior to cognitive impairment and, assess its diagnostic accuracy for individuals at risk of AD.

Methods: 30 cognitively normal individuals thought to be at risk of AD and 30 cognitively normal individuals at no apparent risk to AD, were selected for the study. Platelets collected from peripheral blood were evaluated with western blot for the interested proteins, and statistical associations were assessed between NAL participants and, with cognitive performance.

Results: The APP mature isoform and the ratio of high molecular weight/low molecular weight (HMW/LMW) tau were significantly associated with NAL, while the HMW/LMW tau ratio was significantly inversely correlated with cognitive performance. The accuracy of prediction for distinguishing NAL participants was increased (accuracy = 80%) when the APP mature isoform and HMW/LMW tau ratio were added to the base model of age, gender and *APOE* e4 allele status.

Conclusion: The nature of these associations will expand the current knowledge on platelet-related alterations in individuals at risk of AD. Furthermore, future investigations may enhance the understanding of platelet proteins as potential diagnostic and prognostic biomarkers for early AD prior to cognitive decline.

Declaration

I declare that this thesis, entitled 'Investigating amyloid precursor protein and tau in human platelets as potential blood biomarkers in individuals at risk of Alzheimer's disease' is original research and was written by myself. Any assistance and inputs I received for this thesis have been properly acknowledged for. All sources of information for the formation of this thesis, have been cited for appropriately.

This thesis, created for the 10-month Master of Research degree, has not been previously submitted for a degree or to any other university or research organisation.

The research presented in this thesis has received ethics approval from the Macquarie University Human Research Ethics Committee, reference number 5201701078, amendment number 5201826144153.

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Student Number:

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Abbreviations

Abbreviation	Full Term
α-syn	Alpha-synuclein
AChEI	Acetylcholinesterase inhibitors
AD	Alzheimer's disease
ADAD	Autosomal dominant Alzheimer's disease
ADAM10	A Disintegrin and metalloproteinase
AICD	Amyloid precursor protein intracellular domain
APLP	Amyloid precursor-like proteins
APOE	Apolipoprotein E
APP23	Amyloid precursor protein 23 transgenic mice
APP	Amyloid precursor protein
APPr	Amyloid precursor protein isoform ratio
Aβ	Amyloid-beta
BACE1	Beta-secretase
BCA	Bicinchoninic acid
BNT	Boston Naming Test
BSA	Bovine serum albumin
^{11}C-PiB	Pittsburgh compound B
CAA	Cerebral amyloid angiopathy
CDR	Clinical Dementia Rating
CGS	Citrate glucose saline
CMRglc	Cerebral metabolic rates of glucose
CNS	Central nervous system
COWAT	Controlled Oral Word Association Test
CSF	Cerebrospinal fluid
CTF83	C-terminal fragment 83
CTF99	C-terminal fragment 99
DASS	Depression, Anxiety, Stress Scales
D-KEFS	Delis-Kaplan Executive Function System
DSST	Digit Symbol Substitution Test
ECL	Enhanced chemiluminescence
ELISA	Enzyme-linked immunosorbent assay
EOAD	Early onset Alzheimer's disease
^{18}F-FBB	^{18}F-Florbetaben
FDA	Food and Drug Administration
FDG	2-[^{18}F]fluoro-2-deoxy-D-glucose
GDS	Geriatric depression scale
GSK3β	Glycogen synthase kinase 3β
HMW/LMW	High molecular weight/low molecular weight
HRP	Horseradish peroxidase
HV	Hippocampal volume
IC-MS	Immunoprecipitation coupled mass spectrometry
KARVIAH	Kerr Anglican Retirement Village Initiative in Ageing Health
KPI	Kunitz serine protease inhibitor
LOAD	Late onset Alzheimer's disease
LM	Logical Memory
MAP	Microtubule associated protein

MCI	Mild cognitive impairment
MMSE	Mini Mental State Examination
MoCA	Montreal Cognitive Assessment
MAC-Q	Memory Assessment Clinic-Q
MRI	Magnetic resonance imaging
NMDA	N-Methyl-D-aspartate
NAL	Neocortical amyloid-beta load
NFL	Neurofilament light chain
NFT	Neurofibrillary tangles
P-tau ser202/thr205	Phosphorylated tau at epitope serine 202/threonine 205
P-tau/t-tau	Ratio of the sum of phosphorylated tau at serine202/threonine 205 to total tau
PET	Positron emission tomography
PGE1	Prostaglandin E1
PHF	Paired helical filament
PRMQ	Prospective and Retrospective Memory Questionnaire
PSEN	*Presenilin*
RAVLT	Rey Auditory Verbal Learning Test
RCFT	Rey Complex Figure Test
ROC	Receiver operating characteristic
RT-PCR	Reverse transcriptase-polymerase chain reaction
sAPPα	Soluble α-amyloid precursor protein
SCIQ	Subjective Cognitive Impairment Questionnaire
SDS-PAGE	Sodium dodecyl sulphate- polyacrylamide gel electrophoresis
SIMOA	Single-molecule array
SNP	Single nucleotide genotyping
SPSS	Statistical Package for Social Sciences
SUVR	Standard uptake value ratio
WAIS III	Digit Symbol
WT	Wild type
WTAR	Wechsler Test of Adult Reading

Chapter 1: Introduction

The most common form of dementia is Alzheimer's disease (AD). Currently, an estimated 447,000 Australians are living with dementia, with the number of cases in Australia predicted to rise to over a million by 2058 (1). On a global scale, approximately 50 million people are living with AD, and by 2050, this number is set to escalate to approximately 152 million (2). With the substantial growth of new AD cases every year, it is no surprise that the disease will continue to impart a large socioeconomic burden on the healthcare system. AD, in summary, is a progressive, neurodegenerative disorder with no effective treatment. It is clinically characterised by progressive decline in several cognitive domains, including memory, executive function and behaviour (3). AD-related neuropathology can begin to manifest up to 20 years prior to the onset of clinical symptoms, and once apparent, can progressively decline cognitively, to require complete care within a period usually ranging from 6 to 12 years (4). The current therapeutics for AD are largely ineffective, most likely because the time of treatment administration occurs at a stage where excessive and irreversible neuronal death has already occurred. Therefore, AD treatment investigations are now concentrating on addressing the condition in the preclinical phase, before the evidence of cognitive impairment. To identify individuals for treatment at this early stage, it is crucial that biomarkers which appropriately reflect preclinical AD, are established.

Blood plasma is gaining increasing attention and support for investigation of AD biomarkers. Being easily accessible, multiple proteins in the plasma, such as cytokines and growth factors, have been identified as potential biomarker candidates (5). A particular blood component, platelets, are gaining increasing appeal, as they are known to possess proteins that contribute to the development of the neuropathological hallmarks of AD. Numerous studies have investigated the alterations of these AD platelet proteins in later stages of the disease (6–13); however, none have studied this in platelets of individuals at high risk of AD (preclinical AD), which are characterised by high neocortical amyloid-beta (Aβ) load (NAL+) prior to cognitive decline

Therefore, this literature review seeks to explore the current understandings of AD, its pathological features, current diagnosis and treatments, and existing evidence regarding the use of platelets and its association with AD-related proteins as blood biomarkers for early AD diagnosis. This thesis explores the potential of these proteins as a panel of peripheral blood biomarkers for diagnosing individuals of preclinical AD prior to cognitive decline.

1.1. AD pathology

The pathological hallmarks of AD are only definitively determined upon *post-mortem* brain autopsy by the presence of extracellular Aβ plaques and intracellular tau-comprised neurofibrillary tangles (NFT) within the cortex and hippocampus (14). The pathology of the disease was first recorded in 1906 by a German neuropathologist, Dr Alois Alzheimer, who observed distinct plaques and NFT in the brain of a deceased 50-year-old woman who had experienced memory disturbance, aggression and confusion several years before her death (14).

1.1.1. Senile plaques

Senile plaques within the brain are one of the major hallmarks of AD, and are primarily comprised of extracellular Aβ peptides aggregates (15), as well as other compounds such as inflammatory molecules (16), proteoglycans (17,18) and, metal ions (19,20). Aβ is a 4kDa peptide produced from the enzymatic cleavage of the amyloid precursor protein (APP). There are two forms of Aβ that are observed in senile plaques; $A\beta_{40}$ and $A\beta_{42}$ for which $A\beta_{42}$ is more predominately expressed, demonstrates more neurotoxic effects, and has a higher tendency to aggregate compared to Aβ40 (21). Although Aβ is recognised as a key hallmark in the pathology of AD, the molecular mechanisms leading to the deposition of Aβ, and the manifestation of other pathological and neurophysiological hallmarks, remains controversial. One theory that has gained much attention, and has been extensively supported by research, is the amyloid cascade hypothesis (22), which suggests that Aβ deposition triggers the onset of disease pathogenesis, and is the causative agent for the formation of NFTs, and cognitive decline (Figure 1). This hypothesis also points to the important role of APP processing, which will be discussed further in greater detail.

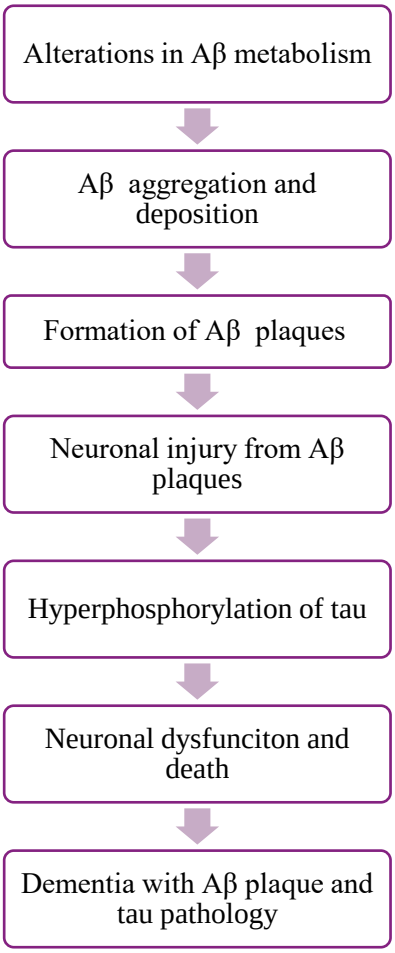


Figure 1.Overview of the amyloid cascade hypothesis. The hypothesis proposes that alterations in Aβ metabolism (as a result from familial and/or sporadic factors) leads to the abnormal deposition of Aβ, and its aggregation into plaques. The presence of Aβ plaques disrupts normal neuronal cell functioning, which in turn results in tau hyperphosphorylation. Tau hyperphosphorylation contributes to further neuronal dysfunction, and widespread cell death through the formation of neurofibrillary tau tangles, eventually resulting in dementia with the Aβ and tau pathological hallmarks. Abbreviations: Aβ, beta amyloid.

1.1.2. Neurofibrillary tangles

Neurofibrillary tangles comprise self-entangling hyperphosphorylated tau and are another major hallmark of AD. Tau is a key microtubule associated protein (MAP) that assists in the assembly and stabilisation of tubulins into microtubules for the development of the cell's cytoskeleton. Tau's microtubule assembling activity is regulated by a normal degree of phosphorylation, however, in AD, tau is excessively phosphorylated, leading to a hyperphosphorylated state (23). Abnormally hyperphosphorylated tau is polymerised into paired helical filaments (PHF) which then aggregates to create the NFT seen in the brain (Figure 2).

The involvement of tau in AD has led to the development of an alternate hypothesis, despite its inclusion in the amyloid cascade hypothesis. The hypothesis is based on the concept that tau hyperphosphorylation

contributes to the final pathway of widespread AD neurodegeneration, with increasing evidence suggesting that this hypothesis provides a closer approximation of explaining the presence of clinical symptoms observed in patients compared to the amyloid cascade hypothesis (24). In AD, hyperphosphorylated tau inhibits normal assembly and stabilisation of microtubules, which induces tau aggregation into paired helical filaments (PHF), and finally, to NFT (25).

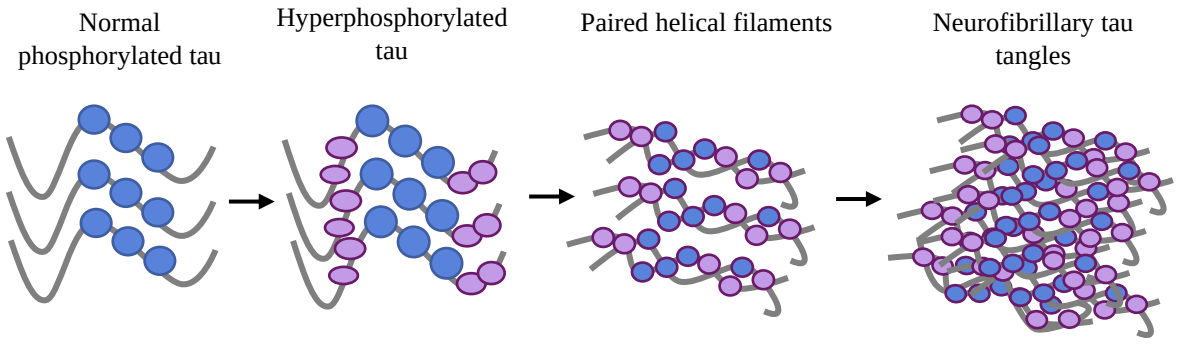


Figure 2. Formation of neurofibrillary tau tangles. In Alzheimer's disease, normal phosphorylated tau becomes hyperphosphorylated, leading to the aggregation of tau into paired helical filaments and eventually creating neurofibrillary tau tangles.

1.1.3. Cerebral amyloid angiopathy

Cerebral amyloid angiopathy (CAA) is another recognised hallmark of AD, and is characterised by the presence of cerebrovascular Aβ deposition within and around cortical and leptomeningeal blood vessel walls (26). The occurrence of CAA is commonly associated with mutations within the Aβ region of the *APP* gene (27). It is also recognised that other proteins are associated with CAA incidence, such as cystatin and the prion protein, and furthermore, in the context of non-related CAA dementia, symptoms of CAA include brain haemorrhage and ischemic brain lesions (28). There are several classifications of CAA depending on the type of deposited amyloid protein, with the sporadic CAA of Aβ protein being the most common in the elderly as well as AD patients (29). The mechanisms by which CAA contributes to neurodegeneration in AD remains unknown, however, one proposed theory suggests that the amyloid that deposits around blood vessels limit the blood supply and leads to oxygen deprivation, which in turn generates oxygen free radicals that demonstrate oxidative stress and cell toxicity. Additionally, the amyloid found in CAA consists mainly of the less toxic $A\beta_{40}$ protein. Furthermore, individuals with CAA pathology have been associated with cognitive impairment, increasing the likelihood of neurodegeneration (30–33).

1.1.4. Alternate hypotheses of AD pathogenesis

Other, less well-known hypotheses of AD pathogenesis have also been postulated, such as the inflammation, cholinergic and oxidative stress hypotheses. The inflammation hypothesis suggests that inflammatory stimuli such as Aβ and NFT, activates glial cells, which produces inflammatory reaction proteins (34). Additionally, activation of glial cells is believed to be capable of producing more of the pathological products of AD, including phosphorylated tau and Aβ (34). The cholinergic hypothesis suggests that the degeneration of cholinergic neurons of the brain contributes significantly to the cognitive dysfunction observed in AD patients (35). The oxidative stress hypothesis implies that cumulative oxidative damage from elements, particularly free radicals, accounts for the slow progression and occurrence of late-onset AD (36).

1.2. Types of AD

AD can be classified into early onset AD (EOAD) and late onset AD (LOAD). Early onset AD typically occurs in individuals aged younger than 65 years and accounts for as much as 10% of all AD cases (37). Autosomal dominant AD (ADAD) mutations in the genes *APP*, *Presenilin 1* (*PSEN1)* and *Presenilin 2* (*PSEN2)* result in familial EOAD, which is relatively rare and accounts for 1-2% of all AD cases (37). Mutations related to the *APP* gene are present near the areas of proteolytic cleavage that leads to the production of Aβ while mutations in the *PSEN1* and *PSEN2* genes (which encode for presenilins 1 and 2 respectively that each make up one of the two secretases involved in APP processing) result in the increased production of the longer $A\beta_{1\text{-}42}$ (38). The age of onset in familial EOAD is often predictable by analysing the age of symptom onset experienced by the parent. However, individuals with *PSEN2* mutations have a greater, and more variable range of age of onset, and have a lower prevalence compared to the *PSEN1* and *APP* mutations (39).

Sporadic AD accounts for the majority of AD cases and is more prevalent in late onset AD (LOAD), with age of onset usually occurring from 65 years and up (40). There is no single deterministic factor for sporadic AD; rather it is believed that most sporadic AD cases arise from a complex interaction of multiple environmental, lifestyle and genetic risk factors, for which are described below.

1.3. Risk factors

The incidence of AD increases with age and is considered the greatest risk factor for sporadic LOAD onset (41). However, age is not the only deterministic factor. Other influences including gender, genetics and a host of modifiable lifestyle factors are thought to be associated.

1.3.1. Genetic and gender factors

As previously stated, the *APP, PSEN1 and PSEN2* genes are major players in the occurrence of autosomal dominant EOAD. In sporadic LOAD, the ε4 allele of the apolipoprotein E (*APOE)* gene is recognised as the major genetic risk factor. In addition to the ε4 allele, there are two other main alleles, namely, ε2 and ε3 (42). Each allele exist at different frequencies, with ε2 being the least common and ε3 being the most common (41). The ε4 allele is more prevalent in AD patients, implying that carriers of this allele are at a greater risk of developing AD. The presence of this allele, however, is not deterministic, and only increases susceptibility of an individual developing the disease.

Numerous epidemiological studies have stated that cognitive decline, neurodegeneration and increased risk of developing dementia occurs more rapidly in females than males once a diagnosis is made (43–45). One proposition for this statement involves the increased longevity of women compared to males, however, this alone does not completely explain the higher frequency and risk of AD observed in women (46). The effects of oestrogen in postmenopausal women is thought to be involved in the gender difference of AD development (47). Oestrogen is believed to confer protective effects on the brain, by slowing neuronal loss, improving blood flow, and modulating *APOE* expression (48). These effects of estrogen are thought to be mediated by its action to reduce the build-up of Aβ in the brain. A deficiency in this hormone is hence speculated as a risk to developing dementia. However, mixed results have been observed, with some studies demonstrating no observable benefits of increasing oestrogen levels on cognitive performance in women (49,50). Hence, the influence of gender on AD risk remains to be elucidated.

1.3.2. Modifiable lifestyle factors

Regular exercise improves the body's susceptibility to oxidative stress and increases energy metabolism, which are crucial towards muscle development and improving cognition (as reviewed in (51)). Such benefits are vital for the prevention or delayed onset of AD. Longitudinal studies have shown an inverse association between physical activity and the risk of cognitive decline in older adults (52,53). Regular walks have been reported to improve cognition, while intense strength training improved overall motor functions, and reduced the risk of AD (54,55). Moreover, lower plasma $A\beta_{40/42}$ were reported in individuals performing higher levels of physical activity (56), while transgenic animal model studies have suggested that physical exercise is implicated in reducing brain Aβ and tau (57). Additionally, frequent engagement in exercise was seen to correlate with low concentrations of phosphorylated tau in the cerebrospinal fluid (CSF) in cognitively normal subjects (58). Evidently, infrequent practice of physical activity suggests an increased risk to the development of AD-related pathological and clinical symptoms.

Diet is another important factor that modulates risk of AD. Consumption of fish and foods rich in antioxidants and vitamins (which play an important role in the reducing oxidative stress) have been reported to lower the risk of AD, while a more western-based diet (comprised of saturated fats and

simple carbohydrates) is thought to contribute to the onset of AD (59). Vitamin C for example, was observed to have lowered the formation of Aβ oligomers and reduce the amounts of oxidative stress in both cell and animal studies (60,61). However, observations from human cohort studies remains controversial, with some investigations showing no difference in the incidence of AD when supplemented with vitamin C, while others claiming that combining vitamin C intake with other vitamins reduces the prevalence and incidence of AD (62,63). The Mediterranean diet has gained much popularity in its association with AD, with one large-scale study demonstrating that higher adherence to the diet was associated with reduced cognitive decline (64). Pathologically, individuals who adhered less to the diet displayed cortical thinning in key AD-related brain regions of the orbitofrontal cortex, entorhinal cortex and posterior cingulate cortex of the left hemisphere compared to participants who adhered more to the regime (65).

The involvement of sleep and AD is not entirely elucidated, however, some evidence from both human and mouse studies have shown that the pathophysiological symptoms that develop in the years prior to cognitive decline, such as brain Aβ, is thought to disrupt sleep (66,67). The findings from these studies imply that sleep disruption is a consequence of developing AD pathology, however, other investigations have proposed that changes in sleep may lead to the elevation of hyperphosphorylated tau (68,69), but whether the latter is mediated by the initial build-up of brain Aβ remains to be determined.

From the evidence gathered on the effects of diet, exercise, and other modifiable risk factors on AD, a 2-year multidomain randomised intervention study known as the Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER), observed the effects of a combined regime of diet, exercise, cognitive training and vascular risk monitoring on individuals at risk of AD (70). It was concluded that a combined regime of said factors could improve or maintain cognition in elderly individuals at risk of the disease, as well as suggesting that intervention at an early stage of AD with non-pharmaceuticals could delay or prevent the onset of AD. It is therefore evident, that the impact of these risk factors should not be ignored.

1.4. Treatments

Treatments currently available for AD have limited efficacy, most likely due to the extensive neurological damage already present at the time of intervention. Although the drugs available provide some relief in the symptoms experienced by patients, and to some extent improve their quality of life, the clinical benefits are not permanent, and are incapable of slowing disease progression. There are two classes of drugs available for treating the symptoms of AD. For mild to moderate AD, a group of medications known as acetylcholinesterase inhibitors (AChEIs) are prescribed. The Food and Drug Administration (FDA) approved four AChEIs; donepezil, galantamine, tacrine and rivastigmine (71), though tacrine has since been discontinued due to major side effects (72). AChEIs work by inhibiting acetylcholinesterase, therefore inhibiting the breakdown of acetylcholine and enhancing neuronal

activity (73). Side effects of AChEI treatment includes gastrointestinal problems, anorexia, diarrhoea and, dizziness (74). For the treatment of moderate to severe AD, N-Methyl-D-aspartate (NMDA) receptor antagonists are recommended. Memantine is a type of NMDA receptor antagonist approved by the FDA, and works by binding to the NMDA receptor, thereby normalising the excessive levels of glutamate that is thought to be associated with cognitive and memory deficits (75). Memantine has similarly displayed mild side effects, mainly dizziness, headaches, hypertension and constipation (76). Therapeutics for targeting the earlier stages of the disease before the onset of clinical and pathological symptoms is of great interest. In order to develop such treatments, it is necessary to accurately and confidently identify individuals who are entering the preclinical stages of AD, so intervention can be achieved as soon as possible to minimise the risk of disease onset or progression.

1.5. Methods of AD diagnosis

As previously stated, definitive AD diagnosis is achieved by *post-mortem* examination of the brain for the presence of Aβ plaques and NFT. However, it is possible to diagnose an individual with probable AD, by administering a brief cognitive and behavioural assessment. The best-known and most routinely used questionnaire for providing an overall brief estimation of one's cognitive state, is the Mini-Mental State Examination (MMSE) (77). However, using an MMSE score alone to provide a diagnosis is not sensitive enough, and hence is often used in conjunction with other, more sophisticated screening tools that have become the current gold standards for diagnosing AD. Additionally, although still in development, measurements of inflammation, lipid metabolism, Aβ, tau and other AD-related markers in the blood, may evolve to become a sensitive panel of peripheral biomarkers for AD diagnosis in the future.

1.5.1. Positron emission tomography

Positron emission tomography (PET) is a type of nuclear imaging technology and enables the visualisation of metabolic processes within the body (78). PET imaging utilises radioligands to bind and detect the proteins/receptor of interest, and in AD, several radiotracers have been used to image brain Aβ plaques (79,80). Ideal tracers for Aβ should be capable of effectively imaging brain Aβ, should be reproducible and, widely accessible. To date, only a handful of Aβ PET tracers have been approved. 2-(1-{6-[(2-[fluorine-18]fluoroethyl)(methyl)amino]-2-naphthyl}-ethylidene)malononitrile ([^{18}F]FDDNP) was the first PET tracer to be reported capable of imaging brain Aβ *in vivo* (81). The tracer, however, has high binding non-specificity, as it may also bind to tau protein (82,83). Another [^{18}F] tracer that has demonstrated highly selective Aβ binding is the [^{18}F] AV-45 tracer compound. In human subjects, the tracer is well-tolerated, and can accurately discriminate AD subjects and controls (84). Yet the most extensively utilised PET tracer for examining Aβ plaques is the Pittsburgh compound B (^{11}C-PiB). Recognised as one of the gold standard methods for diagnosing AD, ^{11}C-PiB is the first tracer to demonstrate clear retention to Aβ deposits and is highly specific in that it does not bind to NFT

or aggregates of alpha-synuclein (α-syn), otherwise termed as Lewy Body complexes. It has been shown previously that ^{11}C-PiB retention is elevated in AD subjects, as well as in cognitively normal individuals, suggesting that individuals of normal cognition with elevated ^{11}C-PiB binding were more likely to develop AD (79,85).

2-[^{18}F]fluoro-2-deoxy-D-glucose (FDG) is another type of PET tracer that is used to measure cerebral metabolic rates of glucose (CMRglc), which represents glucose utilisation activity within the brain. Previous reports have observed reductions in CMRglc in AD patients compared to age-matched controls (86–89). AD-related pathophysiological changes in CMRglc have also been found to be highly associated with familial risk of AD, as well as mild cognitive impairment (MCI) individuals (37,90). These observations have strongly supported the application of FDG-PET tracers as a prognostic marker for early AD diagnosis. However, FDG-PET is not AD-specific, as alterations in CMRglc have also been seen in Parkinson's and Huntington's disease (91,92).

Collectively, PET tracers are of considerable value in providing a definite clinical diagnosis of AD. However, several limitations are evident with PET tracers. These include the expensive nature of the technique, making it not widely available for large-scale clinical utility, as well as the utilisation of radioisotopes, which can negatively impact on one's health and safety if used regularly. Therefore, current methods of diagnosis need to be developed that are more accessible to the wider community.

1.5.2. Magnetic resonance imaging

Magnetic resonance imaging (MRI) in AD is used to assess atrophy and alterations in brain tissue by exploiting an atom's nuclei magnetic properties. The energy released from the strong magnetic field creates a field of signals which allows an image of the brain's density to be created. Because atrophy is regarded as a marker for AD, atrophy-related measures such as grey and white matter, density and, cortical thickness are often assessed when making an AD diagnosis (93). Accelerated rates of atrophy is seen as a predictive factor of AD, as well as in other forms of dementia (94). However, MRI cannot provide an absolute diagnosis of AD.

1.5.3. Cerebrospinal fluid

$A\beta_{42}$, tau and phosphorylated tau (p-tau) are considered gold standard biomarkers for AD (95). The levels of these proteins in the CSF are associated with AD, with studies showing that decreased CSF $A\beta_{42}$ and elevated phosphorylated tau differentiates AD individuals from controls (96). Low concentration of CSF $A\beta_{42}$ was also seen to inversely correlate with brain Aβ build-up (97), while the CSF ratio of tau/$A\beta_{42}$ was shown to be a predictor of cognitive decline in non-demented adults (98). CSF $A\beta_{42}$, CSF tau, along with amyloid detected through imaging technology, are the most clinically acceptable biomarkers for probable AD diagnosis. However, CSF collection is relatively expensive compared to blood collection, requires expert skill, and involves a highly invasive lumbar puncture procedure which is not readily accepted by many patients.

1.6. Blood biomarkers

Over recent times, there are increasing efforts into identifying inexpensive, less invasive and more widely available biomarkers to detect AD, as well as to monitor the long-term effects of therapeutics. Identifying AD biomarkers in the blood can be non-invasive, cheap and easily transferred into a clinical setting. Sources for biomarkers in the blood can be divided into two sections, 1) liquid blood matrix and 2) cell-based blood. Table 1 summarises the advantages and limitations of current biomarkers of AD.

Table 1. Summary of the advantages and limitations of different biomarker sources.

Biomarker source/assay type	Advantages	Disadvantages
Blood-based	Easily available Minimally invasive procedure Cost-effective Multiple analyses Diagnostic and prognostic potential Some components (e.g. platelets) are biochemically similar to neurons	Potential analytical errors
CSF-based	Reflects pathological changes in the brain	Invasive extraction Expensive Potential analytical errors
Aβ PET imaging tracers	Capacity to diagnose early Direct visualisation of brain pathological changes	Expensive Requires sophisticated technology and expertise Not widely available Exposure to radiotracers
MRI	Not invasive No exposure to radioactive elements Compared to PET, is more widely available and less expensive	Not specific to AD Requires sophisticated technology and expertise Not widely available compared to blood tests More expensive compared to blood tests

Table 1 summaries the current and emerging biomarkers for AD diagnosis. Abbreviations: CSF, cerebral spinal fluid; Aβ, amyloid-beta; PET, positron emission tomography; ^{11}C-PiB, Pittsburgh compound B; FDG, 2-[18F] fluoro-2-deoxy-D-glucose; MRI, magnetic resonance imaging.

1.6.1. Liquid blood matrix

Liquid blood matrix consists of plasma or serum. To date, measuring the levels of Aβ and tau in plasma or serum have been inconsistent across studies (99). This is mainly due to the difficulty of measuring these proteins, as they are both present in much lower quantities than in the CSF and in the brain, which has been problematic for reliable measurements when using enzyme-linked immunosorbent assays (100). In 2018, a large-scale collaborative study reported the use of a highly sensitive immunoprecipitation coupled mass spectrometry (IP-MS) assay to measure the levels of plasma Aβ. The study reported that plasma Aβ levels measured by this method predicted brain Aβ levels at an accuracy of 90% (101). However, those results are yet to be replicated in large-scale, independent studies before commercial plasma Aβ measuring kit based on this method is available. Plasma Aβ has also been quantified using single molecule array (SIMOA) technology, wherein a lower ratio of plasma $A\beta_{42}/A\beta_{40}$ was seen in cognitively normal individuals of high brain Aβ compared to those with low Aβ (102). However, while the results appear consistent with other studies (103,104), the accuracy of the association was moderate (76%). More recently though, a new potential biomarker for AD has arisen; namely, neurofilament light chain (NFL). NFL, one of three major subunits in a neurofilament, is a crucial component for axonal cytoskeletal formation (105). It has been shown that NFL concentration in the CSF is substantially higher in patients with AD (106) and other neurological disorders (107,108). Additionally, plasma NFL was found to be inversely correlated with cognitive performance (109). However, despite the promising use of NFL as a biomarker, the protein is not specific to AD, and therefore, cannot be used solely as a means for diagnosing this neurodegenerative disease.

1.6.2. Cell-based sources

The inconsistencies experienced with plasma/serum biomarker profiling has driven attention towards investigating cell-based blood biomarkers. Sources of this type have included lymphocytes and erythrocytes (110–112). AD patients are observed to have impaired endoplasmic reticulum stress and calcium homeostasis in lymphocytes while plasma Aβ binds to erythrocytes over the course of aging, suggesting a pathogenic role of the Aβ/erythrocyte complex (110). There is growing attention however, with another cell-based blood source, namely, platelets, which is discussed below.

1.7. Platelets for AD-related biomarker studies

Structurally, platelets lack a nucleus, however, contain several organelles such as alpha granules which store and release secretory proteins (113). The primary activity of platelets is coagulation; however, it is observed that platelets have a pivotal role in disease pathogenesis (114). Platelets are recognised as a useful *ex vivo* model for studying AD aetiology, as they can be obtained with minimal invasiveness, are inexpensive to process, share similar biochemical properties to neurons and are known to mirror abnormalities witnessed in several neurological disorders (115–117). There are many AD-specific

platelet alterations that hold potential as peripheral biomarkers for AD, including changes in platelet activation, coated platelets, Monoamine oxidase B oxidative activity and, glycogen synthase kinase 3β upregulation (as reviewed in (117)) (Figure 3). APP and tau collectively, represent a large area of research in platelet derived AD biomarkers and will be the subject of discussion for the remaining sections of this review.

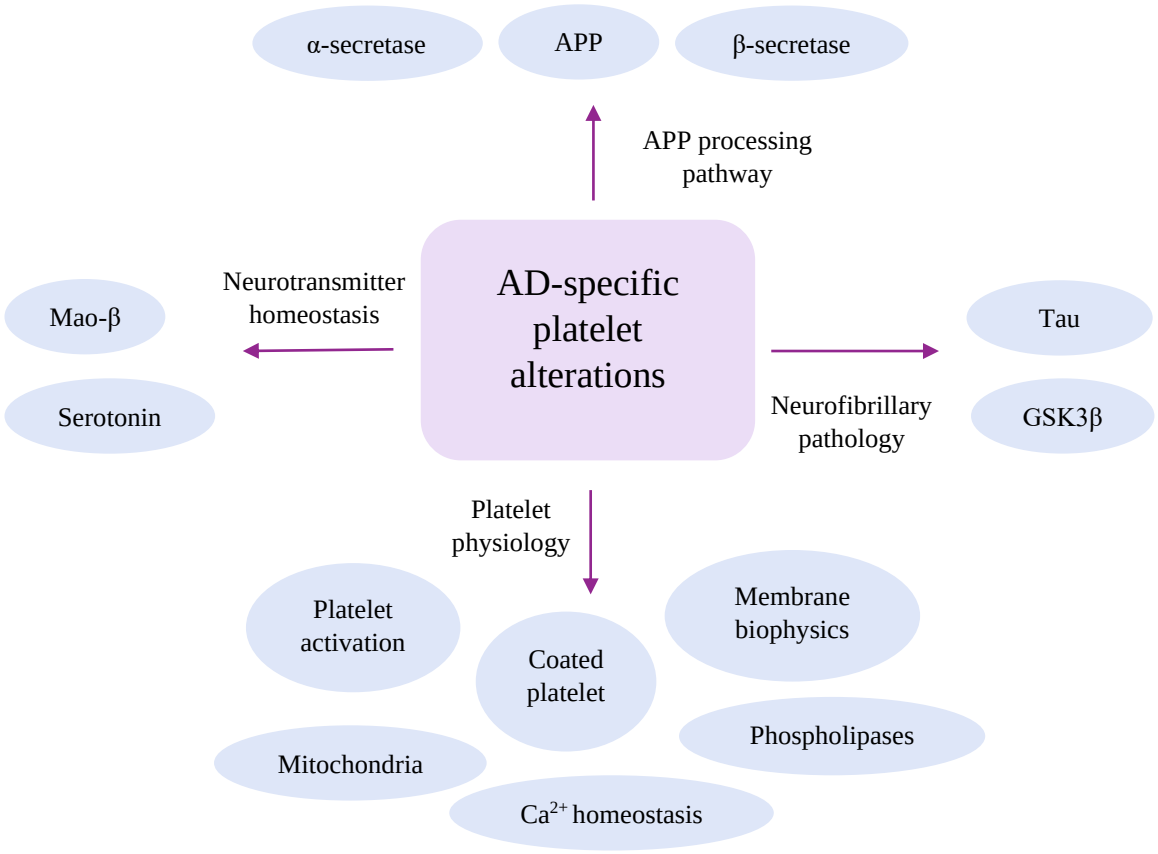


Figure 3. Overview of potential platelet biomarkers for Alzheimer's disease diagnosis. Platelet-specific alterations provide a promising avenue for investigating potential biomarkers of AD. Such avenues include the APP processing pathway, neurofibrillary pathology, platelet physiology and neurotransmitter homeostasis. Abbreviations: AD, Alzheimer's disease; APP, amyloid precursor protein; α-secretase, alpha-secretase; β-secretase, beta-secretase; Aβ, amyloid-beta; GSK3β, glycogen synthase kinase 3β; $Ca2^{2+}$, calcium ions; Mao-β, monoamine oxidase B.

1.7.1. The amyloid precursor protein

Platelets represent more than 90% of circulating APP and are the second highest source of APP protein following the brain. Bush *et al* (113) first demonstrated that platelets contain APP, and that APP is released from platelets during a process called degranulation. Platelets contain their own APP proteolytic machinery and produce similar APP proteolytic products as seen in neuronal cells of the brain (118).

1.7.1.1. Key structural features of the amyloid precursor protein

In mammals, APP is a single-pass transmembrane protein, and is related to a family of proteins that includes amyloid precursor-like proteins (APLP1 and APLP2). These three proteins are processed in similar ways, however, only the APP protein produces the Aβ peptide. APP, when spliced, can generate 8 different isoforms, of which APP695, APP751 and APP770 are the more commonly expressed forms. APP695 is expressed in high levels in the central nervous system, while the other two isoforms are found in lower levels (119). This ratio of expression is reversed in peripheral sources, such as platelets, where the APP751/770 forms are more predominantly expressed compared to APP695. APP 751/770, but not APP695 encodes for a Kunitz serine protease inhibitor (KPI) domain, which is a site that allows the interaction of other proteins with APP in the regulation of various molecular events (118).

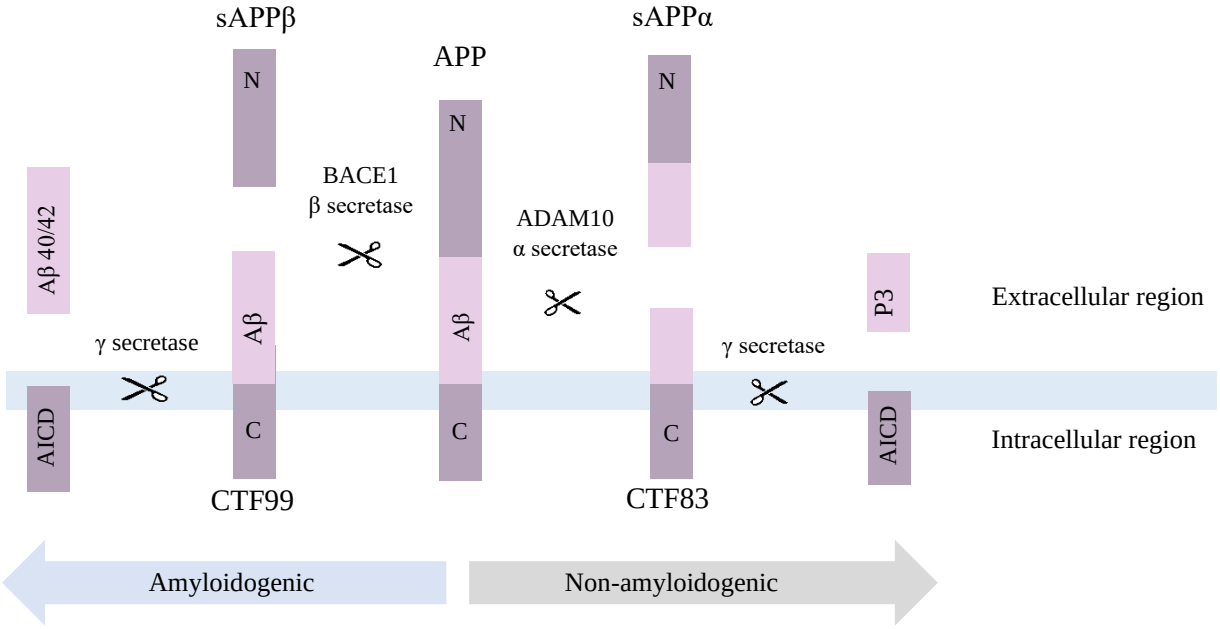


Figure 4. Processing of APP in platelets. Platelet APP can be processed via one of two pathways, wherein the amyloidogenic pathway leads to the formation of the toxic Aβ protein. Abbreviations: APP, amyloid precursor protein; sAPPα, soluble APP-alpha; sAPPβ, soluble APP-beta; CTF99, C-terminal 99 fragment; CTF83, C-terminal 83 fragment; ADAM10 α secretase, a disintegrin and metalloproteinase 10 alpha secretase; BACE1, beta-secretase; γ secretase, gamma secretase; Aβ, amyloid-beta; AICD, APP Intracellular Domain Fragment; N, amino terminus; C, carboxyl-terminus.

1.7.1.2. APP processing

In platelets, the APP protein sits on the cell's surface (120) with the carboxyl (C) – terminal end within the cytosol, and the amino (N) – terminal end lying within the extracellular space. Cleaving APP can be achieved by one of two competing pathways; the non-amyloidogenic or amyloidogenic pathway (Figure 4). In the non-amyloidogenic pathway, APP is cleaved at the C-terminal end by α-secretase that belong to a family of proteinases called "a disintegrin and metalloprotease" (ADAM), wherein the more

common forms involved are ADAM 10 and ADAM 17 (121). This proteolytic cleavage generates a soluble α-APP (sAPPα) fragment that gets released into the extracellular space, as well as a C-terminal fragment tethered to the membrane that is 83 amino-acids in length (CTF83). While the sAPPα fragment retains its structure, the CTF83 fragment is processed further by γ-secretase to produce non-toxic fragments termed p3 and amyloid precursor protein intracellular domain (AICD). AICD has been speculated to have some direct impact on AD pathophysiology, and may hold roles in gene transcription, apoptosis and other cellular mechanisms (122). However, one study reported that AICD hold no apparent significance in the course of AD pathology (123). APP cleavage, via the amyloidogenic pathway results in the generation of extracellular Aβ peptide which is neurotoxic when levels are elevated. Cleavage of APP by the β-APP-cleaving enzyme (BACE1) generates an N-terminus soluble β -APP (sAPPβ) fragment, and a longer C-terminal membrane-bound fragment of 99 amino acids in length (CTF99). Unlike α-secretase, β-secretase does not cleave through the region encoding for Aβ. Therefore, when γ-secretase cleaves the CTF99 fragment, it releases the intact $A\beta_{40/42}$ (118).

1.7.1.3. Platelet APP isoforms and AD

APP plays a key role in the aetiology of AD as being the precursor to Aβ. Therefore, it is necessary to investigate the alterations of APP expression in AD to determine its potential as a peripheral biomarker for AD, before the onset of symptoms. In platelets, there are two main APP isoforms present– a larger, mature APP of 120-130kDa, and a smaller, immature APP of 108-110kDa (124). Patients in the advanced stages of AD have been reported to exhibit decreased levels of APP 120-130kDa in their platelets when compared to controls (125). This finding indicated that the proteolytic processing of APP was defective in AD, leading to increased production of Aβ . Rosenburg and Di Luca both investigated the ratio of APP isoforms 120-130kDa/110-108kDa using western blotting, and in both studies, concluded that the ratios of the APP isoforms was significantly lowered in AD patients compared to controls (126,127). A follow-up study by Rosenburg and colleagues demonstrated that cognitive decline, as measured by MMSE, significantly correlated with reduced the reduced platelet APP isoform ratio. A reduction in platelet APP isoform ratio has also been observed in mild AD, very mild AD, and MCI individuals, with reports of 59%, 47% and 33% ratio decrease respectively compared to controls (100). The results are consistent with the notion that platelet APP isoform ratio alterations may serve as a peripheral biomarker for AD detection prior to development of severe cognitive deficits. Platelet APP isoform ratio have also been considered as a prognostic marker for monitoring the effects of AD therapeutics on AD patients. One study involving mild to moderate AD patients, who were administered donepezil, showed an increase in platelet APP isoform ratio. This increase was more prominent in non-*APOE* ε4 carriers, suggesting that increased platelet APP isoform ratio was influenced by the *APOE* genotype (128). However, since donepezil is not a disease-modifying treatment, the significance of this finding is not clear.

1.7.1.4. Platelet APP processing machinery and AD

APP processing is an important contributor towards to the pathogenesis of AD, therefore understandably, it is necessary to investigate and compare the levels of APP secretases and their products in platelets of both AD and controls to determine any changes between these two groups.

Under normal physiological conditions, APP is preferentially processed through the non-amyloidogenic pathway, with there existing an equal balance between the rate of APP metabolism and Aβ clearance from the brain (129). However, in AD, this equilibrium is disrupted, with APP being processed more favourably by the amyloidogenic pathway (129). Evidence to support this firstly comes from the observations of decreased sAPPα fragments detected in the supernatant of activated platelets within AD platelets compared to controls (7). sAPPα is released from platelet α-granules, and in support of the previous point, activated platelet α-granules were shown to release at least -50% less sAPPα than controls (7). The reduction of sAPPα may be contributing to the negative, downstream effects witnessed in AD aetiology, as sAPPα in the central nervous system (CNS) is thought to possess neuroprotective effects by stabilising the elevated calcium induced by Aβ, which leads to cell injury (109,110). Hence, sAPPα activation appears to be an attractive avenue for therapeutic intervention. Decreased sAPPα levels correlates with lower expression levels of ADAM10 in platelet AD patients compared to controls, as measured longitudinally over the course of the disease (130). This finding has been more recently demonstrated in a 2018 paper, for which the authors similarly demonstrated that ADAM10 was significantly decreased in platelets from AD patients (131). Additionally, studies have also observed a significant correlation of lower platelet ADAM10 with cognitive decline in AD individuals (132).

BACE1 levels have been reported to be elevated in AD brain and CSF (133). In platelets, BACE1 activity, (measured using a fluorogenic substrate assay), revealed a significant increase in AD platelets compared to controls (134). This finding was supported by Johnston *et al*, who similarly reported an increase in BACE1 activity amongst MCI individuals (10). On the contrary, Gorham *et al* did not find any differences in either BACE1 or ADAM 10 activity between MCI patients and controls (9). However, their results may have been influenced by the un-specificity of the commercial protein assay kits used, compared to Johnston's methodology wherein a BACE1-specific inhibitor was used to demonstrate that the protein activity witnessed correlated with BACE1 (10). The presence of sAPPβ fragments in platelets has been previously reported, however, due to its low abundance, comparisons of this fragment between AD and controls have so far remained a challenge (135). The C-terminal fragments CTF83 and CTF99 have been minimally explored in platelets, however in AD transgenic mouse hippocampal regions, CTF99 was accumulated, suggesting its potential implication in neurodegeneration, although further investigations are required (136). There is increasing evidence to indicate that brain AD pathology is reflected in peripheral tissues. For example, Martins et *al* (137) demonstrated increased Aβ production in human skin fibroblast from individuals with mutations in the PS1 gene. Thus, investigating whether CT fragments are present in peripheral tissues particularly APP rich platelets reflecting brain AD pathology, is warranted.

1.7.2. Tau protein

The presence of tau in platelets, and its significance in AD, has received little attention in the field. Nevertheless, key findings in the area have demonstrated significant correlations between platelet tau and cognitive decline, thereby suggesting the potential of platelet tau's utility as a peripheral prognostic biomarker of AD.

1.7.2.1. Tau in AD platelets

As mentioned previously, human tau is involved in the stabilisation and maintenance of microtubule assembly. Normal tau is phosphorylated with 2-3 moles of phosphate, and this phosphorylation level amount allows tau to function optimally (138). However, in AD, tau is hyperphosphorylated, which depresses its normal activity, and leads to PHF polymerisation (138).

Neumann *et al* (139) were the first to demonstrate that tau is present in human platelets. Using immunoblots, they observed several different molecular weight species of tau in AD platelets, ranging from 60kDa to 240kDa. Furthermore, the expression of high molecular weight (HMW, >80kDa) tau species were seen to be more significantly present in the AD individuals than the controls, while the low molecular weight (LMW, <80kDa) tau species were more evident in the controls than AD subjects (139). The authors observed that there was a significant increase in the ratio of the HMW/LMW tau fractions in the AD subjects compared to the age-matched controls. They speculated that the HMW tau fractions were in fact, aggregates of the typical ~60kDa tau species. Additionally, it was observed that as the abundance of HMW tau increased, the LMW tau levels decreased (139). This observation supported the theory of HMW species being formed from LMW tau fractions, as mentioned above. These findings have been corroborated and extended in subsequent studies, as discussed below. One study saw that in addition to a significant difference in platelet HMW/LMW tau ratio between AD and control subjects, there was an association between the tau ratio and brain volume of the medial temporal lobe, cingulate cortex, pulvinar nucleus, frontal cortex and cerebellum (12). Another group demonstrated a strong correlation between platelet tau and the degree of cognitive status (assessed by MMSE) between AD patients and cognitively healthy subjects (8). In addition, they showed that the platelet HMW/LMW tau ratio demonstrated a receiver operating characteristic (ROC) sensitivity and specificity of 76% and 74% respectively, suggesting this ratio as a potential biomarker for detecting and monitoring disease progression. A more recent study similarly saw a significant difference in HMW/LMW tau ratio between AD and control patients, as well as an inverse correlation between MMSE scores and the HMW/LMW tau ratio (140). Conversely, Mukaetova-Ladinska and colleagues did not observe any difference in platelet tau levels between AD and controls. However, they reported an age-dependent correlation of platelet tau protein between younger and older AD participants (11). Additionally, no known studies to date have investigated correlations between platelet tau and cortical tau measured via PET.

Since the discovery of tau in 1975 (141), over 70 different phosphorylated sites spanning across the entire tau protein, are believed to be associated with AD pathology (142). Some of the 70 known sites are found to be hyperphosphorylated in PHFs and NFTs during AD progression, and not in normal, healthy brains (143–145). One such site that has attracted attention is tau phosphorylation at serine 202/threonine 205 (p-tau ser202/thr205). This phosphorylation site has been well characterised as the Braak staging of AD cases based on NFT occurrences (146) using a monoclonal antibody (AT8) that specifically recognises p-tau ser202/thr205 (147). This phosphorylated tau has been studied by many researchers to be an early phosphorylation event in the progression of tau monomers to PHFs, and has therefore been used as a marker for categorising early AD stage prior to tangle formation (146). Based on these observations, p-tau ser202/thr205 is an interesting target to determine its presence and subsequent quantification in platelets.

Although extensive and growing evidence supports the fact that APP, APP isoform ratio, ADAM10, BACE1, tau, HMW/LMW tau and p-tau ser202/thr205, are potential biomarkers for the clinical diagnosis of AD, there are no studies that have explored the presence and levels of these proteins in individuals at high risk of AD prior to cognitive impairment, defined by having high neocortical amyloid-beta. As there is an urgent need for peripheral biomarkers capable of diagnosing AD before the onset of clinical symptoms, the current study primarily investigated whether there was an association between the platelet proteins and neocortical amyloid-beta load (NAL), cognitive performance as assessed by verbal and visual episodic memory, global cognitive performance and subject memory complaints, plasma Aβ and, plasma NFL. Additionally, the study investigated the diagnostic accuracy and predictability of the platelet proteins as potential blood biomarkers for individuals at risk of AD prior to cognitive decline.

1.8. Aims

1. To investigate whether the significant differences in platelet APP, APP isoform ratio 120-130kDa/110-108kDa (APPr), ADAM10, BACE1, tau, HMW/LMW tau ratio and p-tau ser202/thr205, are present between cognitively normal individuals with low neocortical amyloid-beta (NAL-) and individuals with high neocortical amyloid-beta (NAL+).

2. To investigate the association between cognitive performance and platelet APP, APPr, ADAM10 and BACE1 secretases, tau, HMW/LMW tau ratio and, p-tau ser202/thr205.

3. To investigate whether the significant differences in platelet APP, APPr, ADAM10 and BACE1 secretases, tau, HMW/LMW tau ratio and, p-tau ser202/thr205 are present between platelets of subjective memory complainers and non-complainers.

4. To investigate the association between plasma Aβ levels and platelet APP, APPr, ADAM10 and BACE1 secretases, tau, HMW/LMW tau ratio and, p-tau ser202/thr205.

5. To investigate the association between plasma NFL levels and platelet APP, APPr, ADAM10 and BACE1 secretases, tau, HMW/LMW tau ratio and, p-tau ser202/thr205.

6. To determine the diagnostic potential of the platelet proteins as a biomarker for cognitively normal high risk NAL+ individuals.

From these aims, it is hypothesised that the platelet proteins APP, APPr, ADAM10, BACE1, tau, HMW/LMW tau ratio and p-tau ser202/thr205 are:

1. Significantly altered in high-risk, NAL+ individuals compared to low risk, NAL- individuals.
2. Significantly associated with cognitive performance.
3. Significantly altered in subjective memory complainers vs non-complainers.
4. Significantly associated with plasma Aβ levels.
5. Significantly associated with plasma NFL levels.
6. Capable in showing diagnostic potential as a biomarker for identifying cognitively normal high-risk NAL+ individuals.

Chapter 2: Materials and Methods

2.1. Participants

Participants in the current study were from the Kerr Anglican Retirement Village in Ageing Health (KARVIAH) cohort based in Sydney, NSW, wherein participants were recruited in 2015, and blood samples were collected, processed and stored at -80ºC. All volunteers (n=206) were required to meet the inclusion and exclusion criteria.

Volunteers who met the inclusion criteria: were aged between 65-90 years, in good health with no significant cerebral vascular conditions, fluent in English, none or minimal impairments in daily living activities, had adequate hearing and vision, no behavioural and/or functional impairments, no objective memory complaints as determined by the Montreal Cognitive Assessment (MoCA) scores ≥26 and the Mini-Mental State Examination (MMSE) test score ≥26.

Volunteers were excluded from the study if they met at least one of the following exclusion criteria: diagnosed with dementia based on the revised criteria from the National Institute on Aging and Alzheimer's Association (NIA/AA)**,** MoCA score of ≤17 (individuals with a score between 18-25 were discussed for eligibility with relevant practitioners on the basis of their age and educational status), significant functional and/or behavioural problems, presence of any functional psychiatric disorders such as lifelong schizophrenia, medical history of stroke, alcohol or drug abuse during the last two years prior to screening, evidence of depression as classed on the Depression, Anxiety, Stress Scales (DASS), non-fluency in English, cognitive and hearing impairments that influenced cognitive testing, implants that interfered with MRI, e.g. pacemakers, cochlear implants, metallic fragments near spinal cord or eyes, uncontrolled hypertension (systolic blood pressure (BP) > 170 mmHg, or diastolic BP > 100 mmHg).

Of the 206 volunteers, 134 qualified for the study. However, only 105 from the 134 underwent neuroimaging, neuropsychometric testing and blood collection, in which the remaining 29 volunteers either withdrew from the study or did not provide voluntary consent to participate in the full battery of assessments. Of the 105 participants, 100 were considered to be cognitively normal based on their MMSE scores of >26. The 100 qualified volunteers were classified as either NAL+ or NAL-, using a standard uptake value ratio (SUVR) cut-off value of 1.35 (SUVR≥1.35 for NAL+; SUVR≤1.35 for NAL-). From the 100 volunteers, 65 were considered as NAL-, while 35 were classified as NAL+. For the purposes of the current study, the 30 highest NAL+, and 30 lowest NAL- participants were selected by someone other than the investigator. Demographic data was available for all participants and included: date of birth, age, gender, education level, marital status, occupation and retirement history.

Written informed consent was obtained from all KARVIAH participants, and the Bellberry Human Research Ethics Committee, Australia, and the Macquarie University Human Research Ethics Committee, granted approval for the study in 2014 (ethics number – 5201701078). Approval to carry out biomarker studies was obtained in 2016**,** while approval for this current project was obtained late 2018 (amendment number - 5201826144153).

2.2. Neuropsychological testing

A comprehensive battery of well-validated neuropsychological tests were employed by a trained and experienced research assistant. Amongst the collection of tests, the MMSE (77) and MoCA (148) tests were used to define individuals as being cognitively normal, with scores of ≥26 for both tests considered to be normal cognition. Memory complaints and/or concerns were assessed by using the self-reported Memory Assessment Complaint Questionnaire (MAC-Q), wherein participants with a score of ≥26 were classified as memory complainers, and those ≤25 were classified non-complainers (149).

The battery also included the Rey Verbal Learning Test (RAVLT) (150), the Wechsler Logical Memory (LM) I and II (151), Rey Complex Figure Test (RCFT), the Delis-Kaplan Executive Function System (D-KEFS) Category Fluency (Boys Names) Switching (Fruits and Furniture) Tasks (152)**,** the Wechsler Adult Intelligence Scale – Third edition (WAIS-III) Digit Span, the WAIS III Digit Symbol Substitution Test (DSST) (153), the Wechsler Test of Adult Reading (WTAR), the Controlled Oral Word Association (COWAT) (154) and, the Boston Naming Test (BNT) (155). The composite scores for verbal and visual episodic memory were generated from the mean of the z-scores obtained from RAVLT List A, RAVLT short delay, RAVLT long delay, LM I, LMII, RCFT 3min and RCFT 30min, while composite scores for working memory and executive function were created from the mean of the z-scores obtained from the Digit Span Backward, DSST, and D-KEFS category and switching tasks (109). A global composite score was generated from the mean z scores of MMSE, verbal and visual episodic memory and, working memory and executive function (109). Brief descriptions of the neuropsychological tests used to generate the composite scores are as follows: RAVLT evaluates a diverse set of functions, including short-term auditory and verbal memory, rate of learning and, retention of information; the Wechsler Logical Memory provides detailed assessments on verbal memory functioning including immediate memory and general memory; the RCFT provides assessment on recognition memory; the DSST is part of the WAIS-III, and measures cognitive and psychomotor speed, visual scanning, attention and executive functions; the D-KEFS also involves the assessment on executive function.

2.3. Neuroimaging

Neuroimaging was performed at Macquarie Medical Imaging in Sydney, within three months following blood collection, on the 60 participants who were included in this study and were considered cognitively normal based on their MMSE scores. The participants were subjected to PET scans by receiving a slow intravenous injection of the ^{18}F-Florbetaben (FBB) ligand over the course of 30secs, while in a rested position. Following 50min post-injection, images were developed over a 20min scan. Neocortical amyloid-beta load was calculated as a mean SUVR based on the ratio of the frontal, superior parietal, lateral temporal, lateral occipital and anterior and posterior cingulate regions, to the cerebellum (as the ligand uptake reference region), using imaging processing software CapAIBL (156,157).

All 60 participants underwent MRI using a General Electric (GE) 3 Tesla scanner (Model 750W) following a protocol previously described (158). The images acquired were processed using CapAIBL and were used to calculate hippocampal volume. The results were normalised with the total intracranial volume comprising CSF, grey matter and, white matter. Both the PET scans and MRI imaging were performed by other members of the Martins research group in 2015.

2.4. *APOE* genotyping

APOE genotype was determined from purified genomic DNA extracted from 0.5ml of whole blood. Each participant was genotyped for the presence of the three *APOE* variants, ε2, ε3 and ε4. This was achieved using TaqMan single nucleotide genotyping (SNP) assays for rs7412 (C 904973) and rs429358 (C 3084793) according to the manufacturer's instructions (AB Applied Biosystems by Life Technologies, Scoresby, VIC, Australia) (159,160). Briefly, a 384 well plate containing single samples and duplicate control genotypes (ε2/ε2, ε3/ε3 and ε4/ε4) was set up. To each well, 2-20 ng of DNA in 2 µl volume was dispensed, followed by 4.15 µl of a master mix solution, consisting of 2X genotyping master mix, 40X genotyping assay probe and distilled water. The plate was mixed and centrifuged briefly, then run in a qRT-PCR machine as a standard absolute quantification assay. The plate was then analysed using the accompanying the SDS software. *APOE* genotyping was completed in 2015 by another member of the Martins research team.

2.5. Platelet and plasma fractionation

Participants fasted for a minimum of 10hrs prior to blood collection, wherein 80ml of whole blood was drawn using standard serological methods and collected in tubes containing either EDTA or lithium heparin. Immediately following blood collection, platelets and plasma were fractionated. For plasma, both EDTA and lithium heparin tubes were centrifuged at 200g and 20˚C for 10 mins, and the supernatant (platelet rich plasma) was transferred into new tubes. The platelet rich plasma supernatant

and the remaining red blood cell pellets in the original tubes were both centrifuged again at 800g and 20˚C for 15 mins. The supernatant (plasma) from both tubes were collected, combined, aliquoted into Cryovial tubes, and immediately frozen at -80˚C. For platelet fractionation, the pellet of platelets at the bottom of the tube that contained the platelet rich plasma were carefully resuspended in 5ml of solution containing citrate glucose saline (CGS) and Prostaglandin E1 (PGE1). The resuspended pellet was then spun at 1300g for 15 min at 20˚C. The solution was removed and 500µl of phosphate buffered saline (PBS) with PGE1 was added. The platelet resuspension was aliquoted into Cryovials and immediately stored at -80˚C. Blood processing was performed in 2015 by another member of the Martins research team. The platelet and plasma aliquots fractionated from the blood collected in the EDTA tubes were used in the current study.

2.6. Measure of platelet APP, tau and related proteins

Western blot was employed to detect APP, ADAM10 and BACE1 secretase, tau, and p-tau ser202/thr205 in human platelets and mouse brain samples, while the ImageJ processing program was used to quantify the proteins of interest.

2.6.1. Sample preparation

All selected platelet samples underwent two rounds of freeze-thaw cycles for cell lysis. To begin, platelet samples were removed from -80˚C and allowed to thaw, therefore achieving the first freeze-thaw. Once thawed, 190 µl of platelet sample was transferred to separate tubes, and 19 µl of cOmplete™, Mini, EDTA-free Protease Inhibitor Cocktail (Merck Australia, catalogue number - 4693159001 Roche,) was added to each platelet sample and vortexed thoroughly. The samples with inhibitor were placed back into the freezer, and once frozen, were removed and allowed to once again thaw, completing the second freeze-thaw. Samples were next sonicated for 30 secs in a bath sonicator, then aliquoted into 20 µl volumes and finally stored at -80˚C for future analysis. The remaining protease inhibitor-free samples similarly underwent two freeze-thaw cycles, sonicated for 30 secs, aliquoted into 20 µl volumes, and stored at -80˚C. Mouse brain tissue collected from both 8-month-old C57BL/6J wild type (WT) mice and 8-month-old APP23 transgenic mice (APP23) (kindly donated by the Dementia Research Centre, Macquarie University, Australia) were likewise prepared with protease inhibitor, aliquoted into 5 µl fractions, and stored at -80˚C. For the current study, the platelet and mouse brain samples with protease inhibitor, were used for the analysis of the platelet proteins. The mice brain samples were used as positive controls to validate the antibodies for this study. Internal controls were created by combining 20 µl of each protease-inhibitor-rich platelet sample, aliquoted into 20 µl fractions and stored at 80˚C. This ‘internal control master mix’ was used to normalise for day-to-day variations, differences in imaging exposure times, and antibody incubation times.

2.6.2. Measuring total protein concentration

Total protein concentration for each sample (human platelets and mouse brains) were determined with the Pierce™ BCA Protein Assay Kit (ThermoFisher Scientific, Australia; catalogue number – 23227). The BCA assay utilises a formulation based on bicinchoninic acid (BCA) for the colorimetric detection and quantification of total protein. Reagents and bovine serum albumin standards (BSA) standards were prepared according to the manufacture's protocol. Firstly, a 1:100 dilution was made for each human platelet and mouse brain sample of unknown concentrations containing the protease inhibitor cocktail, by diluting it in phosphate-buffered saline (PBS) and vortexing it thoroughly. Using a 96-well plate, 25 µl of each diluted unknown sample and BSA standards were pipetted into separate wells in duplicates. Next, 200 µl of BCA Working Reagent, comprising of BCA reagent A and BCA reagent B in a 1:50 ratio, was added to each well. The plate was then covered with aluminium foil, mixed briefly on a plate shaker for 30 secs and incubated at 37˚C for 30 mins. Following incubation, the plate was allowed to cool to room temperature (as seen in Figure 5) and the absorbance of each unknown sample and BSA standard was measured using the PHERAstar FS plate reader (BMG Labtech, Germany) at a 562 nm wavelength. The more purple solution indicated more concentrated sample.

A standard curve with a range of 20-2000 µg/ml was generated by plotting the average of the blank-corrected absorbances for each BSA standard against its corresponding concentration in ug/ml. The standard curve was then used to determine the total protein concentration of each unknown sample.

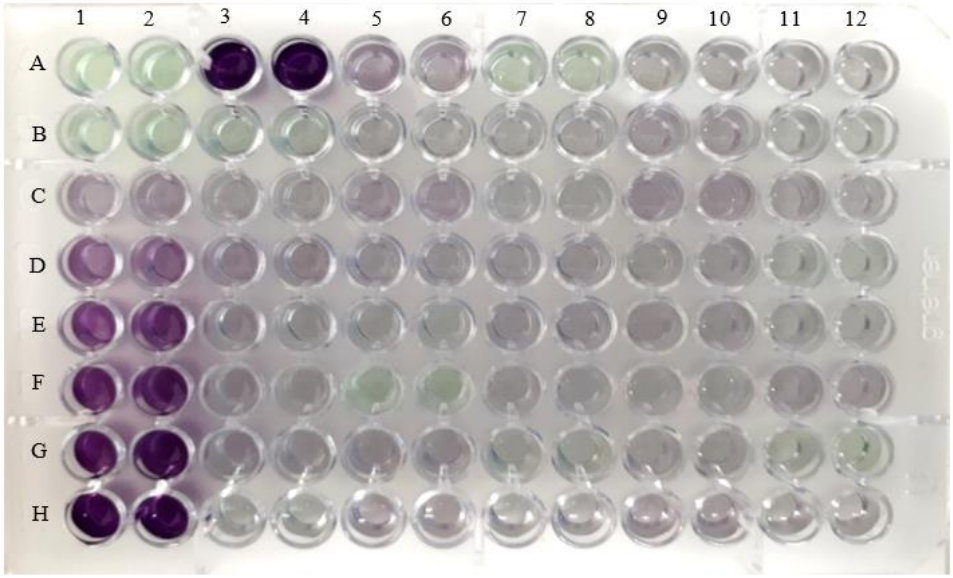


Figure 5. A BCA assay for platelet samples. Image of a 96-well plate taken after the incubation step. Diluted standards and unknown samples were run in duplicates for each standard and sample, with the standard series occupying columns 1, 2 and wells A3 + A4.

2.6.3. SDS-PAGE and transfer of proteins from gel to membrane

20 ug of human platelet and WT mouse brain, were used to detect APP, ADAM10 and, BACE1, while 40 ug of human platelet and WT mouse brain were used to detect tau and p-tau ser202/thr205. For all target proteins, 5 µg of APP23 was used.

All samples were prepared for SDS-PAGE, by firstly combining samples with calculated volumes of NuPAGE™ LDS Sample Buffer (4X) (ThermoFisher Scientific, Australia; catalogue number – NP0007), NuPAGE™ Sample Reducing Agent (10X) (ThermoFisher Scientific, Australia; catalogue number - NP0009) and 1X phosphate-buffered saline (PBS). The prepared samples were vortexed thoroughly then briefly spun down in a microcentrifuge at 13,000 rpm. The homogenised samples were next heated at 90˚C for 10 mins, then briefly centrifuged at 13,000 rpm to collect condensed sample. Homogenised samples were loaded into a NuPAGE™ 4-12% Bis-Tris Protein Gel (ThermoFisher Scientific, Australia; catalogue number - NP0336BOX) and fractionated firstly at 80V for 30 mins, then 120V for another 1.45 hrs. Samples for detecting APP, ADAM10 and BACE1 were separated using 1X dilution of NuPAGE MOPS (20X), while samples for detecting tau and p-tau ser202/thr205 were separated using 1X NuPAGE MES buffer (20X). The Novex™ Sharp Pre-stained Protein Standard (ThermoFisher Scientific, Australia; catalogue number - LC5800) was used as the molecular weight ladder. All platelet samples were tested in duplicates, and duplicates of the platelet internal controls were included in every western blot.

Following SDS-PAGE, the fractionated proteins were wet transferred onto a 0.45 µm nitrocellulose membrane (ThermoFisher Scientific, Australia; catalogue number – LC2006), using transfer buffer comprised of 10X tris-glycine solution, 20% w/v methanol and Milli-Q water. Transfer of separated samples for APP, ADAM10 and BACE1 were performed at 250 mA for 2.5 hrs at 4˚C, while separated proteins for tau and p-tau ser202/thr205 were transferred at 250 mA for 16 hrs in 4˚C. A magnetic stirrer was used to ensure even distribution of transfer buffer ions during the running of the wet transfers.

2.6.4. Western blot

Following wet transfer, the nitrocellulose blots were stained with Ponceau S dye to check if transfer was successful. After the stain was removed, the blots were blocked with 5% w/v skim-milk diluted in 1X tris-buffer saline (TBS) for 1hr at room temperature. The blots were then incubated in the following primary antibodies (Table 2): 22C11 for the APP isoforms (1:30,000, Chemicon®, catalogue number – MAB348), ADAM10 for ADAM10 secretase (1:3000, Chemicon®, catalogue number - AB19026), BACE1 for BACE1 secretase (1:1000, Affinity Bio, Golden, CO; catalogue number – PA1-757), Tau-5 for tau (1:1000, Calbiochem®, catalogue number - 577801), AT8 for p-tau ser202/thr205 (1:1000, ThermoFisher Scientific, catalogue number - MN1020) and β-actin as the loading control (1:40,000 for 20ug of loaded protein and, 1:80,000 for 40ug of loaded protein; Abcam, catalogue number – ab8227). The antibodies 22C11 and AT8 were incubated for 16 hrs at 4˚C, while ADAM10, BACE1, Tau-5 and β-actin were left to incubate for 2 hrs at room temperature. Following primary antibody incubations,

blots were washed vigorously three times in 1X TBS with 0.1% Tween 20 (TBST) for 10 mins each, except for blots detecting APP with the 22C11 antibody, for which an additional 10 min wash was included. After washing, all blots were incubated in horseradish peroxidase (HRP)-conjugated IgG secondary antibodies for 1 hr at room temperature. Blots for 22C11, Tau-5 and AT8 were incubated in Amersham ECL Mouse IgG, HRP-linked whole antibody (from sheep) (1:5000, GE Healthcare Life Sciences, catalogue number – NA931), while blots detecting ADAM10, BACE1 and β-actin were incubated in Amersham ECL Rabbit IgG, HRP-linked whole antibody (from donkey) (1:20,000, GE Healthcare Life Sciences, catalogue number – NA934). After incubation, all blots were washed in 1X TBST following the same conditions as above, followed by two 5 min washes in 1X TBS to remove any remaining tween. Blots were then developed with enhanced chemiluminescence (ECL) substrates in a 1:1 ratio (Bio-Rad; catalogue number - 1705061) and imaged using Bio-Rad's ChemiDoc™ MP Imaging System. The images were compiled and formatted using the Image Lab Software (version 5.1, Bio-Rad) and finally converted to TIF files for further analysis.

Table 2. Antibodies for western blot analysis

Primary antibody	Antibody type	Epitope	Dilution
Anti-Alzheimer Precursor Protein A4, clone 22C11	Monoclonal	Amino acid residues 66-81 of APP (N-terminal)	1:30,000
Anti-ADAM 10 antibody	Polyclonal	Amino acid residues 732-748 (C-terminal) of human ADAM10	1:3000
BACE1 polyclonal antibody	Polyclonal	Amino acid residues 485-501 (C-terminal) of human beta-secretase	1:1000
Anti-Tau Mouse monoclonal antibody (Tau-5)	Monoclonal	Within the central region	1:1000
Phospho-Tau (Ser202/Thr205) monoclonal antibody (AT8)	Monoclonal	Phosphorylated tau at amino acid residues Ser202/Thr205	1:1000
Anti-beta Actin antibody	Polyclonal	Amino acid residues 1-100 of human beta-actin	1:40,000 for 20 µg 1:80,000 for 40 µg

Table 2 describes the antibodies used in this study. Abbreviations: APP, amyloid precursor protein; ADAM10, A Disintegrin and metalloproteinase; BACE1, beta-secretase; ser202/thr205, serine 202/threonine 205

2.6.5. Western blot quantification with ImageJ software

Quantification of platelet APP, ADAM10 and BACE1 secretases, tau and, p-tau ser202/thr205 were performed using the ImageJ software (IJ 1.46r, National Institute of Health**),** where the integrated density of each protein was measured.

1. To begin, the rectangular selection tool was used to enclose the first protein band in the row (Figure 6). The size of the selected area was made sure to enclose the largest protein band in the same row.

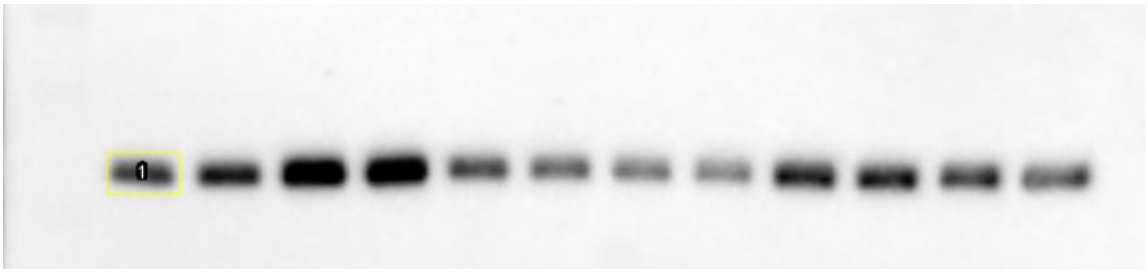


Figure 6. Example of an imaged western blot being quantified with ImageJ. The yellow box around the first band represents the selection to be used for all remaining bands in the same row.

2. The same rectangular selection was used to enclose all remaining bands within the same row (Figure 7).

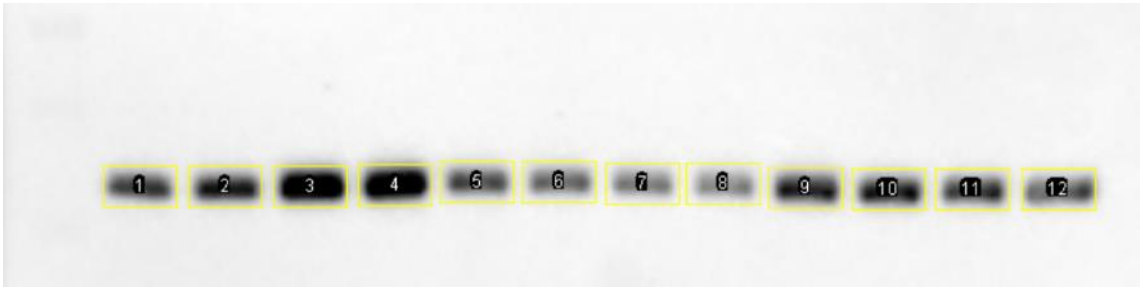


Figure 7. Example of an imaged western blot being quantified with ImageJ. The same yellow box selection for every band across the same row of proteins.

3. Once all bands were selected, a background selection was taken using the same rectangular frame, by placing the selection in an area on the western blot not covered with protein bands, stains or artefacts (Figure 8).

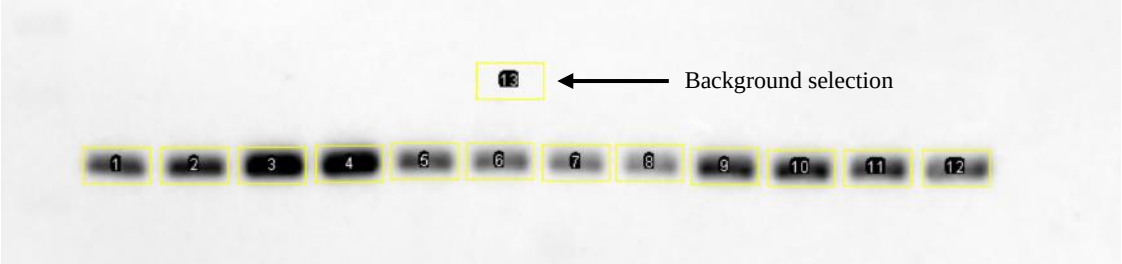


Figure 8. Example of an imaged western blot being quantified with ImageJ. The yellow box above the row of protein bands represents the background selection.

4. The integrated density was then measured for all of the protein bands and the background.
5. This process was repeated for all remaining rows of proteins, making sure that a separate, single selection frame was defined for every different row of protein bands.
6. Once all measurements were made, the background measurement was subtracted from the integrated density value for each individual sample protein band, and the averages of each background-subtracted duplicated sample was taken.
7. The sample protein bands were then normalised to its respective internal control of the same row. The normalised value was used as the final value for statistical analyses.

2.6.6. Plasma amyloid-beta and neurofilament light chain quantification

The ultra-sensitive Single Molecule Array (SIMOA, Quanterix) platform was used to measure the concentrations of plasma $A\beta_{40}$ and $A\beta_{42}$ and plasma NFL (161). Measurements were performed according to the manufacturer's instructions. Calibrators for plasma $A\beta_{40,}$ $A\beta_{42}$ and NFL were run in duplicates, while sample dilutions (which were run in singlicates) were diluted fourfold for plasma NFL and $A\beta_{42,}$ and eightfold for $A\beta_{40.}$ Duplicates of two quality control (QC) samples were run at the beginning and end of each assay plate for NFL. The concentration of one QC sample was 12.1pg/ml, with a repeatability and intermediate precision of 20.2%, while the other QC sample had a concentration of 115.8pg/ml, a repeatability of 14.6% and an intermediate precision of 14.9% (161).

In short, for the measurement of plasma Aβ, plasma samples collected previously were diluted, combined and incubated with paramagnetic beads coated in an antibody specific to the N-terminus of both $A\beta_{40}$ and $A\beta_{42}$, as well as two different biotinylated antibodies specific to the C-terminus of Aβ. After washing, the captured beads were incubated with streptavidin β-galactosidase conjugate. The beads were washed again, then resuspended in β-D-galactopyranoside substrate solution. For the measurement of plasma NFL, paramagnetic beads coated in a capture antibody specific to the conserved rod domain of NFL, were similarly diluted, combined and incubated with plasma samples. After washing and an initial incubation with blocking solution, the beads were washed again and incubated in a biotinylated detection antibody. Detection and measurement of plasma $A\beta_{40,}$ $A\beta_{42}$ and NFL were performed on the SIMOA disc (162).

2.6.7. Statistical analyses

All statistical analyses were accomplished using the Statistical Package for Social Sciences (SPSS) (Version 26). Two-tailed significance at an alpha of 0.05 was used for all analyses.

Descriptive statistics for the continuous variables age, MMSE scores, education years, left and right hippocampal volumes and NAL, which included the means and standard deviations, were calculated for the NAL- and NAL+ groups. Chi-square tests were utilised for gender, *APOE* ε4 allele status and subjective memory complainers (SMC) between NAL- and NAL+ groups.

The sum of the identified tau bands (t-tau), and the sum of the identified p-tau ser202/thr205 bands (p-tau) were calculated, and the ratio of p-tau/t-tau was generated. The ratio of the APP isoforms 105-115kDa with 80-100kDa (APPr) was also calculated, as well as the ratio of high molecular weight tau bands >80kDa (HMW) to low molecular weight tau bands <80kDa (LMW) (HMW/LMW).

Continuous variables were assessed for normality and equality of variances using distribution histograms and the Levene's test, where a bell curve distribution on the histogram and a *p* value of >0.05 on the Levene's was deemed to be normally distributed and met assumptions to employ parametric tests. Non-normal data was log-transformed. General linear models (GLM) were employed to compare APP, APPr, ADAM10, BACE1, tau, t-tau, HMW/LMW tau, p-tau ser202/thr205, p-tau and p-tau/t-tau between groups of interest (NAL- vs NAL+, and SMC- vs SMC+) with and without adjusting for covariates age, gender and *APOE* ε4 allele status. Spearman's bivariate correlations were used to assess correlations of APP, APPr, ADAM10, BACE1, tau, t-tau, HMW/LMW tau ratio, p-tau ser202/thr205, p-tau and p-tau/t-tau ratio with 1) NAL, 2) cognitive performance assessed by verbal and visual episodic memory, working memory and executive function and global cognitive performance, 3) plasma $A\beta_{40}$, $A\beta_{42}$ and, $A\beta_{42/40}$ ratio, and 4) plasma NFL levels.

Receiver operating characteristic (ROC) curves were generated using R within RStudio for all interested proteins, to determine their diagnostic ability for NAL+.

Chapter 3: Results

3.1. Participant demographics

The demographic characteristics of study participants is presented in Table 3. There were no significant differences in gender, age, MMSE scores, SMC, left hippocampal and right hippocampal volumes between the NAL- and NAL+ groups. There was a statistically significant difference in the frequency of *APOE* ε4 carriers (p=0.003) between the NAL- and NAL+ groups, where the NAL+ group had a higher frequency of ε4 allele carriers (n=14/17), compared to the NAL- group (n=3/17).

Table 3. Participant demographics

	Overall cohort (n=60)	**NAL-, <1.35 (n=30)**	**NAL+, ≥1.35 (n=30)**	***p***
Gender (M/F)	16/44	10/20	6/24	0.382
Age (years, mean ± SD)	78.13 ± 5.28	77.00 ± 5.29	79.27 ± 5.11	0.097
n *APOE* ε4 carriers (%)	17 (28.33)	3 (10)	14(46.67)	**0.003***
MMSE (mean ± SD)	28.60 ± 1.17	28.5 ± 1.20	28.7 ± 1.15	0.512
SMC, (*yes/no)*	13/47	5/25	8/22	0.532
NAL SUVR	1.43 ± 0.39	1.08 ± 0.04	1.77 ± 0.24	-
Left HV %	0.196 ± 0.019	0.196 ± 0.018	0.195 ± 0.020	0.816
Right HV %	0.201 ± 0.019	0.202 ± 0.019	0.199 ± 0.019	0.565

Table 3. Demographic characteristics for the study participants. Comparisons of participant demographic characteristics were made between NAL- (SUVR < 1.35) and NAL+ (SUVR ≥ 1.35). *P*-value was significant between NAL- and NAL+ groups for *APOE* ε4 allele status (p=0.003). *p<0.05. Abbreviations: NAL, neocortical amyloid-beta load; *APOE,* Apolipoprotein E; MMSE, Mini-Mental State Examination; SUVR, standard uptake value ratio; HV, hippocampal volume; SMC, subjective memory complainer.

3.2. Description of platelet APP, ADAM10, BACE1 and tau

The presence and intensity of the platelet proteins APP, ADAM10, BACE1 and tau were determined with western blots and quantified with ImageJ.

3.2.1. APP, ADAM10 and BACE1 in platelet samples

In the platelets, three APP isoforms were observed (Figure 9A); a faint, ~105-115kDa fragment and, two darker and lower molecular weight bands of ~ 80kDa and 100kDa (113,127). In the WT mouse brain sample, no bands were observed (Figure 9B), while in the APP23 mouse brain sample, two APP fragments of ~ 80-100kDa and ~105-115kDa were detected (Figure 9B). The platelet samples showed β-actin bands of ~40kDa (Figure 9C). The WT mouse brain sample showed a β-actin band at ~40kDa, while the APP23 mouse brain sample revealed a fainter ~40kDa β-actin. (Figure 9D), The β-actin band for the APP23 mouse brain sample was observed more clearly after increasing the contrast (Figure 9E).

In the platelets, a single ADAM10 band of ~ 65kDa was identified (130,163,164) (Figure 10A). In the WT and APP23 mouse brain samples, two intense ADAM10 bands at ~75kDa and ~80kDa were observed, as well as a lighter ~60kDa band (Figure 10B). The platelet samples showed β-actin bands of ~40kDa (Figure 10C). The WT mouse brain sample showed a β-actin band at ~40kDa, while the APP23 mouse brain sample revealed a fainter ~40kDa β-actin. (Figure 10D), The β-actin band for the APP23 mouse brain sample was observed more clearly after increasing the contrast (Figure 10E).

Multiple BACE1 bands in the platelets, which consistently appeared across all samples, were detected from varying imaging exposure times; bands at ~200kDa (3-10secs) (Figure 11A), ~160kDa (3-50secs) (Figure 11B), ~110kDa (3-50secs) (Figure 11B), ~80kDa (3-50secs) (Figure 11B) and ~60kDa (120-160secs) (Figure 11C). The WT and APP23 mouse brain samples showed three BACE1 bands at ~80kDa, ~75kDa and ~60kDa (Figure 11E). The platelet samples showed β-actin bands of ~40kDa (Figure 11D). The WT mouse brain sample showed a β-actin band at ~40kDa, while the APP23 mouse brain sample revealed a fainter ~40kDa β-actin. (Figure 11F), The β-actin band for the APP23 mouse brain sample was observed more clearly after increasing the contrast (Figure 11G).

3.2.2. Tau in platelet samples

Multiple immunoreactive tau bands (139)) were observed across all platelet samples, ranging from ~30kDa to ~200kDa (Figure 12A). Of particular interest were tau fragments ~200kDa, ~110kDa, ~80kDa, ~60kDa, ~55kDa, ~50kDa and, ~33kDa in size, which appeared to be consistently expressed across all platelet samples (Figure 12A). The WT and APP23 mouse brain samples showed an intense band at ~50kDa, and a lighter band at ~60kDa (Figure 12B). The platelet samples showed β-actin bands of ~40kDa (Figure 12C). The WT mouse brain sample showed a β-actin band at ~40kDa, while the

APP23 mouse brain sample revealed a fainter ~40kDa β-actin. (Figure 12D), The β-actin band for the APP23 mouse brain sample was observed more clearly after increasing the contrast (Figure 12E).

3.2.3. Phosphorylated tau in platelet samples

Multiple bands of varying molecular weights were observed for p-tau ser202/thr205 (Figure 13A). Of particular interest were bands of approximate molecular weights 200kDa, 80kDa, 55kDa and 33kDa, which appeared to be consistently expressed across all platelet samples (Figure 13A). In the WT and APP23 mouse brain samples, a single, intense band at ~55kDa was seen (Figure 13B). The platelet samples showed β-actin bands of ~40kDa (Figure 13C). The WT mouse brain sample showed a β-actin band at ~40kDa, while the APP23 mouse brain sample revealed a fainter ~40kDa β-actin. (Figure 13D), The β-actin band for the APP23 mouse brain sample was observed more clearly after increasing the contrast (Figure 13E).

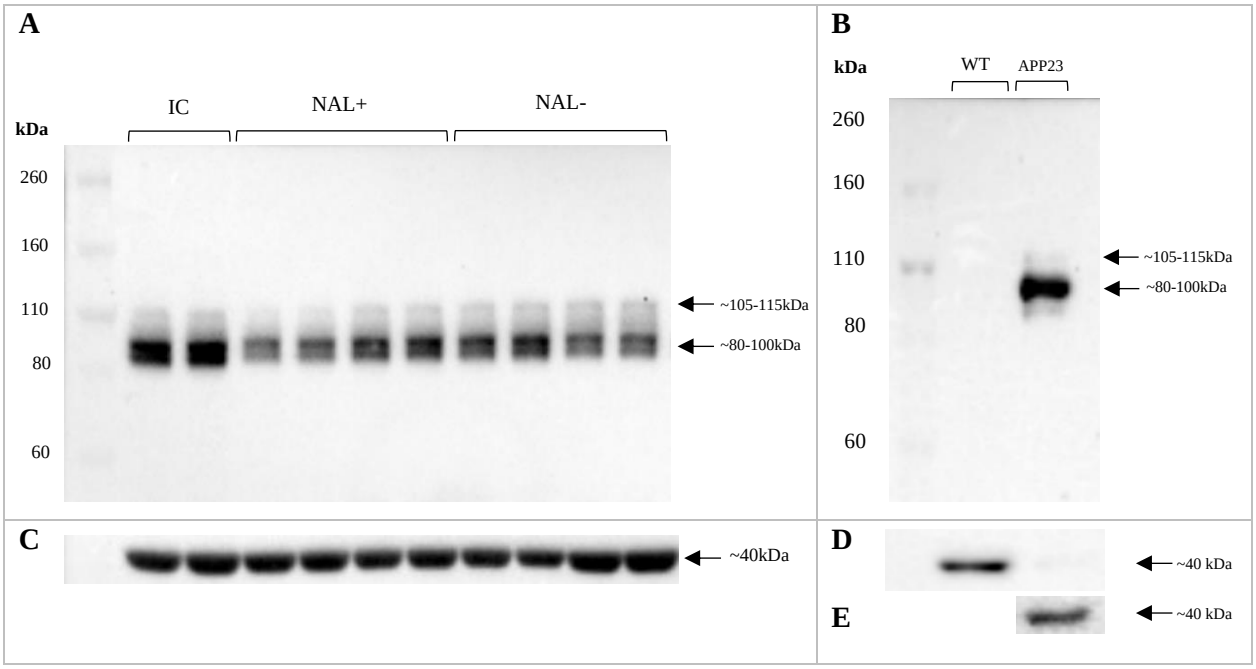


Figure 9. Representative western blots for APP isoforms. All APP isoforms were detected with the 22C11 antibody (Merck). The C57BL/6J WT (20µg) and APP23 (5µg) mouse brain samples were included as positive controls. A) In the platelet samples (20µg), a large faint band at ~105-115kDa and two smaller darker bands at ~80-100kDa were detected. B) The WT mouse brain sample showed no bands, while the APP23 mouse brain sample showed a strong band at ~80-100kDa, and a fainter, larger band at ~110kDa. C) The platelet samples showed β-actin bands at ~40kDa. D) The WT mouse brain sample showed a β-actin band at ~40kDa, while the APP23 mouse brain sample revealed a fainter ~40kDa β-actin. E) β-actin band was observed more clearly in the APP23 mouse brain sample after increasing the contrast. It should be noted that β-actin is not a great loading control in mouse brain samples. Abbreviations: IC, internal control; NAL+, high neocortical amyloid-beta; NAL-, low neocortical amyloid-beta; APP, amyloid precursor protein; WT, wild type; APP23, amyloid precursor protein 23 transgenic mouse; β-actin, beta-actin.

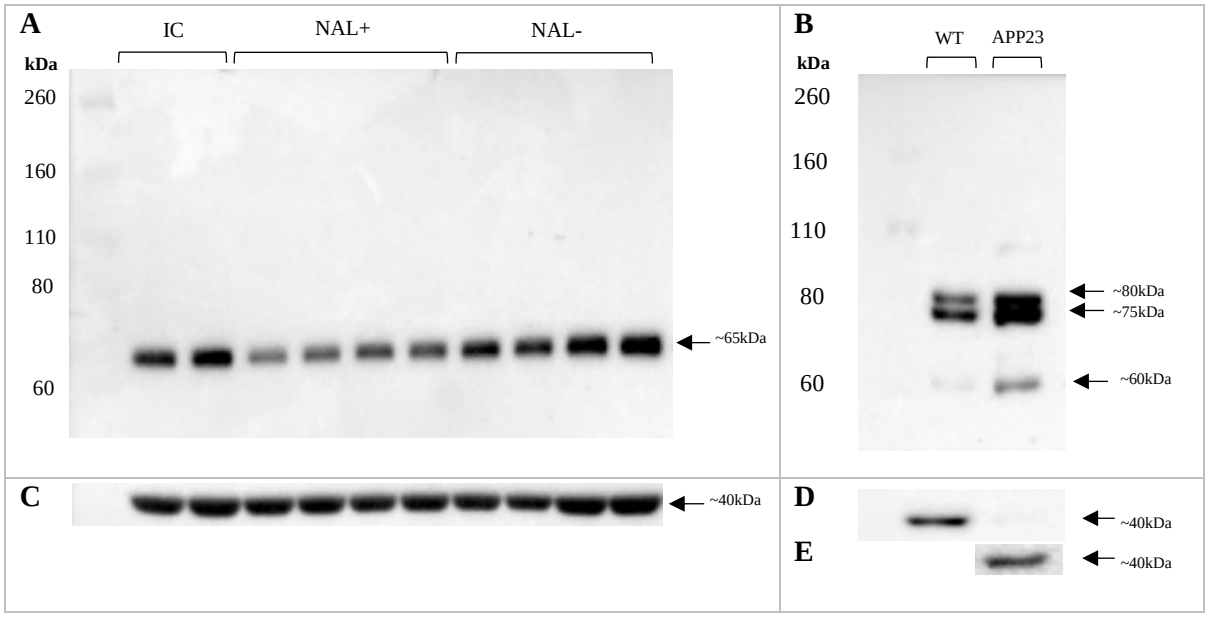


Figure 10. Representative western blots for ADAM10. Bands were detected with the ADAM10 antibody (Merck). The C57BL/6J WT and APP23 mouse brain samples were included as positive controls. A) The platelet samples observed a single band at ~65kDa. B) The WT mouse brain sample (20µg) and APP23 mouse brain samples (5µg) observed three bands at ~80kDa, ~75kDa and ~60kDa. C) The platelet samples showed β-actin bands at ~40kDa. D) The WT mouse brain sample showed a β-actin band at ~40kDa, while the APP23 mouse brain sample revealed a fainter ~40kDa β-actin. E) β-actin band was observed more clearly in the APP23 mouse brain sample after increasing the contrast. It should be noted that β-actin is not a great loading control in mouse brain samples. Abbreviations: IC, internal control; NAL+, high neocortical amyloid-beta; NAL-, low neocortical amyloid-beta; ADAM10, A Disintegrin and metalloproteinase; WT, wild type; APP23, amyloid precursor protein 23 transgenic mouse; β-actin, beta-actin.

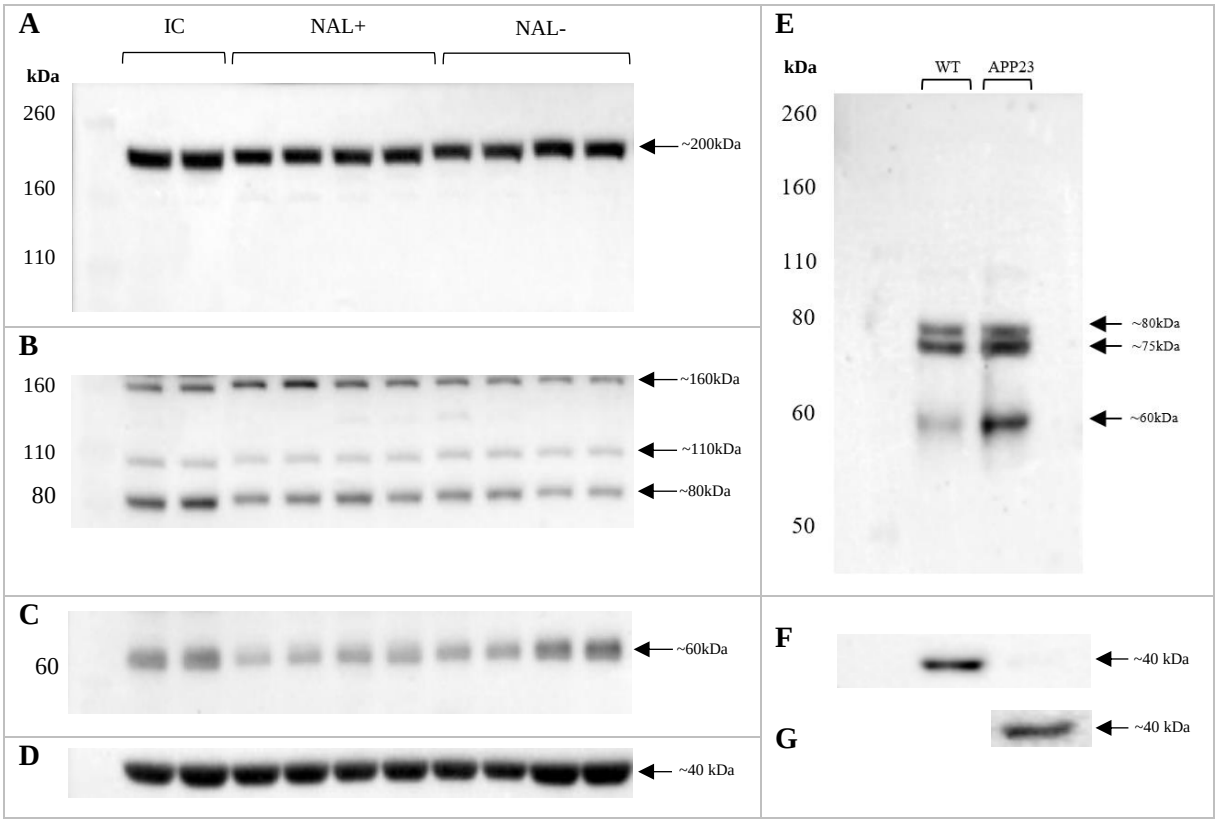


Figure 11. Representative western blots for BACE1. All BACE1 bands were detected with the BACE1 antibody (Thermo Fisher) at different exposure times. The C57BL/6J WT (20µg) and APP23 (5µg) mouse brain samples were included as positive controls. The platelet samples (20µg) showed bands at different exposure times: A) ~200kDa (3-10secs), B) ~160kDa, ~110kDa and ~80kDa (40-60secs), and C) ~60kDa (80-120secs). D) The platelet samples showed β-actin bands at ~40kDa. E) The WT and APP23 mouse brain samples showed bands at ~80kDa, ~75kDa and ~60kDa. F) The WT mouse brain sample showed a β-actin band at ~40kDa, while the APP23 mouse brain sample revealed a fainter ~40kDa β-actin. G) β-actin band was observed more clearly in the APP23 mouse brain sample after increasing the contrast. It should be noted that β-actin is not a great loading control in mouse brain samples. Abbreviations: IC, internal control; NAL+, high neocortical amyloid-beta; NAL-, low neocortical amyloid-beta; BACE1, beta-secretase; WT, wild type; APP23, amyloid precursor protein 23 transgenic mouse; β-actin, beta-actin.

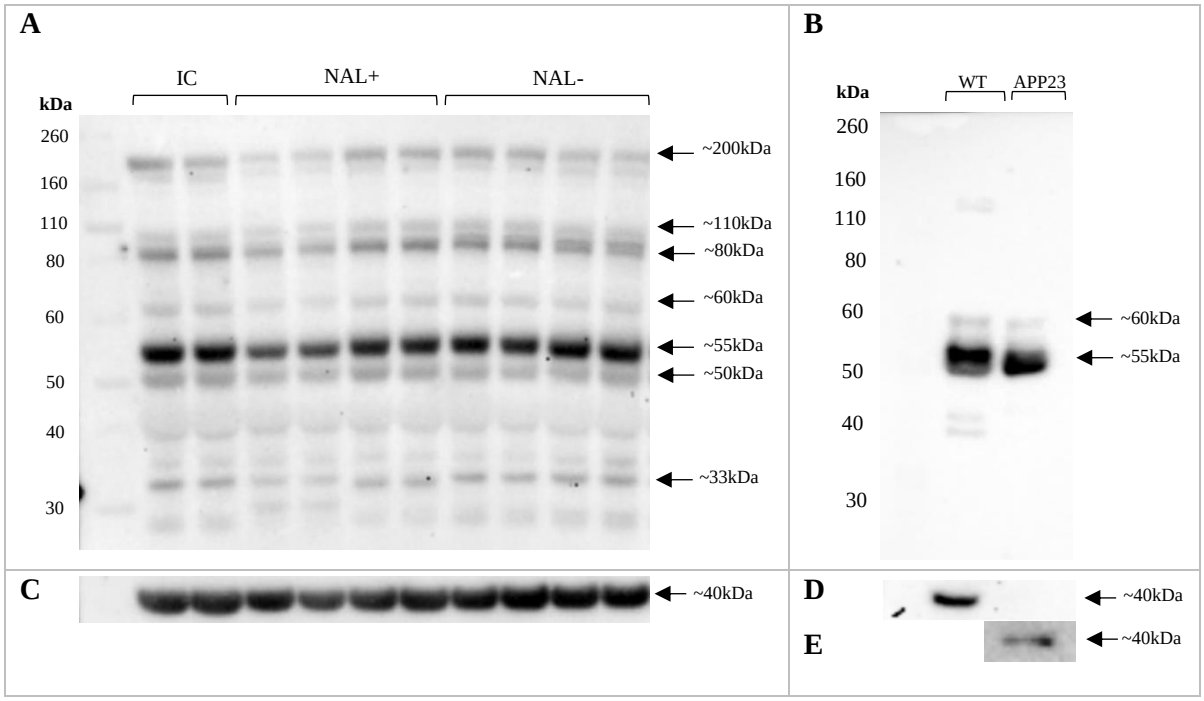


Figure 12. Representative western blots for tau. All tau bands were detected with the Tau-5 antibody (Merck). C57BL/6J WT (40µg) and APP23 (5µg) mouse brain samples were included as positive controls. A) The platelet samples (40µg) showed consistent bands at ~200kDa, ~110kDa, ~80kDa, ~60kDa, ~55kDa, ~50kDa and, ~33kDa. B) The WT and APP23 mouse brain samples showed tau bands at ~60kDa and ~55kDa. C) The platelet samples showed β-actin bands at ~40kDa. D) The WT and APP23 mouse brain samples showed a β-actin a band at ~40kDa, while the APP23 mouse brain sample showed a fainter β-actin ~40kDa. E) β-actin band was observed more clearly in the APP23 mouse brain sample after increasing the contrast. It should be noted that β-actin is not a great loading control in mouse brain samples. Abbreviations: IC, internal control; NAL+, high neocortical amyloid-beta; NAL-, low neocortical amyloid-beta; WT, wild type; APP23, amyloid precursor protein 23 transgenic mouse; β-actin, beta-actin.

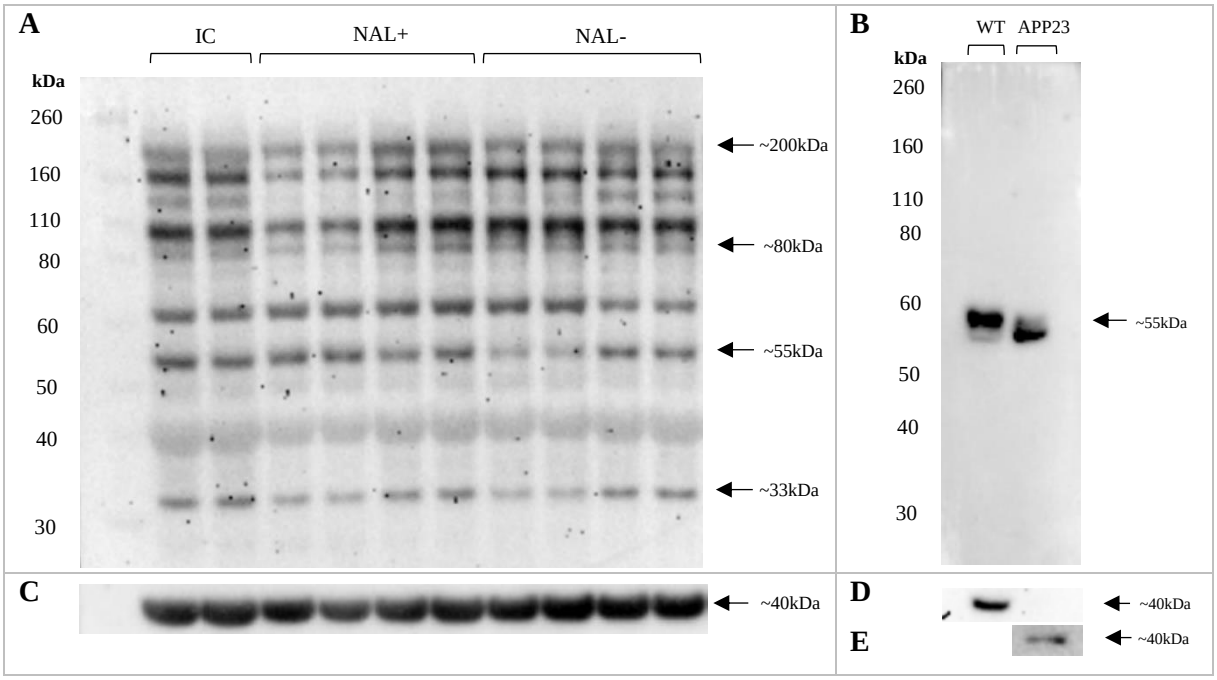


Figure 13. Representative western blots for p-tau ser202/thr205. All p-tau ser202/thr205 bands were detected with the AT8 antibody (Thermo Fisher). The C57BL/6J WT (40µg) and APP23 (5µg) mouse brain samples were included as positive controls. A) The platelet samples (40µg) showed consistent bands at ~200kDa, ~80kDa, ~55kDa, and, ~33kDa. B) The WT and APP23 mouse brain samples showed a p-tau ser202/thr205 band at ~55kDa. . C) The platelet samples showed β-actin bands at ~40kDa. D) The WT and APP23 mouse brain samples showed a β-actin band at ~40kDa, while the APP23 mouse brain sample showed a fainter β-actin ~40kDa. E) β-actin band was observed more clearly in the APP23 mouse brain sample after increasing the contrast. It should be noted that β-actin is not a great loading control in mouse brain samples. Abbreviations: IC, internal control; NAL+, high neocortical amyloid-beta; NAL-, low neocortical amyloid-beta; WT, wild type; APP23, amyloid precursor protein 23 transgenic mouse; p-tau ser202/thr205, phosphorylated tau at serine 202/threonine 205; β-actin, beta-actin.

3.3. Associations of platelet proteins with AD risk factors age, gender and *APOE* ε4 allele status

Statistical analyses were performed to determine whether the AD risk factors age, gender and *APOE* ε4 allele status were significantly associated with the platelet proteins. No significant associations were found between the platelet proteins and age (Appendix A). However, with gender (Table 4, Figure 14), APP 105-115kDa (p=0.005), APPr (p=0.006), BACE ~60kDa (p=0.003), tau ~50kDa (p=0.010), p-tau ser202/thr205 ~200kDa (p<0.0001) and p-tau ser202/thr205 ~33kDa (p=0.025) were significantly lower in the females compared to the males. Tau ~55kDa (p=0.055) and BACE1 ~80kDa (p=0.092) saw a trend of lower mean levels in the females over males, while t-tau (p=0.054) and p-tau (p=0.065) showed trends of increased mean levels in females compared to males.

With *APOE* ε4 allele carrier status, only BACE1 ~200kDa was significantly lower in the *APOE* ε4 allele carriers compared to the non-carriers (p=0.010), while a trend of increased mean levels of BACE1 ~110kDa (p=0.088) was observed in the *APOE* ε4 allele carriers (Table 5, Figure 15). The significance remained for BACE1 ~200kDa after adjusting for covariates age and gender (p^a=0.014), while the trend also continued for BACE1 ~110kDa (p^a=0.061).

Table 4. Comparison of platelet proteins between males and females

Platelet proteins	Total (n=60)	Male (n=16)	Female (n=44)	*p*
APP 105-115kDa	0.37 ± 0.61	0.78 ± 1.07	0.22 ± 0.15	***0.005**
APP 80-100kDa	2.17 ± 1.71	2.90 ± 2.78	1.90± 1.01	0.309
APPr	0.l6 (0.16)	0.26 (0.24)	0.12 (0.10)	***0.006**
ADAM10	1.52 ± 0.65	1.59 ± 0.53	1.50 ± 0.70	0.648
BACE1 ~200kDa	2.04 ± 0.30	2.11 ± 0.27	2.02 ± 0.31	0.322
BACE1 ~160kDa	0.56 ± 0.26	0.52 ± 0.29	0.58 ± 0.25	0.481
BACE1 ~110kDa	0.80 ± 0.30	0.83 ± 0.23	0.79 ± 0.33	0.636
BACE1 ~80kDa	0.72 ± 0.39)	0.86 ± 0.47	0.67 ± 0.34	0.092
BACE1 ~60kDa	0.69 ± 0.37	0.92 ± 0.38	0.60 ± 0.33	***0.003**
Tau ~ 200kDa	0.91 ± 0.38	1.04 ± 0.34	0.86 ± 0.38	0.108
Tau ~ 110kDa	0.29 ± 0.18	0.34 ± 0.14	0.28 ± 0.19	0.290
Tau ~ 80kDa	0.50 ± 0.28	0.56 ± 0.26	0.48 ± 0.28	0.364
Tau ~ 55kDa	1.88 ± 0.81	2.21 ± 0.95	1.75 ± 0.73	0.055
Tau ~ 50kDa	0.51 ± 0.21	0.64 ± 0.28	0.46 ± 0.16	***0.010**
Tau ~ 33kDa	0.45 ± 0.26	0.55 ± 0.35	0.41 ± 0.22	0.168
HMW/LMW tau	0.59 ± 0.21	0.55 ± 0.14	0.60 ± 0.23	0.412
T-tau	5.16 ± 2.15	4.57 ± 1.80	5.91 ± 2.17	0.054
P-tau ser202/thr205 ~ 200kDa	1.03 ± 0.48	1.51 ± 0.52	0.91 ± 0.39	*****<0.0001**
P-tau ser202/thr205 ~80kDa	0.81 ± 0.43	0.67 ± 0.20	0.85 ± 0.47	0.210
P-tau ser202/thr205 ~ 55kDa	1.01 ± 0.39	1.03 ± 0.29	1.01 ± 0.42	0.888
P-tau ser202/thr205 ~33kDa	0.25 ± 0.15	0.34 ± 0.16	0.23 ± 0.15	***0.025**
P-tau	3.10 ± 1.13	2.57 ± 0.92	3.26 ± 1.14	0.065
P-tau/t-tau	0.57 ± 0.17	0.59 ± 0.20	0.57 ± 0.16	0.791

Table 4. Comparison of platelet proteins between males and females. APP 105-115kDa, APPr, BACE1 ~60kDa, tau ~50kDa, p-tau ser202/thr205 ~200kDa and, p-tau ser202/thr205 ~33kDa are significantly lower in females compared to males. All data are mean ± standard deviation. *$p<0.05$, ***$p<0.0001$. Abbreviations: NAL, neocortical amyloid-beta load; APP, Amyloid precursor protein; APPr, amyloid precursor protein ratio; ADAM10, a Disintegrin and metalloproteinase; BACE1, beta-secretase. HMW/LMW, high molecular weight/low molecular weight; t-tau, sum of all tau bands; p-tau ser202/thr205, phosphorylated tau at serine202/threonine205; p-tau; sum of all phosphorylated tau bands at serine 202/threonine 205 bands; p-tau/t-tau, sum of phosphorylated tau at serine 202/threonine 205/sum of all tau bands.

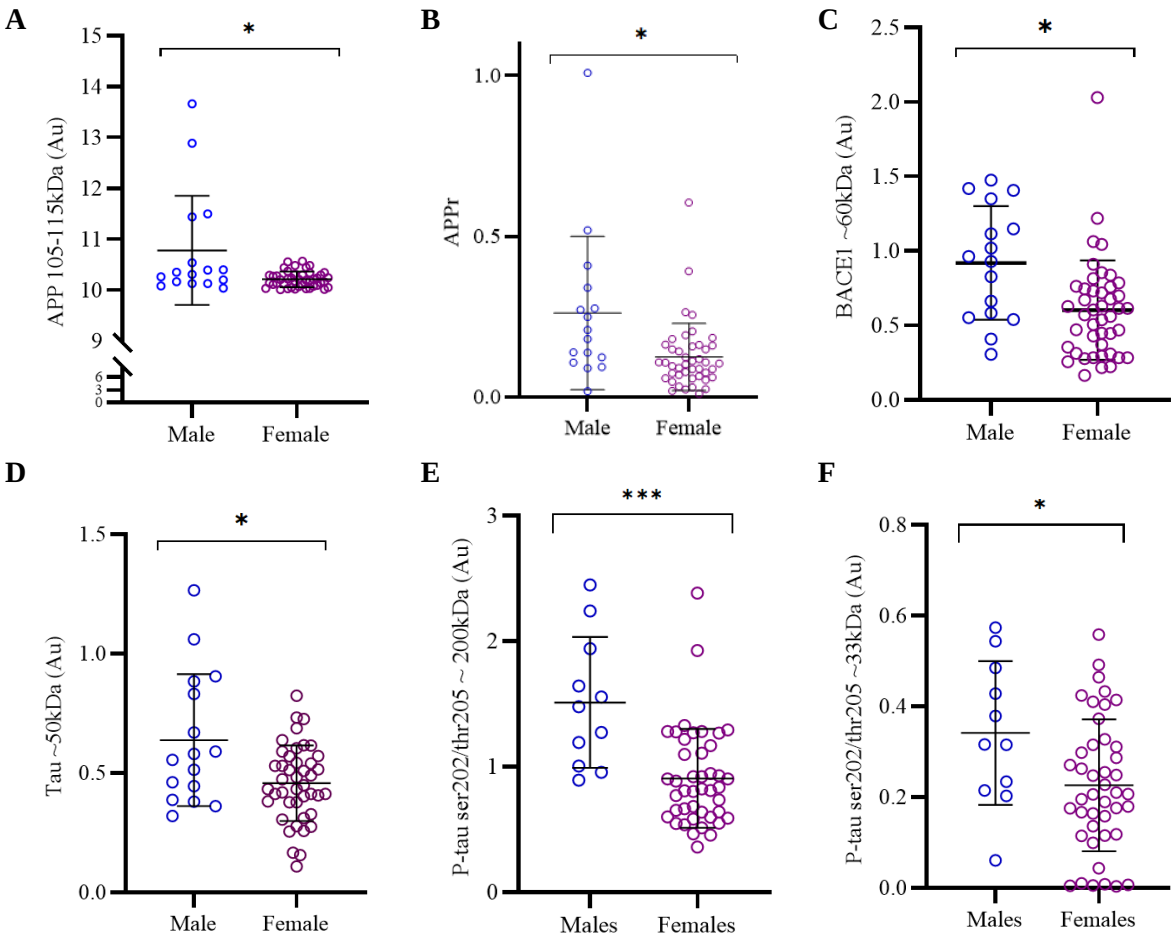


Figure 14. Comparison of platelet proteins between males and females. Significantly lower levels of A) APP 105-115kDa (p=0.005), B) APPr (p=0.006), C) BACE1 ~60kDa (p=0.003), D) tau ~50kDa, E) p-tau ser202/thr205 ~200kDa (p=<0.0001) and F) p-tau ser202/thr205 ~33kDa (p=0.025) were seen in females compared to males. *p<0.05, ***p<0.0001. Abbreviations: APP, Amyloid precursor protein; APPr, Amyloid precursor protein ratio; BACE1, beta-secretase; p-tau ser202/thr205, phosphorylated tau at serine202/threonine205; Au, Arbitrary units.

Table 5. Associations of platelet proteins with APOE ε4 allele status

Platelet proteins	Total (n=60)	*APOE* ε4 – (n=43)	*APOE* ε4 + (n=17)	*p*	p^a
APP 105-115kDa	0.37 ± 0.61	0.42 ± 0.71	0.23 ± 0.14	0.279	0.547
APP 80-100kDa	2.17 ± 1.71	2.19 ± 1.92	2.10 ± 1.04	0.849	0.871
APPr	0.16 ± 0.16	0.18 ± 0.18	0.12 ± 0.08	0.263	0.482
ADAM10	1.5 ± 0.65	1.53 ± 0.66	1.50 ± 0.67	0.908	0.949
BACE1 ~200kDa	2.04 ± 0.30	2.11 ± 0.32	1.89 ± 0.17	***0.010**	***0.014**
BACE1 ~160kDa	0.56 ± 0.26	0.54 ± 0.24	0.62 ± 0.31	0.290	0.367
BACE1 ~110kDa	0.80 ± 0.30	0.75 ± 0.22	0.92 ± 0.44	0.088	0.061
BACE1 ~80kDa	0.72 ± 0.39	0.70 ± 0.38	0.77 ± 0.41	0.527	0.308
BACE1 ~60kDa	0.69 ± 0.37	0.70 ± 0.39	0.65 ± 0.34)	0.642	0.888
Tau ~ 200kDa	0.91 ± 0.38	0.94 ± 0.40	0.084 ± 0.31	0.361	0.595
Tau ~ 110kDa	0.29 ± 0.18	0.27 ± 0.11	0.37 ± 0.28	0.111	0.137
Tau ~ 80kDa	0.50 ± 0.28	0.48 ± 0.24	0.56 ± 0.35	0.321	0.211
Tau ~ 55kDa	1.88 ± 0.81	1.91 ± 0.85	1.79 ± 0.72	0.590	0.938
Tau ~ 50kDa	0.51 ± 0.21	0.53 ± 0.23	0.44 ± 0.15	0.152	0.407
Tau ~ 33kDa	0.45 ± 0.26	0.48 ± 0.30	0.39 ± 0.14	0.245	0.366
T-tau	5.61 ± 2.15	5.71 ± 2.32	5.39 ± 1.75	0.614	0.872
HMW/LMW tau	0.59 ± 0.21	0.57 ± 0.20	0.63 ± 0.22	0.315	0.414
P-tau ser202/thr205 ~ 200kDa	1.03 ± 0.48	1.04 ± 0.47	1.01 ± 0.53	0.825	0.792
P-tau ser202/thr205 ~80kDa	0.81 ± 0.43	0.84 ± 0.46	0.76 ± 0.36	0.517	0.411
P-tau ser202/thr205 ~ 55kDa	1.01 ± 0.39	1.04 ± 0.42	0.96 ± 0.33	0.537	0.635
P-tau ser202/thr205 ~33kDa	0.25 ± 0.15	0.26 ± 0.16	0.23 ± 0.13	0.456	0.692
P-tau	3.10 ± 1.12	3.17 ± 1.160	2.95 ± 1.06	0.510	0.701
P-tau/t-tau	0.57 ± 0.17	0.58 ± 0.18	0.56 ± 0.15	0.679	0.913

Table 5. Associations of platelet proteins with *APOE* ε4 allele status. BACE1 ~200kDa is significantly lower in *APOE* ε4 (*APOE* ε4 +) carriers compared to non-carriers (*APOE* ε4 –). **p*<0.05. Abbreviations: NAL, neocortical amyloid-beta load; APP, Amyloid precursor protein; APPr, amyloid precursor protein ratio; ADAM10, a Disintegrin and metalloproteinase; BACE1, beta-secretase, HMW/LMW, high molecular weight/low molecular weight; t-tau, sum of all tau bands; p-tau ser202/thr205, phosphorylated tau at serine202/threonine205; p-tau, sum of all phosphorylated tau bands at serine 202/thr205; p-tau/t-tau, sum of phosphorylated tau bands at serine 202/threonine 205/sum of all tau bands.

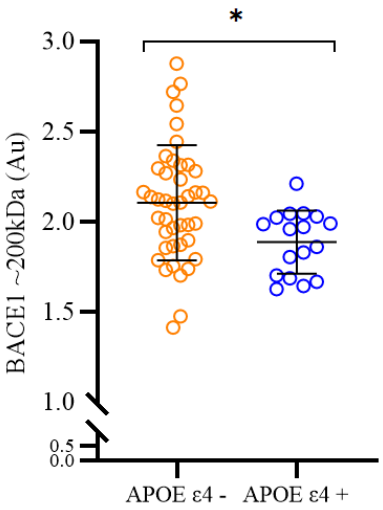


Figure 15. Comparison of APOE ε4 allele status with BACE1 ~200kDa. A) BACE1 ~200kDa is significantly lower in *APOE* ε4 carriers compared to non-carriers (p=0.010). p*<0.05. Abbreviations: BACE1, beta-secretase; APOE, Apolipoprotein E; Au, Arbitrary units.

3.4. Comparison of platelet proteins between NAL- and NAL+ groups

Associations of platelet proteins between NAL- and NAL+ have been presented in Table 6 and are graphically depicted in Figure 16. APPr was not significantly different between NAL- and NAL+ groups and instead showed a non-significant trend of lower mean levels of APPr in the NAL+ group (p=0.092). However, this trend disappeared after adjusting for the covariates age, gender and *APOE* ε4 allele status (p^a=0.156). The APP 105-115kDa upper isoform was significantly lower in the NAL+ group compared to the NAL- group (p=0.024). This significance remained after adjusting for the covariates (p^a=0.035). ADAM10 showed a non-significant trend of lower mean levels in the NAL+ groups (p=0.087), with the trend remaining after covariate adjustments (p^a=0.092). The BACE1 band of ~200kDa was significantly lower in the NAL+ group (p=0.020), however, this significance disappeared after adjusting for the covariates (p^a=0.255).

When the tau bands were compared between NAL- and NAL+ participants, tau ~50kDa band and tau ~33kDa both presented significantly lower mean levels in the NAL+ groups compared to NAL- (p=0.036 and p=0.015). However, only the ~33kDa band remained significant after covariate adjustment (p^a=0.027). When the ratio of HMW/LMW tau was analysed, there was a significant increase in the mean levels in the NAL+ group compared to the NAL- group (p=0.018), even after adjusting for covariates (p^a=0.030).

P-tau ser202/thr205 ~200kDa showed significantly lower levels in the NAL+ group compared to the NAL- group (p=0.032), while p-tau ser202/thr205 ~80kda band was not significantly different between the two groups (p=0.127). However, when adjusted for covariates, p-tau ser202/thr205 ~200kDa became non-significant (p^a=0.345), while the ~80kDa band showed a p-value of p^a=0.033. P-tau showed a trend of decreased mean levels in the NAL+ group (p=0.091), but this trend was removed after adjusting for the covariates (p^a=0.155).

Table 6. Associations of platelet proteins between NAL- and NAL+

Platelet Proteins	Total (n=60)	NAL- (<1.35) (n=30)	NAL+ (≥1.35) (n=30)	*p*	p^a
APP 105-115kDa	0.36 ± 0.61	0.54 ± 0.82	0.19 ± 0.13	***0.024**	***0.035**
APP 80-100kDa	2.17 ± 1.70	2.54 ± 2.16	1.79 ± 0.99	0.271	0.149
APPr	0.16 ± 0.16	0.20 ± 0.21	0.12 ± 0.09	0.092	0.156
ADAM10	1.52 ± 0.65	1.67 ± 0.57	1.38 ± 0.71	0.087	0.092
BACE1 ~200kDa	2.04 ± 0.30	2.13 ± 0.35	1.95 ± 0.22	***0.020**	0.255
BACE1 ~160kDa	0.56 ± 0.26	0.51 ± 0.21	0.62 ± 0.29	0.130	0.112
BACE1 ~110kDa	0.80 ± 0.30	0.79 ± 0.23	0.81 ± 0.37	0.768	0.228
BACE1 ~80kDa	0.72 ± 0.39	0.75 ± 0.42	0.69 ± 0.36	0.567	0.370
BACE1 ~60kDa	0.69 ± 0.37	0.71 ± 0.35	0.66 ± 0.40	0.592	0.228
Tau ~ 200kDa	0.90 ± 0.38	0.92 ± 0.39	0.90 ± 0.37	0.859	0.767
Tau ~ 110kDa	0.30 ± 0.18	0.26 ± 0.11	0.33 ± 0.23	0.168	0.388
Tau ~ 80kDa	0.50 ± 0.28	0.50 ± 0.26	0.51 ± 0.30	0.902	0.731
Tau ~ 55kDa	1.88 ± 0.81	1.99 ± 0.89	1.77 ± 0.73	0.294	0.341
Tau ~ 50kDa	0.51 ± 0.21	0.57 ± 0.24	0.45 ± 0.16	***0.036**	0.096
Tau ~ 33kDa	0.45 ± 0.26	0.54 ± 0.32	0.36 ± 0.15	***0.015**	***0.027**
T-tau	5.61 ± 2.15	5.98 ± 2.35	5.19 ± 1.86	0.181	0.331
HMW/LMW tau	0.59 ± 0.21	0.52 ± 0.16	0.65 ± 0.23	***0.018**	***0.030**
P-tau ser202/thr205 ~ 200kDa	1.03 ± 0.48	1.16 ± 0.48	0.88 ± 0.46	***0.032**	0.345
P-tau ser202/thr205 ~80kDa	0.81 ± 0.43	0.90 ± 0.48	0.72 ± 0.34	0.127	***0.033**
P-tau ser202/thr205 ~ 55kDa	1.01 ± 0.39	1.02 ± 0.37	1.01 ± 0.42	0.931	0.880
P-tau ser202/thr205 ~33kDa	0.25 ± 0.15	0.27 ± 0.18	0.22 ± 0.12	0.242	0.715
P-tau	3.10 ± 1.13	3.35 ± 1.13	2.83 ± 1.08	0.091	0.155
P-tau/t-tau	0.57 ± 0.17	0.59 ± 0.18	0.56 ± 0.16	0.500	0.492

Table 6 shows the comparison of platelet proteins between NAL- and NAL+ participants. Significantly lower mean levels in NAL+ are seen with APP 105-115kDa, BACE1 ~200kDa, tau ~50kDa, tau ~33kDa and, p-tau ser202/thr205 ~200kDa. Significantly higher mean levels of HMW/LMW tau ratio are seen in NAL+ compared to NAL-. *$p<0.05$, p^a = after adjusting for covariates age, gender and *APOE* ε4 allele status. Abbreviations: NAL, neocortical amyloid-beta load; NAL-, low neocortical amyloid-beta load; NAL+, high neocortical amyloid-beta load; APP, Amyloid precursor protein; APPr, amyloid precursor protein ratio; ADAM10, a Disintegrin and metalloproteinase; BACE1, beta-secretase; HMW/LMW, high molecular weight/low molecular weight; t-tau, sum of all tau bands; p-tau ser202/thr205, phosphorylated tau at serine202/threonine205; p-tau, sum of all phosphorylated tau bands at serine 202/threonine 205; p-tau/t-tau, sum of phosphorylated tau bands at serine 202/threonine 205/sum of all tau bands.

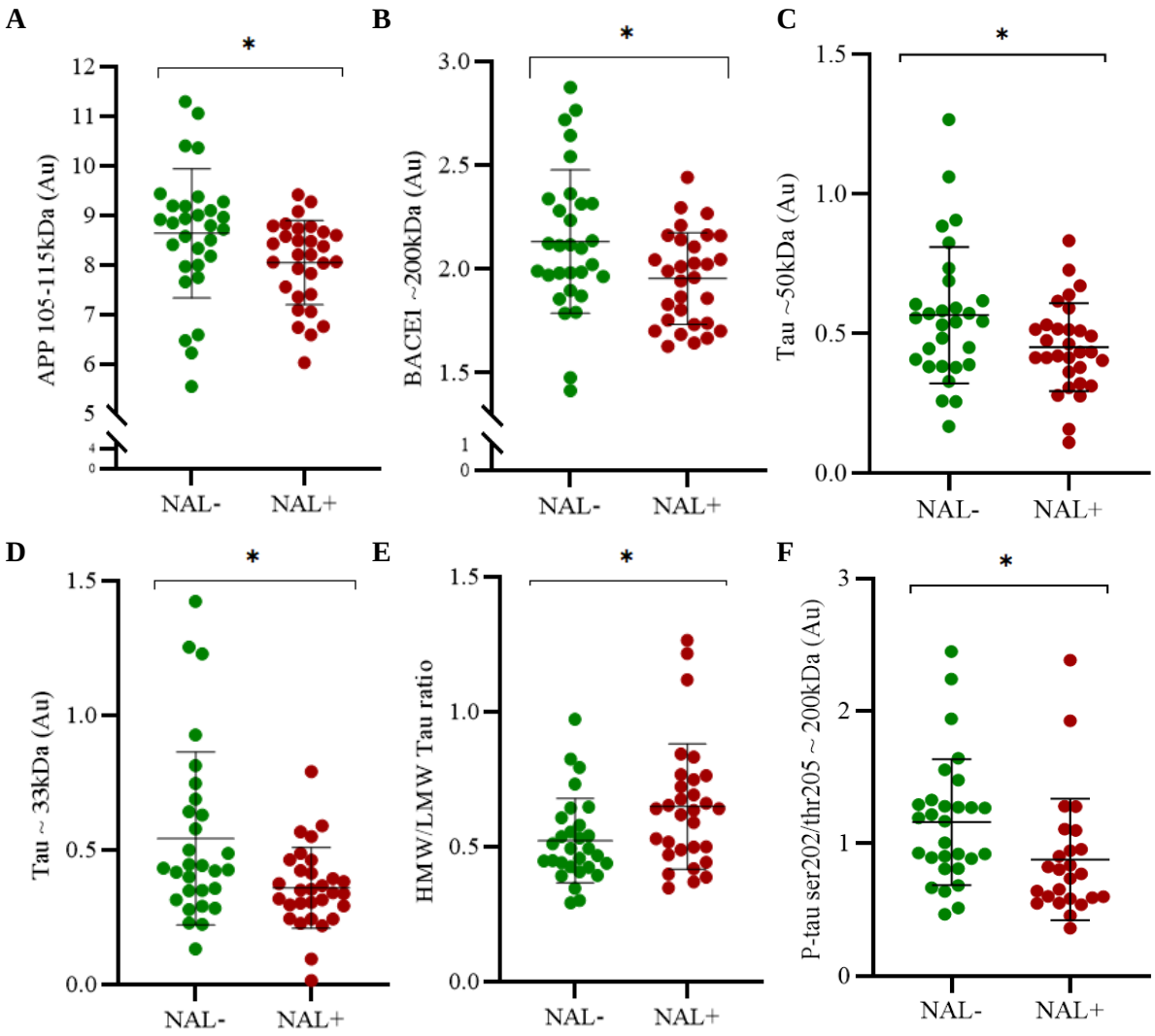


Figure 16. Comparison of platelet proteins between NAL- and NAL+ groups. Box plots show significantly lower mean levels in NAL+ for A) APP 105-115kDa (p=0.024), B) BACE1 ~200kDa (p=0.020), C) Tau ~50kDa (p=0.036), D) Tau ~33kDa (p=-0.015) and, F) p-tau ser202/thr205 ~200kDa (p=0.032). Significantly higher mean levels of E) HMW/LMW tau ratio (p=0.018) are seen in NAL+ compared to NAL-. p*<0.05. Abbreviations: APP, amyloid precursor protein; APPr, APP 105-115kDa/108-110kDa ratio; BACE1, beta-secretase; HMW/LMW, high molecular weight/low molecular weight; p-tau ser202/thr205, phosphorylated tau at serine 202/threonine 205; NAL, neocortical amyloid-beta load; NAL-, low neocortical amyloid-beta load; NAL+, high neocortical amyloid-beta load; Au, Arbitrary units.

3.5. Correlation between platelet proteins and NAL

Correlations are described in Table 7 and are graphically depicted in Figure 17. The APP 105-115kDa isoform showed an inverse correlation with NAL (p=0.008), as did the APPr (p=0.005) and the BACE1 ~200kDa band (p=0.012). BACE1 ~60kDa saw a negative trend (p=0.081) with NAL. Tau ~50kDa was inversely correlated with NAL (p=0.0015) as was tau ~33kDa (p=0.024). The HMW/LMW tau ratio saw a positive correlation with NAL (p=0.003). P-tau ser202/thr205 ~200kDa band showed an inverse correlation with NAL (p=0.001). When the platelet proteins were stratified based on NAL (Figure 18), APP 105-115kDa (p=0.001), APPr (p=0.011), tau ~50kDa, (p=0.001) and p-tau ser202/thr205 ~200kDa

(p=0.003) were inversely correlated with NAL within the NAL-, group. HMW/LMW tau ratio was positively correlated with NAL within the NAL- group (p=0.022) No significant correlations were observed between the platelet proteins and NAL within the NAL+ group.

Table 7. Correlations between platelet proteins and NAL (SUVR)

Platelet Proteins	**$r_s(p)$**
APP 105-115kDa	**-0.337 *(0.008)**
APP 80-100kDa	-0.139 (0.289)
APPr	**-0.357 *(0.005)**
ADAM10	-0.210 (0.108)
BACE1 ~200kDa	**-0.333 *(0.012)**
BACE1 ~160kDa	0.158 (0.228)
BACE1 ~110kDa	-0.165 (0.209)
BACE1 ~80kDa	-0.107 (0.415)
BACE1 ~60kDa	-0.227 (0.081)
Tau ~ 200kDa	-0.049 (0.715)
Tau ~ 110kDa	0.055 (0.679)
Tau ~ 80kDa	0.008 (0.954)
Tau ~ 55kDa	-0.153 (0.249)
Tau ~ 50kDa	**-0.316 *(0.015)**
Tau ~ 33kDa	**-0.293 *(0.024)**
T-tau	-0.144 (0.299)
HMW/LMW tau	**0.383 *(0.003)**
P-tau ser202/thr205 ~ 200kDa	**-0.440 *(0.001)**
P-tau ser202/thr205 ~80kDa	-0.053 (0.706)
P-tau ser202/thr205 ~ 55kDa	-0.010 (0.944)
P-tau ser202/thr205 ~ 33kDa	-0.182 (0.187)
P-tau	-0.215 (0.199)
P-tau/t-tau	-0.090 (0.518)

Table 7 shows the correlations of platelet proteins with NAL. Significant correlations are seen with APP 105-115kDa, APPr, BACE1 ~200kDa, tau ~50kDa, tau ~33kDa, HMW/LMW tau ratio and, p-tau ser202/thr205 ~200kDa. *p<0.05. Abbreviations: NAL, neocortical amyloid-beta load; SUVR, standardised uptake value ratio; APP, amyloid precursor protein; APPr, APP 105-115kDa/108-110kDa ratio; ADAM10, a Disintegrin and metalloproteinase; BACE1, beta-secretase; HMW/LMW, high molecular weight/low molecular weight; t-tau, sum of all tau bands; p-tau ser202/thr205, phosphorylated tau at serine 202/threonine 205; p-tau, sum of all phosphorylated tau bands at ser202/thr205; p-tau/t-tau, sum of phosphorylated tau bands at serine 202/threonine 205/sum of all tau bands.

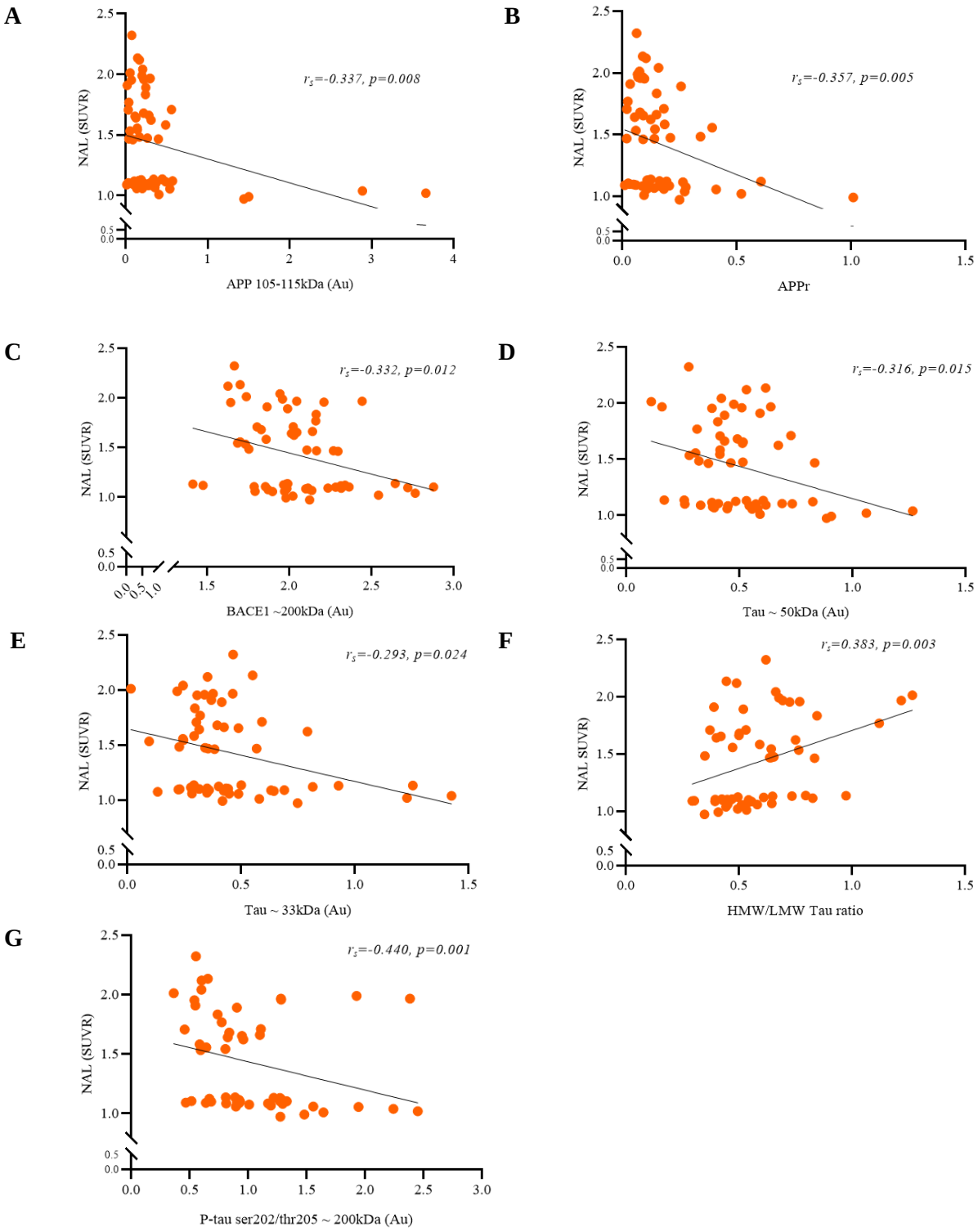
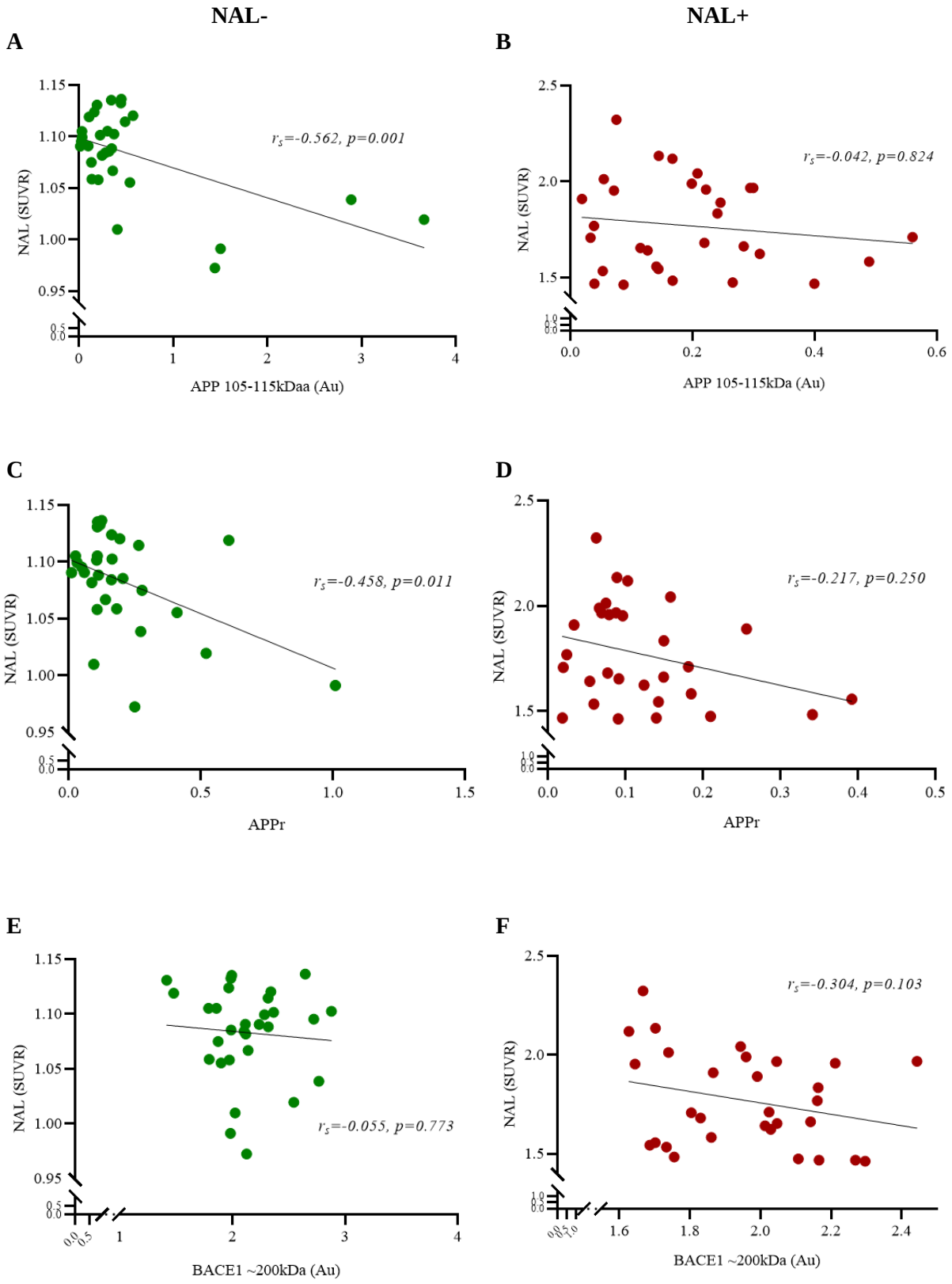
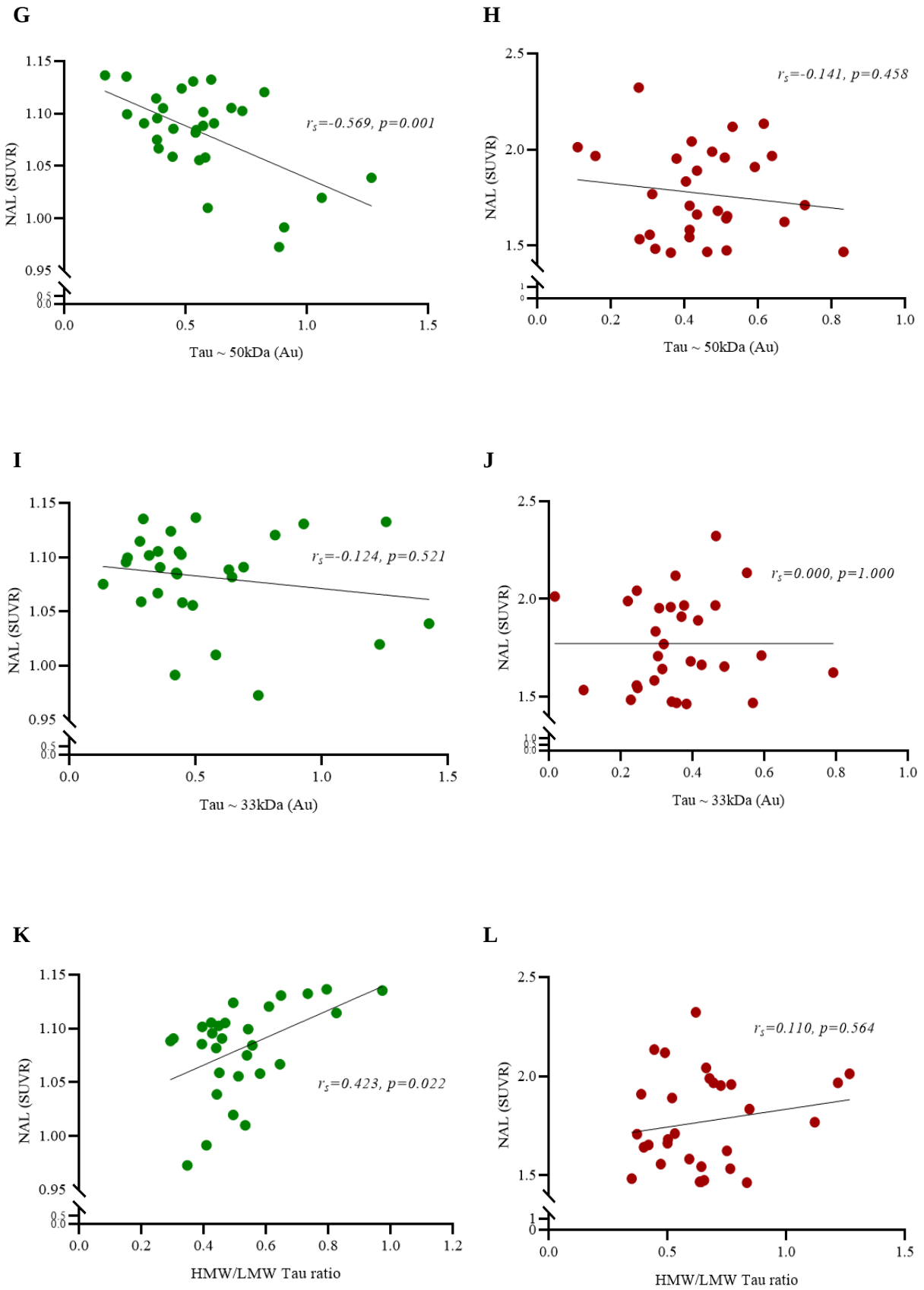


Figure 17. Correlation of NAL with platelet proteins in all participants. Significant negative correlations with NAL are observed with A) APP 105-115kDa, B) APPr, C) BACE1 ~200kDa, D) tau ~50kDa, E) tau ~33kDa and, G) p-tau ser202/thr205 ~200kDa. A significant positive correlation is seen with F) HMW/LMW tau ratio and NAL. Abbreviations: NAL, neocortical amyloid-beta load; SUVR, standardised uptake value ratio; APP, amyloid precursor protein; APPr, APP 105-115kDa/108-110kDa ratio; BACE1, beta-secretase; HMW/LMW, high molecular weight/low molecular weight; p-tau ser202/thr205, phosphorylated tau at serine 202/threonine 205; Au, Arbitrary units.


NAL-
NAL+
A
B
C
D
E
F
NAL (SUVR)
APP 105-115kDaa (Au)
APP 105-115kDa (Au)
APPr
BACE1 ~200kDa (Au)
r_s=-0.562, p=0.001
r_s=-0.042, p=0.824
r_s=-0.458, p=0.011
r_s=-0.217, p=0.250
r_s=-0.055, p=0.773
r_s=-0.304, p=0.103


G
H
I
J
K
L
NAL (SUVR)
Tau ~ 50kDa (Au)
Tau ~ 33kDa (Au)
HMW/LMW Tau ratio
r_s=-0.569, p=0.001
r_s=-0.141, p=0.458
r_s=-0.124, p=0.521
r_s=0.000, p=1.000
r_s=0.423, p=0.022
r_s=0.110, p=0.564

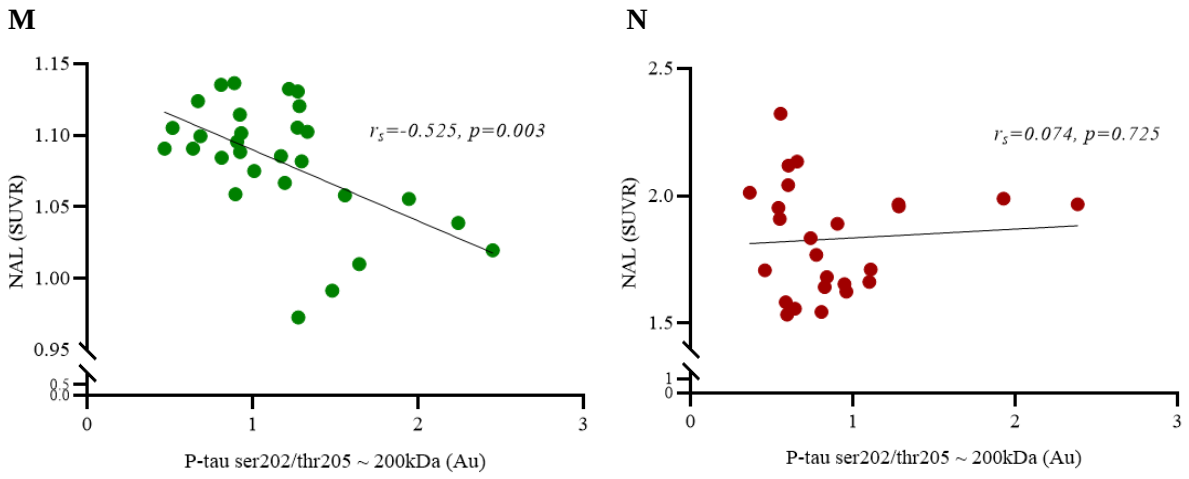


Figure 18. Correlations between platelet proteins and NAL, after stratification of participants based on NAL status. Significant correlations were seen in the NAL- group between NAL and A) APP 105-115kDa, C) APPr, G) tau ~50kDa, K) HMW/LMW tau ratio and, M) p-tau ser202/thr205 ~200kDa. No significant correlations were observed in the NAL+ group for the platelet proteins (B, D, F, H, J, L, N). Abbreviations: NAL, neocortical amyloid-beta load; SUVR, standardised uptake value ratio; APP, amyloid precursor protein; APPr, amyloid precursor protein isoform ratio; BACE1, beta-secretase; p-tau ser202/thr205, phosphorylated tau at site serine 202/threonine 205; HMW/LMW, high molecular weight/low molecular weight; Au, Arbitrary units.

3.6. Evaluation of platelet proteins in distinguishing between NAL- and NAL+

APP 105-115kDa, tau ~33kDa and HMW/LMW tau ratio were evaluated as potential indictors to predict NAL status. A 'base model' was generated, which included the AD risk factors age, gender and *APOE* ε4 allele status. This 'base model' was compared with the model comprising of the 'base model' + the inclusion of APP 105-115kDa, tau ~33kDa and HMW/LMW tau ratio (Figure 19). The area under the receiver operating characteristic (ROC) curve (AUC) for the 'base + APP 105-115kDa, tau ~33kDa and HMW/LMW tau ratio model' (AUC = 83.44%, accuracy = 80%, specificity = 80% at sensitivity = 80%) outperformed the 'base model' (AUC = 77%, accuracy = 73.35%, specificity = 66.7% at sensitivity = 80%) in distinguishing NAL+ from NAL- participants.

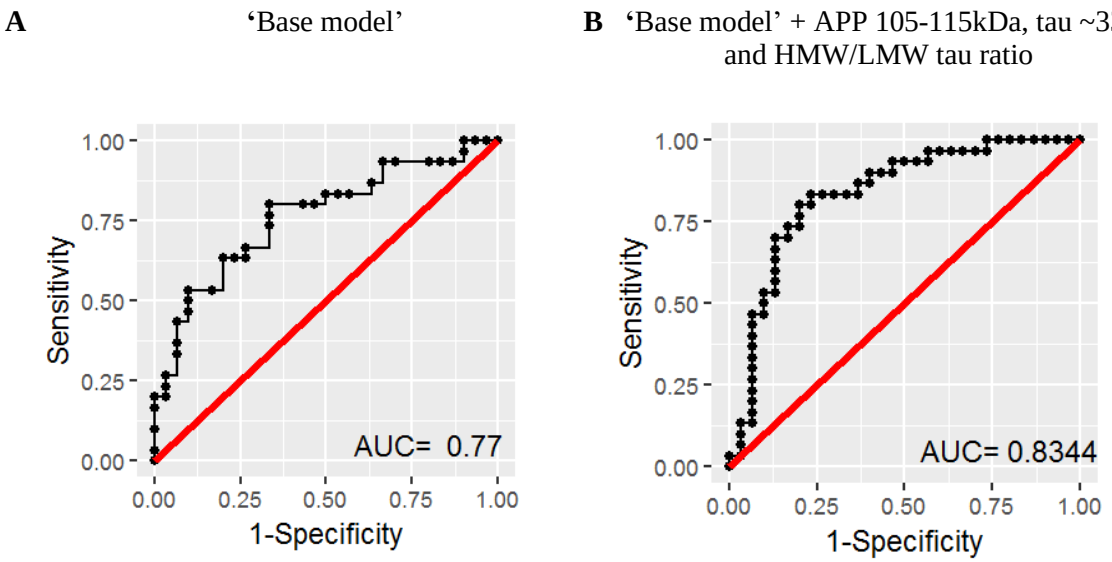


Figure 19. Receiver operating characteristic curves for the prediction of NAL- vs NAL+ participants. The area under the ROC curve (AUC) for the 'base + APP 105-115kDa, tau ~33kDa and HMW/LMW tau ratio model' (AUC = 83.44%, accuracy = 80%, specificity = 80% at sensitivity = 80%) outperformed the 'base model' (AUC = 77%, accuracy = 73.35%, specificity = 66.7% at sensitivity = 80%) in distinguishing NAL+ from NAL- participants. Abbreviations: APP, amyloid precursor protein; HMW/LMW, high molecular weight/low molecular weight; NAL, neocortical amyloid-beta load; ROC, receiver operating characteristic.

3.7. Correlations between platelet proteins and cognitive performance

Cognitive performance was assessed using 1) the verbal and visual episodic memory and, 2) the working memory and executive function domains. Additionally, global cognitive performance comprising MMSE, verbal and visual episodic memory and, working memory and executive function, was assessed.

3.7.1. Correlations between platelet proteins and verbal and visual episodic memory

There were significant inverse correlations between tau ~80kDa (p=0.018), tau ~110kDa (p=0.027) and the HMW/LMW tau ratio (p=0.043) with verbal and visual episodic memory (Table 8, Figure 20). Following stratification of the data into NAL- and NAL+, no significant correlations were seen between the platelet proteins and verbal and visual episodic memory. A negative trend, however, was observed with tau ~80kDa (r_s=-0.333, p=0.072).

Table 8. Correlations between platelet proteins and verbal and visual and episodic memory

Platelet Proteins	$r_s(p)$
ADAM10	0.084 (0.538)
APP 105-115kDa	-0.050 (0.717)
APP 80-100kDa	-0.055 (0.686)
APPr	0.043 (0.751)
BACE1 ~200kDa	0.135 (0.319)
BACE1 ~160kDa	-0.197 (0.146)
BACE1 ~110kDa	-0.103 (0.451)
BACE1 ~80kDa	-0.110 (0.421)
BACE1 ~60kDa	-0.092 (0.499)
Tau ~ 200kDa	-0.012 (0.925)
Tau ~ 110kDa	**-0.288 *(0.027)**
Tau ~ 80kDa	**-0.308 *(0.018)**
Tau ~ 55kDa	0.000 (1.000)
Tau ~ 50kDa	-0.061 (0.648)
Tau ~ 33kDa	-0.159 (0.230)
T-tau	-0.123 (0.375)
HMW/LMW tau	**-0.264 *(0.043)**
P-tau ser202/thr205 ~ 200kDa	-0.024 (0.861)
P-tau ser202/thr205 ~80kDa	-0.179 (0.195)
P-tau ser202/thr205 ~ 55kDa	-0.086 (0.535)
P-tau ser202/thr205 ~ 33kDa	-0.119 (0.391)
P-tau	-0.121 (0.382)
P-tau/t-tau	0.004 (0.979)

Table 8 shows the correlations between platelet proteins and verbal and visual episodic memory. The composite z-scores of verbal and visual episodic memory were calculated from the mean of the z-scores of RAVLT List A, RAVLT short delay, RAVLT long delay, LM I, LMII, RCFT 3 min and RCFT 30 min. Significant correlations were present with tau ~110kDa, tau ~80kDa and HMW/LMW tau ratio *p<0.05. Abbreviations: APP, amyloid precursor protein; APPr, APP 105-115kDa/108-110kDa ratio; ADAM10, a Disintegrin and metalloproteinase; BACE1, beta-secretase; HMW/LMW, high molecular weight/low molecular weight; t-tau, sum of all tau bands; p-tau ser202/thr205, phosphorylated tau at serine 202/threonine 205; p-tau, sum of all phosphorylated tau bands at serine202/threonine 205; p-tau/t-tau, sum of phosphorylated tau bands at serine 202/threonine 205/sum of all tau bands; LT, Rey Auditory Verbal Learning Test; LM, Logical Memory; RCFT, Rey Complex Figure Test.

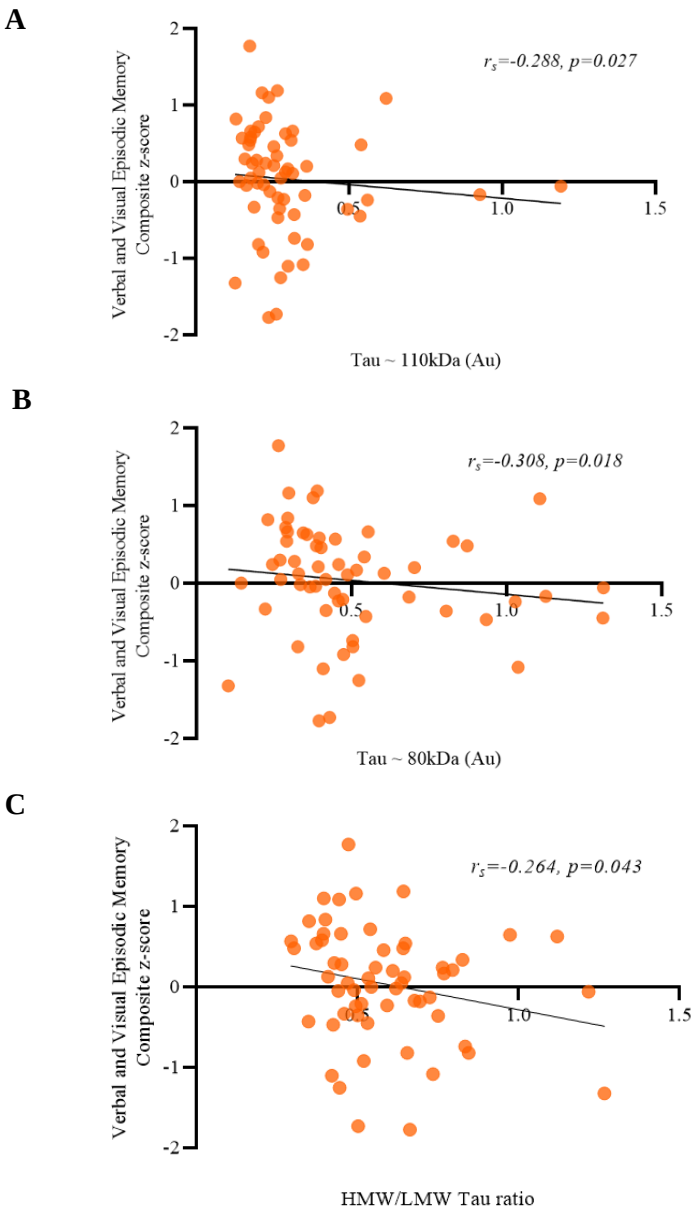


Figure 20. Correlations between platelet proteins and verbal and visual episodic memory. The composite z-scores of verbal and visual episodic memory was calculated from the mean of the z-scores of RAVLT List A, RAVLT short delay, RAVLT long delay, LM I, LMII, RCFT 3 min and RCFT 30 min. Verbal and visual episodic memory inversely correlates with A) tau ~110kDa, B) tau ~80kDa and, C) HMW/LMW tau ratio. Abbreviations: HMW/LMW, high molecular weight/low molecular weight; RAVLT, Rey Auditory Verbal Learning Test; LM, Logical Memory; RCFT, Rey Complex Figure Test; Au, Arbitrary units.

3.7.2. Correlation between platelet proteins and working memory and executive function

No significant correlations were seen between platelet proteins and working memory and executive function in any of the study participants (Appendix B).

3.7.3. Correlation between platelet proteins and global cognitive performance

Overall global cognitive performance was assessed from the global composite z-scores calculated from the mean z-scores of MMSE, verbal and visual episodic memory, and working memory and executive function. Only the HMW/LMW tau ratio showed a significant inverse correlation with global cognitive performance (p=0.047) (Table 9, Figure 21). When the data was stratified into NAL- and NAL+, the HMW/LMW tau ratio did not significantly correlate with global cognitive performance in either NAL- or NAL+.

Table 9. Correlations of global cognitive performance with platelet proteins

Platelet Proteins	r_s (*p*)
APP 105-115kDa	0.030 (0.817)
APP 80-100kDa	0.027 (0.839)
APPr	0.114 (0.387)
ADAM10	0.116 (0.377)
BACE1 ~200kDa	0.163 (0.212)
BACE1 ~160kDa	-0.095 (0.470)
BACE1 ~110kDa	-0.049 (0.710)
BACE1 ~80kDa	0.033 (0.803)
BACE1 ~60kDa	0.025 (0.852)
Tau ~ 200kDa	0.093 (0.482)
Tau ~ 110kDa	-0.166 (0.209)
Tau ~ 80kDa	-0.191 (0.147)
Tau ~ 55kDa	0.096 (0.470)
Tau ~ 50kDa	0.020 (0.881)
Tau ~ 33kDa	-0.049 (0.713)
T-tau	0.019 (0.892)
HMW/LMW tau	**-0.259 *(0.047)**
P-tau ser202/thr205 ~ 200kDa	0.133 (0.337)
P-tau ser202/thr205 ~80kDa	-0.139 (0.317)
P-tau ser202/thr205 ~ 55kDa	-0.046 (0.744)
P-tau ser202/thr205 ~ 33kDa	-0.084 (0.547)
P-tau	-0.019 (0.891)
P-tau/t-tau	-0.005 (0.973)

Table 9 shows the correlation of global cognitive performance with platelet proteins. Global cognitive performance was assessed from the global composite z-scores calculated from the mean z-scores of MMSE, verbal and visual episodic memory, and working memory and executive function. Only HMW/LMW tau ratio was significantly inversely correlated with global cognitive performance. *p<0.05. Abbreviations: APP, amyloid precursor protein; APPr, APP 105-115kDa/108-110kDa ratio; ADAM10, a Disintegrin and metalloproteinase; BACE1,beta-secretase; HMW/LMW, high molecular weight/low molecular weight; t-tau, sum of all tau bands; p-tau ser202/thr205, phosphorylated tau at serine 202/threonine 205; p-tau, sum of all phosphorylated tau bands at serine202/threonine 205; p-tau/t-tau, sum of phosphorylated tau bands at serine 202/threonine 205/sum of all tau bands.

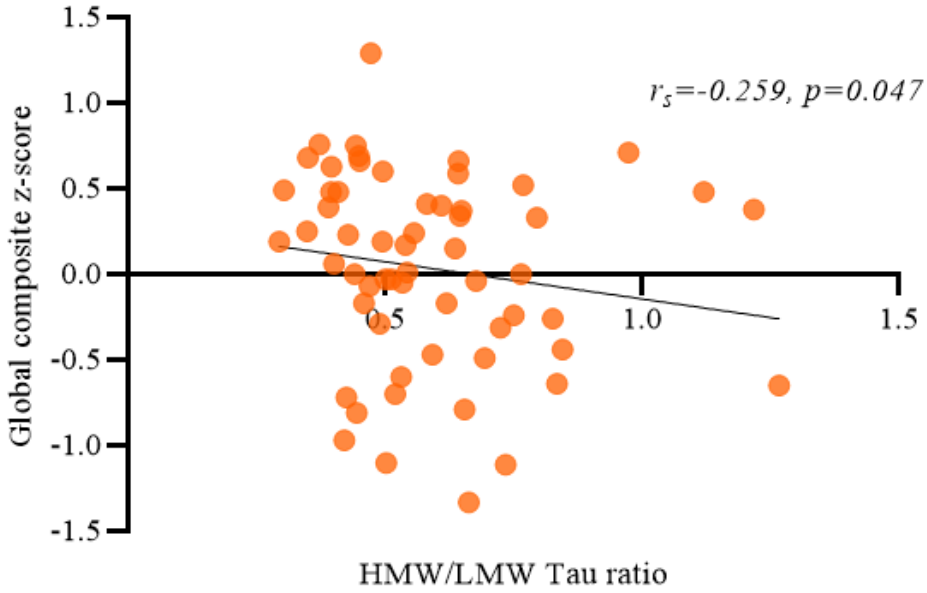


Figure 21. Correlation between HMW/LMW tau ratio and global cognitive performance. Global cognitive performance was assessed from the global composite z-scores calculated from the mean z-scores of MMSE, verbal and visual episodic memory, and working memory and executive function. HMW/LMW tau ratio significantly inversely correlated with global cognitive performance. Abbreviations: HMW/LMW, high molecular weight/low molecular weight.

3.8. Associations of platelet proteins between SMC- and SMC+

Associations are described in Table 10 and graphically presented in Figure 22. Tau ~55kDa (p=0.004), t-tau (p=0.018), p-tau ser202/thr205 ~200kDa (p=0.047) and p-tau (p=0.022) were significantly higher in the SMC+ group and, remained significant after adjusting for covariates age, gender and *APOE* ε4 allele status. Trends of higher mean levels in the SMC+ group were observed for tau ~80kDa and tau ~50kDa (p=0.053 and p=0.052 respectively). However, after adjusting for age, gender and *APOE* ε4 allele status, both tau ~80kDa and tau ~50kDa displayed significantly higher mean levels in the SMC+ group (p=0.027 and p=0.033 respectively). P-tau ser202/thr205 ~55kDa showed a trend of higher mean levels in the SMC+ group (p=0.061), with this trend remaining after adjusting for the covariates (p=0.072).

Table 10. Associations of platelet proteins between SMC- and SMC+

Platelet proteins	Total (n=60)	SMC- (n=13)	SMC+ (n=47)	*p*	*p*[a]
APP 105-115kDa	0.39 ± 0.63	0.23 ± 0.15	0.43 ± 0.69	0.353	0.346
APP 80-100kDa	2.22 ± 1.75	1.80 ± 0.93	2.33 ± 1.89	0.373	0.366
APPr	0.17 ± 0.16	0.14 ± 0.09	0.17 ± 0.18	0.527	0.556
ADAM10	1.56 ± 0.66	1.57 ± 0.72	1.55 ± 0.65	0.921	0.909
BACE1 ~200kDa	2.05 ± 0.31	2.06 ± 0.31	2.05 ± 0.31	0.920	0.744
BACE1 ~160kDa	0.56 ± 0.26	0.53 ± 0.25	0.57 ± 0.27	0.617	0.550
BACE1 ~110kDa	0.81 ± 0.31	0.72 ± 0.16	0.83 ± 0.33	0.274	0.194
BACE1 ~80kDa	0.73 ± 0.40	0.66 ± 0.34	0.75 ± 0.41	0.489	0.421
BACE1 ~60kDa	0.71 ± 0.38	0.67 ± 0.35	0.72 ± 0.39	0.699	0.667
Tau ~ 200kDa	0.91 ± 0.38	0.78 ± 0.22	0.95 ± 0.40	0.288	0.198
Tau ~ 110kDa	0.29 ± 0.18	0.23 ± 0.05	0.31 ± 0.20	0.143	0.062
Tau ~ 80kDa	0.50 ± 0.28	0.37 ± 0.10	0.54 ± 0.30	0.053	***0.027**
Tau ~ 55kDa	1.88 ± 0.81	1.35 ± 0.43	2.03 ± 0.84	***0.004**	***0.002**
Tau ~ 50kDa	0.51 ± 0.21	0.41 ± 0.15	0.54 ± 0.22	0.052	***0.033**
Tau ~ 33kDa	0.45 ± 0.26	0.35 ± 0.15	0.48 ± 0.28	0.123	0.130
T-tau	5.61 ± 2.15	4.27 ± 1.08	5.96 ± 2.23	***0.018**	***0.012**
HMW/LMW tau	0.59 ± 0.21	0.66 ± 0.25	0.57 ± 0.19	0.158	0.181
P-tau ser202/thr205 ~ 200kDa	1.03 ± 0.48	0.77 ± 0.35	1.10 ± 0.49	***0.047**	***0.025**
P-tau ser202/thr205 ~80kDa	0.81 ± 0.43	0.64 ± 0.50	0.86 ± 0.41	0.145	0.170
P-tau ser202/thr205 ~ 55kDa	1.01 ± 0.39	0.82 ± 0.34	1.06 ± 0.39	0.061	0.072
P-tau ser202/thr205 ~33kDa	0.25 ± 0.15	0.19 ± 0.14	0.27 ± 0.16	0.122	0.141
P-tau	3.10 ± 1.12	2.41 ± 1.06	3.28 ± 1.08	***0.022**	***0.006**
P-tau/t-tau	0.57 ± 0.17	0.55 ± 0.16	0.58 ± 0.17	0.651	0.673

Table 10 shows the comparisons of platelet proteins between SMC- and SMC+. Tau ~55kDa, t-tau, p-tau ser202/thr205 ~200kDa and, p-tau were significantly higher in the SMC+ group compared to the SMC- group, before and after adjusting for covariates age, gender and *APOE* ε4 allele status. Tau ~80kDa and tau ~50kDa were significant only after adjusting for covariates. Abbreviations: APP, amyloid precursor protein; APPr, APP 105-115kDa/108-110kDa ratio; ADAM10, a Disintegrin and metalloproteinase; BACE1, beta-secretase; HMW/LMW, high molecular weight/low molecular weight; t-tau, sum of all tau bands; p-tau ser202/thr205, phosphorylated tau at serine 202/threonine 205**;** p-tau, sum of all phosphorylated tau bands at serine202/threonine 205; p-tau/t-tau, sum of phosphorylated tau bands at serine 202/threonine 205/sum of all tau bands; SMC-, non-subjective memory complainers; SMC+, subjective memory complainers.

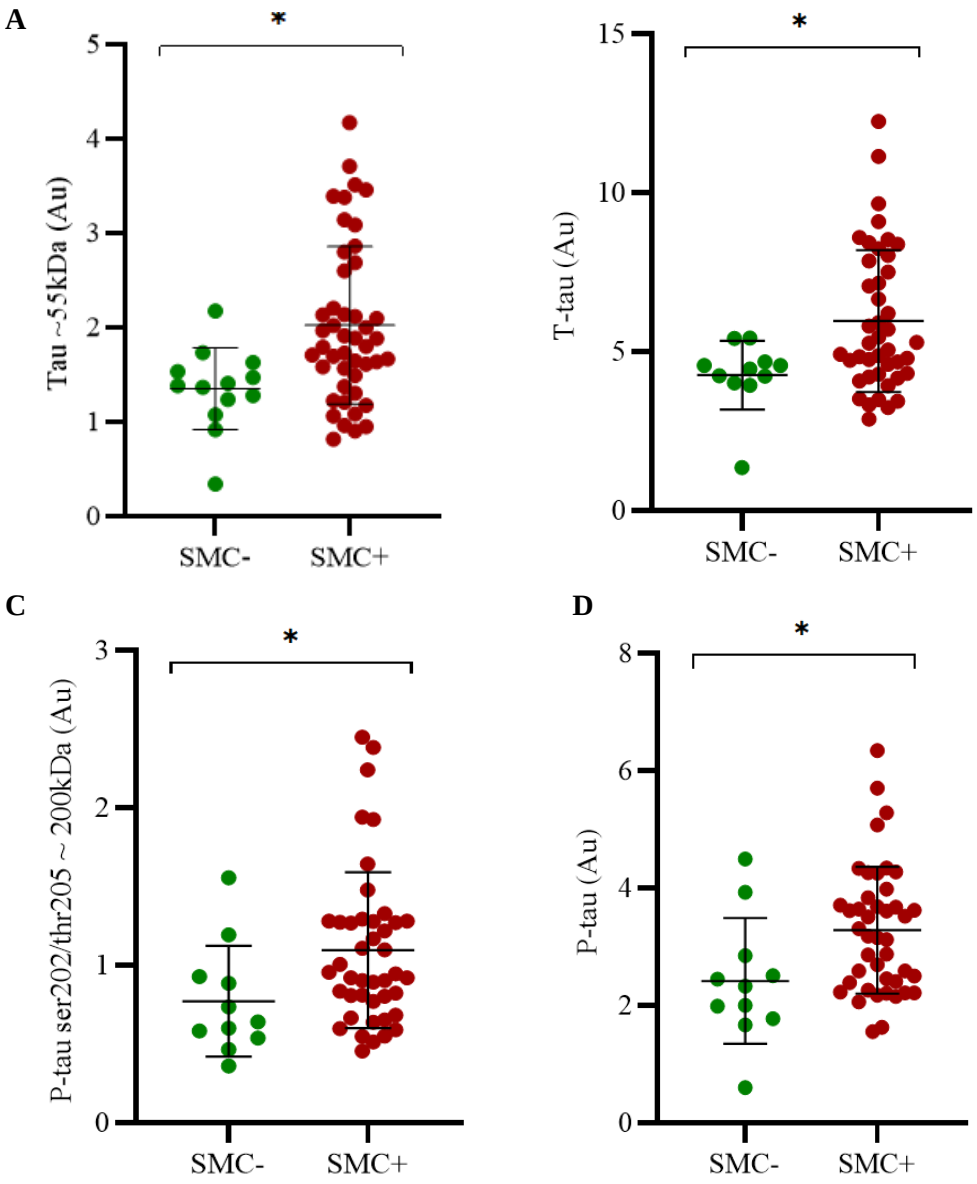


Figure 22. Associations of platelet proteins between SMC+ and SMC-. Significantly higher mean levels in the SMC+ group were seen with A) tau ~55kDa (p=0.004) B) t-tau (p=0.018), C) p-tau ser202/thr205 ~200kDa (p=0.047) and D) p-tau (p=0.022). Abbreviations: t-tau, sum of all tau bands; p-tau ser202/thr205, phosphorylated tau at serine 202/threonine 205; p-tau; sum of all phosphorylated tau bands at serine 202/threonine 205; SMC-, non-subjective memory complainers; SMC+, subjective memory complainers; Au, Arbitrary units.

3.9. Correlations between platelet proteins and plasma Aβ

Correlations are presented in Table 11. No significant correlations were observed between platelet proteins and plasma $A\beta_{40}$ and $A\beta_{42}$. However, an inverse trend was seen between p-tau ser202/thr205 ~55kDa (p=0.065) and plasma $A\beta_{42}$. P-tau ser 202/thr205 ~55kDa presented a significant inverse correlation with plasma $A\beta_{42}$,/ $A\beta_{40}$ ratio (p=0.029) (Figure 23). When the data was stratified into NAL- and NAL+, p-tau ser202/thr205 ~55kDa presented a non-significant inverse trend with plasma $A\beta_{42}$,/ $A\beta_{40}$ in NAL+ (r_s=-0.385, p=0.069).

Table 11. Correlations between platelet proteins and plasma Aβ

Platelet Proteins	Plasma $A\beta_{40}$ – r_s (*p*)	Plasma $A\beta_{42}$ – r_s (*p*)	Plasma $A\beta_{42}$,/$A\beta_{40}$ – r_s (*p*)
APP 105-115kDa	-0.081 (0.543)	-0.035 (0.793)	0.009 (0.947)
APP 80-100kDa	-0.159 (0.232)	-0.089 (0.506)	-0.028(0.838)
APPr	-0.012 (0.929)	0.022 (0.869)	0.037 (0.789)
ADAM10	-0.052 (0.696)	0.030 (0.825)	0.045 (0.744)
BACE1 ~200kDa	0.085 (0.528)	0.006 (0.964)	-0.088 (0.519)
BACE1 ~160kDa	0.139 (0.298)	0.028 (0.835)	-0.172 (0.206)
BACE1 ~110kDa	-0.170 (0.201)	-0.123(0.357)	0.020 (0.882)
BACE1 ~80kDa	-0.040 (0.768)	-0.089 (0.505)	-0.197 (0.146)
BACE1 ~60kDa	0.061 (0.648)	0.141 (0.292)	0.045 (0.743)
Tau ~ 200kDa	0.100 (0.461)	0.127 (0.346)	0.049 (0.723)
Tau ~ 110kDa	0.118 (0.383)	0.148 (0.272)	-0.022 (0.873)
Tau ~ 80kDa	0.088 (0.517)	0.096 (0.479)	-0.051 (0.712)
Tau ~ 55kDa	0.025 (0.855)	-0.079 (0.557)	-0.205 (0.133)
Tau ~ 50kDa	-0.008 (0.950)	0.059 (0.663)	-0.014 (0.921)
Tau ~ 33kDa	-0.067 (0.623)	0.074 (0.585)	0.149 (0.277)
T-tau	-0.103 (0.469)	-0.146 (0.303)	-0.121 (0.403)
HMW/LMW tau	0.094 (0.487)	0.202 (0.131)	0.129 (0.348)
P-tau ser202/thr205 ~ 200kDa	-0.114 (0.420)	0.023 (0.873)	0.143 (0.323)
P-tau ser202/thr205 ~80kDa	0.141 (0.319)	0.110 (0.438)	-0.152 (0.292)
P-tau ser202/thr205 ~ 55kDa	-0.139 (0.324)	-0.258 (0.065)	**-0.309 *(0.029)**
P-tau ser202/thr205 ~ 33kDa	0.021 (0.882)	0.016 (0.909)	0.012 (0.934)
P-tau	-0.034 (0.809)	-0.010 (0.946)	-0.095 (0.512)
P-tau/t-tau	0.120 (0.397)	0.087 (0.540)	-0.014 (0.923)

Table 11 shows correlations between platelet proteins and plasma Aβ. P-tau ser202/thr205 ~55kDa is significantly correlated with plasma $A\beta_{42}$,/$A\beta_{40}$. *p<0.05. Abbreviations: APP, amyloid precursor protein; APPr, APP 105-115kDa/108-110kDa ratio; ADAM10, a Disintegrin and metalloproteinase; BACE1, beta-secretase; HMW/LMW, high molecular weight/low molecular weight; t-tau, sum of all tau bands; p-tau ser202/thr205, phosphorylated tau at serine 202/threonine 205; p-tau, sum of all phosphorylated tau bands at serine202/threonine 205; p-tau/t-tau, sum of phosphorylated tau bands at serine 202/threonine 205/sum of all tau bands.

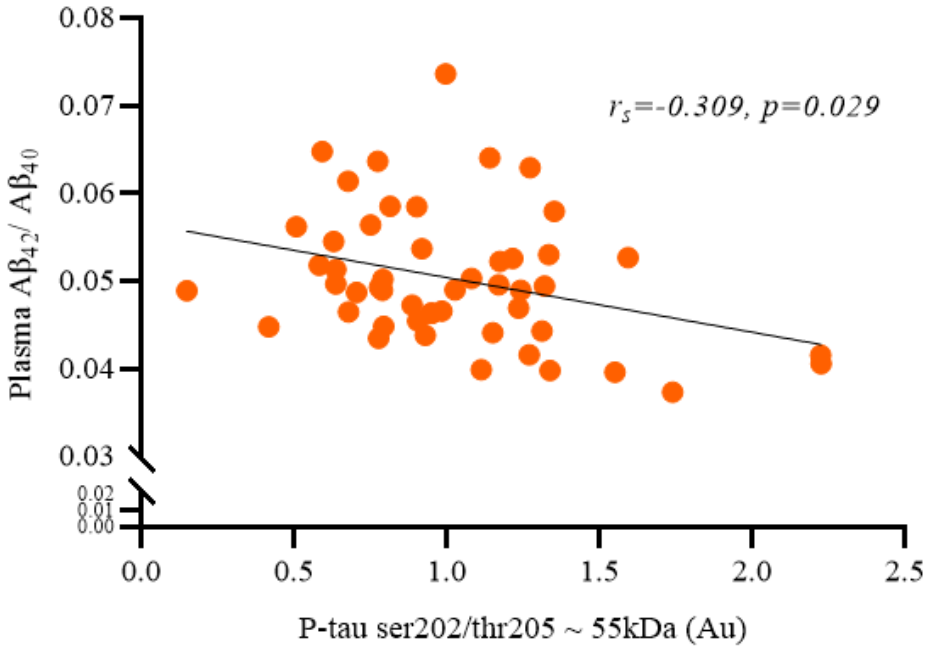


Figure 23. Correlation between p-tau ser202/thr205 ~55kDa with plasma Aβ42,/ Aβ40 ratio. A significant negative correlation was shown between plasma $A\beta_{42}$,/$A\beta_{40}$ and p-tau ser202/thr205 ~55kDa. Abbreviations: Aβ, amyloid-beta; p-tau ser202/thr205, phosphorylated tau at serine 202/threonine 205; Au, Arbitrary units.

3.10. Correlations between platelet proteins and plasma NFL

No significant correlations or trends were observed between plasma NFL and the platelet proteins (Appendix C).

Chapter 4: Discussion

The current study aimed to investigate whether the selected platelet proteins known to be involved in the molecular pathology of AD, namely APP, APPr, ADAM10, BACE1, tau, t-tau, HMW/LMW tau ratio, p-tau ser202/thr205, p-tau and p-tau/t-tau ratio are associated with neocortical amyloid-beta load, cognitive performance and self-reported memory complaints, and how they compare with known potential plasma biomarkers such as amyloid-beta and neurofilament light chain levels. To summarise the most significant findings from this thesis, APP 105-115kDa and tau ~33kDa were significantly lower in the NAL+ group compared to the NAL- group, while HMW/LMW tau ratios were significantly higher in the NAL+ group compared to the NAL- group before and after adjusting for major AD risk factors namely age, gender and *APOE* ε4 allele status. Interestingly, platelet proteins APP 105-115kDa, tau ~33kDa and HMW/LMW tau ratio, in combination with AD risk factors age, gender and *APOE* ε4 allele status, distinguished between the NAL+ and NAL- individuals with an accuracy, sensitivity and specificity of 80%. Further on comparing platelet proteins between memory complainers and non-complainers, significantly higher mean levels of tau ~55kDa and p-tau ser202.thr205 ~200kDa were observed in the memory complainers before and after adjusting for the covariates age, gender and *APOE* ε4 allele status. Upon investigating the association between the platelet proteins and cognitive performance, the HMW/LMW tau ratio inversely correlated significantly with verbal and visual episodic memory and, overall global cognitive performance. Lastly, on investigating associations between platelet proteins and the extensively studied blood marker, plasma $A\beta_{42}/A\beta_{40}$ ratio, a significant inverse correlation with p-tau ser202/thr205 ~55kDa was observed.

No studies to the investigator's knowledge, has explored APP and APPr in individuals considered to be at high risk of AD, categorised by neocortical Aβ load. However, previous findings have shown that APPr (ratio of the upper APP band to the lower APP band) is decreased in MCI and AD compared to healthy controls, indicating that this platelet APP ratio is altered in the early stages of the disease (124,126,130,135,165). This difference in the ratio between groups could imply that either the upper APP band is decreased in MCI/AD compared to healthy controls or, the lower APP band is increased in MCI/AD compared to healthy controls. In the current study, the former is observed, as the APP 105-115kDa upper band showed a significant decrease in the NAL+ group over the NAL- group, even after adjusting for covariates age, gender and *APOE* ε4 allele status. However, the significantly reduced mean levels of the APP 105-115kDa isoform between NAL groups, may come as a reflection of the significant gender differences observed. Hence, the gender disparity in this current study needs to be addressed in a larger cohort with a more even distribution of genders, such as the Australian Imaging, Biomarker & Lifestyle Study of Ageing (AIBL) cohort of over 1200 participants followed longitudinally every 18 months over 13 years, to determine whether this promising finding is truly significant. Additionally, the 22C11 monoclonal antibody used in the current study to detect the APP isoforms, is not specific to APP

alone, as it can also bind to the homologous amyloid precursor-like proteins (APLP) (166). Therefore, the APP isoforms detected in the present study need to be confirmed with a more specific APP antibody, such as the Y188 antibody (167). Furthermore, the molecular weight range of the APP 105-115kDa isoform differs slightly from previous literature, where reports of a higher isoform of APP ~120-130kDa were made (110,145). However, the pattern of APP separation in this study remains the same, such that there is a higher fainter band, and two lower, darker bands (110,114). Discrepancies in the APP isoform molecular weights observed between previous studies and the current project, may be related to the variation in gels, running buffer and, molecular weight ladder used for SDS-PAGE.

Although APPr was not significantly reduced in the NAL+ group over the NAL- group, there was a decreased trend in the NAL+ group, which appears to follow the expected direction of lower APPr in MCI/AD individuals. The decrease in the mature platelet APP 105-115kDa and APPr, may be attributed to alterations in mature APP processing in AD. This can be supported by previous reports that have shown that APP trafficking is different in AD compared to controls, for which AD patients withhold more APP at the platelet membrane surface (125). Alternatively, long term tissue storage could have possibly resulted in degradation of the higher molecular weight bands. This possibility can be addressed using freshly obtained blood platelets.

In contrast to the present study, Bush *et al* (168) detected no significant differences in platelet APPr with the 22C11 antibody between sporadic AD and normal controls. However, it should be acknowledged, that the study was published almost 30 years ago, and therefore, it is possible that not all patients diagnosed as AD were AD patients, given the introduction of the gold standard biomarkers of the disease is fairly recent (169) and clinical diagnosis per se can range from 70 to 80% accuracy at best. Further contradictions to the present study's observation of reduced APP and APPr in NAL+, can be described in the investigations of platelet APPr in familial AD cases. For example, platelet APPr was studied in a cohort of *PSEN1* mutation carriers who were cognitively normal at the time, but were expected to develop AD in 3-25 years from the time of the study (170). The authors speculated that, because *PSEN1* mutation carriers have elevated brain Aβ years before cognitive decline (171), platelet APPr would be reduced. However interestingly, APPr was not significantly different between *PSEN1* groups. While the authors expected this outcome (seeing as how the results from their study were analogous to a previous sporadic AD study for which the degree of APP alterations was proportional to cognitive performance (6)), another study involving *PSEN2* mutation carriers suggested that *PSEN2* mutation carriers did not have reduced platelet APPr before or after clinical AD (172). Therefore, the differences in platelet APPr levels between those observed in the familial studies, and the current study, may be attributed to the slight differences in APP processing between sporadic and familial AD cases, and not from alterations in *APP* splicing (170). In support of this theory, reverse-transcription polymorphism chain reaction (RT-PCR) experiments with platelet messenger RNA (mRNA) levels of APP,695, APP751 and APP770 showed no difference in the three APP mRNA levels between sporadic AD and healthy controls (126).

Furthermore, correlation analyses revealed that APPr was significantly inversely correlated with the continuous NAL variable measured as a standardised uptake value ratio. In other words, decreasing APPr correlated with increasing NAL. The same significance was also observed for APP 105-115kDa. Although this aligns with the study's hypothesis, it should be highlighted that when the data were stratified by NAL groups to account for the clustering of data points observed, significance observed for both APP 105-115kDa and APPr were only present in the NAL- group. Plausible reasons to explain this may relate firstly, to the sample sizes (n=30). Secondly, the correlations observed for both APP 105-115kDa and APPr before stratifying the data, were extremely skewed. This skewness was perhaps driving the significance towards the NAL- group, as the same pattern of data distribution was seen in the NAL- group.

ADAM10 observed a trend of decreased mean levels in NAL+ compared to NAL- and was still prevalent after adjusting for age, gender and *APOE* ε4 allele status. Although not significant, this trend agrees with previous literature that states platelet ADAM10 is reduced in AD as compared to healthy controls (7,130,164). However, a key difference between the current study's findings to those previously witnessed, lie importantly with the classification of the investigated cohort. The studies that saw significant ADAM10 reductions, included individuals who were more advanced from the preclinical stage, as opposed to the KARVIAH cohort, wherein the subjects were preclinical and did not demonstrate alterations in cognitive performance.

The current study detected multiple BACE1 immunoreactive bands when analysed by western blot. The band at ~60kDa most likely represents the full length, immature form of BACE1 found in platelets (130). No reports to the investigator's knowledge thus far, however, have described the presence of the other detected BACE1 bands in platelets. Yet, in a study examining the expression of BACE1 in transfected kidney cell lines, a BACE1 band of ~70kDa was observed (175), which may reflect the band at ~80kDa. BACE1 is known to contain several *N*-linked glycosylation sites (176), therefore, it was proposed that the ~70kDa band (and therefore the ~80kDa band in this study) is a *N*-linked glycosylated BACE1 product. This study observed faint BACE1 immunoreactive species including the ~60kDa and ~80kDa bands, in comparison to a much larger, and intriguing BACE1 band of ~200kDa. No reports have previously described this BACE1 band in platelets. One theory to potentially explain this fascinating BACE1 species, postulates that the fainter, lower BACE1 proteins observed in this study may be aggregating to create the higher, and more intense BACE1 ~200kDa (177). BACE1 ~200kDa was seen to be significantly lower in NAL+ when compared to NAL-, disagreeing with the initial hypothesis, and the general assumption that BACE1 is elevated in sporadic AD patients of platelets and other biological tissue (178,179). However, because this band deviates from the expected molecular weight, it can be suggested that this hypothetically aggregated form may be disrupting, or inhibiting, the secretase's proteolytic activity of cleaving APP towards the toxic Aβ. In support of this idea, a recent study similarly demonstrated a high molecular weight BACE1 band that migrated at ~200kDa in microsomes from wild type mouse brain (177). The ~200kDa BACE1 complex reported in that study also suggested that this was an aggregation of the normal, lower molecular weight BACE1 secretase. Interestingly, the study

also identified a much larger BACE1/γ-secretase megacomplex of ~500kDa. On comparing the proteolytic activity of both BACE complexes, the BACE1 ~200kDa complex showed reduced activity when compared to the ~500kDa megacomplex. This provides for a plausible explanation for the BACE1 ~200kDa observed in this study, where perhaps the decreased levels of this BACE1 protein in the NAL+ group may reflect decreased APP proteolytic activity in the NAL+ group compared to the NAL- group. This HMW BACE1 species may also be a BACE1 trimer that has been previously seen in living HEK293T cells at ~200kDa (180). However, future studies to investigate the true identity of the BACE1 ~200kDa need to be performed. This may perhaps include utilising an anti-Presenilin 1 antibody that specifically recognises the Presenilin complexes that constitute the γ-secretase, to clarify whether or not the BACE1 ~200kDa complex contains γ-secretase. Additionally, mass-spectrometry would be beneficial in elucidating the exact molecular weight of the BACE1 ~200kDa complex. Perhaps a more convincing explanation for this unusually HMW BACE1 species, rests in the polyclonal nature of the BACE1 antibody utilised in this study. Polyclonal antibodies offer greater sensitivity, however, tend to demonstrate lower specificity, as they are capable of recognising different proteins with similar epitopes (181). However, a previous study that used this same BACE1 antibody in their analysis of platelet BACE1, did not report BACE1 protein species greater than 60kDa in MCI participants (130). Yet it can be argued that the representative western blot for BACE1 provided in that study was cropped, questioning whether they observed HMW BACE1, but did not report on it. While this may be possible, the non-specificity of the polyclonal BACE1 antibody can be challenged upon observing the immunodetection of BACE1 from the WT and APP23 mouse brain samples included in this study. Both mouse brain samples showed a band at ~60kDa, which most likely represents the immature precursor BACE1 protein, and a larger ~80kDa band representing the mature BACE1 (182). Therefore, the antibody appears specific, hence leaving to contemplate the identity of the BACE1 ~200kDa species. However, it should be noted that upon adjusting for age, gender and *APOE* ε4 allele status, the significant difference of this BACE1 protein between the NAL groups, disappeared, most likely as a result of the significant effect *APOE* ε4 allele status had on BACE1 ~200kDa. This leads to speculate whether this BACE1 ~200kDa retains any biological significance in the APP processing pathway, and AD pathology.

Microtubule assembly in platelets is postulated to maintain the cell's integrity, structure and discoid shape (183). It is thus conceivable, that tau phosphorylation and oligomerisation can destabilise and disassemble the platelet microtubule's infrastructure. In light of this, the current study investigated whether tau was associated with NAL. Corroborating previous studies (8,139,140) multiple tau immunoreactive species with differing molecular weights ranging between 33kDa and 200kDa were detected in all samples within the current study. Bands of particular interest included LMW tau bands of ~33kDa, ~50kDa, ~55kDa and ~60kDa, as well as HMW tau bands of ~80kDa, 110kDa and ~200kDa. Interestingly, only the lowest detected tau bands (~33kDa and ~50kDa), were significantly lower in the NAL+ group compared to the NAL- group. However, the ~50kDa tau species lost significance following adjustment for age, gender and *APOE* ε4 allele status. Nevertheless, while none of the HMW tau bands

appeared to be significantly associated with NAL, most of the HMW species demonstrated higher mean levels in the NAL+ group. However, the LMW platelet tau observed in this study were significantly reduced in the NAL+ group, leading to speculate why this may be so, given that CSF tau is considerably higher in AD patient's compared to controls (184). However, it could be posited that the HMW tau oligomers correspond to aggregates of the LMW tau bands (139), which may explain for the decreased levels of the LMW tau species in the NAL+ group compared to the NAL- group, as well as the significant inverse correlations observed for both tau ~50kDa and ~33kDa with NAL.

Although the HMW tau in this current study were not significantly different between NAL groups, previous reports of platelet tau have implied that mean levels of HMW tau were significantly higher in AD individuals compared to controls (8,139,140). The same pattern of significance has also been described in other biological tissue, such as the hippocampus and entorhinal cortex of human AD brains (185) and CSF (186). Similarly, LMW tau has been observed to be decreased in AD platelets (8,139,140) and detected in CSF of AD patients (187). Contributing to these intriguing findings, the levels of platelet HMW/LMW tau ratio was significantly higher in NAL+ compared to NAL-, which is in agreeance with the study's initial hypothesis. This significance was still evident even after adjusting for covariates, suggesting that the AD risk factors age, gender and *APOE* ε4 allele status did not influence the observed finding. Similar observations of increased HMW/LMW tau ratio have also been seen in platelets (8,139,140), and in human AD brain tissue (185), leading to speculate the potential clinical utility of this ratio, and the singular tau species as preclinical AD indicators. Yet, similar to the reasoning hypothesised for APP and BACE1, the unexpected significant correlation of HMW/LMW tau ratio observed in the NAL- group when the data were stratified, may be explained by 1) the small sample size, or 2) the change in data distribution. However, given that tau is regarded as a longitudinal marker for AD progression (188–190), and brain Aβ pathology (NAL) is considered as a reliable cross-sectional marker to identify AD (191,192), further investigations of the correlation between NAL and the tau species and the HMW/LMW tau ratio, should be considered, to elucidate these platelet proteins as potential indicators for cognitive state.

Alterations in memory presents one of the earliest cognitive changes in ageing, and is pathologically relevant with AD (193). Verbal and visual episodic memory is described as a long-term memory subset, where information can be stored for an infinite amount of time (194). Working memory, on the other hand, refers to the storage of information required to perform immediate and complex cognitive tasks (195). An average of both memory domains, with the addition of MMSE, creates a measure for overall global cognitive performance. Because neuropsychological testing is considered one of the initial assessments for diagnosing AD, it appeared logical to investigate whether there was an association between measures of cognitive performance and the platelet proteins. Tau ~80kDa, tau ~110kDa and the HMW/LMW tau ratio inversely correlated with verbal and visual episodic memory, while HMW/LMW tau ratio inversely correlated with overall global cognitive performance. These findings, in particular the HMW/LMW tau ratio, are in line with two previous studies wherein one study reported that the HMW/LMW the tau ratio in platelets was positively associated with brain atrophy (12), while

the other study reported that the HMW/LMW tau ratio in platelets was positively associated with disease severity (8). Whilst the data appear promising from these studies, and seem to agree with the current results, a difference exist between the studies with the techniques used to determine cognitive performance. While the current thesis measured cognitive severity based on the average z-scores of verbal and visual episodic memory and working memory and executive function, using a combination of the neuropsychological tests MMSE, RAVLT, LM I and II, DSST and D-KEFS, Farias et al. (8) evaluated the severity of cognition of the study's participants using MMSE and the global deterioration scale (196). Additionally, while the current study used the AD risk factors age, gender and *APOE* ε4 allele status as potential predictors of cognitive state, Farias *et al* (8) used 'years of education'. Years of education as an AD risk factor remains highly debated, with some literature suggesting lower education is associated with increased dementia risk, whilst others debated the association (as reviewed in (197)). Therefore, the legitimacy of the reported correlation and the predictability of platelet HMW/LMW tau ratio as a biomarker for AD in that study, requires further investigations.

To the authors current knowledge, no previous studies have explored the relationship between platelet tau and cognitive performance as measured by verbal and visual episodic memory and, working memory and executive function. However, it has been extensively shown that tau accumulation in the medial temporal lobe of the brain strongly associates with episodic memory in cognitively unimpaired individuals classified with high brain amyloid-beta load (198–200). Additionally, it was reported that MCI and AD individuals showed a decline in working memory compared to controls (201). Furthermore, because tau is regarded as a longitudinal measure of neurodegeneration severity, and cognitive decline advances with increasing AD severity (188–190), the association between platelet tau and cognitive performance was expected.

Abnormally phosphorylated tau is a main characteristic in AD development, and therefore may offer a comparable, if not, improved diagnostic tool for AD, compared to tau (191). Particularly, p-tau ser202/thr205, detected with the well characterised monoclonal AT8 antibody that is used to assign Braak stages based on NFT occurrences (146). Hence, the association between NAL and phosphorylated tau was investigated in the current study. The p-tau ser202/thr205 ~200kDa band was significantly lower in NAL+ compared to NAL-. However, the significance disappeared after adjusting for age, gender and *APOE* ε4 allele status, for which the significant association observed between p-tau ser202/thr205 ~200kDa and gender, was likely responsible for this disappearance. In contrast, p-tau ser202/thr205 ~80kDa, was significantly lower in NAL+ after adjusting for the same covariates. When the p-tau ser202/thr205 species were correlated with NAL, only the p-tau ser202/thr205 ~200kDa species was significantly inversely correlated, however, like with the previously mentioned platelet proteins, the significant relationship was observed only in the NAL- following data stratification by NAL+/- status. This may again be due to the discontinuity of the data when divided, the small sample size, and the correlation between two variables speculated to have different pathogenic mechanisms in AD. The AT8 antibody used to detect p-tau at site ser202/thr205, has been previously reported to show an immunoreactive band ranging between ~50-80kDa in human brain tissue (202,203). Tau species with

molecular weights similar to the aforementioned were observed in the study, such as the ~55kDa and ~80kDa. A higher, ~200kDa p-tau ser202/thr205 band detected by AT8 in this study, has similarly been observed in AD brains (185). No literature identified by the investigator thus far, has examined the presence, or potential alterations of p-tau ser202/thr205 in human platelets using western blot analysis. One study however, examined p-tau ser202/thr205 in platelets of AD patients compared to normal controls using ELISA (11). In contrast to the current study, p-tau ser202/thr205 was found to have similar levels in both the AD and control participants. Such differences in study outcomes may be related to the type of protein assay used. Results obtained by ELISA are a signal that reflects the sum of all protein species detected by a single antibody and is therefore unable to identity the molecular weights of each protein detected. In contrast, western blots have the advantage of providing a visual representation of the proteins of interest and can therefore more confidently identify which proteins may be of relevance to the project's aims.

While p-tau ser202/thr205 is used to assign Braak stages based on NFT occurrences (146), it has been postulated that this phosphorylated tau may not be a reliable marker for early AD classification. This hypothesis can be supported by a previous study that showed p-tau ser202/thr205 was only significantly increased in the Braak stages V/VI (203), insinuating that p-tau ser202/thr205 may be elevated in the more severe stages of the disease, and therefore may be more suitable as a late-stage AD biomarker. Phosphorylated tau at serine 181 (p-tau181) contrastingly, has been shown to be significantly higher in the CSF of cognitively normal Aβ positive participants compared to cognitively normal Aβ negative individuals (204). Therefore, it may be assumed that decreased levels of p-tau ser202/thr205 could not be observed in the current study, due to the cognitively normal state of the participants. However, alterations of p-tau ser202/thr205 could potentially be observed in the later stages of the disease for this cohort. Therefore, it would be worthwhile to explore the levels of p-tau181 in the current study's cohort to examine whether this phosphorylated tau is increased in the cognitively normal NAL+ group compared to the cognitively normal NAL- group. Additionally, it would be beneficial to explore the levels of p-tau ser202/thr205 in the current study's cohort longitudinally, to observe whether p-tau ser202/thr205 alters with declining cognition.

P-tau ser202/thr205 ~55kDa inversely correlated with plasma $A\beta_{42}/A\beta_{40}$ ratio. Although the correlation was modest (r_s =-0.309), the significance is questionable, as this p-tau immunoreactive species was not significantly associated with NAL, cognitive performance, or any other variable investigated. Therefore, it is possible that this observation may be a false positive due to multiple statistical tests or, may have arisen from the smaller sample size and methodological technicalities of plasma Aβ quantification. However, it has been previously reported that high CSF p-tau and low plasma Aβ were observed in MCI subjects (205), while another study found that lower plasma $A\beta_{42}/A\beta_{40}$ ratio were associated with elevated tau deposition in the bilateral frontal lobe (206). Although none of these previous findings were observed in platelets, let alone involved the ser202/thr205 phosphorylated tau, it provides some degree of support for the present finding. On another note, the significance of this inverse correlation with plasma $A\beta_{42}/A\beta_{40}$ ratio, may be attributed to the more ultrasensitive single molecule array (SIMOA)

assay used to measure plasma $A\beta_{42}$/ $A\beta_{40}$ (207). The findings for this currently remain speculative, and further studies are required to understand why p-tau ser202/thr205 ~55kDa and plasma $A\beta_{42}$/ $A\beta_{40}$ correlate, or why a false positive was potentially observed.

Subjective memory complaint represents concerns reported by study participants, patients or their informants believing their cognitive functions have declined, and such worries are more common amongst the elderly (208). As the KARVIAH study participants assessed in this study comprised of older age individuals with 22% SMCs, the current study compared platelet proteins between SMC- and SMC+. It was observed that tau ~55kDa, t-tau, p-tau ser202/thr205 ~200kDa and p-tau were significantly higher in the SMC+ compared to the SMC- before and after adjusting for covariates, implying the significance observed was independent of the AD risk factors. No studies known to the investigator have explored the direct relationship between SMC and platelet tau and p-tau ser202//thr205. However, various studies have demonstrated that SMC are potentially capable of predicting cognitive decline in the elderly considered to be cognitively normal (209,210). Additionally, a number of longitudinal studies have suggested that SMC is a symptom of pre-MCI, and may be a useful indicator for predicting dementia onset (209,211,212).

However, while the current study observed significant associations between SMC with the platelet proteins, it should be noted that the use of SMC as a variable possesses two main limitations. The first limitation is that a self-reported assessment (MAC-Q) was used to classify individuals as SMCs and was therefore subject to information bias. Secondly, the number of SMC+ (n=47) in this study greatly outnumbered those as SMC- (n=13). Therefore, the significance observed may have been unintentionally skewed towards the SMC+. Nevertheless, considering tau, t-tau and p-tau are thought of as longitudinal markers that reflect disease severity, it can be suggested that inverse correlations between SMC and t-tau and p-tau may provide an indication of AD onset in individuals at risk of AD, and as a way to monitor cognitive decline. However, future studies of a longitudinal design will be required to provide a further understanding of the relationship between SMC and platelet proteins as a measure for cognitive decline.

Taken together, the platelet proteins found to be most significantly associated with NAL (APP 105-115kDa, tau ~33kDa and HMW/LMW tau ratio), showed a higher accuracy, specificity and sensitivity when combined with a base model comprising of the AD risk factors age, gender and *APOE* ε4 allele status (80%), compared to the base model alone (73.35%). The findings suggest that the combination of these selected proteins with AD risk factors may provide diagnostic potential for distinguishing NAL+ individuals from NAL- individuals. Although not including tau, a previous study interpreted the diagnostic potential of APPr in distinguishing MCI and AD (213). The authors concluded that APPr may potentiate as a surrogate marker for AD progression, however, acknowledged that the diagnostic accuracy (74%) was relatively low, and therefore required further investigations in larger cohorts. However, while the current study reported greater specificity and sensitivity than that of study previously mentioned, it should be noted that the study design was a longitudinal one, whereas the

current study was cross-sectional. Therefore, this difference in experimental design may have influenced the levels of proteins observed, therefore, affecting its overall diagnostic potential.

This study may be limited firstly, in the modest sample size used (NAL+, n=30 and NAL-, n=30). Yet, given the scope of this study with respect to time limitations, the numbers were sufficient to generate pilot data. However, it is acknowledged that a larger sample size would provide more robust findings and would perhaps be able to distinguish any potential false positives observed in this study. Additionally, the current study did not adjust for multiple comparisons in the analyses and determined statistical significance on a less stringent *p* value of 0.05, rather than a more constricted *p* value of 0.001. However, due to the study's small sample size, such considerations were not feasible, and as such, future studies with larger cohorts should address these factors.

Another possible limitation in the current study's design may rest in the fact that the samples analysed were selected from the extremities of both NAL- and NAL+ groups. Although this was done to ensure that the maximum amount of significance was observed, it may have unintentionally skewed the data. In doing so, potential data points that could have provided greater significance to the observed findings, were left out. However, all the participants screened in this study have an MMSE ≥26 , therefore, the range of cognitive differences was highly restricted, leading to emphasise the fact that the significance between cognition and platelet proteins, may still hold importance. Knowingly, future studies should include larger cohorts with more equal numbers of NAL- and NAL+ individuals.

It is also recognised that this study was of cross-sectional design, which restricted investigations of platelet proteins to a single time-point only in individuals determined to be at high risk of AD (i.e. NAL+), or low risk (NAL-). A longitudinal exploration of the platelet proteins for the study participants, and especially cognitive performance over time, will be more informative.

In relation to the methodology of the study, western immunoblotting is an established technique for the detection and semi-quantification of proteins. Its strength lies in its ability to specifically detect a single analyte from a complex mass of proteins, and is routinely implemented in all laboratories due to its technical simplicity and inexpensiveness (214). However, it should be acknowledged that the feasibility of performing a western blot in a clinical setting, is not ideal, with the process taking 1-2 days to complete. Additionally, western blot has low throughput, due to the limited number of samples loaded per gel. This therefore is undesirable for large-scale sample screening. Furthermore, the technique is subject to potential false positives and quantification errors, but this limitation can be overcome with the inclusion of appropriate standards. Taking these limitations into account, many of the multiple immunoreactive tau and p-tau ser202/thr205 species observed in this current study, may be a result of unspecific antibody binding. Particularly, the AT8 antibody, known to recognise the ser202/thr205 site of p-tau, was found by one study to have a similar phosphorylation profile with other p-tau sites, such as p-tau threonine 231, p-tau serine 199, p-tau tyrosine 18 and p-tau serine 422 (203). This suggests that antibody cross-reactivity may have occurred, as the p-tau sites described are in close proximity to one another (203). One solution to assess specificity would be to absorb out immunoreactivity with a specific

peptide to which the antibody was raised against. However, the WT and APP23 mouse brain positive controls, only demonstrated one dark band at ~55kDa, leading to speculate whether the p-tau ser202/thr205 signals observed in the platelets, demonstrate AD biological relevance.

With regards to tau, the antibody used was the same in previous tau AD platelet papers (8,139,140). However, transcriptomic studies on normal human platelets reported no evidence of tau in the platelet proteome (215,216) leading to suspect that perhaps the immunoreactive tau bands observed are not tau, and might represent artefacts. Yet, an independent group who investigated the presence and levels of age-related proteins in platelets from healthy human subjects, detected the presence of tau (217). It should also be noted, that the identification of tau in platelets from the abovementioned studies (not including the transcriptomic studies), together with the current study, used western blot measurement method. It is therefore imperative that other methods of quantification be implemented to validate the identities of the tau immunoreactive species, before studying further platelet tau as a potential biomarker for individuals at potential risk of AD. Techniques such as mass spectrometry or SIMOA, may provide a more sensitive and specific means of identification, however, the clinical practicality of western blotting and mass spectrometry will not be ideal, if it is to be implemented as a population wide screening method for detection of tau and the other platelet proteins. Therefore, more sensitive assays for the specific antigens of interest in platelets using the SIMOA platform may need to be developed.

Conclusion

The increasing population of elderly individuals has markedly increased the worldwide prevalence of AD cases. Despite this, there is yet no strong peripheral biomarker capable of definitively diagnosing AD *ante*-mortem that is inexpensive, non-invasive, reliable and readily available for population wide-screening. The findings from this thesis presents several platelet proteins that appear as potentially promising biological indicators for diagnosing preclinical AD categorised by cognitively normal high risk NAL+ individuals. In addition to its diagnostic potential, it can be envisioned that the platelet proteins may provide clinical relevance in monitoring therapeutic interventions and lifestyle habits. However, it must be recognised that future studies using mass spectrometry, need to be performed to validate the tau immunoreactive species identified in the current project. Additionally, further clarifications of the platelet proteins and its involvement with cognitive severity, need to be explored in larger sample sizes of longitudinal designs. Nevertheless, the current study has undoubtedly expanded the existing knowledge on platelet protein-related alterations in individuals at potential risk of AD, and has additionally provided further insights into the pathophysiological mechanisms contributing to the progression of AD.

References

1. Brown L, Hansnata E, La HA. Economic cost of dementia in Australia 2016-2056. 2017.
2. International D. World Alzheimer Report 2018 - The state of the art of dementia research: New frontiers; World Alzheimer Report 2018 - The state of the art of dementia research: New frontiers.
3. Weller J, Budson A. Current understanding of Alzheimer's disease diagnosis and treatment. Vol. 7, F1000Research. F1000 Research Ltd; 2018.
4. Price JL, Morris JC. Tangles and plaques in nondemented aging and "preclinical" Alzheimer's disease. Ann Neurol. 1999/03/11. 1999;45(3):358–68.
5. Olson L, Humpel C. Growth factors and cytokines/chemokines as surrogate biomarkers in cerebrospinal fluid and blood for diagnosing Alzheimer's disease and mild cognitive impairment. Vol. 45, Experimental Gerontology. 2010. p. 41–6.
6. Baskin F, Rosenberg RN, Iyer L, Hynan L, Cullum CM. Platelet APP isoform ratios correlate with declining cognition in AD. Neurology. 2000 May 23;54(10):1907–9.
7. Colciaghi F, Borroni B, Pastorino L, Marcello E, Zimmermann M, Cattabeni F, et al. [alpha]-Secretase ADAM10 as well as [alpha]APPs is reduced in platelets and CSF of Alzheimer disease patients. Mol Med. 2002/06/25. 2002;8(2):67–74.
8. Farias G, Perez P, Slachevsky A, Maccioni RB. Platelet tau pattern correlates with cognitive status in Alzheimer's disease. J Alzheimers Dis. 2012/04/13. 2012;31(1):65–9.
9. Gorham P, Bark N, Meaney S, Bjorkhem I, Crisby M. Platelet Alpha- and Beta- Secretase Activities are not Significantly Affected by Dementia or Mild Cognitive Impairment in Swedish Patients. Curr Alzheimer Res. 2010;
10. Johnston JA, Liu WW, Coulson DTR, Todd S, Murphy S, Brennan S, et al. Platelet β-secretase activity is increased in Alzheimer's disease. Neurobiol Aging. 2008;
11. Mukaetova-Ladinska EB, Abdell-All Z, Andrade J, da Silva JA, Boksha I, Burbaeva G, et al. Platelet Tau Protein as a Potential Peripheral Biomarker in Alzheimer's Disease: An Explorative Study. Curr Alzheimer Res. 2018/04/07. 2018;15(9):800–8.
12. Slachevsky A, Guzman-Martinez L, Delgado C, Reyes P, Farias GA, Munoz-Neira C, et al. Tau Platelets Correlate with Regional Brain Atrophy in Patients with Alzheimer's Disease. J Alzheimers Dis. 2016/12/03. 2017;55(4):1595–603.
13. Tang K, Hynan LS, Baskin F, Rosenberg RN. Platelet amyloid precursor protein processing: a bio-marker for Alzheimer's disease. J Neurol Sci. 2005/10/26. 2006;240(1–2):53–8.
14. Hippius H, Neundörfer G. The discovery of Alzheimer's disease. Dialogues Clin Neurosci. 2003;5(1):101–8.
15. Glenner GG, Wong CW. Alzheimer's disease: Initial report of the purification and characterization of a novel cerebrovascular amyloid protein. Biochem Biophys Res Commun. 1984 May 16;120(3):885–90.
16. Haga S, Akai K, Ishii T. Demonstration of microglial cells in and around senile (neuritic) plaques in the Alzheimer brain. An immunohistochemical study using a novel monoclonal antibody. Acta Neuropathol. 1989;77(6):569–75.
17. Perry G, Siedlak SL, Richey P, Kawai M, Cras P, Kalaria RN, et al. Association of heparan sulfate proteoglycan with the neurofibrillary tangles of Alzheimer's disease. J Neurosci. 1991

Nov;11(11):3679–83.

18. Snow AD, Willmer J, Kisilevsky R. Sulfated glycosaminoglycans: a common constituent of all amyloids? Lab Invest. 1987 Jan;56(1):120–3.

19. Smith MA, Harris PLR, Sayre LM, Perry G. Iron accumulation in Alzheimer disease is a source of redox-generated free radicals. Proc Natl Acad Sci U S A. 1997 Sep 2;94(18):9866–8.

20. Lovell MA, Robertson JD, Teesdale WJ, Campbell JL, Markesbery WR. Copper, iron and zinc in Alzheimer's disease senile plaques. J Neurol Sci. 1998 Jun 11;158(1):47–52.

21. Yan Y, Wang C. Abeta42 is more rigid than Abeta40 at the C terminus: implications for Abeta aggregation and toxicity. J Mol Biol. 2006 Dec 15;364(5):853–62.

22. Hardy JA, Higgins GA. Alzheimer's disease: the amyloid cascade hypothesis. Science. 1992 Apr 10;256(5054):184–5.

23. Bennett DA, Schneider JA, Wilson RS, Bienias JL, Arnold SE. Neurofibrillary Tangles Mediate the Association of Amyloid Load with Clinical Alzheimer Disease and Level of Cognitive Function. Arch Neurol. 2004;

24. Kametani F, Hasegawa M. Reconsideration of Amyloid Hypothesis and Tau Hypothesis in Alzheimer's Disease . Vol. 12, Frontiers in Neuroscience . 2018. p. 25.

25. Chirita CN, Congdon EE, Yin H, Kuret J. Triggers of Full-Length Tau Aggregation: A Role for Partially Folded Intermediates. Biochemistry. 2005;44(15):5862–72.

26. Pantoni L. Cerebral small vessel disease: from pathogenesis and clinical characteristics to therapeutic challenges. Vol. 9, The Lancet Neurology. 2010. p. 689–701.

27. Rovelet-Lecrux A, Hannequin D, Raux G, Le Meur N, Laquerrière A, Vital A, et al. APP locus duplication causes autosomal dominant early-onset Alzheimer disease with cerebral amyloid angiopathy. Nat Genet. 2006 Jan;38(1):24–6.

28. Panegyres PK, Kwok JBJ, Schofield PR, Blumbergs PC. A Western Australian kindred with Dutch cerebral amyloid angiopathy. J Neurol Sci. 2005 Dec 15;239(1):75–80.

29. Yamada M. Predicting cerebral amyloid angiopathy-related intracerebral hemorrhages and other cerebrovascular disorders in Alzheimer's disease. Front Neurol. 2012;APR.

30. Boyle PA, Yu L, Nag S, Leurgans S, Wilson RS, Bennett DA, et al. Cerebral amyloid angiopathy and cognitive outcomes in community-based older persons. Neurology. 2015 Dec 1;85(22):1930–6.

31. Arvanitakis Z, Leurgans SE, Wang Z, Wilson RS, Bennett DA, Schneider JA. Cerebral amyloid angiopathy pathology and cognitive domains in older persons. Ann Neurol. 2011 Feb;69(2):320–7.

32. Neuropathology Group. Medical Research Council Cognitive Function and Aging Study. Pathological correlates of late-onset dementia in a multicentre, community-based population in England and Wales. Neuropathology Group of the Medical Research Council Cognitive Function and Ageing Study (MRC CFAS). Lancet (London, England). 2001 Jan 20;357(9251):169–75.

33. Pfeifer LA, White LR, Ross GW, Petrovitch H, Launer LJ. Cerebral amyloid angiopathy and cognitive function: The HAAS autopsy study. Neurology. 2002 Jun 11;58(11):1629–34.

34. McGeer PL, McGeer EG. Inflammation, autotoxicity and Alzheimer disease. Neurobiol Aging. 22(6):799–809.

35. Bartus R, Dean R, Beer B, Lippa A. The cholinergic hypothesis of geriatric memory dysfunction. Science (80-). 1982 Jul 30;217(4558):408–14.

36. Coyle JT, Puttfarcken P. Oxidative stress, glutamate, and neurodegenerative disorders. Science (80-). 1993;262(5134):689–95.

37. Bateman RJ, Xiong C, Benzinger TLS, Fagan AM, Goate A, Fox NC, et al. Clinical and biomarker changes in dominantly inherited Alzheimer's disease. N Engl J Med. 2012;

38. Campion D, Pottier C, Nicolas G, Le Guennec K, Rovelet-Lecrux A. Alzheimer disease: Modeling an Aβ-centered biological network. Molecular Psychiatry. 2016.

39. Youn YC, Bagyinszky E, Kim HR, Choi BO, An SS, Kim SY. Probable novel PSEN2 Val214Leu mutation in Alzheimer's disease supported by structural prediction. BMC Neurol. 2014;

40. Bekris LM, Yu CE, Bird TD, Tsuang DW. Review article: Genetics of Alzheimer disease. Vol. 23, Journal of Geriatric Psychiatry and Neurology. 2010. p. 213–27.

41. Farrer LA, Cupples AL, Kukull W a, Mayeux R, Myers RH, Pericak-vance M a, et al. Effects of Age , Sex , and Ethnicity on the Association Between Apolipoprotein E Genotype and Alzheimer Disease. JAMA J Am Med Assoc. 1997;

42. Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, et al. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science (80-). 1993;

43. Hebert LE, Weuve J, Scherr PA, Evans DA. Alzheimer disease in the United States (2010-2050) estimated using the 2010 census. Neurology. 2013;

44. Lin FC, Chuang YS, Hsieh HM, Lee TC, Chiu KF, Liu CK, et al. Early statin use and the progression of Alzheimer disease: A total population-based case-control study. Med (United States). 2015;

45. Sinforiani E, Citterio A, Zucchella C, Bono G, Corbetta S, Merlo P, et al. Impact of gender differences on the outcome of alzheimer's disease. Dement Geriatr Cogn Disord. 2010;

46. Nebel RA, Aggarwal NT, Barnes LL, Gallagher A, Goldstein JM, Kantarci K, et al. Understanding the impact of sex and gender in Alzheimer's disease: A call to action. Alzheimer's Dement. 2018;

47. Shumaker SA, Legault C, Rapp SR, Thal L, Wallace RB, Ockene JK, et al. Estrogen Plus Progestin and the Incidence of Dementia and Mild Cognitive Impairment in Postmenopausal Women - The Women's Health Initiative Memory Study: A Randomized Controlled Trial. J Am Med Assoc. 2003;

48. Diaz Brinton R, Chen S, Montoya M, Hsieh D, Minaya J, Kim J, et al. The women's health initiative estrogen replacement therapy is neurotrophic and neuroprotective. Neurobiol Aging. 2000;

49. Henderson VW, Paganini-Hill A, Miller BL, Elble RJ, Reyes PF, Shoupe D, et al. Estrogen for Alzheimer's disease in women: Randomized, double-blind, placebo-controlled trial. Neurology. 2012;

50. Mulnard RA, Cotman CW, Kawas C, Van Dyck CH, Sano M, Doody R, et al. Estrogen replacement therapy for treatment of mild to moderate Alzheimer disease: A randomized controlled trial. J Am Med Assoc. 2000;

51. Northey JM, Cherbuin N, Pumpa KL, Smee DJ, Rattray B. Exercise interventions for cognitive function in adults older than 50: a systematic review with meta-analysis. Br J Sport Med. 2017/04/26. 2018;52(3):154–60.

52. Abe K, AS B, Yu L, PA B, RS W, DA B. Total daily physical activity and the risk of AD and cognitive decline in older adults...Neurology. 2012 Apr 24;78(17):1323-9. Neurology. 2012;

53. Buchman, A.S., Boyle, P.A., Yu, L., Shah, R.C., Wilson, R.S., & Bennett DA, Buchman AS, Boyle PA, Yu L, Shah RC, Wilson RS, et al. Total daily physical activity and the risk of AD and cognitive decline in older adults. Neurology. 2012;

54. Suttanon P, Hill KD, Said CM, Williams SB, Byrne KN, LoGiudice D, et al. Feasibility, safety

and preliminary evidence of the effectiveness of a home-based exercise programme for older people with Alzheimer's disease: a pilot randomized controlled trial. Clin Rehabil. 2013 May;27(5):427–38.

55. Venturelli M, Scarsini R, Schena F. Six-month walking program changes cognitive and ADL performance in patients with Alzheimer. Am J Alzheimers Dis Other Demen. 2011 Aug;26(5):381–8.

56. Brown BM, Peiffer JJ, Taddei K, Lui JK, Laws SM, Gupta VB, et al. Physical activity and amyloid-β plasma and brain levels: results from the Australian Imaging, Biomarkers and Lifestyle Study of Ageing. Mol Psychiatry. 2013 Aug;18(8):875–81.

57. Brown BM, Peiffer J, Rainey-Smith SR. Exploring the relationship between physical activity, beta-amyloid and tau: A narrative review. Vol. 50, Ageing Research Reviews. Elsevier Ireland Ltd; 2019. p. 9–18.

58. Liang KY, Mintun MA, Fagan AM, Goate AM, Bugg JM, Holtzman DM, et al. Exercise and Alzheimer's disease biomarkers in cognitively normal older adults. Ann Neurol. 2010 Sep;68(3):311–8.

59. Hu N, Yu J-T, Tan L, Wang Y-L, Sun L, Tan L. Nutrition and the risk of Alzheimer's disease. Biomed Res Int. 2013;2013:524820.

60. Montilla-López P, Muñoz-Agueda MC, Feijóo López M, Muñoz-Castañeda JR, Bujalance-Arenas I, Túnez-Fiñana I. Comparison of melatonin versus vitamin C on oxidative stress and antioxidant enzyme activity in Alzheimer's disease induced by okadaic acid in neuroblastoma cells. Eur J Pharmacol. 2002 Sep 20;451(3):237–43.

61. Murakami K, Murata N, Ozawa Y, Kinoshita N, Irie K, Shirasawa T, et al. Vitamin C restores behavioral deficits and amyloid-β oligomerization without affecting plaque formation in a mouse model of Alzheimer's disease. J Alzheimers Dis. 2011;26(1):7–18.

62. Luchsinger JA, Tang M-X, Shea S, Mayeux R. Antioxidant vitamin intake and risk of Alzheimer disease. Arch Neurol. 2003 Feb;60(2):203–8.

63. Zandi PP, Anthony JC, Khachaturian AS, Stone S V, Gustafson D, Tschanz JT, et al. Reduced risk of Alzheimer disease in users of antioxidant vitamin supplements: the Cache County Study. Arch Neurol. 2004 Jan;61(1):82–8.

64. Scarmeas N, Stern Y, Tang M-X, Mayeux R, Luchsinger JA. Scarmeas et al- (2006) Mediterranean diet and risk for Alzheimer's disease.- Annals of Neurology . Ann Neurol. 2006;

65. Mosconi L, Murray J, Tsui WH, Li Y, Davies M, Williams S, et al. Mediterranean Diet and Magnetic Resonance Imaging-Assessed Brain Atrophy in Cognitively Normal Individuals at Risk for Alzheimer's Disease. J Prev Alzheimer's Dis. 2014;

66. Kang JE, Lim MM, Bateman RJ, Lee JJ, Smyth LP, Cirrito JR, et al. Amyloid-beta dynamics are regulated by orexin and the sleep-wake cycle. Science (80-). 2009/09/26. 2009;326(5955):1005–7.

67. Spira AP, Gamaldo AA, An Y, Wu MN, Simonsick EM, Bilgel M, et al. Self-reported Sleep and β-Amyloid Deposition in Community-Dwelling Older AdultsSelf-reported Sleep and β-Amyloid DepositionSelf-reported Sleep and β-Amyloid Deposition. JAMA Neurol. 2013;70(12):1537–43.

68. Rothman SM, Herdener N, Frankola KA, Mughal MR, Mattson MP. Chronic mild sleep restriction accentuates contextual memory impairments, and accumulations of cortical Aβ and pTau in a mouse model of Alzheimer's disease. Brain Res. 2013 Sep 5;1529:200–8.

69. Di Meco A, Joshi YB, Praticò D. Sleep deprivation impairs memory, tau metabolism, and synaptic integrity of a mouse model of Alzheimer's disease with plaques and tangles. Neurobiol Aging. 2014 Aug;35(8):1813–20.

70. Ngandu T, Lehtisalo J, Solomon A, Levalahti E, Ahtiluoto S, Antikainen R, et al. A 2 year multidomain intervention of diet, exercise, cognitive training, and vascular risk monitoring versus control to prevent cognitive decline in at-risk elderly people (FINGER): a randomised controlled trial. Lancet. 2015;

71. Kumar A, Singh A. Pharmacological Reports Review article A review on Alzheimer ' s disease pathophysiology and its management : an update. Pharmacol Rep. 2015;

72. Mehta M, Adem A, Sabbagh M. New Acetylcholinesterase Inhibitors for Alzheimer's Disease. Int J Alzheimers Dis. 2012;2012:1–8.

73. Findeis MA. The role of amyloid β peptide 42 in Alzheimer's disease. Pharmacology and Therapeutics. 2007.

74. Tayeb HO, Yang HD, Price BH, Tarazi FI. Pharmacotherapies for Alzheimer's disease: beyond cholinesterase inhibitors. Pharmacol Ther. 2012 Apr;134(1):8–25.

75. Olivares D, Deshpande VK, Shi Y, Lahiri DK, Greig NH, Rogers JT, et al. N-methyl D-aspartate (NMDA) receptor antagonists and memantine treatment for Alzheimer's disease, vascular dementia and Parkinson's disease. Curr Alzheimer Res. 2012;

76. van Marum RJ. Update on the use of memantine in Alzheimer's disease. Neuropsychiatr Dis Treat. 2009;5:237–47.

77. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state": A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res. 1975;12(3):189–98.

78. Brust P, van den Hoff J, Steinbach J. Development of 18F-labeled radiotracers for neuroreceptor imaging with positron emission tomography. Neuroscience Bulletin. 2014.

79. Klunk WE, Engler H, Nordberg A, Wang Y, Blomqvist G, Holt DP, et al. Imaging brain amyloid in Alzheimer's disease with Pittsburgh Compound-B. Ann Neurol. 2004/03/03. 2004;55(3):306–19.

80. Mathis CA, Bacskai BJ, Kajdasz ST, McLellan ME, Frosch MP, Hyman BT, et al. A lipophilic thioflavin-T derivative for positron emission tomography (PET) imaging of amyloid in brain. Bioorg Med Chem Lett. 2002;12(3):295–8.

81. Agdeppa ED, Kepe V, Liu J, Flores-Torres S, Satyamurthy N, Petric A, et al. Binding characteristics of radiofluorinated 6-dialkylamino-2-naphthylethylidene derivatives as positron emission tomography imaging probes for beta-amyloid plaques in Alzheimer's disease. J Neurosci. 2001 Dec 15;21(24):RC189.

82. Noda A, Murakami Y, Nishiyama S, Fukumoto D, Miyoshi S, Tsukada H, et al. Amyloid imaging in aged and young macaques with [11C]PIB and [18F]FDDNP. Synapse. 2008 Jun;62(6):472–5.

83. Thompson PW, Ye L, Morgenstern JL, Sue L, Beach TG, Judd DJ, et al. Interaction of the amyloid imaging tracer FDDNP with hallmark Alzheimer's disease pathologies. J Neurochem. 2009 Apr;109(2):623–30.

84. Wong DF, Rosenberg PB, Zhou Y, Kumar A, Raymont V, Ravert HT, et al. In vivo imaging of amyloid deposition in Alzheimer disease using the radioligand 18F-AV-45 (flobetapir F 18). J Nucl Med. 2010 Jun;51(6):913–20.

85. Rowe CC, Ng S, Ackermann U, Gong SJ, Pike K, Savage G, et al. Imaging beta-amyloid burden in aging and dementia. [Neurology. 2007] - PubMed result. Neurology. 2007;

86. Alexander GE, Chen K, Pietrini P, Rapoport SI, Reiman EM. Longitudinal PET Evaluation of Cerebral Metabolic Decline in Dementia: A Potential Outcome Measure in Alzheimer's Disease Treatment Studies. Am J Psychiatry. 2002 May;159(5):738–45.

87. de Leon MJ, Ferris SH, George AE, Reisberg B, Christman DR, Kricheff II, et al. Computed tomography and positron emission transaxial tomography evaluations of normal aging and

Alzheimer's disease. J Cereb Blood Flow Metab. 1983 Sep;3(3):391–4.

88. Foster NL, Chase TN, Mansi L, Brooks R, Fedio P, Patronas NJ, et al. Cortical abnormalities in Alzheimer's disease. Ann Neurol. 1984 Dec;16(6):649–54.

89. Friedland RP, Brun A, Budinger TF. Pathological and positron emission tomographic correlations in Alzheimer's disease. Lancet (London, England). 1985 Jan 26;1(8422):228.

90. Mosconi L, De Santi S, Li J, Tsui WH, Li Y, Boppana M, et al. Hippocampal hypometabolism predicts cognitive decline from normal aging. Neurobiol Aging. 2008 May;29(5):676–92.

91. Poston KL, Eidelberg D. 18F-fluorodeoxyglucose PET in the evaluation of parkinson disease. PET Clinics. 2010.

92. Shin H, Kim MH, Lee SJ, Lee KH, Kim MJ, Kim JS, et al. Decreased metabolism in the cerebral cortex in early-stage huntington's disease: A possible biomarker of disease progression? J Clin Neurol. 2013;

93. Knight MJ, McCann B, Kauppinen RA, Coulthard EJ. Magnetic Resonance Imaging to Detect Early Molecular and Cellular Changes in Alzheimer's Disease. Front Aging Neurosci. 2016;8:139.

94. Chan D, Fox NC, Scahill RI, Crum WR, Whitwell JL, Leschziner G, et al. Patterns of temporal lobe atrophy in semantic dementia and Alzheimer's disease. Ann Neurol. 2001 Apr;49(4):433–42.

95. Blennow K, Hampel H. CSF markers for incipient Alzheimer's disease. Vol. 2, Lancet Neurology. Lancet Publishing Group; 2003. p. 605–13.

96. Blennow K, Hampel H. CSF markers for incipient Alzheimer's disease. Lancet Neurol. 2003/09/25. 2003;2(10):605–13.

97. Strozyk D, Blennow K, White LR, Launer LJ. CSF Abeta 42 levels correlate with amyloid-neuropathology in a population-based autopsy study. Neurology. 2003 Feb 25;60(4):652–6.

98. Fagan AM, Roe CM, Xiong C, Mintun MA, Morris JC, Holtzman DM. Cerebrospinal fluid tau/β-amyloid42 ratio as a prediction of cognitive decline in nondemented older adults. Arch Neurol. 2007 Mar;64(3):343–9.

99. Toledo JB, Shaw LM, Trojanowski JQ. Plasma amyloid beta measurements - a desired but elusive Alzheimer's disease biomarker. Alzheimers Res Ther. 2013;5(2):8.

100. Padovani A, Borroni B, Colciaghi F, Pettenati C, Cottini E, Agosti C, et al. Abnormalities in the Pattern of Platelet Amyloid Precursor Protein Forms in Patients With Mild Cognitive Impairment and Alzheimer Disease. Arch Neurol. 2002;59(1):71–5.

101. Nakamura A, Kaneko N, Villemagne VL, Kato T, Doecke J, Doré V, et al. High performance plasma amyloid-β biomarkers for Alzheimer's disease. Nature. 2018;554:249.

102. Chatterjee P, Elmi M, Goozee K, Shah T, Sohrabi HR, Dias CB, et al. Ultrasensitive Detection of Plasma Amyloid-β as a Biomarker for Cognitively Normal Elderly Individuals at Risk of Alzheimer's Disease. J Alzheimer's Dis. 2019 Aug 16;1–9.

103. Nakamura A, Kaneko N, Villemagne VL, Kato T, Doecke J, Dore V, et al. High performance plasma amyloid-beta biomarkers for Alzheimer's disease. Nature. 2018/02/09. 2018;554(7691):249–54.

104. Ovod V, Ramsey KN, Mawuenyega KG, Bollinger JG, Hicks T, Schneider T, et al. Amyloid β concentrations and stable isotope labeling kinetics of human plasma specific to central nervous system amyloidosis. Alzheimers Dement. 2017 Aug;13(8):841–9.

105. Gaetani L, Blennow K, Calabresi P, Di Filippo M, Parnetti L, Zetterberg H. Neurofilament light chain as a biomarker in neurological disorders. Journal of Neurology, Neurosurgery and Psychiatry. BMJ Publishing Group; 2019.

106. Zetterberg H, Skillbäck T, Mattsson N, Trojanowski JQ, Portelius E, Shaw LM, et al. Association of Cerebrospinal Fluid Neurofilament Light Concentration With Alzheimer Disease Progression. JAMA Neurol. 2016 Jan;73(1):60–7.

107. Bäckström DC, Eriksson Domellöf M, Linder J, Olsson B, Öhrfelt A, Trupp M, et al. Cerebrospinal Fluid Patterns and the Risk of Future Dementia in Early, Incident Parkinson Disease. JAMA Neurol. 2015 Oct;72(10):1175–82.

108. Scherling CS, Hall T, Berisha F, Klepac K, Karydas A, Coppola G, et al. Cerebrospinal fluid neurofilament concentration reflects disease severity in frontotemporal degeneration. Ann Neurol. 2014 Jan;75(1):116–26.

109. Chatterjee P, Goozee K, Sohrabi HR, Shen K, Shah T, Asih PR, et al. Association of Plasma Neurofilament Light Chain with Neocortical Amyloid-β Load and Cognitive Performance in Cognitively Normal Elderly Participants. J Alzheimers Dis. 2018;63(2):479–87.

110. Kiko T, Nakagawa K, Satoh A, Tsuduki T, Furukawa K, Arai H, et al. Amyloid β Levels in Human Red Blood Kiko, T., Nakagawa, K., Satoh, A., Tsuduki, T., Furukawa, K., Arai, H., & Miyazawa, T. (2012). Amyloid β Levels in Human Red Blood Cells. PLoS ONE. http://doi.org/10.1371/journal.pone.0049620Cells. PLoS One. 2012;

111. Nakagawa K, Kiko T, Kuriwada S, Miyazawa T, Kimura F, Miyazawa T. Amyloid β induces adhesion of erythrocytes to endothelial cells and affects endothelial viability and functionality. Biosci Biotechnol Biochem. 2011;75(10):2030–3.

112. Ngoungoure VLN, Schluesener J, Moundipa PF, Schluesener H, Foguem C, Kamsu-Foguem B, et al. Increased protein and lipid oxidative damage in mitochondria isolated from lymphocytes from patients with Alzheimer's disease: insights into the role of oxidative stress in Alzheimer's disease and initial investigations into a potential biomarker for this. Am J Pathol. 2015;24(1):8–20.

113. Bush AI, Martins RN, Rumble B, Moir R, Fuller S, Milward E, et al. The amyloid precursor protein of Alzheimer's disease is released by human platelets. J Biol Chem. 1990/09/15. 1990;265(26):15977–83.

114. Sevush S, Jy W, Horstman LL, Mao WW, Kolodny L, Ahn YS. Platelet activation in Alzheimer disease. Arch Neurol. 1998 Apr;55(4):530–6.

115. Behari M, Shrivastava M. Role of platelets in neurodegenerative diseases: a universal pathophysiology. Int J Neurosci. 2013 May;123(5):287–99.

116. Pletscher A, Laubscher A. Blood platelets as models for neurons: uses and limitations. J Neural Transm Suppl. 1980;(16):7–16.

117. Veitinger M, Varga B, Guterres SB, Zellner M. Platelets, a reliable source for peripheral Alzheimer's disease biomarkers? Acta Neuropathol Commun. 2014 Jun 16;2:65.

118. Zhang Y, Thompson R, Zhang H, Xu H. APP processing in Alzheimer's disease. Mol Brain. 2011;4:3.

119. O'Brien RJ, Wong PC. Amyloid Precursor Protein Processing and AlO'Brien, R. J., & Wong, P. C. (2011). Amyloid Precursor Protein Processing and Alzheimer's Disease. Annual Review of Neuroscience, 34(1), 185–204. https://doi.org/10.1146/annurev-neuro-061010-113613zheimer's Dise. Annu Rev Neurosci. 2011;34(1):185–204.

120. Lorenzen A, Samosh J, Vandewark K, Anborgh PH, Seah C, Magalhaes AC, et al. Rapid and Direct Transport of Cell Surface APP to the Lysosome defines a novel selective pathway. Mol Brain. 2010;3(1):11.

121. Lammich S, Kojro E, Postina R, Gilbert S, Pfeiffer R, Jasionowski M, et al. Constitutive and regulated alpha-secretase cleavage of Alzheimer's amyloid precursor protein by a disintegrin metalloprotease. Proc Natl Acad Sci U S A. 1999;

122. Müller T, Meyer HE, Egensperger R, Marcus K. The amyloid precursor protein intracellular

domain (AICD) as modulator of gene expression, apoptosis, and cytoskeletal dynamics-Relevance for Alzheimer's disease. Vol. 85, Progress in Neurobiology. 2008. p. 393–406.

123. Ghosal K, Vogt DL, Liang M, Shen Y, Lamb BT, Pimplikar SW. Alzheimer's disease-like pathological features in transgenic mice expressing the APP intracellular domain. Proc Natl Acad Sci U S A. 2009;106(43):18367–72.

124. Di Luca M, Pastorino L, Cattabeni F, Zanardi R, Scarone S, Racagni G, et al. Abnormal pattern of platelet APP isoforms in Alzheimer disease and Down syndrome. Arch Neurol. 1996 Nov;53(11):1162–6.

125. Davies TA, Long HJ, Sgro K, Rathbun WH, McMenamin ME, Seetoo K, et al. Activated Alzheimer disease platelets retain more beta amyloid precursor protein. Neurobiol Aging. 18(2):147–53.

126. Di Luca M, Pastorino L, Bianchetti A, Perez J, Vignolo LA, Lenzi GL, et al. Differential level of platelet amyloid beta precursor protein isoforms: an early marker for Alzheimer disease. Arch Neurol. 1998/09/18. 1998;55(9):1195–200.

127. Rosenberg RN, Baskin F, Fosmire JA, Risser R, Adams P, Svetlik D, et al. Altered amyloid protein processing in platelets of patients with Alzheimer disease. Arch Neurol. 1997/02/01. 1997;54(2):139–44.

128. Borroni B, Colciaghi F, Pastorino L, Archetti S, Corsini P, Cattabeni F, et al. ApoE genotype influences the biological effect of donepezil on APP metabolism in Alzheimer disease: evidence from a peripheral model. Eur Neuropsychopharmacol. 2002 Jun;12(3):195–200.

129. Vetrivel KS, Thinakaran G. Amyloidogenic processing of beta-amyloid precursor protein in intracellular compartments. Neurology. 2006 Jan 24;66(2 Suppl 1):S69-73.

130. Colciaghi F, Marcello E, Borroni B, Zimmermann M, Caltagirone C, Cattabeni F, et al. Platelet APP, ADAM 10 and BACE alterations in the early stages of Alzheimer disease. Neurology. 2004 Feb 10;62(3):498–501.

131. Bram JM de F, Talib LL, Joaquim HPG, Sarno TA, Gattaz WF, Forlenza OV. Protein levels of ADAM10, BACE1, and PSEN1 in platelets and leukocytes of Alzheimer's disease patients. Eur Arch Psychiatry Clin Neurosci. 2018 May 29;

132. Manzine PR, Barham EJ, Vale F de AC do, Selistre-de-Araújo HS, Iost Pavarini SC, Cominetti MR. Correlation between mini-mental state examination and platelet ADAM10 expression in Alzheimer's disease. J Alzheimers Dis. 2013;36(2):253–60.

133. Holsinger RMD, McLean CA, Beyreuther K, Masters CL, Evin G. Increased expression of the amyloid precursor β-secretase in Alzheimer's disease. Ann Neurol. 2002;

134. Liu WW, Todd S, Craig D, Passmore AP, Coulson DTR, Murphy S, et al. Elevated platelet beta-secretase activity in mild cognitive impairment. Dement Geriatr Cogn Disord. 2007;24(6):464–8.

135. Evin G, Li Q-X. Platelets and Alzheimer's disease: Potential of APP as a biomarker. World J psychiatry. 2012 Dec 22;2(6):102–13.

136. Lauritzen I, Pardossi-Piquard R, Bauer C, Brigham E, Abraham J-D, Ranaldi S, et al. The -Secretase-Derived C-Terminal Fragment of APP, C99, But Not A , Is a Key Contributor to Early Intraneuronal Lesions in Triple-Transgenic Mouse Hippocampus. J Neurosci. 2012;

137. Martins RN, Turner BA, Carroll RT, Sweeney D, Kim KS, Wisniewski HM, et al. High levels of amyloid-beta protein from S182 (Glu246) familial Alzheimer's cells. Neuroreport. 1995 Dec 29;7(1):217–20.

138. Köpke Tung YC, Shaikh S, Alonso ADC, Iqbal K, Grundke-Iqbal I, Köpke E, et al. Microtubule-associated protein tau: Abnormal phosphorylation of a non-paired helical filament pool in Alzheimer disease. J Biol Chem. 1993;

139. Neumann K, Farías G, Slachevsky A, Perez P, Maccioni RB, Farias G, et al. Human platelets tau: a potential peripheral marker for Alzheimer's disease. J Alzheimers Dis. 2011/03/04. 2011;25(1):103–9.

140. Guzmán-Martínez L, Tapia JP, Farías GA, González A, Estrella M, Maccioni RB. The Alz-tau Biomarker for Alzheimer's Disease: Study in a Caucasian Population. J Alzheimers Dis. 2019;67(4):1181–6.

141. Weingarten MD, Lockwood AH, Hwo SY, Kirschner MW. A protein factor essential for microtubule assembly. Proc Natl Acad Sci U S A. 1975;72(5):1858–62.

142. Tenreiro S, Eckermann K, Outeiro TF. Protein phosphorylation in neurodegeneration: Friend or foe? Vol. 7, Frontiers in Molecular Neuroscience. Frontiers Research Foundation; 2014.

143. Lund H, Cowburn RF, Gustafsson E, Strömberg K, Svensson A, Dahllund L, et al. Tau-tubulin kinase 1 expression, phosphorylation and co-localization with phospho-Ser422 tau in the Alzheimer's disease brain. Brain Pathol. 2013 Jul;23(4):378–89.

144. Goedert M, Jakes R, Crowther RA, Six J, Lübke U, Vandermeeren M, et al. The abnormal phosphorylation of tau protein at Ser-202 in Alzheimer disease recapitulates phosphorylation during development. Proc Natl Acad Sci U S A. 1993 Jun 1;90(11):5066–70.

145. Bramblett GT, Goedert M, Jakes R, Merrick SE, Trojanowski JQ, Lee VM. Abnormal tau phosphorylation at Ser396 in Alzheimer's disease recapitulates development and contributes to reduced microtubule binding. Neuron. 1993 Jun;10(6):1089–99.

146. Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. Vol. 82, Acta Neuropathologica. Springer-Verlag; 1991. p. 239–59.

147. Goedert M, Jakes R, Vanmechelen E. Monoclonal antibody AT8 recognises tau protein phosphorylated at both serine 202 and threonine 205. Neurosci Lett. 1995/04/21. 1995;189(3):167–9.

148. Nasreddine ZS, Phillips NA, Bédirian V, Charbonneau S, Whitehead V, Collin I, et al. The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. J Am Geriatr Soc. 2005;

149. Crook TH, Feher EP, Larrabee GJ. Assessment of memory complaint in age-associated memory impairment: the MAC-Q. Int psychogeriatrics. 1992;4(2):165–76.

150. Estévez-González A, Kulisevsky J, Boltes A, Otermín P, García-Sánchez C. Rey verbal learning test is a useful tool for differential diagnosis in the preclinical phase of Alzheimer's disease: Comparison with mild cognitive impairment and normal aging. Int J Geriatr Psychiatry. 2003 Nov;18(11):1021–8.

151. Wechsler D. A Standardized Memory Scale for Clinical Use. J Psychol Interdiscip Appl. 1945;19(1):87–95.

152. Delis DC, Kaplan E, Kramer JH. Delis-Kaplan Executive Function System (D-KEFS). San Antonio, TX: The Psychological Corporation; 2001.

153. Wechsler D. Wechsler Adult Intelligence Scale – III (WAIS-III) Administration and Scoring Manual. San Antonio, TX: The Psychological Corporation; 1997.

154. Patterson J. COWAT. In: Kreutzer J, DeLuca J, Caplan B, editors. Encyclopedia of Clinical Neuropsychology. Springer New York; 2011. p. 727–727.

155. Kaplan E, Goodglass H, Weintraub S. Boston naming test. Philadelphia: Lea & Febiger; 1983.

156. Bourgeat P, Villemagne VL, Dore V, Brown B, Macaulay SL, Martins R, et al. Comparison of MR-less PiB SUVR quantification methods. Neurobiol Aging. 2015 Jan;36 Suppl 1:S159-66.

157. Zhou L, Salvado O, Dore V, Bourgeat P, Raniga P, Macaulay SL, et al. MR-less surface-based amyloid assessment based on 11C PiB PET. PLoS One. 2014;9(1):e84777.

158. Goozee K, Chatterjee P, James I, Shen K, Sohrabi HR, Asih PR, et al. Alterations in erythrocyte fatty acid composition in preclinical Alzheimer's disease. Sci Rep. 2017 Dec 1;7(1).

159. Goozee K, Chatterjee P, James I, Shen K, Sohrabi HR, Asih PR, et al. Elevated plasma ferritin in elderly individuals with high neocortical amyloid-β load. Mol Psychiatry. 2018 Aug 1;23(8):1807–12.

160. Koch W, Ehrenhaft A, Griesser K, Pfeufer A, Müller J, Schömig A, et al. TaqMan systems for genotyping of disease-related polymorphisms present in the gene encoding apolipoprotein E. Clin Chem Lab Med. 2002 Nov;40(11):1123–31.

161. Chatterjee P, Zetterberg H, Goozee K, Lim CK, Jacobs KR, Ashton NJ, et al. Plasma neurofilament light chain and amyloid-β are associated with the kynurenine pathway metabolites in preclinical Alzheimer's disease. J Neuroinflammation. 2019 Dec 10;16(1):186.

162. Janelidze S, Stomrud E, Palmqvist S, Zetterberg H, van Westen D, Jeromin A, et al. Plasma β-amyloid in Alzheimer's disease and vascular disease. Sci Rep. 2016;6:26801.

163. Schuck F, Wolf D, Fellgiebel A, Endres K. Increase of α-Secretase ADAM10 in Platelets Along Cognitively Healthy Aging. J Alzheimers Dis. 2016;50(3):817–26.

164. Manzine PR, de França Bram JM, Barham EJ, do Vale F de AC, Selistre-de-Araújo HS, Cominetti MR, et al. ADAM10 as a biomarker for Alzheimer's disease: a study with Brazilian elderly. Dement Geriatr Cogn Disord. 2013;35(1–2):58–66.

165. Marksteiner J, Humpel C. Platelet-derived secreted amyloid-precursor protein-β as a marker for diagnosing Alzheimer's disease. Curr Neurovasc Res. 2013;10(4):297–303.

166. Yasuoka K, Hirata K, Kuraoka A, He JW, Kawabuchi M. Expression of amyloid precursor protein-like molecule in astroglial cells of the subventricular zone and rostral migratory stream of the adult rat forebrain. J Anat. 2004 Aug;205(2):135–46.

167. Del Turco D, Paul MH, Schlaudraff J, Hick M, Endres K, Müller UC, et al. Region-specific differences in amyloid precursor protein expression in the mouse hippocampus. Front Mol Neurosci. 2016 Nov 29;9(NOV2016).

168. Bush AI, Martins RN, Rumble B, Moir R, Fuller S, Milward E, et al. The amyloid precursor protein of Alzheimer's disease is released by human platelets. J Biol Chem. 1990 Sep 15;265(26):15977–83.

169. Shaw LM, Vanderstichele H, Knapik-Czajka M, Figurski M, Coart E, Blennow K, et al. Qualification of the analytical and clinical performance of CSF biomarker analyses in ADNI. Acta Neuropathol. 2011 May;121(5):597–609.

170. Baskin F, Rosenberg RN, Iyer L, Schellenberg GD, Hynan L, Nee LE. Platelet APP isoform ratios in asymptomatic young adults expressing an AD-related presenilin-1 mutation. J Neurol Sci. 2001 Jan 15;183(1):85–8.

171. Poorkaj P, Sharma V, Anderson L, Nemens E, Alonso ME, Orr H, et al. Missense mutations in the chromosome 14 familial Alzheimer's disease presenilin 1 gene. Hum Mutat. 1998;11(3):216–21.

172. Pastorino L, Colciaghi F, Marcon G, Borroni B, Cottini E, Cattabeni F, et al. Presenilin 2 Mutation Does Not Influence Expression and Concentration of APP Forms in Human Platelets. Mol Med. 2000 Oct 1;6(10):816–24.

173. Chen M. The Maze of APP Processing in Alzheimer's Disease: Where Did We Go Wrong in Reasoning? Front Cell Neurosci. 2015;9:186.

174. Chen M, Nguyen HT. Our "energy-Ca(2+) signaling deficits" hypothesis and its explanatory potential for key features of Alzheimer's disease. Front Aging Neurosci. 2014;6:329.

175. Vassar R, Bennett BD, Babu-Khan S, Kahn S, Mendiaz EA, Denis P, et al. β-Secretase

cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. Science (80-). 1999 Oct 22;286(5440):735–41.

176. Haniu M, Denis P, Young Y, Mendiaz EA, Fuller J, Hui JO, et al. Characterization of Alzheimer's β-secretase protein BACE: A pepsin family member with unusual properties. J Biol Chem. 2000 Jul 14;275(28):21099–106.

177. Liu L, Ding L, Rovere M, Wolfe MS, Selkoe DJ. A cellular complex of BACE1 and γ-secretase sequentially generates Aβ from its full-length precursor. J Cell Biol. 2019 Feb 1;218(2):644–63.

178. Yang LB, Lindholm K, Yan R, Citron M, Xia W, Yang XL, et al. Elevated β-secretase expression and enzymatic activity detected in sporadic Alzheimer disease [1]. Vol. 9, Nature Medicine. 2003. p. 3–4.

179. Li R, Lindholm K, Yang LB, Yue X, Citron M, Yan R, et al. Amyloid β peptide load is correlated with increased β-secretase activity in sporadic Alzheimer's disease patients. Proc Natl Acad Sci U S A. 2004 Mar 9;101(10):3632–7.

180. Liebsch F, Aurousseau MRP, Bethge T, McGuire H, Scolari S, Herrmann A, et al. Full-length cellular -secretase has a trimeric subunit stoichiometry, and its sulfur-rich transmembrane interaction site modulates cytosolic copper compartmentalization. J Biol Chem. 2017;292(32):13258–70.

181. Ansar W, Ghosh S. Monoclonal Antibodies: A Tool in Clinical Research. Indian J Clin Med. 2013 Jan;4:IJCM.S11968.

182. Cole SL, Vassar R. The Alzheimer's disease β-secretase enzyme, BACE1. Mol Neurodegener. 2007;2(1).

183. Patel-Hett S, Richardson JL, Schulze H, Drabek K, Isaac NA, Hoffmeister K, et al. Visualization of microtubule growth in living platelets reveals a dynamic marginal band with multiple microtubules. Blood. 2008 May 1;111(9):4605–16.

184. Sunderland T, Linker G, Mirza N, Putnam KT, Friedman DL, Kimmel LH, et al. Decreased beta-amyloid1-42 and increased tau levels in cerebrospinal fluid of patients with Alzheimer disease. JAMA. 289(16):2094–103.

185. Zhou Y, Shi J, Chu D, Hu W, Guan Z, Gong CX, et al. Relevance of phosphorylation and truncation of tau to the etiopathogenesis of Alzheimer's disease. Front Aging Neurosci. 2018 Feb 6;10(FEB).

186. Sjögren M, Davidsson P, Tullberg M, Minthon L, Wallin A, Wikkelso C, et al. Both total and phosphorylated tau are increased in Alzheimer's disease. J Neurol Neurosurg Psychiatry. 2001 May;70(5):624–30.

187. Johnson G V, Seubert P, Cox TM, Motter R, Brown JP, Galasko D. The tau protein in human cerebrospinal fluid in Alzheimer's disease consists of proteolytically derived fragments. J Neurochem. 1997 Jan;68(1):430–3.

188. Toledo JB, Xie SX, Trojanowski JQ, Shaw LM. Longitudinal change in CSF Tau and Aβ biomarkers for up to 48 months in ADNI. Acta Neuropathol. 2013 Nov;126(5):659–70.

189. Jack CR, Wiste HJ, Schwarz CG, Lowe VJ, Senjem ML, Vemuri P, et al. Longitudinal tau PET in ageing and Alzheimer's disease. Brain. 2018 May 1;141(5):1517–28.

190. Hanseeuw BJ, Mormino BC, Becker A, Sepulcre J, Papp K V., Schultz AP, et al. Longitudinal tau accumulation is associated with cognitive decline in normal elderly. Alzheimer's Dement. 2017 Jul;13(7):P134–6.

191. Vlassenko AG, Benzinger TLS, Morris JC. PET amyloid-beta imaging in preclinical Alzheimer's disease. Vol. 1822, Biochimica et Biophysica Acta - Molecular Basis of Disease. 2012. p. 370–9.

192. Hanseeuw BJ, Betensky RA, Jacobs HIL, Schultz AP, Sepulcre J, Becker JA, et al. Association of Amyloid and Tau with Cognition in Preclinical Alzheimer Disease: A Longitudinal Study. JAMA Neurol. 2019;

193. Grady CL. Cognitive neuroscience of aging. Ann N Y Acad Sci. 2008 Mar;1124:127–44.

194. Tromp D, Dufour A, Lithfous S, Pebayle T, Després O. Episodic memory in normal aging and Alzheimer disease: Insights from imaging and behavioral studies. Vol. 24, Ageing Research Reviews. Elsevier Ireland Ltd; 2015. p. 232–62.

195. Cowan N. Chapter 20 What are the differences between long-term, short-term, and working memory? Vol. 169, Progress in Brain Research. 2008. p. 323–38.

196. Reisberg B, Ferris SH, De Leon MJ, Crook T. The global deterioration scale for assessment of primary degenerative dementia. Am J Psychiatry. 1982;139(9):1136–9.

197. Sharp ES, Gatz M. Relationship between education and dementia: An updated systematic review. Vol. 25, Alzheimer Disease and Associated Disorders. 2011. p. 289–304.

198. Buckley RF, Hanseeuw B, Schultz AP, Vannini P, Aghjayan SL, Properzi MJ, et al. Region-Specific Association of Subjective Cognitive Decline With Tauopathy Independent of Global β-Amyloid Burden. JAMA Neurol. 2017;74(12):1455–63.

199. Schöll M, Lockhart SN, Schonhaut DR, O'Neil JP, Janabi M, Ossenkoppele R, et al. PET Imaging of Tau Deposition in the Aging Human Brain. Neuron. 2016 Mar 2;89(5):971–82.

200. Maass A, Lockhart SN, Harrison TM, Bell RK, Mellinger T, Swinnerton K, et al. Entorhinal tau pathology, episodic memory decline, and neurodegeneration in aging. J Neurosci. 2018 Jan 17;38(3):530–43.

201. Van Geldorp B, Heringa SM, Van Den Berg E, Olde Rikkert MGM, Biessels GJ, Kessels RPC. Working memory binding and episodic memory formation in aging, mild cognitive impairment, and Alzheimers dementia. J Clin Exp Neuropsychol. 2015 May 28;37(5):538–48.

202. Rankin CA, Sun Q, Gamblin TC. Pseudo-phosphorylation of tau at Ser202 and Thr205 affects tau filament formation. Brain Res Mol Brain Res. 2005 Jul 29;138(1):84–93.

203. Neddens J, Temmel M, Flunkert S, Kerschbaumer B, Hoeller C, Loeffler T, et al. Phosphorylation of different tau sites during progression of Alzheimer's disease. Acta Neuropathol Commun. 2018 Jun 29;6(1):52.

204. Höglund K, Kern S, Zettergren A, Börjesson-Hansson A, Zetterberg H, Skoog I, et al. Preclinical amyloid pathology biomarker positivity: Effects on tau pathology and neurodegeneration. Transl Psychiatry. 2017;7(1).

205. Maccioni RB, Lavados M, Guillón M, Mujica C, Bosch R, Farías G, et al. Anomalously phosphorylated tau and Aβ fragments in the CSF correlates with cognitive impairment in MCI subjects. Neurobiol Aging. 2006 Feb;27(2):237–44.

206. Risacher SL, Fandos N, Romero J, Sherriff I, Saykin AJ, Apostolova LG. Association between plasma Aβ levels and cerebral amyloid and tau deposition. Alzheimer's Dement. 2018 Jul;14(7):P1551.

207. Janelidze S, Stomrud E, Palmqvist S, Zetterberg H, Van Westen D, Jeromin A, et al. Plasma β-amyloid in Alzheimer's disease and vascular disease. Sci Rep. 2016 May 31;6.

208. Jonker C, Geerlings MI, Schmand B. Are Memory Complaints Predictive for Dementia? Int J Geriatr Psychiatry. 2000 Nov;15(11):983–91.

209. Jessen F, Wolfsgruber S, Wiese B, Bickel H, Mösch E, Kaduszkiewicz H, et al. AD dementia risk in late MCI, in early MCI, and in subjective memory impairment. Alzheimers Dement. 2014 Jan;10(1):76–83.

210. Geerlings MI, Jonker C, Bouter LM, Adèr HJ, Schmand B. Association between memory complaints and incident Alzheimer's disease in elderly people with normal baseline cognition.

Am J Psychiatry. 1999 Apr;156(4):531–7.

211. Jessen F, Wiese B, Bachmann C, Eifflaender-Gorfer S, Haller F, Kölsch H, et al. Prediction of Dementia by Subjective Memory Impairment. Arch Gen Psychiatry. 2014 Apr;67(4):414–22.

212. Reisberg B, Prichep L, Mosconi L, John ER, Glodzik-Sobanska L, Boksay I, et al. The pre-mild cognitive impairment, subjective cognitive impairment stage of Alzheimer's disease. Alzheimers Dement. 2008 Jan;4(1 Suppl 1):S98–108.

213. Zainaghi IA, Talib LL, Diniz BS, Gattaz WF, Forlenza O V. Reduced platelet amyloid precursor protein ratio (APP ratio) predicts conversion from mild cognitive impairment to Alzheimer's disease. J Neural Transm. 2012 Jul;119(7):815–9.

214. Kurien BT, Dorri Y, Dillon S, Dsouza A, Scofield RH. An overview of Western blotting for determining antibody specificities for immunohistochemistry. Methods Mol Biol. 2011;717:55–67.

215. Rowley JW, Oler AJ, Tolley ND, Hunter BN, Low EN, Nix DA, et al. Genome-wide RNA-seq analysis of human and mouse platelet transcriptomes. Blood. 2011 Oct 6;118(14).

216. McRedmond JP, Park SD, Reilly DF, Coppinger JA, Maguire PB, Shields DC, et al. Integration of proteomics and genomics in platelets. A profile of platelet proteins and platelet-specific genes. Mol Cell Proteomics. 2004 Feb;3(2):133–44.

217. Daniele S, Pietrobono D, Fusi J, Lo Gerfo A, Cerri E, Chico L, et al. α-synuclein aggregated with tau and β-amyloid in human platelets from healthy subjects: Correlation with physical exercise. Front Aging Neurosci. 2018 Jan 30;10(JAN).

Appendix

Appendix A. Correlations of platelet proteins with age

Platelet proteins	r_s (*p*)
APP 105-115kDa	0.005 (0.971)
APP 80-100kDa	0.042 (0.747)
Total APP	0.020 (0.878)
APPr	-0.097 (0.463)
ADAM10	-0.018 (0.894)
BACE1 ~200kDa	-0.083 (0.528)
BACE1 ~160kDa	-0.052 (0.693)
BACE1 ~110kDa	-0.020 (0.881)
BACE1 ~80kDa	0.067 (0.609)
BACE1 ~60kDa	-0.003 (0.985)
Tau ~ 200kDa	0.013 (0.920)
Tau ~ 110kDa	0.096 (0.468)
Tau ~ 80kDa	0.177 (0.180)
Tau ~ 55kDa	0.151 (0.252)
Tau ~ 50kDa	0.037 (0.780)
Tau ~ 33kDa	-0.132 (0.321)
T-tau	0.045 (0.746)
HMW/LMW tau	0.085 (0.524)
P-tau ser202/thr205 ~ 200kDa	-0.100 (0.473)
P-tau ser202/thr205 ~80kDa	0.036 (0.798)
P-tau ser202/thr205 ~ 55kDa	0.206 (0.136)
P-tau ser202/thr205 ~33kDa	0.062 (0.658)
P-tau	0.055 (0.692)
P-tau/t-tau	-0.008 (0.952)

Appendix A shows the correlation of age with platelet proteins. No significant associations were seen with the age and the platelet proteins. Abbreviations: NAL, neocortical beta-amyloid load; APP, Amyloid precursor protein; APPr, amyloid precursor protein ratio; ADAM10, a Disintegrin and metalloproteinase; BACE1, beta-secretase. HMW/LMW, high molecular weight/low molecular weight; t-tau, sum of all tau bands; p-tau ser202/thr205, phosphorylated tau at serine202/threonine205; p-tau, sum of all phosphorylated tau bands at serine202/threonine 205; p-tau/t-tau, sum of all phosphorylated tau bands at serine 202/threonine 205/sum of all tau bands.

Appendix B. Correlations of platelet proteins with the composite z-scores of working memory and executive function

Platelet Proteins	r_s (*p*)
APP 105-115kDa	0.055 (0.686)
APP 80-100kDa	0.064 (0.641)
APPr	0.075 (0.581)
ADAM10	0.041 (0.762)
BACE1 ~200kDa	0.141 (0.301)
BACE1 ~160kDa	0.038 (0,780)
BACE1 ~110kDa	-0.069 (0.612)
BACE1 ~80kDa	0.049 (0.717)
BACE1 ~60kDa	0.040 (0.768)
Tau ~ 200kDa	0.012 (0.930)
Tau ~ 110kDa	-0.124 (0.349)
Tau ~ 80kDa	-0.107 (0.421)
Tau ~ 55kDa	-0.007 (0.959)
Tau ~ 50kDa	-0.010 (0.939)
Tau ~ 33kDa	0.022 (0.870)
T-tau	0.013 (0.928)
HMW/LMW tau	-0.117 (0.379)
P-tau ser202/thr205 ~ 200kDa	0.208 (0.131)
P-tau ser202/thr205 ~80kDa	-0.007 (0.958)
P-tau ser202/thr205 ~ 55kDa	-0.105 (0.449)
P-tau ser202/thr205 ~ 33kDa	-0.048 (0.733)
P-tau	0.065 (0.641)
P-tau/t-tau	0.108 (0.435)

Appendix B shows the correlations between platelet proteins and a composite score for working memory and executive function. No significant correlations were seen the composite z-scores of working memory and executive function with the platelet proteins. The composite z-scores for working memory and executive function were calculated from the summation of z-scores of Digit span backward, DSST, and D-KEFS Category Fluency and Switching Tasks. Abbreviations: APP, amyloid precursor protein; APPr, APP 105-115kDa/108-110kDa ratio; ADAM10, a Disintegrin and metalloproteinase;BACE1, beta-secretase; HMW/LMW, high molecular weight/low molecular weight; t-tau, sum of all tau bands; p-tau ser202/thr205, phosphorylated tau at site serine 202/threonine 205; p-tau, sum of all phosphorylated tau bands at serine202/threonine 205; p-tau/t-tau, sum of all phosphorylated tau bands at serine 202/threonine 205/sum of all tau bands; DSST, Digit Symbol Substitution Test; D-KEFS, Delis-Kaplan Executive Function System.

Appendix C: Correlations of plasma NFL with platelet proteins

Platelet Proteins	r_s (*p*)
APP 105-115kDa	-0.184 (0.159)
APP 80-100kDa	-0.156 (0.234)
APPr	-0.155 (0.237)
ADAM10	-0.213 (0.103)
BACE1 ~200kDa	-0.013 (0.920)
BACE1 ~160kDa	-0.089 (0.497)
BACE1 ~110kDa	-0.187 (0.152)
BACE1 ~80kDa	-0.083 (0.529)
BACE1 ~60kDa	-0.118 (0.370)
Tau ~ 200kDa	0.062 (0.640)
Tau ~ 110kDa	0.190 (0.148)
Tau ~ 80kDa	0.160 (0.227)
Tau ~ 55kDa	0.104 (0.433)
Tau ~ 50kDa	0.137 (0.301)
Tau ~ 33kDa	-0.061 (0.648)
T-tau	0.017 (0.901)
HMW/LMW tau	0.122 (0.359)
P-tau ser202/thr205 ~ 200kDa	-0.069 (0.619)
P-tau ser202/thr205 ~80kDa	0.199 (0.148)
P-tau ser202/thr205 ~ 55kDa	0.208 (0.131)
P-tau ser202/thr205 ~ 33kDa	0.204 (0.126)
P-tau	0.189 (0.171)
P-tau/t-tau	0.181 (0.191)

Appendix C shows the correlations of plasma NFL with the platelet proteins. No significant correlations or trends were observed. Abbreviations: APP, amyloid precursor protein; APPr, APP 105-115kDa/108-110kDa ratio; ADAM10, a Disintegrin and metalloproteinase; BACE1,beta-secretase; HMW/LMW, high molecular weight/low molecular weight; t-tau, sum of all tau bands; p-tau ser202/thr205, phosphorylated tau at site serine 202/threonine 205; p-tau, sum of all phosphorylated tau bands at serine202/threonine 205; p-tau/t-tau, sum of all phosphorylated tau bands at serine 202/threonine 205/sum of all tau bands; NFL, neurofilament light chain.

Appendix (Ethics Approval) of this thesis has been removed as it may contain sensitive/confidential content