5-2022

Biomarkers in the Detection of Alzheimer's Disease

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by

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A Scholarly Project

Submitted to the Graduate Faculty of the University of North Dakota

in partial fulfillment of the requirements for the degree of

Master of Physician Assistant Studies

Grand Forks, North Dakota

May 2022

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Acknowledgements

I would like to thank my advisor and contributing author Jay Metzger, MPAS, for his guidance in this project. I appreciate the recommendations and support provided by instructors Mindy Staveteig, PA-C and Daryl Sieg, PA-C through this journey. I would like to express gratitude to Dr. Kelly Krogh for his expertise, guidance, and recommendations throughout this project. Lastly, I would like to thank my family and friends for their endless support.

Abstract

Alzheimer's Disease (AD) is a progressive form of dementia that affects memory, cognition, and functional ability. Although there are diagnostic tests being used to aid in the clinical diagnosis of AD, the only definitive method of diagnosis is through post-mortem biopsy of the brain. The purpose of this project and literature review is to investigate the most effective biomarkers when clinically evaluating AD. The main biomarkers of investigation include positron emission tomography (PET) imaging, cerebral spinal fluid (CSF), and blood-based biomarkers. The role of the apolipoprotein E (APOE4) gene as a genetic influence on AD was also evaluated within this literature review. Studies regarding information on CSF and plasma biomarkers that were dated prior to 2016 were excluded. Studies regarding PET imaging dated prior to 2011 were also excluded. The research conducted in this review indicates that an individual with a positive APOE4 genotype has a higher risk of developing AD in comparison to those with APOE2 and APOE3 genotypes. Amyloid PET imaging and CSF biomarkers demonstrate immense potential in offering diagnostic hope of AD. Of the CSF biomarkers, the Aβ-1-42/T-tau and Aβ-1-42/P-tau181 ratios may be the most reliable in recognizing AD. Plasma biomarkers Aβ 42/40 and plasma p-tau 181 have shown extreme promise in presumed AD cases. The PrecivityAD™ blood test is the first available blood test used to identify AD pathology, by use of plasma Aβ 42/40 and APOE genotype.

Keywords: Alzheimer's Disease, amyloid beta, tau, PET, cerebral spinal fluid, blood, APOE-ε4

Biomarkers in the Detection of Alzheimer's Disease

Alzheimer's Disease (AD) is a progressive form of dementia that affects many individuals worldwide. This disease is characterized by many neurological changes that may lead to alterations in memory, language, behavior, and the ability of an individual to care for themselves. AD is frequently clinically diagnosed, and often times, is not diagnosed until an individual has significant symptoms of disease progression. AD is most often associated with increasing age; however, a portion of the population affected by AD are diagnosed with early onset dementia. This literature review evaluates the efficacy of various biomarkers used in the diagnosis of AD. Current biomarkers of research include cerebral spinal fluid (CSF) via lumbar puncture, positron emission tomography (PET) imaging, and blood-based biomarkers.

Statement of the Problem

Alzheimer's Disease is often clinically diagnosed once symptoms of the disease are significant. It can also be difficult to differentiate AD from other forms of dementia, especially in the early stages of disease. Although no curative treatments for AD exist at this time, there are currently medications that can be utilized through various stages of the disease to help control and delay symptoms. Earlier detection, intervention, and symptom control of the disease could ultimately improve patient outcomes. In order for curative or preventative options to be of benefit in the future, there must be methods suitable for early detection of the disease. This review focuses on the question of whether these biomarkers can be of benefit in the diagnosis of this disease.

Research Question

Are there biomarkers that are efficacious in the detection of Alzheimer's Disease?

Research Methods

A comprehensive literature review was performed using electronic databases including PubMed, AccessMedicine, Clinical Key, and CINHAL. Search criteria included the MeSH terms Alzheimer's Disease and biomarker. Key words included CSF, blood, PET, amyloid beta, tau, diagnosis, mild cognitive impairment, and APOE-eε4. The MeSH words Alzheimer's Disease and biomarker were paired with key terms as separate search engines in order to find studies regarding the specific biomarker and its efficacy in the diagnosis of AD. The search engine was limited to the last five years of research. In total, the literature review encompassed approximately 2,460 articles. The review excluded studies with a sample size less than 75 and those that used non-human subjects. Studies/trials regarding information on CSF and plasma biomarkers that were dated prior to 2016 were excluded. Studies regarding PET imaging dated prior to 2011 were also excluded. Research regarding the pathophysiology of AD was not limited to a certain timeline. The literature review included clinical trials, meta-analysis, randomized controlled trials, and systematic reviews. The articles were reviewed and assessed for pertinence regarding the topic and narrowed using quality review methods, disregarding bias, and duplicate articles.

Literature Review

Pathophysiology of AD

Alzheimer's Disease is a form of dementia that causes neurodegenerative changes of the brain that ultimately lead to progressive cognitive decline. Initial symptoms of AD typically begin with changes in memory. These changes progress overtime to include language, visuospatial, and executive dysfunction (Seeley & Miller, 2018). There are two forms of AD which include early onset AD and late onset AD. The development of late onset AD is thought to be multifactorial, involving both genetic and environmental factors. The primary genetic factor that is thought to play a role in late onset AD is a positive carrier status of the ε4 allele on the apolipoprotein E (APOE) gene (Seeley & Miller, 2018). The development of early onset AD typically occurs prior the age 65 and is due to mutations on genes including amyloid precursor protein (APP) gene and the presenilin genes (1 and 2). These mutations ultimately cause over production of Aβ in the brain. Early onset familial AD accounts for less than 1% of cases, while the late onset form of AD contributes to most cases (>95%) (Masters et al., 2015).

There are two key pathological features of AD including the deposition of β-amyloid plaques in the brain and neurofibrillary tangles of tau protein (Weller & Budson, 2018). The deposition of β-amyloid plaques and neurofibrillary tangles eventually leads to neuronal dysfunction and loss, ultimately causing macroscopic atrophy of brain tissue (Lane et al., 2018). Amyloid plaques are misfolded amyloid-β with either 40 or 42 amino acids, known as Aβ-40 and Aβ-42. Of the two, Aβ-42 is more abundant due to structural components. Neurofibrillary tangles are paired helical filaments of tau proteins (Lane et al., 2018). In AD, there is an inverse relationship between amyloid levels in the brain and CSF: as amyloid plaque deposition increases, levels in CSF decrease. CSF tau levels parallel the increased levels seen in the brain in

a typical case of AD (Lane et al., 2018). Although there are many areas of the brain affected in AD, the areas responsible for basic cognitive function and memory are most often affected by the deposition of β-amyloid plaques and neurofibrillary tangles. Initially, this primarily includes the cerebral cortex and hippocampus (Lane et al., 2018). Although the definitive diagnosis of AD requires post-mortem biopsy of the brain, there are emerging biomarkers that may aid in the diagnosis of AD prior to post-mortem evaluation (Weller & Budson, 2018).

The role of APOE-ε4 and AD

ApoE is a protein that is abundant within the human brain. This protein plays a role in lipid transport, energy production, signaling, inflammation, and metabolism (Uddin et al., 2019). There are three different alleles including APOE2, APOE3, APOE4 that each encode for various proteins within the body (ApoE2, ApoE3, ApoE4). The exact genetic mechanisms of the APOE gene and AD are still being researched. When evaluating the prevalence of the APOE alleles, the APOE3 allele has the highest frequency overall approximating at 77.8%, while APOE2 (8.4%) and APOE4 (13.7%) occur less frequently (Liu et al., 2013). In AD, the prevalence of APOE4 increases up to 40% suggesting that there may be a link between APOE4 and AD (Liu et al., 2013). Individuals that are heterozygous for the APOE4 allele have a three times higher risk of developing AD, while those who are homozygous for the APOE4 allele have up to 12 times the risk of AD. The APOE2/APOE2 and APOE2/APOE3 genotypes have a more protective role against AD (Kirmess et al., 2021). ApoE is thought to play a role in the aggregation and clearance of Aβ, neurodegenerative characteristics of tau pathology, and pro-inflammatory markers in the brain (Uddin et al., 2019). According to Liu et al. (2013), amyloid plaque deposition is most apparent in people between the ages of 50-59 years with approximately 40%

of APOE4 carriers having evidence of amyloid plaque deposition versus approximately 8% of non-carriers.

To further assess the variation of APOE, Lautner et al. (2017) evaluated the correlation of APOE status and CSF levels of Aβ-42. In amyloid pathology, CSF Aβ-42 levels are typically low. Previous research has been done with this correlation in more elderly populations. Lautner et al. specifically evaluated the CSF Aβ-42 levels in conjunction with APOE status in various age groups without known amyloid pathology. This study had a total of 716 (n=716) cognitively healthy individuals who ranged in age from 17-99 years. Participants were split into three subgroups based on age including those less than 45 years (n=237), 46-64 years (n=242), and those over 65 years (n=237). After genotyping of the participants, it was found that 506 participants (70.7%) did not have an APOE4 allele, 190 participants were APOE4 heterozygous (26.5%), and 20 participants were APOE4 homozygous (2.8%) (Lautner et al., 2017).

When evaluating Aβ-42 levels within CSF and APOE4 carrier status, Lautner et al. found that lower concentrations of CSF Aβ-42 were associated with a positive APOE4 status ($p<0.001$). There was no significant correlation between CSF Aβ-42 levels and individuals under 45 years old; however, a statistically significant correlation was seen between CSF levels and those 46-64 years ($p<0.001$) and those over 65 years ($p<0.001$). In individuals that were APOE heterozygous and APOE negative, it was found that CSF Aβ-42 levels increased over time followed by a decrease in levels. The mean age at which CSF Aβ-42 reached maximum levels followed by a decline was 43 years (95% CI 17–48) for APOE4 heterozygous individuals and 50 years (95% CI 42-54) for APOE negative individuals. In contrast, CSF Aβ-42 levels in APOE homozygous individuals did not initially increase and rather stayed at lower levels (Lautner et al., 2017).

Overall, Lautner et al. concluded that the Aβ pathology seen in AD may begin in middle age individuals who carry the APOE4 allele. Although this study had a large sample size, only a small number of individuals carried a homozygous status for the APOE4 allele. Lautner et al. recognized that future studies should be done with participants of the same setting rather than a cross sectional approach.

Amyloid PET Imaging as a Biomarker for the Diagnosis of AD

Amyloid PET imaging has emerged as a widely used method to visualize amyloid plaques in vivo in patients with presumed AD. It is the only FDA approved method for evaluation of brain amyloid in presumed AD cases (Kirmess et al., 2021). Given that amyloid plaque deposition in the brain is a primary characteristic of AD, having the ability to detect amyloid deposition via imaging aids in proper clinical diagnosis of AD. There are currently three approved radioligands that can be used for PET imaging including ^{18}F-Florbetaben (Neuraceq), ^{18}F-Florbetapir (Amyvid), and ^{18}F-Flutemetamol (Vizamyl). These ligands have a high affinity for amyloid in the brain and have a longer half-life than tracers that have previously been utilized offering benefit for more thorough evaluation in presumed AD cases (Anand & Sabbagh, 2017).

Doraiswamy et al. (2012) evaluated the efficacy of ^{18}F-Florbetapir PET in detecting risk of cognitive decline in individuals through a longitudinal study over 18 months. The study involved 151 individuals split into groups consisting of those with MCI (n=51), those with diagnosed AD (n=31), and a healthy control group (n=69). Each PET scan was reviewed and given a semiquantitative (0-4) and binary qualitative classification Aβ(+/-), followed by calculated standardized uptake value ratio (SUVR) to determine overall PET status (Doraiswamy

et al., 2012). In addition to PET imaging, participants also underwent both cognitive and functional evaluations which will not be covered in detail for the purpose of this review.

The following results showed the percent of individuals in each group subset that were classified as Aβ+ ($p<0.0001$) during baseline florbetapir PET image evaluation: 14% of the healthy control group, 37% of the MCI group, and 68% of the AD group (Doraiswamy et al., 2012). When evaluating overall change in status over the 18-month period of time, Doraiswamy et al. found that eight people of the MCI group converted to an AD status. Seven individuals of the MCI group converted to a cognitively healthy status. Of the MCI that converted to AD, a higher percentage were Aβ+ status (29.4%) versus those that were Aβ- status (10.3%), although not statistically significant ($p=0.0996$) (Doraiswamy et al., 2012).

Overall, Doraiswamy et al. (2012) concluded that individuals with higher levels of Aβ PET are at higher risk of cognitive/functional decline, suggesting that ^{18}F-Florbetapir PET may be a "predictive biomarker" in at risk individuals. The sample size and the duration of evaluation were limitations of this study. Neurodegenerative changes occur over long periods of time so, the cognitive tests that were performed in this study would represent more accurate results had the study been over a longer duration. Thus, Doraiswamy et al. reported that this study is an insufficient representation of cognitive decline in an Aβ+ status.

Like the study completed by Doraiswamy et al. (2012), Johnson et al. (2013) evaluated the performance of ^{18}F-Florbetapir PET imaging in the diagnosis of AD. The study by Johnson et al. included 184 participants from a total of 24 centers. Participants were split into three categories including a control group (n=79), those with MCI (n=60), and those with AD (n=45). In addition to the evaluation of ^{18}F-Florbetapir PET imaging, Johnson et al. correlated PET findings to participant age and carrier status of APOE. PET interpretation was done through

semiquantitative visual reading and by a binary classification scoring (either visually positive or negative for amyloid beta). For a quantitative measure of ^{18}F-Florbetapir uptake, standardized uptake values (SUV) were created for various areas within the brain creating thresholds for positive results. Johnson et al. found that the ^{18}F-Florbetapir uptake was highest in the AD group followed by the MCI group and lowest for the control group for all diagnostic methods including visual readings ($p<0.0001$), binary Aβ(+/-) classification ($p<0.0001$) and standardized uptake value ratios ($p<0.0001$). ^{18}F-Florbetapir uptake was then examined in various areas of the brain including precuneus, frontal, temporal, parietal, anti-cingulate, and post-cingulate with all areas showing the highest uptake in the AD group and lowest uptake in the control group ($p<0.0001$ for all 6 areas of the brain evaluated). Looking more specifically at the relationship of binary classification and quantitative findings, 76% of the AD group, 38% of the MCI group, and 14% of the control group were classified as Aβ+ through visual binary classification scoring while 84% of the AD group, 42% of the MCI and 23% of the control group were classified as Aβ + through quantitative values (SUVR >1.10) (Johnson et al., 2013).

Additional factors that were evaluated included age, gender, education, race, APOE carrier status, and cognitive testing. Of these factors, age correlated with the SUVR in the control group ($p<0.005$) but not in the MCI($p=0.21$) or AD group ($p<0.97$) (Johnson et al., 2013). Regarding APOE status, there was significant correlation between SUVR and APOE in the AD group ($p=0.0017$) and the MCI group ($p<0.0001$); however, no statistical significance was found in the control group. There was no statistical significance when looking at gender, education, or race. Johnson et al. recognized that recruitment of participants and potential variation in screening factors could have played a role in the variation of participant amyloid classification (Johnson et al., 2013).

In a study completed by Lin et al. (2016), PET imaging using tracer ^{18}F-Florbetapir (AV-45/Amyvid) was evaluated to aid in the diagnostics of AD in regard to amyloid deposition and cerebral blood flow perfusion to the brain. Each participant underwent dual PET imaging which included an initial injection of a tracer known as perfusion 18F-AV-45 (pAV-45) to evaluate overall cortical perfusion followed by an injection of amyloid 18F-AV-45 (18F-AV-45) to evaluate amyloid deposition. The study included 82 participants (n=82) split into three different groups including healthy controls (n=14), those with MCI (n=44), and those with diagnosed AD (n=24). Participants of the MCI and AD groups that demonstrated Aβ negative results were not included in the study (Lin et al., 2016).

Lin et al. (2016) reported the results regarding perfusion utilizing the pAV-45 tracer by surface plots using 3D imaging. Regarding overall brain perfusion using the pAV-45 tracer, the healthy control group showed equal perfusion throughout the brain cortices (frontal, temporal, and occipital) for both Aβ positive and negative individuals, whereas the MCI and AD groups had more perfusion deficits. The MCI group was split into three subsets, and the AD group was split into two subsets. When looking more specifically into the three MCI groups, the MCI-1 and MCI-2 had perfusion similar to the control groups, while the MCI-3 group had perfusion that appeared closer to the AD-1 group. The MCI-3 group had moderately reduced perfusion throughout most cortices of the brain which was similar to the results of both the AD-1 and AD-2 groups which had slightly more extensive perfusion deficits. The 18F-AV-45 tracer used to evaluate amyloid deposition status was also reported using surface plots and 3D imaging. The healthy control groups demonstrated little amyloid deposition, while increased deposition was seen in both the MCI and AD groups, with more of an increase seen in the AD groups (Lin et al., 2016).

CSF as a Biomarker for the Diagnosis of AD

CSF directly interacts with extracellular fluid in the brain which is why it is a promising biomarker source in the detection of AD (Blennow et al., 2015). Through various studies, it has been recognized that there is an inverse relationship between CSF levels of Aβ42 and the deposition of amyloid plaques within the brain, while tau CSF levels parallel tau levels within the brain. Overall, hallmark CSF findings for individuals with AD include low levels of Aβ42 and high levels of p-tau and t-tau (Blennow et al., 2015).

To further evaluate the efficacy of CSF biomarkers, Hansson et al. (2018) examined whether CSF biomarkers Aβ (1–42), pTau/Aβ (1–42), and tTau/Aβ (1–42) were in conjunction with clinical presentation of disease and PET imaging results utilizing the Elecsys CSF immunoassay. A total of (n=646) participants were included in the validation of this immunoassay. The determined cutoff values included Aβ (1–42) 880 pg/mL, pTau/Aβ (1–42) 0.028, and tTau/Aβ (1–42) 0.33. The results of this study demonstrated that tTau/Aβ (1–42) and pTau/Aβ (1–42) ratios were most concordant with the PET findings over Aβ (1–42) levels. Overall, the p-tau/Aβ (1–42) ratio demonstrated the highest accuracy overall (Hansson et al., 2018).

Struyfs et al. (2015) further evaluated the efficacy of CSF amyloid-β biomarkers in detecting AD. This study specifically looked at the additive benefit that biomarkers Aβ 1-37, Aβ 1-38, and Aβ 1-40 may have in the diagnosis of AD. The CSF findings of Aβ 1-37, Aβ 1-38, and Aβ 1-40 were compared to Aβ 1-42, T-tau, and P-tau181P, as these have historically been used to enhance the diagnostic accuracy of AD. CSF was obtained from 200 participants including 50 individuals with AD, 50 individuals with non-AD dementia, 50 individuals with MCI, and 50 control patients. For the purpose of this review, the non-AD dementia subsets will not be covered

in detail. The study also examined the role of the biomarkers in question and their correlation with disease severity by testing with a mini mental status exam (MMSE), as well as their correlation with APOE- ε4 carrier status. Struyfs et al. found that MMSE scores moderately correlated with biomarkers Aβ 1-37, Aβ 1-38, and Aβ 1-40, with p values of 0.000, 0.003, and 0.002 respectively. In regard to APOE- ε4 carrier status, Struyfs et al. found that the Aβ 1-42 biomarker level was significantly lower in APOE- ε4 carriers versus non carriers ($p<0.001$). The statistical findings of the Aβ 1-37, Aβ 1-38, and Aβ 1-40 biomarkers when comparing APOE- ε4 carriers versus non carriers showed no significant differences (Struyfs et al., 2015).

To examine biomarker accuracy, Struyfs et al. (2015) utilized ROC curve analysis using sensitivity and specificity. Results demonstrated that there were no significant findings when differentiating AD and MCI. When comparing the AD group versus the control, results demonstrated the following: Aβ-1-42/T-tau (sensitivity of 93.9% and a specificity of 92.0%) and Aβ-1-42/P-tau181 (sensitivity of 91.8% and a specificity of 86.0%). Results for MCI group versus the control group demonstrated Aβ-1-42/T-tau (sensitivity of 83.7% and a specificity of 90.0%) and Aβ-1-42/ Aβ-1-40 (sensitivity of 91.8% and a specificity of 84.0%). The diagnostic performance of individual biomarkers Aβ 1-37, Aβ 1-38, and Aβ 1-40 were evaluated by looking at their ratios with Aβ-1-42. The Aβ-1-42/ Aβ-1-37 ratio showed significance ($p<0.05$) when comparing MCI versus control, MCI versus non-AD dementias, and AD versus non-AD dementias. The Aβ-1-42/ Aβ-1-38 ratio showed significance when comparing AD versus non-AD dementias ($p=0.049$), and MCI versus non-AD dementias ($p=0.000$). The Aβ-1-42/ Aβ-1-40 ratio showed significance when comparing MCI versus controls ($p=0.002$), MCI versus non-AD dementias ($p=0.000$). These findings indicated that CSF isoforms may be beneficial in increasing

the diagnostic accuracy of Aβ 1-42 and promoting benefit when differentiating AD from some non-AD forms of dementia (Struyfs et al., 2015).

Blood as a Biomarker for the Diagnosis of AD

Research on blood-based biomarkers in the clinical detection of AD has increased immensely due to the need for less invasive, cost-effective methods for detection. Due to the pathophysiology of AD, plasma Aβ and plasma tau levels have been of specific interest.

Plasma Aβ as a Biomarker

The blood test known as PrecivityAD™, was developed and evaluated by C2N Diagnostics (Kirmess et al., 2021). This blood test quantifies plasma Aβ42/Aβ40 levels and evaluates APOE genotype. To date, this is the only blood test available for use for the evaluation of AD (About PrecivityAD™, 2020). Although not FDA approved, the blood test, PrecivityAD™, is available in 49 states (About PrecivityAD™, 2020).

C2N Diagnostics has investigated the role of plasma amyloid in AD. The following study in this literature review further evaluates research completed by C2N Diagnostics leading to the development of PrecivityAD™. The Mass Spectrometry (MS) blood-based assay was developed to evaluate plasma Aβ42, plasma Aβ40, and APOE genotype (West et al., 2021). Plasma was collected in six cohorts, totaling 414 (n=414) participants. Approximately 59% of participants were female and 41% male with age ranging from 45-93 years. Prior to evaluation of plasma samples by C2N Diagnostics, amyloid PET imaging and CSF biomarkers were used to determine brain amyloid status in each participant. Regarding APOE genotype, 7.7% of participants were classified as E2/E3, 2.1% as E2/E4, 52.1% as E3/E3, 34.4% as E3/E4, and 4.2% E4/E4. Overall, 39% of participants were considered amyloid positive based on PET imaging. Within the amyloid positive group, approximately 32.3% of individuals did not demonstrate an E4 allele,

50.9% were heterozygous with one E4 allele, and 16.8% homozygous with two E4 alleles (West et al., 2021). Plasma Aβ42 and Aβ40 were evaluated individually and as a ratio of Aβ42/Aβ40, as the ratio has historically been a better indicator of amyloid positivity for both plasma and CSF evaluation. West et al. found that the plasma Aβ42/ Aβ40 ratio was lower in the amyloid positive group ($p<0.0001$). Results demonstrated a 75% accuracy between amyloid positivity and plasma Aβ42/ Aβ40 accuracy. When incorporating Aβ42/ Aβ40 plasma ratio with APOE status and age, accuracy of detecting amyloid status improved with an AUC=0.90 with p=3.2X10^-55 (West et al., 2021).

C2N Diagnostics has further evaluated the efficacy of PrecivityAD™. Blood from a total of 686 individuals (n=686), ranging from 60-91 years of age, were evaluated using this blood test (About PrecivityAD™, 2020). Participants had cognitive impairment or presumed AD, and each underwent amyloid PET imaging. Of the 686 participants, 378 were considered to be amyloid positive based on neuroimaging. Results demonstrated an 86% sensitivity and 92% specificity for the PrecivityAD™ blood test (About PrecivityAD™, 2020).

Plasma Tau as a Biomarker

The following studies discussed in the literature review have placed an emphasis on the evaluation of plasma p-tau 181 as a diagnostic biomarker for AD. In a study completed by Janelidze et al. (2020), 526 participants were split into two cohorts. The first cohort, composed of 182 individuals, had Tau PET imaging in addition to serum evaluation. The second cohort, composed of 344 participants, was followed over a longer period of time (8 years) to monitor the progression of AD. Each cohort was further broken down into groups including those that were cognitively unimpaired, those with MCI, those with AD, and those with non-AD neurodegenerative diseases. A portion of each cohort (129 from cohort 1 and 324 from cohort 2)

underwent additional Aβ PET imaging to compare the serum p-tau 181 levels to Aβ brain pathology (Janelidze et al., 2020).

Janelidze et al. (2020) found that plasma p-tau 181 levels correlated with CSF p-tau 181 levels in both cohorts ($p<0.001$). Sensitivities, specificities, and AUC of plasma p-tau 181 versus CSF p-tau 181 levels were evaluated in regard to Tau PET imaging. Results demonstrated that CSF levels were slightly better in predicting abnormal Tau PET results. When evaluating p-tau 181 levels with that of Aβ PET imaging, elevated p-tau 181 serum levels correlated with increased Aβ PET for cohort 1 and 2 for individuals that were Aβ positive ($p<0.001$). There were no significant findings in the individuals that were Aβ negative. Janelidze et al. also examined whether plasma p-tau 181 could effectively differentiate controls and preclinical AD and AD vs non-AD neurodegenerative disorders. Plasma p-tau 181 differentiated control individuals and preclinical AD ($p<0.001$) as well as AD and non-AD disorders ($p<0.001$). Overall, Aβ positive individuals had higher p-tau 181 levels than in individuals that were Aβ negative for both cohorts ($p<0.05$). Janelidze et al. tracked plasma p-tau 181 levels over an eight-year period of time in 332 participants of cohort 2. Results demonstrated that plasma p-tau levels were higher in those who progressed to AD compared to those who never developed dementia at all ($p<0.001$) and compared to those who progressed to dementia from other causes ($p<0.001$) (Janelidze et al., 2020). Overall, individuals with an Aβ positive status who progressed to AD had higher levels of plasma p-tau 181 compared to individuals with an Aβ negative status who did not progress to AD (Janelidze et al., 2020).

The diagnostic accuracy of plasma p-tau 181 in differentiating cognitively healthy individuals, MCI, AD, and non-AD dementia was also evaluated in a study completed by Karikari et al. (2020). There were 1,131 participants in total ($n=1,131$) split into four cohorts.

Cohort one (n=37) included cognitively healthy individuals and those diagnosed with AD. The second and third cohort 989 individuals from outside studies. Within these groups, there were healthy controls (average age 23 years), MCI, AD, and those with frontotemporal dementia. Individuals within these cohorts underwent baseline cognitive and functional evaluation, CSF biomarker levels drawn, and PET imaging. The last cohort included 105 participants from primary care, ranging from normal cognitive status to those with undiagnosed neurological conditions (Karikari et al., 2020).

Karikari et al. (2020) found that CSF and plasma biomarker levels were in conjunction with one another ($p<0.0001$) for both the first and second cohorts. Within the first cohort, there was a 2-3-fold elevation of plasma in AD individuals compared to the healthy controls ($p<0.0001$). When evaluating the second cohort, Karikari et al. found that the plasma p-tau 181 levels were highest in participants that were classified as an Aβ positive PET AD status ($p<0.0001$). There was also noted to be higher plasma p-tau levels in healthy control individuals that had Aβ positive PET status or MCI with either Aβ (+/-) status ($p<0.05$). Regarding the third cohort, plasma p-tau 181 levels were lowest in healthy controls with an Aβ negative status, while the highest plasma p-tau 181 levels were in those with an Aβ positive AD status compared to all other groups ($p<0.0001$). Similarly, plasma p-tau 181 levels in the fourth cohort were lowest in healthy controls, with a progressive increase with MCI and AD (recall this group did not have additional CSF and PET exams) (Karikari et al., 2020).

Karikari et al. further evaluated the fourth cohort (primary care group) using area under the curve values (AUC) and accuracy. The plasma p-tau 181 assay was able to distinguish AD from the young (100% of the time). The assay also performed well when differentiating healthy elderly controls versus AD (AUC=84.44%, with accuracy being >90%). The plasma p-tau 181

assay was not able to distinguish AD from MCI (AUC=55.0%). Both Aβ and tau PET imaging was obtained in the second and third cohort, with plasma p-tau 181 levels corresponding to Aβ PET with an AUC=76.14%-88.09% and tau PET with an AUC=82.37%-93.11% (Karikari et al., 2020).

In a study completed by Lantero et al. (2020), the efficacy of plasma p-tau 181 was evaluated in predicating AD in the years prior to death and differentiating it from other forms of dementia. This was a longitudinal study that evaluated a cohort of individuals that included healthy controls, MCI, AD, mixed AD, and non-AD pathology. There was a total of 115 participants (n=115). Participants were followed until post-mortem, at which time confirmatory diagnosis was completed to determine neurocognitive status. Plasma p-tau 181 levels and overall patient evaluation were obtained at approximately 8 years (time point 1), 4 years (time point 2), and 2 years (time point 3) prior to post-mortem status (Lantero et al., 2020).

Lantero et al. (2020) found that the AD group had significantly higher p-tau 181 levels than the control group at time point 1 ($p=0.001$), time point 2 ($p<0.0001$), and time point 3 ($p<0.0001$); however, there were no significant findings when comparing the AD and the MCI group. Of the clinically diagnosed AD population, 75% had confirmed post-mortem biopsy results consistent with AD. Lantero et al. then evaluated the plasma p-tau 181 levels of all participants in comparison to post-mortem diagnosis without regard to clinical diagnosis. Results of this at all three timepoints demonstrated that individuals given an AD status after post-mortem biopsy had higher plasma p-tau 181 levels than those confirmed as a "control" status ($p<0.0001$) and those confirmed as non-AD pathology ($p<0.0001$). Lantero et al. utilized ROC analysis to assess the ability of plasma p-tau 181 in differentiating AD and non-AD pathology. When evaluating p-tau 181 levels at 8 years prior to post-mortem status, plasma p-tau 181

demonstrated to be more accurate in differentiating AD and non-AD pathology (AUC= 97.4%) and controls (AUC= 92.1%) (Lantero et al., 2020). When evaluating mixed AD pathologies, plasma p-tau 181 revealed less reliable results with an AUC=57.3% (Lantero et al., 2020).

Overall, Lantero et al. concluded that higher plasma p-tau 181 levels correlate with a post-mortem AD classification and that plasma p-tau 181 levels may be useful for detecting individuals with AD years prior to a post-mortem diagnosis. The study demonstrated that plasma p-tau 181 may also be useful in distinguishing AD from non-AD pathologies but less reliable if there is mixed AD pathology (Lantero et al., 2020).

Historically, elevated plasma total tau (t-tau) has an association with cognitive impairment and decline; however, it does not have a specific association with AD. Unlike t-tau, p-tau 181 is thought to have a stronger association with AD (Mielke et al., 2018). In the study completed by Mielke et al. (2018), plasma p-tau 181 and total tau levels were evaluated in relation to AD, PET imaging, and cortical thickness. The study included a total of 269 participants; 172 having intact cognition, 57 with MCI, and 40 participants with clinical AD. Patient age, sex, and APOE status were also taken into account during statistical analysis (Mielke et al., 2018).

Mielke et al. (2018) found that AD patients had higher levels of t-tau in comparison to the MCI group ($p=0.029$) and to the control group ($p<0.001$). No significant differences were noted between the control group and MCI group. Regarding p-tau 181, levels were elevated in the AD group in comparison to the control group ($p<0.001$) but not compared to the MCI group ($p=0.251$). Mielke et al. evaluated the correlation of the tau plasma biomarkers and Aβ PET status. Overall analysis demonstrated that p-tau 181 offered more accuracy with detecting increased Aβ PET compared to total tau ($p<0.01$). P-tau 181 levels were comparable to age and

APOE prediction status for AD ($p<0.05$). Overall, the study concluded that both plasma p-tau 181 and t-tau are elevated in AD. Plasma p-tau 181 levels showed to be more congruent with Aβ PET status than plasma t-tau (Mielke et al., 2018).

Discussion

To date, neuroimaging in combination with clinical findings, are the primary tools in the clinical diagnosis of AD. With amyloid PET imaging being the only FDA approved method for brain amyloid evaluation, it is essential to find less invasive, reliable, and cost-effective methods for detection. Extensive research being conducted has placed emphasis on understanding disease components and utilizing advanced technology to evaluate potential biomarkers in AD.

This literature review has demonstrated that the presence of the APOE4 allele is the primary genetic component of AD. Research indicates that an individual homozygous for the APOE4 is at higher risk for developing AD than an individual either heterozygous or negative for the APOE4 allele (Kirmess et al., 2021). Although this genetic marker cannot be used for a confirmatory diagnosis, utilizing APOE4 status in conjunction with other biomarkers may provide benefit in a final diagnosis of AD. Research has offered significant evidence that PET imaging with the use of tracers can be extremely beneficial in aiding in the clinical diagnosis of AD. Patterns show that individuals that have a confirmed amyloid beta positive status have increased uptake of PET tracer, indicating a higher deposition of pathologic amyloid plaques (Lin et al., 2016). Although amyloid PET imaging is FDA approved for brain amyloid evaluation, it is expensive, time consuming, and involves utilizing radioactive tracer. In regard to utilizing CSF as a biomarker, findings reveal low Aβ levels and high tau levels are hallmark to that of AD (Lane et al., 2018). It appears that CSF ratios of Aβ-1-42/T-tau and Aβ-1-42/P-tau181 may provide the most reliable information in regard to AD status (Struyfs et al., 2015).

Individual biomarkers including Aβ 1-37, Aβ 1-38, and Aβ 1-40 may provide additional benefit in AD diagnostics (Struyfs et al., 2015). When considering CSF as a biomarker, it is important to remember that obtaining CSF is largely invasive and expensive. The development of potential blood-based biomarkers has been a huge breakthrough in the evaluation of AD. Both plasma Aβ and plasma tau have been of interest and show immense potential for AD diagnosis. These tests are less invasive, more accessible to patients and clinicians, and cost friendly in comparison to other biomarkers. Plasma tau offers potential in future testing as elevated plasma ptau-181 levels statistically correlate with a positive amyloid PET status (Janelidze et al., 2020). Plasma amyloid beta ratios have shown to be lower in individuals with an amyloid positive PET status (West et al., 2021). The PrecivityAD™ blood test, evaluating Aβ42, Aβ40, and APOE status is now available for use in most states and has shown to have promising accuracy with an 86% sensitivity and 92% specificity efficacy rate (About PrecivityAD™, 2020).

Continued research and utilization of these biomarkers may provide an earlier clinical diagnosis in presumed AD cases. Earlier detection ultimately leads to timely intervention. Having the ability to use sufficient biomarkers to diagnose this disease may be beneficial in the future if or when a preventative or curative treatment option becomes available.

Applicability to Clinical Practice

The information within this literature review will help clinicians have a better understanding of the various methods that can be utilized in the clinical diagnosis of AD. It is essential for providers to be able to recognize the early symptoms of disease. This literature review provides knowledgeable information about new and upcoming technology that may make testing and diagnosing AD more accurate. As research advances and new treatment options become available, having biomarkers that are less invasive and cost effective will make the

diagnosis of the disease more attainable. In addition, if a patient has a confirmed clinical diagnosis, the use of biomarkers may provide benefit in tracking disease progression making changes to treatment plans. Having diagnostic tests to confirm AD earlier will allow for exceptional patient centered care, interprofessional collaboration, and early intervention.

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