Review

Role of Fluid Biomarkers and PET Imaging in Early Diagnosis and its Clinical Implication in the Management of Alzheimer’s Disease

Shahul Hameed⁠, Jong-Ling Fuh⁠, Vorapun Senanarong, Esther Gunaseli M. Ebenezer, Irene Looi, Jacqueline C. Dominguez, Kyung Won Park, Ananda Krishna Karanam and Oliver Simon

Department of Neurology, National Neuroscience Institute, Singapore General Hospital, Singapore
Duke NUS Medical School, Singapore
Department of Neurology, Neurological Institute, Taipei Veterans General Hospital, Taipei, Taiwan
Faculty of Medicine, National Yang-Ming University School of Medicine, Taipei, Taiwan
Division of Neurology, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand
Faculty of Medicine, University Kula Lumpur, Royal College of Medicine Perak, Ipoh, Malaysia
Clinical Research Centre, Hospital Seberang Jaya, Penang, Malaysia
Department of Medicine, Hospital Seberang Jaya, Penang, Malaysia
Institute for Neurosciences, St. Luke’s Medical Center, Metro Manila, Philippines
Department of Neurology and Cognitive Disorders and Dementia Center, Institute of Convergence Bio-Health, Dong-A University College of Medicine, Busan, Republic of Korea
Novartis Healthcare Private Limited, Hyderabad, India
Novartis (Singapore) Pte. Ltd., Singapore, Singapore

Accepted 3 January 2020

Abstract. Clinical diagnosis of Alzheimer’s disease (AD) is based on symptoms; however, the challenge is to diagnose AD at the preclinical stage with the application of biomarkers and initiate early treatment (still not widely available). Currently, cerebrospinal fluid (CSF) amyloid-β 42 (Aβ42) and tau are used in the clinical diagnosis of AD; nevertheless, blood biomarkers (Aβ42 and tau) are less predictive. Amyloid-positron emission tomography (PET) imaging is an advancement in technology that uses approved radioactive diagnostic agents (florbetapir, flutemetamol, or florbetaben) to estimate Aβ neuritic plaque density in adults with cognitive impairment evaluated for AD and other causes of cognitive decline. There is no cure for AD to date—the disease progression cannot be stopped or reversed; approved pharmacological agents (donepezil, galantamine, and rivastigmine; memantine) provide symptomatic treatment. However, the disease-modifying therapies are promising; aducanumab and CAD106 are in phase III trials for the early stages of AD. In conclusion, core CSF biomarkers reflect pathophysiology of AD in the early and late stages; the application of approved radiotracers have potential in amyloid-PET brain imaging to detect early AD.

Keywords: Alzheimer’s disease, biomarker, blood, cerebrospinal fluid, early diagnosis, positron emission tomography

*Correspondence to: Shahul Hameed, National Neuroscience Institute, Department of Neurology, Singapore General Hospital, Outram Road, Singapore, Singapore. E-mail: shahul.hameed@singhealth.com.sg.
INTRODUCTION

Dementia is a progressive heterogeneous syndrome leading to cognitive decline [1, 2], thereby interfering with individuals’ abilities to perform daily activities independently [3] and affecting their quality of life [4]. According to Alzheimer’s Disease International [5], an estimated 46.8 million people lived with dementia in 2015 (Asia: 22.9 million, Europe: 10.5 million, United States: 9.4 million, and Africa: 4.0 million), and this number is expected to increase to 131.5 million by 2050. Dementia is overwhelming for patients, family members as well as their caregivers, and there is a need for healthcare professionals to raise awareness among caregivers and improve the quality of care for patients [6]. Currently, the symptomatic treatment of patients with dementia preserves functional independence, thereby improving their quality of life [7]. The total estimated economic cost of dementia worldwide is US$ 817.9 billion, representing 1.09% of the global gross domestic product [5]. With advances in technology, the diagnosis of dementia at early stages and early therapeutic intervention could reduce the health and social care costs.

Alzheimer’s disease (AD) is a multifactorial neurodegenerative disorder [8] and the leading cause of dementia in older individuals [9, 10]. Other common types of dementia include: vascular dementia [11, 12], Parkinson’s disease dementia [13, 14], Lewy body dementia [15, 16], and frontotemporal dementia [17, 18]. The continuum of AD covers progression of disease from the asymptomatic to symptomatic phases (cognitive decline), through a preclinical phase identified by biomarkers that detect underlying neuropathophysiologic changes without clinical manifestations [19]. Clinically, AD is characterized by a progressive decline in the cognitive function [20] that interferes with the daily activities [21]. In AD, the cognitive impairment is a result of the neuronal cell death [22, 23], and mainly due to the loss of the neocortical synapses involved in cognition [24]. A major known risk factor for dementia due to AD is the advancing age [25], whereas another important risk factor is the apolipoprotein E (APOE) ε4 genotype [26]. Worldwide, an older population with an age of ≥65 years is increasing from an estimated 617.1 million in 2015 (total world population of 7.3 billion) to 998.7 and 1,565.8 million in 2030 (total world population: 8.3 billion) and 2050 (total world population: 9.4 billion), respectively; the population aged ≥65 years will rise with once a year average increase of 27.1 million from 2015 to 2050 [27]. One of the reasons for an increasing older population could be an improvement in life expectancy, which in turn increases the incidence of AD. Family members and caregivers play a critical role in maintaining the quality of life and improving the care of individuals living with AD dementia [28].

Currently, the amyloid cascade hypothesis and tau hypotheses are recognized in the pathogenesis of AD [29]. The amyloid-β (Aβ) peptide and tau (an axonal protein) are well-established predictors in AD pathogenesis [30]. The neuropathological hallmark of AD is the extracellular Aβ protein fragment (plaques) accumulation outside of the neurons and aggregation of the tau protein (tangles) within the neurons [31, 32]. According to the Aβ cascade hypothesis [31], the imbalance in the metabolism of amyloid-β protein precursor (AβPP) results in monomeric Aβ through proteolytic processing by the β-site AβPP cleaving enzyme-1 (BACE1) within the endosomes and by intramembrane processing by γ-secretase [33]. The potential therapeutic strategy is, therefore, to decrease Aβ peptide formation [34–36]. Further, the Aβ monomers misfold and aggregate resulting in an abnormal elevation of Aβ oligomers [37, 38] accumulating outside of the neuron that could trigger a cascade of cellular events, including hyperphosphorylation of tau (p-Tau) [39] accompanied by mitochondrial dysfunction [40, 41]. According to the mitochondrial cascade hypothesis, the dysfunction of mitochondria leads to the formation of Aβ plaques, neurofibrillary tangles, synaptic degradation, and neuronal apoptosis in the late-onset, sporadic AD [42–44]. The Translocase of Outer Mitochondrial Membrane 40 (TOMM40) gene affects the mitochondrial dysfunction cascade in AD. TOMM40, located on human chromosome 19 (5’-upstream of the APOE gene), has received increasing attention as a promising AD biomarker. TOMM40 regulates Aβ influx into mitochondria independently or by interacting with APOE-dependent mechanisms, resulting in the cell to undergo downstream apoptotic processes through reactive oxygen species generation [45]. In addition, persistent neuroinflammation plays a key role in AD pathogenesis as well as progression [46, 47]. In-depth understanding of the molecular mechanism could help identify new disease-modifying therapies (DMTs).

To date, limited knowledge is available regarding the pathogenesis of AD. The success of preventive strategies relies on understanding the time-course of AD and identifying individuals at risk of AD at
the earliest stages (who have no significant signs of neurodegeneration) with the application of sensitive biomarkers. The challenge, however, remains with screening individuals at risk for AD prior to the onset of cognitive decline during the “preclinical” stages where there is a greater potential for the use of DMTs. This paper, therefore, aims to review the role of biomarkers in early diagnosis and its clinical implication in the management of AD; the pharmacological treatment options are summarized. A literature search of English language articles on “Alzheimer’s Disease”, “biomarkers” and “treatment” through electronic databases (PubMed or Ovid) published before November 2019 was performed. Additional searches were performed through the clinical trial registry (ClinicalTrials.gov) for unpublished studies. Studies identified during the literature search were assessed for relevance based on the titles, abstracts, and/or the full text of the retrieved articles.

ALZHEIMER’S DISEASE DIAGNOSTIC CRITERIA AND BIOMARKER CLASSIFICATION SYSTEM

In 1984, the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association (NINCDS–ADRDA) developed criteria for the clinical diagnosis of AD based on clinicopathologic correlations [48]. The criteria included “probable AD” and “possible AD” (diagnosed clinically), and “definite AD” confirmed upon neuropathological investigations. The probabilistic AD diagnosis is within the clinical context with no definitive biomarker for diagnosis. In 2013, the American Psychiatric Association published the 5th edition of the Diagnostic and Statistical Manual of Mental Disorders [49] and introduced the new term “neurocognitive disorders”. Although “dementia” is a “major neurocognitive disorder” according to DSM-5, the current diagnostic term “dementia” is an acceptable alternative [50]. The DSM-5 characterizes “major neurocognitive disorder” as a disturbance in one or more cognitive domains [50]: complex attention; executive function; learning and memory; language; perceptual-motor function; and social cognition. For major neurocognitive disorders due to AD, there should be a decline in at least two cognitive domains (one should be learning and memory) according to the DSM-5 criteria, whereas the learning and memory deficit is sufficient for the diagnosis of mild neurocognitive disorders due to AD. The DSM-5 criteria are designed for clinicians and focus on the clinical diagnosis. The clinical diagnosis of AD is usually after the onset of symptoms, by which point most neurons are affected; the goal is, therefore, to diagnose before the onset of clinical symptoms.

The recent paradigm shift in diagnosis helps the early detection of AD before the occurrence of clinical symptoms. The International Working Group (IWG) criteria [51–54] allows more accurate diagnosis of AD than the NINCDS–ADRDA criteria, even at the prodromal stage. This new diagnostic framework (defined as a dual clinicobiological entity) has shifted towards neurobiological measures of AD. The diagnosis is achieved using the clinical manifestations of AD as well as via confirmation of AD pathology in vivo through biomarkers (pathophysiological and topographical markers) [52]. According to the IWG criteria, preclinical AD includes both an “asymptomatic at-risk state for AD” and “presymptomatic AD”, whereas “prodromal AD” includes a symptomatic pre-dementia phase of AD (mild cognitive impairment [MCI] category) [51–54].

The National Institute on Aging–Alzheimer’s Association (NIA–AA) workgroup proposed a diagnostic conceptualization of AD that will allow for the most effective DMT [55]. The criteria focus on the AD pathophysiological continuum with distinct cognitive staging [55–57]. The NIA–AA research framework defines AD biologically to recognize the disease progression that leads to cognitive impairment [56]. The NIA–AA classifies individuals with AD in to “probable AD dementia”, “possible AD dementia”, and “probable or possible AD dementia” with evidence of the AD pathophysiological processes [57]. The term “mild cognitive impairment due to AD” was used to refer to the symptomatic pre-dementia phase of AD [58]. Preclinical AD precedes MCI, and screening for individuals with preclinical AD thereby provides an opportunity for DMT to change the course of the disease and evaluate the application of novel biomarkers. The preclinical AD stages include “asymptomatic cerebral amyloidosis”; “amyloid positivity plus evidence of synaptic dysfunction and/or early neurodegeneration”; and “amyloid positivity and neurodegeneration plus cognitive decline” [59]. The NIA–AA and IWG use biomarkers for the diagnosis of AD, in contrast to the NINCDS–ADRDA criteria. Both NIA–AA and IWG criteria use similar terminology to define the AD continuum: “preclinical AD”, “MCI due to AD”
(NIA–AA) or “prodromal AD” (IWG) and “AD dementia” [60].

Finally, A/T/N is a binary classification system [61] related to biomarkers, which differentiates p-Tau and total (t)-Tau. There are 7 major AD biomarkers divided into 3 binary categories (each rated positive or negative) based on pathophysiology. “A” corresponds with the Aβ biomarker (amyloid positron emission tomography [PET] or CSF Aβ42), “T” corresponds with the tau pathology biomarker (CSF p-Tau or tau PET), and “N” corresponds with the quantitative or topographic biomarker of neurodegeneration or neuronal injury (CSF t-Tau, fluorodeoxyglucose (FDG)-PET, or structural MRI).

**APOE E4 GENE VARIANT AS A RISK FACTOR FOR ALZHEIMER’S DISEASE**

In humans, the APOE gene allelic variants include ε2, ε3, and ε4 of which the APOE ε4 allele is the prevalent risk factor that is related to AD. Individuals with two copies of the APOE ε4 allele have an increased risk of developing AD (12-fold) compared with those with 1 copy (3-fold) [62]. The association between APOE ε4 and the incidence of AD has been demonstrated in many population-based studies. The results from a meta-analysis [63] showed a stronger association between the APOE genotype and AD (ε3/ε4: odds ratio [OR], 5.6; ε4/ε4: OR, 33.1) in Japanese subjects compared with Caucasians (ε3/ε4: OR, 2.7 to 3.2, ε4/ε4: OR, 12.5 to 14.9); however, the APOE ε4 and AD association was weaker among African Americans (ε3/ε4: OR, 1.1; ε4/ε4, OR, 5.7) and Hispanics (ε3/ε4: OR, 2.2; ε4/ε4: OR, 2.2). A systematic review [64] showed that APOE ε4 carrier frequencies varied, with the highest regional prevalence estimates in Northern Europe (ε4/ε4: 61.3%, 95% confidence interval [CI] 55.9–66.7; ε4/ε4 : 14.1%, 95% CI 12.2–16.0) and the lowest regional estimates were in Asia (ε4/ε4: 41.9%, 95%CI 38.5–45.3; ε4/ε4: 7.7%, 95% CI 5.8–9.6) or Southern Europe/Mediterranean countries (ε4/ε4: 40.5%, 95% CI 36.8–44.1; ε4/ε4 prevalence: 4.6%, 95%CI 2.7–6.4). A meta-analysis [65] in the Chinese population showed a positive association between the APOE ε4 allele carriers and AD (OR, 3.93; 95% CI 3.37–4.58; p < 0.00001). The carriers of the homozygous APOE ε4/ε4 and heterozygous APOE ε4/ε3 alleles have a significant association with AD (OR, 11.76 and 3.08, respectively; both p < 0.00001). Generally, the prevalence of AD is higher in women possibly due to a longer life expectancy [66]. A meta-analysis [67] of 27 studies (57,979 participants), however, showed that both men and women with APOE ε3/ε4 genotype had similar risks of AD between 55 and 85 years of age (OR 3.09 and 3.31, respectively); whereas women had a higher risk of AD than men between 65 and 75 years (OR: 4.37 and 3.14, respectively). Studies have shown an association between the ε4 allele and cognitive decline. From the Alzheimer’s Disease Neuroimaging Initiative study, 399 subjects (cognitively normal = 109, amnestic subjects with MCI = 192, AD = 98) were used to evaluate the effect of APOE ε4 on biomarkers of neurodegeneration [68]; the results showed a clear APOE ε4 dose-dependent effect on CSF Aβ1–42 levels within each clinical group. The results from a large multicenter study of 716 cognitively healthy individuals (aged 17–99 years) showed age-dependent effects of the APOE ε4 allele on the onset of preclinical AD as CSF Aβ1–42 concentrations started to decline at 50 years of age in APOE ε4 allele negative individuals, at 43 years of age in those carrying one APOE ε4 allele, and even earlier in individuals carrying 2 APOE ε4 alleles [69]. The Generation Scotland: Scottish Family Health Study (N = 18,337) showed the association of additive effects of APOE ε4 with lower scores on logical memory (β = –0.095, p = 0.003), verbal fluency (β = 0.075, p = 0.023), and digit symbol tests (β = –0.087, p = 0.004) in individuals aged >60 years [70]. Taken together, individuals who carry APOE ε4 allele may have increased risk of developing AD, increased rate of age-dependent cognitive decline, and decreased memory performance compared with non-carriers. Currently, the clinical use of APOE ε4 genotyping is being tested and could be used to screen asymptomatic individuals, but is not recommended outside of research settings.

**BIOMARKERS FOR EARLIER DIAGNOSIS OF AD DEMENTIA**

According to Hulka and colleagues, biomarkers (biological markers) are “cellular, biochemical or molecular alterations that are measurable in biological media such as human tissues, cells, or fluids” [71]. Biomarkers provide insight into underlying mechanisms, disease progression, prognosis, regression, response to therapy, and accurate early diagnosis for early treatment [71]. Clinically, AD is diagnosed based on symptoms and the challenge is to diagnose AD at the preclinical stage with the application of
biomarkers and initiate early treatment. The clinical diagnostics of AD are currently probabilistic. At present, the biomarkers are available in certain countries only, and the newer treatment options emerge for early AD and help in the definitive diagnosis.

**Fluid biomarkers (CSF and blood) for the clinical diagnosis of Alzheimer’s disease**

The CSF is in contact directly with the extracellular spaces of the brain and the metabolism of proteins (e.g., Aβ and tau) in the brain is therefore reflected within the CSF. Hence, Aβ42, t-Tau, and p-Tau are the core biomarkers used as diagnostic tools in AD [72]. The results from a number of longitudinal studies have suggested that altered CSF Aβ42 is predictive of AD. Studies have demonstrated that high levels of CSF tau and low CSF Aβ42 are predictive of AD and their application in the pre-dementia clinical studies could help to include suitable subjects for the assessment of treatment benefit against the risk. A study in patients with (n = 33) and without (n = 11) dementia of the Alzheimer’s type showed that increased dementia severity was correlated with decreased concentrations of soluble AβPP and Aβ protein and increased CSF tau [73]. Results from a retrospective study [74] of 21 patients with probable AD showed a significant decrease in the concentrations of CSF Aβ42 (265 ± 156 versus 746 ± 238 ng/l) but an increase in t-Tau (803 ± 553 versus 297 ± 129 ng/l) and p-Tau (95.9 ± 57.5 versus 49.5 ± 21.2 ng/l) compared with the control population (all p < 0.001). During the 5- and 6-year follow-up, 8 out of 21 and 11 out of 21 patients who died had significantly lower levels of CSF Aβ42 compared with those alive ((mean ± standard deviation [SD] 170.6 ± 80.7 versus 323.6 ± 164.1 ng/l; p = 0.011) and (mean ± SD: 193.6 ± 84.4 versus 344.3 ± 180.9 ng/l; p = 0.041), respectively. A follow-up study [75] (range: 4.0–6.8 years) showed that patients with MCI at baseline who developed AD (MCI-AD, n = 57) had a significant decrease in CSF Aβ42 (mean [SD] 324 [101] [MCI-AD] versus 700 [181] [controls] or 551 [188] ng/l [stable MCI], both p < 0.0001) and CSF Aβ42/p-Tau181 ratio compared with controls or those with stable MCI (mean [SD] 3.7 [1.6] [MCI-AD] versus 12.5 [4.7] [controls] or 9.5 [3.8] [stable MCI], both p < 0.0001), whereas CSF p-Tau181 (mean [SD] 95 [29] [MCI-AD] versus 61 [17] [controls] or 62 [16] ng/l [stable MCI], both p < 0.0001) and CSF t-Tau (mean [SD] 816 [426] [MCI-AD] versus 326 [157] [controls] or 340 [212] ng/l [stable MCI], both p < 0.0001) significantly increased compared with controls or those with stable MCI. The study showed that pathological CSF was a strong risk factor for the development of AD with an adjusted hazard ratio [95%CI] for t-Tau and Aβ42 of 17.7 (5.33–58.9; p < 0.0001), CSF p-Tau181 and Aβ42 of 16.8 (5.02–56.5; p < 0.0001), and t-Tau and Aβ42/p-Tau181 of 19.8 (5.99–65.7; p < 0.0001). Results from a two-part study (cross-sectional and prospective cohort studies; N = 750) [76] showed that 330 patients progressed to clinical dementia from MCI and 420 of were on stable MCI for at least 2 years of follow-up. Of the 330 patients with MCI, 271 were diagnosed with AD and 59 with other dementias. Of the 271 patients with incipient AD, the CSF Aβ42 levels were significantly lower than controls (median [range]: 356 [96–1075] versus 675 [182–1897] ng/l; p < 0.001), whereas the p-Tau and t-Tau levels were significantly higher than controls (median [range]: 81 [15–183] versus 51 [16–156] ng/l and 582 [83–2174] versus 280 [42–915] ng/l, respectively; both p < 0.001). The positive and negative likelihood ratio values for Aβ42; p-Tau; and t-Tau were 2.3 (95%CI 2.0–2.6) and 0.32 (95%CI 0.28–0.36); 1.6 (95%CI, 1.4–1.8) and 0.34 (95%CI, 0.31–0.37); as well as 1.9 (95%CI 1.7–2.2) and 0.26 (95%CI 0.23–0.29), respectively. The area under the receiver operating characteristic (ROC) curve for Aβ42, p-Tau, and t-Tau was 0.78 (95%CI 0.75–0.82), 0.76 (95%CI 0.72–0.80), and 0.79 (95%CI 0.76–0.83), respectively. In a cross-sectional case-control study [77] Chinese patients (N = 48) with AD had significantly higher levels of CSF tau (median [interquartile range] 660.22 [394.65] versus 224.61 [132.66] pg/ml) and p-Tau (78.13 [44.35] versus 35.53 [20.53] pg/ml) compared with non-demented controls (both p < 0.001). Patients with AD had significantly lower CSF Aβ42 levels than non-demented controls (median [interquartile range] 278.11 [181.64] versus 458.90 [417.55] pg/ml; p < 0.022). Moreover, patients with AD had significantly lower Aβ42–t-Tau (median [interquartile range] 19.8 (5.99–65.7; p < 0.0001), and Aβ42–p-Tau ratios compared with non-demented controls (median [interquartile range] 3.69 [3.82] versus 19.54 [10.71]; p < 0.001). The results of the first study assessing the Aβ42/Aβ40 ratio [78] showed that patients with MCI at baseline who developed AD (MCI-AD, n = 57) had a significant decrease in CSF Aβ42/Aβ40 ratio and Aβ42 concentration than those with stable MCI or controls (Aβ42/Aβ40 ratio: mean ± SD 0.78 ± 0.19 [MCI-
sus controls (N = 8315) and lower baseline CSF Aβ42 levels in patients with AD compared with controls and those with other neurological disorders (OND) (316.1 ± 105.7 [AD] versus 676.0 ± 175.1 [control] and 565.8 ± 187.9 pg/ml [OND]; p < 0.001); conversely there were higher t-Tau (583.0 ± 286.4 [AD] versus 212.5 ± 67.3 [control] and 227.9 ± 120.0 pg/ml [OND]; p < 0.001) and p-Tau (73.8 ± 28.8 [AD] versus 41.9 ± 12.8 [control] and 37.0 ± 15.4 pg/ml [OND]; p < 0.001) levels in patients with AD compared with controls and OND. The areas under the curve were more accurate for t-Tau/Aβ42 and pTau/Aβ42 ratios: 0.99 (for both biomarker ratios) and 0.94 (for both biomarker ratios) for AD dementia versus control and AD dementia versus OND, respectively. Recently, a large, multicentric cohort study [80] (N = 3565) assessed the relationship between CSF Aβ42 and CSF tau. Of the 3565 patients, 947 had a normal biomarker levels (A-N-), 1299 had an AD profile (A+N+), 789 patients were amyloid positive (A+N-), and 527 had the suspected non-AD pathophysiology profile positive for neurodegeneration (A-N+). The findings from this study showed that 36% of patients who were amyloid positive evolved to AD profile (A+N+).

Findings from a recent systematic review and meta-analysis [81] of fluid biomarkers (CSF and blood) showed AD to control Aβ42 ratios below one (except for one) with average ratio of 0.56 (AD patients = 9949, controls = 6841) and AD to control t-tau as well as p-tau ratios above one with average ratio of 2.54 (AD patients = 11341, controls = 7086) and 1.88 (AD patients = 7498, controls = 5126), respectively (all p < 0.0001). These core biomarkers also differentiated between cohorts with MCI due to AD and those with stable MCI with an average ratio of 0.67 for CSF Aβ42 (AD MCI = 352, stable MCI = 610), 1.72 for p-tau (AD MCI = 307, stable MCI = 570), and 1.76 for t-tau (AD MCI = 251, stable MCI = 501). Moreover, results from a meta-analysis (Version 2.1, June 2018) [82] showed lower CSF Aβ42 levels in patients with AD (N = 11,277) versus controls (N = 8315) and lower baseline CSF Aβ42 levels in those with MCI to develop AD (MCI-AD N = 526) versus stable MCI (MCI-stable N = 881), with an overall effect size (weighted average of the individual effect sizes) of 0.559 and 0.663, respectively (both p < 0.0001). Conversely, CSF t-tau levels were higher in patients with AD (N = 12,503) versus controls (N = 8145) and baseline CSF t-tau levels were higher in those with MCI to develop AD (MCI-AD N = 481) versus stable MCI (MCI-stable N = 841), with an overall effect size of 2.480 and 1.730, respectively (both p < 0.0001). Although the CSF Aβ42 and tau have sensitivity and specificity, there is a need for other biomarkers for early diagnosis of AD. Recently, results from a meta-analysis [83] (129 papers) showed that in early AD there was an increase in the levels of CSF t-tau as well as CSF p-tau and a decrease in CSF Aβ42 levels. Currently, the diagnosis of AD is made from the clinical observation of cognitive decline; however, definitive AD is confirmed postmortem from microscopic observation of the brain tissue.

Novel biomarkers available in clinical samples such as blood are being discovered for the early diagnosis of AD. However, studies have shown that the free blood plasma Aβ is less predictive for the clinical diagnosis of AD and there is no correlation between the blood and CSF Aβ42 concentrations [84, 85]. Plasma tau as a biomarker for the clinical diagnosis of AD is not supported as the correlations between high plasma tau as well as higher CSF tau and lower CSF Aβ42 were mild and differed between cohorts [86]. In addition, results from a meta-analysis (Version 2.1, June 2018) [82] showed no difference in plasma Aβ42 in patients with AD (N = 2336) versus controls (N = 4452) and did not differ in baseline plasma Aβ42 levels in those with MCI to develop AD (MCI-AD N = 308) versus stable MCI (MCI-stable N = 379), with an overall effect size (weighted average of the individual effect sizes) of 1.031 (p = 0.38718) and 0.807 (p = 0.32403), respectively. Conversely, plasma levels of t-tau were higher in patients with AD (N = 447) versus controls (N = 552) with an overall effect size of 1.788 (p = 0.00550), with considerable variability in the studies. Recently, immunoprecipitation and mass spectrometry techniques have been used to measure levels of high-performance plasma Aβ biomarkers in the blood [87]. The results showed that AβPP669–711/Aβ1–42 and Aβ1–40/Aβ1–42 ratios as well as their composites are clinically useful plasma biomarkers. However, there is a need for other noninvasive biomarkers to detect AD and sensitive techniques to measure such proteins at low...
concentrations. Recently, plasma neurofilament light (NFL) is proposed as a blood-based biomarker, and studies have suggested its potential to predict the course of AD. A highly sensitive technology, the single-molecule array (Simoa) platform was used to measure the plasma NFL to assess its application as a noninvasive biomarker to detect AD [88]. The results from this prospective case-control study (cognitively healthy controls = 193, MCI = 197 patients, AD with dementia = 180 patients) showed that there was a correlation between plasma NFL and CSF NFL (Spearman $\rho = 0.59$, $p < 0.001$). Compared with controls (mean, 34.7 ng/l) there was increase in plasma NFL in patients with MCI (mean, 42.8 ng/l) and patients with AD dementia (mean, 51.0 ng/l) ($p < 0.001$). Although high plasma NFL levels were associated with cognitive decline, there was no difference in plasma NFL levels between Aβ-positive patients with progressive MCI and those with stable MCI. Moreover, findings from a recent study [89] showed that plasma NFL levels were significantly different across the diagnostic groups: AD (50.9 pg/ml) > amnestic MCI (43.0 pg/ml) > cognitively normal (34.7 pg/ml) (all $p < 0.001$), but with substantial overlap thereby limiting its application as a diagnostic biomarker. A prospective study [90] of women (N = 5309) from the prospective epidemiological risk factor study showed the high levels of Tau-A and Tau-C (truncated tau) biomarkers in the serum were associated with a lower risk of AD (Tau-A: HR [95% CI] 0.71 [0.52–0.98]; Tau-C: 0.78 [0.60–1.03]). Recently, a study using immuno-infrared assay [91] showed the ability of amide I blood biomarker to detect AD on average 8 years before onset of the clinical symptoms (ESTHER study). The assay distinguished AD from controls with a sensitivity of 71% and specificity of 91% for ESTHER study and a sensitivity of 69% and specificity of 86% for the BioFINDER study. Recently, a study using Quanterix Simoa-HD1 tau platform [92] showed that the plasma pTau181 was a more sensitive and specific predictor of elevated brain Aβ than total tau, and that plasma pTau181 may be used as a biomarker of AD pathology. A study of two-step immunoassay that measured concentrations of Aβ38, Aβ40, and Aβ42 in the human blood plasma showed that Aβ42/Aβ40 ratio is promising biomarker candidate of AD [93]. The areas under the ROC curves were 0.87 and 0.80 for the Aβ42/Aβ40 ratio and Aβ42/Aβ38 ratio, respectively. A study quantified plasma t-tau, p-tau, and Aβ1–42 in 76 patients (cognitively normal, $n = 52$; MCI, $n = 9$; AD dementia, $n = 15$) and examined the degree of brain tau deposition as observed using tau-PET [94]. The study showed that in plasma t-tau/Aβ1–42 ratio was highly predictive of brain tau deposition, with high t-tau/amyloid-β1–42 AUC value of 0.890 (sensitivity, 80%; specificity, 91%) than 0.802 for t-tau (sensitivity, 93%; specificity, 63%) or 0.766 for plasma p-tau/Aβ1–42 (sensitivity, 93%; specificity, 51%) or 0.731 for plasma p-tau (sensitivity, 93%; specificity, 49%). A study used immunoprecipitation and liquid chromatography-mass spectrometry assay measured the levels of plasma and CSF of Aβ42/Aβ40 in cognitively normal individuals (N = 158) [95]. The study provided class II evidence that plasma Aβ42/Aβ40 was predictive of the brain amyloidosis, with area under the ROC curves of 0.88 and high correspondence with CSF p-tau181/Aβ42 (AUC 0.85). Recently, findings from a study using Elecsys immunoassays (BioFINDER cohort, $n = 842$; independent validation cohort, $n = 237$) showed the area under the ROC curve of 0.80 for plasma Aβ42 and Aβ40 to predict Aβ positivity in BioFINDER compared with 0.86 in the independent validation cohorts [96]. Currently there are no validated blood-based biomarkers for AD in clinical use. The advantage of blood-based biomarkers is that they are less invasive and more cost-effective than the CSF biomarkers (which involve lumbar puncture and CSF collection); however, the advent of new techniques could enable early diagnosis of AD, effectively screen patient populations, and measure treatment effect in the clinical studies.

**PET imaging biomarkers (Aβ-PET and tau PET) for clinical diagnosis of Alzheimer’s disease**

The Aβ-PET is a molecular imaging tool that uses radiotracers to picture the accumulation of Aβ plaque within an AD brain and monitors disease progression. At the moment, Aβ-PET imaging or measuring CSF Aβ levels are the available options for the clinical diagnosis of Aβ deposition in AD. Florbetapir [97] (Amyvid™) was the first approved radioactive diagnostic agent followed by Flutemetamol [98] (Vizamyl™) and Florbetaben [99] (NeuraCept™) indicated for PET imaging of the brain to estimate the density of Aβ neuritic plaque in adults with cognitive impairment who are being evaluated for AD and other causes of cognitive decline. Florbetapir F 18 is a sterile, non-pyrogenic radioactive diagnostic agent that binds to Aβ aggregates. Results from the first phase III study (N = 152) [100] showed good correlation (primary analysis cohort
of 29 patients) between the whole brain flurbetapir-PET visual image scores and cortical Aβ pathology at autopsy as measured by immunohistochemistry (Bonferroni ρ, 0.78 [95%CI 0.58–0.89]; p < 0.001) and silver stain neuritic plaque score (Bonferroni ρ, 0.71 [95%CI 0.47–0.86]; p < 0.001). Moreover, the prospective cohort study (59 primary analysis participants) [101] for patients who had autopsies within 2 and 1 years of cerebral PET imaging with flurbetapir to detect moderate to frequent neuritic Aβ plaques showed a sensitivity of 92% (36 out of 39; 95%CI 78–98) and 96% (27 of 28; 95%CI 80–100), respectively, as well as a specificity of 100% (20 out of 20; 95%CI 80–100) and 100% (18 out of 18; 95%CI 78–100), respectively. This study distinguished patients with moderate to frequent plaques (Aβ positive) from those with no or sparse plaques (Aβ negative). Flutemetamol F18 is a sterile, non-pyrogenic, radioactive diagnostic agent that binds to Aβ aggregates. Results from the phase III study (N = 176; 68 evaluable brains: 37% Aβ negative and 63% Aβ positive) [102] showed high sensitivity without computed tomography of 81%–93% (median, 88%; majority, 86%) and high specificity of 44%–92% (median, 88%; majority, 92%) to detect neuritic Aβ plaque with PET imaging using [18F] flutemetamol. Flurbetaben F18 is a sterile, non-pyrogenic radioactive diagnostic agent that binds to Aβ aggregates. Results from a pivotal histopathology phase III study (N = 216; 74 deceased subjects, 46 out of 47 Aβ positive; 24 out of 27 Aβ negative) [103] showed high sensitivity of 97.9% (95%CI 93.8–100) and specificity of 88.9% (95%CI 77.0–100) to detect neuritic Aβ plaques with the visual analysis consistent with quantitative assessment using flurbetaben PET (sensitivity: 89.4% [95%CI 80.6–98.2] and specificity: 92.3% [95%CI 82.1–100]). The amyloid load in an AD brain can be measured using PET, which has played a key role in clinical diagnosis.

Tau PET imaging is sensitive and detects early cognitive changes in the preclinical AD than Aβ-PET imaging [104, 105]. Although tau PET imaging provides novel insights into AD progression, there are several challenges because tau proteins form intracellular aggregates (tangles) [106] and radiotracers for these proteins have to cross the blood–brain barrier [107]; moreover, tau proteins undergo post-translational modifications [108] and available in six isoforms [109]. Efforts are ongoing to develop specific radiotracers for tau PET imaging [110]. Currently, tau radiotracers are not available for clinical use, and so far [18F] flortaucipir (Avid Radiopharmaceuticals/Eli Lilly) is the most validated tau PET radiotracer. A cross-sectional study [111] in 719 patients (n = 179, AD dementia [100% Aβ positive]; n = 254, non-AD neurodegenerative disorder [23.8% Aβ positive], n = 126, MCI [65.9% Aβ positive]; n = 160, cognitively normal controls [26.3% Aβ positive]) had shown that the [18F] flortaucipir PET had distinguished AD dementia from all non-AD neurodegenerative disorders in the medial-basal and lateral temporal cortex (89.9% sensitivity and 90.6% specificity [SUVR 1.34]). A case study [112] was performed to validate the use of [18F] flortaucipir PET to detect in vivo tau pathology in an individual with early onset AD (PSEN1 mutation). This study showed that in vivo retention of [18F] flortaucipir was correlated with postmortem tau pathology in the AD brain: density of tau-positive neurites (AT8: rs = 0.87; p < 0.001; Gallyas: rs = 0.92; p < 0.001), intrasomal tau tangles (AT8: rs = 0.65; p = 0.01; Gallyas: rs = 0.84; p < 0.001) and total tau burden (AT8: rs = 0.84; p < 0.001; Gallyas: rs = 0.82; p < 0.001), but not with the Aβ pathology. Recently, a small Phase III study [113] was performed in 156 patients (aged ≥ 50 years) who had projected life expectancy of ≤ 6 months and in those consented to brain donation at autopsy. This study assessed the relationship between antemortem [18F] flortaucipir PET imaging and tau pathology in AD at autopsy. Of 156 patients who underwent [18F] flortaucipir PET imaging, 67 were evaluated postmortem. In this study [18F] flortaucipir demonstrated statistically significant sensitivity and specificity to detect tau pathology of Braak Stage V/VI and high level of total AD neuropathologic change as defined by NIA-AA criteria [114].

PHARMACOLOGICAL SYMPTOMATIC TREATMENT FOR ALZHEIMER’S DISEASE AND UPCOMING DISEASE-MODIFYING THERAPIES

Symptomatic treatment for Alzheimer’s disease

The cholinergic hypothesis has yielded approved drugs for treating AD and has been pivotal for studies in dementia. According to the cholinergic hypothesis [115], the degeneration of cholinergic neurons and a decrease in cholinergic neurotransmission in the brain leads to cognitive deficits in patients with AD. At present, there is no cure for AD and the progression of the disease cannot be stopped or reversed; however, pharmacolog-
ical treatment (cholinesterase inhibitors [ChEIs] [116] and N-methyl-D-aspartate [NMDA] receptor antagonists [117]) provides symptomatic relief [2]. Current symptomatic AD treatment options approved by the US Food and Drug Administration include the ChEIs donepezil, galantamine, and rivastigmine as well as the NMDA receptor antagonist, memantine. The ChEIs donepezil and galantamine have acetylcholinesterase-inhibiting activity; whereas rivastigmine is a dual acetylcholinesterase–butyrylcholinesterase inhibitor [118]. Although the current pharmacological drug options provide symptomatic improvement, there is a need for treatment at the presymptomatic phase of the disease with disease-modifying effects.

In the brain of a patient with AD, there is a decrease in acetylcholine levels. A strategy to treat AD is inhibiting ChEI to hydrolyze the neurotransmitter acetylcholine into choline at the cholinergic synapses resulting in increased brain acetylcholine levels and leading to cognitive benefits of treatment compared with placebo. Donepezil (Aricept®) is a reversible acetylcholinesterase inhibitor indicated for the treatment of mild, moderate, and severe AD [119]. In the double-blind, randomized controlled studies there were improvements in cognition as measured by the Alzheimer’s Disease Assessment Scale-Cognitive subscale (ADAS-Cog) in patients with AD treated with donepezil compared with those who received placebo [120–122]. Galantamine (Razadyne ER® and Razadyne®) is a competitive reversible acetylcholinesterase inhibitor indicated for the treatment of mild-to-moderate dementia of the Alzheimer’s type [123]. Double-blind, randomized controlled studies in patients with AD showed improvements in cognition as measured by ADAS-Cog in those treated with galantamine compared with placebo [124–127]. Rivastigmine is a reversible ChEI available as a capsule (Exelon®) or patch (Exelon Patch®). Oral rivastigmine is indicated for the treatment of mild-to-moderate dementia of the Alzheimer’s type [128], whereas transdermal rivastigmine is indicated for mild, moderate, and severe dementia of the Alzheimer’s type [129]. Rivastigmine is also indicated for mild-to-moderate dementia associated with Parkinson’s disease [128, 129]. In the brain of AD patients there is a decrease in acetylcholine levels, and rivastigmine increases brain acetylcholine levels by dual inhibition of acetylcholinesterase–butyrylcholinesterase, which is responsible for acetylcholine hydrolysis [118]. In double-blind, randomized controlled studies improvements in cognition as measured by ADAS-Cog were observed in patients with AD treated with rivastigmine compared with those who received placebo [130, 131]. Glutamate is the main excitatory neurotransmitter that activates NMDA receptors of the central nervous system contributing to AD symptoms. Memantine uncompetitively binds to the NMDA receptor open-channel with moderate affinity and to exert its therapeutic effect. Memantine is an orally active NMDA receptor antagonist indicated for the treatment of moderate to severe dementia of the Alzheimer’s type [132]. Results from the double-blind, randomized controlled studies in patients with AD showed improvements in cognition as measured by ADAS-Cog in those treated with memantine compared with placebo [133, 134]. Generally, in AD clinical studies cognitive change in patients with AD is measured using ADAS-Cog, which is a standard primary outcome where the cognitive defect is severe. However, in the early stages of AD (prodromal) there is a mild decline in cognition, and these changes are difficult to measure. Recently, a new sensitive outcome measure, the AD Composite Score (ADCOMS) was developed to assess the cognitive decline in early AD trials and detect treatment effects [135].

Recently a meta-analysis [136] was performed (36 studies) including 6611 patients with AD to assess the efficacy and safety of donepezil, galantamine, rivastigmine, and memantine in symptomatic AD treatment. Results showed significant changes in cognition with active treatment versus placebo. The changes in cognition as assessed by ADAS-cog showed standardized mean differences of –0.28 (95%CI [–0.39,–0.16], p < 0.00001), –0.49 (95%CI [–0.56,–0.43]; p < 0.00001), –0.65 (95%CI [–1.06,–0.23]; p = 0.002) and –0.12 (95%CI [–0.24,–0.01], p = 0.03) for donepezil, galantamine, rivastigmine, and memantine, respectively. The findings from meta-analysis showed delay for at least 52 weeks in the progression of cognitive impairment in patients with AD treated with symptomatic treatment with ChEIs (donepezil, rivastigmine, galantamine) and N-methyl-D-aspartate receptor antagonist (memantine).

**Upcoming disease-modifying therapies for Alzheimer’s disease**

Pharmacological treatment of AD with approved ChEIs and memantine lessen cognitive symptoms with no effect on disease progression; therefore, there is a need for promising DMTs to delay progression or
| Studies            | Sample size | Follow-up       | Biomarker(s)       | Cut-off definition                                                                 | Sensitivity | Specificity |
|--------------------|-------------|-----------------|-------------------|-------------------------------------------------------------------------------------|-------------|-------------|
| Wallin et al. [74] | N = 50      | 5- and 6-year   | ↓ CSF Aβ42        | Cut-off in healthy controls                                                         | CSF Aβ42: 86% (18/21) | CSF Aβ42: 88% (21/24) |
|                    | 21 probable AD patients | | ↑ CSF t-Tau       | CSF Aβ42: <427 ng/l                                                             | CSF t-Tau: 86% (18/21) | CSF t-Tau: 88% (21/24) |
|                    | 24 controls  | | ↑ CSF p-Tau       | CSF Aβ42: <445 ng/l                                                             | CSF p-Tau: 60% (12/20) | CSF p-Tau: 88% (21/24) |
| Hansson et al. [75]| 180 MCI patients | 4.0–6.8 years   | ↓ CSF Aβ42-p-Tau181 | Cut-off for pathological CSF: t-Tau: >350 ng/l                                     | CSF Aβ42 and t-Tau: 95% | CSF Aβ42 and t-Tau: 83% |
|                    | 137 CSF was collected (56 stable MCI 57 MCI-AD 21 MCI other 3 died before 4 years follow-up) | | ↑ CSF t-Tau       | Aβ42-p-Tau181: >60 ng/l                                                          | CSF t-Tau: 86% (18/21) | CSF p-Tau: 88% (21/24) |
|                    | | | ↑ CSF p-Tau       | Aβ42-p-Tau181: <6.5                                                           | CSF p-Tau: 88% (21/24) | |
| Hansson et al. [78]| 137 MCI patients CSF was collected | 4.0–6.8 years   | ↓ CSF Aβ42        | Cut-off values for pathological CSF: Aβ42: ≤0.64 ng/ml                            | CSF Aβ42: 93% (95% CI 82–98) | CSF Aβ42: 53% (95% CI 41–64) |
|                    | | | ↓ CSF Aβ42/Aβ40 | Aβ42-Aβ40 ratio: 87% (95% CI 76–95)                                          | CSF Aβ42-Aβ40: 78% (95% CI 67–86) |
| Mattsson et al. [76]| 750 patients with MCI 529 with AD | 2–11 years      | Incipient AD       | CSF Aβ42: 79% (215 of 271; 95% CI 74–84)                                          | CSF t-Tau: 99% (95% CI 61–69) |
|                    | | | ↓ CSF Aβ42       | CSF Aβ42: ≤482 ng/l                                                          | CSF p-Tau: 84% (227 of 270; 95% CI, 80–88) |
|                    | | | ↑ CSF t-Tau      | t-Tau: ≥320 ng/l                                                              | CSF t-Tau: 86% (232 of 271; 95% CI 62–90) |
|                    | | | ↑ CSF p-Tau      | p-Tau: ≥52 ng/l                                                               | CSF t-Tau: 86% (232 of 271; 95% CI 62–90) |
| Shea et al. [77]   | N = 48      | 4.0–6.8 years   | ↓ CSF Aβ42        | t-Tau: >325.7 pg/ml                                                               | CSF t-Tau: 91% | |
|                    | 24 AD patients | | ↑ CSF tau          | p-Tau: >44.25 pg/ml                                                            | CSF p-Tau: 92% | |
|                    | 12 non-demented control | | ↑ CSF p-Tau181      | Aβ42: ≤357.1 pg/ml                                                             | CSF t-Tau: 99% (95% CI 83–88) |
|                    | 12 Non-AD dementia | | ↓ Aβ42-t-Tau       | Aβ42: >331.2 pg/ml                                                             | CSF p-Tau: 84% (227 of 270; 95% CI, 80–88) |
|                    | | | ↓ Aβ42-p-Tau      | Aβ42-p-Tau: <9.84                                                           | CSF t-Tau: 86% (232 of 271; 95% CI 62–90) |
| Park et al. [79]   | 71 controls | | ↓ CSF Aβ42        | AD dementia versus control                                                    | AD dementia versus control |
|                    | 76 patients with AD dementia | | ↑ CSF t-Tau     | Aβ42: <481 pg/ml                                                               | Aβ42: 94% | |
|                    | 47 OND with cognitive decline | | ↑ CSF p-Tau     | t-Tau: >326 pg/ml                                                              | t-Tau: 96% |
|                    | | | | p-Tau: >57 pg/ml                                                           | t-Tau: 96% |
|                    | | | | t-Tau/Aβ42: >0.55                                                          | p-Tau: 90% |
|                    | | | | p-Tau/Aβ42: >0.10                                                          | p-Tau: 90% |
|                    | | | | AD dementia versus OND                                                    | AD dementia versus OND |
|                    | | | | Aβ42: 93% | AD dementia versus OND |
|                    | | | | t-Tau: 83% | AD dementia versus OND |
|                    | | | | Aβ42: 93% | AD dementia versus OND |
|                    | | | | p-Tau: 86% | AD dementia versus OND |
|                    | | | | p-Tau/Aβ42: 93% | AD dementia versus OND |
|                    | | | | p-Tau/Aβ42: 93% | AD dementia versus OND |
|                    | | | | p-Tau/Aβ42: 93% | AD dementia versus OND |
|                    | | | | p-Tau/Aβ42: 93% | AD dementia versus OND |

AD, Alzheimer’s disease; CI, confidence interval; CSF, cerebrospinal fluid; MCI, mild cognitive impairment; OND, other neurological disorders.
prevents AD. There were a total of 112 agents in development for AD treatment in phase I (n = 23 agents in 25 trials), phase II (n = 63 agents in 75 trials), and phase III (n = 26 agents in 35 trials) stages [137]. Of these, the majority were DMTs (63%), but only a few disease-modifying compounds are promising and undergoing phase III trials. More recently, Cummings and his colleagues [138] reviewed clinicaltrials.gov for AD clinical studies and provided an update on AD drug development pipeline. In the 2019 pipeline, there were a total of 132 agents in the AD clinical trials (31 phase I studies: 30 agents, 83 phase II studies: 74 agents, 42 phase III studies: 28 agents) than 112 agents observed in the 2018 pipeline [137]. Of the 132 agents, 96 (73%) were intended for disease modification in AD clinical trials.

Aducanumab (BIIB037) is a human monoclonal antibody that selectively binds to aggregated forms of Aβ [139] and reduces Aβ plaques in AD [140]. Results from the interim analysis of the PRIME study (ClinicalTrials.gov identifier NCT01677572 [141]) supported the development of aducanumab [140]. ENGAGE (ClinicalTrials.gov identifier NCT02477800 [142]; estimated to enroll 1605 patients) and EMERGE (ClinicalTrials.gov identifier NCT02484547 [143]; estimated to enroll 1605 patients) are two ongoing randomized, double-blind phase III clinical studies to evaluate the effect of aducanumab compared with placebo (primary endpoint: Clinical Dementia Rating-Sum of Boxes [CDR-SB] score) in patients with early stages of AD. Recently, results based on new analysis of EMERGE a Phase III study [144] in patients with early AD exposed to high dose aducanumab showed a significant reduction of clinical decline in CDR-SB scores at 78 weeks from baseline (23% versus placebo, p = 0.01). CAD106 is a second-generation active Aβ immunotherapy designed to induce antibody production against Aβ1–6 peptide fragments, avoiding the Aβ-specific T-cell response [145]; whereas, CNP520 is an orally active β-secretase (BACE-1) inhibitor that reduces Aβ-peptide production [146]. The generation program is testing CNP520 and CAD106 in two pivotal studies of participants at risk for the onset of AD clinical symptoms (Generation Study 1 [estimated to enroll 1340 patients]: ClinicalTrials.gov identifier: NCT02565511 [147] [CNP520 and CAD106], and Generation Study 2 [estimated to enroll 2000 patients]: ClinicalTrials.gov Identifier: NCT03131453 [148] [CNP520]) using dual primary outcome measures including 1) time to diagnosis of MCI due to AD or dementia due to AD and 2) change in the Alzheimer’s Prevention Initiative Composite Cognitive (APCC) Test Score. These outcome measures were developed as sensitive instruments to evaluate treatment effects and assess the cognitive decline in individuals at risk of progression of AD. Further, the investigation of the BACE1 inhibitor CNP520 was discontinued in two pivotal Phase II/III studies in the Alzheimer’s Prevention Initiative Generation Program [149].

CONCLUSIONS

In the clinical practice, diagnosis of AD is mainly based on the observation of cognitive decline, and definitive AD is confirmed upon histological examination of the brain tissue. The advances in neuroimaging and the application of AD biomarkers help in better understanding of early pathological changes in AD brain. This review paper identified Aβ42 and tau are the core CSF biomarkers used as clinical diagnostic tools in AD, and that the application of approved radiotracers (florbetapir, flutemetamol, or florbetaben) for amyloid-PET brain imaging serves as a robust tool to detect early stages of AD.

The CSF Aβ42 and tau are biomarkers reflecting brain pathology and the alterations in concentrations of these proteins indicate early and late stages of AD. Increasing evidence shows that the CSF biomarkers (Aβ42 and tau) have a high sensitivity and specificity profile, and therefore have clinical utility in prediction of AD stages. Currently, the progression of AD cannot be stopped or reversed; however, the DMTs are promising and undergoing phase III trials for the early stages of AD. Contemporary AD management should advocate in identifying biomarkers for pre-dementia diagnosis and recommend DMTs to possibly reverse the pathology.

ACKNOWLEDGMENTS

This review article is sponsored by Novartis (Singapore) Pte. Ltd. The publication processing fees were funded by Novartis (Singapore) Pte. Ltd. The authors thank Stephen Christopher for reviewing this manuscript and providing helpful suggestions.

All authors of this review article met the International Committee of Medical Journal Editors (ICMJE) criteria for authorship and approved the final version to be published.
CONFLICT OF INTEREST

Oliver Simon and Ananda Krishna Karanam are employees of Novartis. The remaining authors have nothing to disclose.

REFERENCES

[1] Gale SA, Acar D, Daffner KR (2018) Dementia. Am J Med 131, 1161-1169.
[2] Scott KR, Barrett AM (2007) Dementia syndromes: Evaluation and treatment. Expert Rev Neurother 7, 407-422.
[3] Prizer LP, Zimmerman S (2018) Progressive support for activities of daily living for persons living with dementia. Gerontologist 58, S74-87.
[4] Jing W, Willis R, Feng Z (2016) Factors influencing quality of life of elderly people with dementia and care implications: A systematic review. Arch Gerontol Geriatr 66, 23-41.
[5] Prince M, Wimo A, Guerchet M, Ali G-C, Leino-Kilpi H, Prince M (2014) Neuroimaging of vascular dementia. Lancet Neurol 13, 23-41.
[6] O'Brien RJ, Wong PC (2011) Amyloid precursor protein (APP) processing and Alzheimer's disease. Trends Mol Med 17, 141-152.
[7] Hardy J, Selkoe DJ (2002) The amyloid hypothesis of Alzheimer's disease: Progress and problems on the road to therapeutics. Science 297, 353-356.
[8] Serrano-Pozo A, Frosch MP, Masliah E, Hyman BT (2011) Neuropathological alterations in Alzheimer disease. Neuropathol Appl Neurobiol 37, 1-12.
[9] O'Brien RJ, WC (2011) Amyloid precursor protein processing and Alzheimer's disease. Annu Rev Neurosci 34, 185-204.
[10] Storey E, Cappai R (1999) The amyloid precursor protein of Alzheimer's disease and the Aβ peptide. Neuropathol Appl Neurobiol 25, 81-97.
[11] Hayden EY, Teplow DB (2013) Amyloid β-protein oligomers and Alzheimer's disease. Alzheimers Res Ther 5, 60.
and molecular mechanism of neurofibrillary degeneration. J Alzheimers Dis 33, S123-139.

[40] Eckert A, Schmitt K, Gotz J (2011) Mitochondrial dysfunction - the beginning of the end in Alzheimer’s disease? Separate and synergistic modes of tau and amyloid-β toxicity. Alzheimers Res Ther 3, 15.

[41] Reddy PH (2011) Abnormal tau, mitochondrial dysfunction, impaired axonal transport of mitochondria, and synaptic deprivation in Alzheimer's disease. Brain Res 1415, 136-148.

[42] Swerdlow RH, Khan SM (2004) A “mitochondrial cascade hypothesis” for sporadic Alzheimer’s disease. Med Hypotheses 63, 8-20.

[43] Swerdlow RH, Khan SM (2009) The Alzheimer’s disease mitochondrial cascade hypothesis: An update. Exp Neurol 218, 308-315.

[44] Swerdlow RH, Burns, JM, Khan, SM (2010) The Alzheimer’s disease mitochondrial cascade hypothesis. J Alzheimers Dis 20, S265-279.

[45] Ferencz B, Karlsson S, Kalpouzos G (2012) Promising genetic biomarkers of preclinical Alzheimer’s disease: The influence of APOE and TOMM40 on brain integrity [Article ID 421452]. Int J Alzheimers Dis 2012, 421-452.

[46] Bronzuoli MR, Iacomino A, Steardo L, Scuderi C (2016) Targeting neuroinflammation in Alzheimer's disease. J Inflamm Res 9, 199-208.

[47] Heneka MT, Carson MJ, El Khoury J, Landreth GE, Brosseron F, Feinstein DL, Jacobs AH, Wyss-Coray T, Vitorica J, Ransohoff RM, Herrup K, Breitner JC, Cole GM, Golenohara ML, Perry VH, Holmes C, Bazan NG, Brooks DJ, Hunot S, Joseph B, Deigendesch N, Garaschuk O, Boddeke E, Dinarello CA, Breitner JC, Cole GM, Golenbock DT, Kummer MP (2015) Neuroinflammation in Alzheimer’s disease. Lancet Neurol 14, 388-405.

[48] McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM (1984) Clinical diagnosis of Alzheimer’s disease: Report of the NINCDS-ADRDA work group under the auspices of department of health and human services task force on Alzheimer’s Disease. Neurology 34, 939-944.

[49] American Psychiatric Association (2013) Diagnostic and Statistical Manual of Mental Disorders. American Psychiatric Association, Washington DC.

[50] Sachdev PS, Blacker D, Blazer DG, Ganguli M, Jeste DV, Paulsen JS, Petersen RC (2014) Classifying neurocognitive disorders: The DSM-5 approach. Nat Rev Neurol 10, 634-642.

[51] Dubois B, Feldman HH, Jacova C, Dekosky ST, Barberger-Gateau P, Cummings J, Delacourte A, Galasko D, Gauthier S, Jicha GA, Morris JC, Morris RC, Rossor MN, Scheltens P, Carrillo MC, Thies B, Phelps CH (2011) The diagnosis of mild cognitive impairment due to Alzheimer’s disease: Recommendations from the National Institute on Aging-Alzheimer’s Association workgroups on diagnostic guidelines for Alzheimer’s disease. Alzheimers Dement 7, 257-262.

[52] Albert MS, DeKosky ST, Dickson D, Dubois B, Feldman HH, Fox NC, Gamst A, Holtzman DM, Jagust WJ, Petersen RC, Snyder PJ, Carrillo MC, Thies B, Phelps CH (2011) The diagnosis of mild cognitive impairment due to Alzheimer’s disease: Recommendations from the National Institute on Aging-Alzheimer’s Association workgroups on diagnostic guidelines for Alzheimer’s disease. Alzheimers Dement 7, 263-269.

[53] Albert MS, DeKosky ST, Dickson D, Dubois B, Feldman HH, Fox NC, Gamst A, Holtzman DM, Jagust WJ, Petersen RC, Snyder PJ, Carrillo MC, Thies B, Phelps CH (2011) The diagnosis of mild cognitive impairment due to Alzheimer’s disease: Recommendations from the National Institute on Aging-Alzheimer’s Association workgroups on diagnostic guidelines for Alzheimer’s disease. Alzheimers Dement 7, 270-279.

[54] Dubois B, Feldman HH, Jacova C, Dekosky ST, Gauthier S, Selkoe D, Bate-
[61] Jack CR Jr, Bennett DA, Blennow K, Carrillo MC, Feldman HH, Frisoni GB, Hampel H, Jagust WJ, Johnson KA, Knopman DS, Petersen RC, Scheltens P, Sperling RA, Dubois B (2016) A/T/N: An unbiased descriptive classification scheme for Alzheimer disease biomarkers. Neurology 87, 539-547.

[62] Verghese PB, Castellano JM, Holtzman DM (2011) Apolipoprotein E in Alzheimer’s disease and other neurological disorders. Lancet Neurol 10, 241-252.

[63] Farrer LA, Cupples LA, Haines JL, Hyman B, Kukull WA, Mayeux R, Myers RH, Pericak-Vance MA, Risch N, van Duijn CM (1997) Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer disease meta analysis consortium. JAMA 278, 1349-1356.

[64] Ward A, Crean S, Mercaldi CJ, Collins JM, Boyd D, Cook MN, Arrighi HM (2012) Prevalence of apolipoprotein E4 genotype and homozygotes (APOE e4/4) among patients diagnosed with Alzheimer’s disease: A systematic review and meta-analysis. Neuroepidemiology 38, 1-17.

[65] Liu M, Bian C, Zhang J, Wen F (2014) Apolipoprotein E gene polymorphism and Alzheimer’s disease in Chinese population: A meta-analysis. Sci Rep 4, 4383.

[66] Mielke MM, Vemuri P, Rocca WA (2014) Clinical epidemiology of Alzheimer’s disease: Assessing sex and gender differences. Clin Epidemiol 6, 37-48.

[67] Neu SC, Pa J, Kukull W, Beekly D, Kuzma A, Gangadha-Ran P, Wang LS, Romero K, Arlendic SP, Redolfi A, Orlandi D, Frisoni GB, Au R, Devine S, Auerbach S, Espinosa A, Boada M, Ruiz A, Johnson SC, Koscik R, Wang JI, Hsu WC, Chen YL, Toga AW (2017) Apolipoprotein E genotype and sex risk factors for Alzheimer disease: A meta-analysis. JAMA Neurol 74, 1178-1189.

[68] Vemuri P, Wiste HJ, Weigand SD, Knopman DS, Shaw LM, Trojanowski JQ, Aisen PS, Weiner M, Petersen RC, Jack CR Jr; Alzheimer’s Disease Neuroimaging Initiative (2010) Effect of apolipoprotein E on biomarkers of amyloid load and neuronal pathology in Alzheimer disease. Ann Neurol 67, 308-316.

[69] Lautner R, Insel PS, Skillback T, Olsson B, Landén M, Frisoni GB, Herukka SK, Hampel H, Wallin A, Minthon L, Hansson O, Blennow K, Mattsson N, Zetterberg H (2017) Preclinical effects of APOE e4 on cerebrospinal fluid Aβ42 concentrations. Alzheimers Res Ther 9, 87.

[70] Marioni RE, Campbell A, Scotland G, Hayward C, Porteous DJ, Deary IJ (2016) Differential effects of the APOE ε4 on cerebrospinal fluid β-amyloid peptide and tau. PLoS One 14, 1-13.

[71] Olsson B, Lautner R, Andreasson U, Øhrfelt A, Portelli E, Bjerke M, Höltät M, Rosén C, Olsson C, Strobel G, Wu E, Dakin K, Petzold M, Blennow K, Zetterberg H (2016) CSF and blood biomarkers for the diagnosis of Alzheimer’s disease: A systematic review and meta-analysis. Lancet Neurol 15, 673-684.

[72] Alzforum Alzbiomarker Version 2.0 (2017) http://www.alzforum.org/alzbiomarker. Accessed January 8, 2020.

[73] Gurjeet Kaur Virk, Anne Poljak, Nady Braidy, Perrinnder S, Sachdev (2018) CSF and blood biomarkers of early-onset Alzheimer’s disease: A systematic review and meta-analysis [poster p3-226] Alzheimers Dement 14, P1158.

[74] Hansson O, Zetterberg H, Vanmechelen E, Scheltens P, Blankenstein MA (2010) Evaluation of plasma Aβ40 and Aβ42 as predictors of conversion to Alzheimer’s disease in patients with mild cognitive impairment. Neurobiol Aging 31, 357-367.

[75] Lovheim H, Elgh F, Johansson A, Zetterberg H, Blennow K, Hallmans G, Eriksson S (2017) Plasma concentrations of free amyloid β cannot predict the development of Alzheimer’s disease. Alzheimers Dement 13, 778-782.

[76] Mattsson N, Zetterberg H, Janelidze S, Insel PS, Andreasson U, Stomrud E, Palmqvist S, Baker D, Tan Hehir CA, Jeromin A, Hanlon D, Song L, Shaw LM, Trojanowski JQ, Weiner MW, Hansson O, Blennow K; ADNI Investigators (2016) Plasma tau in Alzheimer disease. Neurology 87, 1827-1835.

[77] Nakamura A, Kaneko N, Villemagne VL, Kato T, Doeecke J, Doré V, Fowler C, Li QX, Martins R, Rowe C, Tomita T, Matsuzaki K, Ishii K, Ishii K, Arahata Y, Iwashita S, Ito K, Tanaka K, Masters CL, Yanagisawa K (2018) High per-
formance plasma amyloid-β biomarkers for Alzheimer’s disease. *Nature* 554, 249-254.

[88] Mattsson N, Andreasson U, Zetterberg H, Blennow K; Alzheimer’s Disease Neuroimaging Initiative (2017) Association of plasma neurofilament light with neurodegeneration in patients with Alzheimer disease. *JAMA Neurol* 74, 557-566.

[89] Zhou W, Zhang J, Ye F, Xu G, Su H, Su Y, Zhang X; Alzheimer’s Disease Neuroimaging Initiative (2017) Plasma neurofilament light chain levels in Alzheimer’s disease. *Neurosci Lett* 650, 60-64.

[90] Neergaard JS, Dragsbak K, Christiansen C, Karstad MA, Brix S, Henriksen K (2018) Two novel blood-based biomarker candidates measuring degradation of tau are associated with dementia: A prospective study. *PLoS One* 13, 1-13.

[91] Nabers A, Perina L, Lange J, Mons U, Schartner J, Guldénhaupt J, Saum KU, Janelidze S, Holleczech B, Rajescu D, Hansson O, Gerwert K, Brenner H (2018) Amyloid blood biomarker detects Alzheimer’s disease. *EMBO Mol Med* 10 1-11.

[92] Miecke MM, Hagen CE, Xu J, Chai X, Vemuri P, Lowe VJ, Airey DC, Knopman DS, Roberts RO, Machulda MM, Jack CR Jr, Petersen RC, Dage JL (2018) Plasma phosphorylated tau181 increases with Alzheimer’s disease clinical severity and is associated with tau- and amyloid-positron emission tomography. *Alzheimers Dement* 14, 989-997.

[93] Shahpandas-Kroner H, Klafki HW, Bauer C, Schuchhardt J, Hüttenrauch M, Stazi M, Bouter C, Wirths O, Vogelsgang J, Wiltfang J (2018) A two-step immunoassay for the simultaneous assessment of Aβ38, Aβ40 and Aβ42 in human blood plasma supports the Aβ42/Aβ40 ratio as a promising biomarker candidate of Alzheimer’s disease. *Alzheimers Res Ther* 10, 121.

[94] Park JC, Han SH, Yi D, Byun MS, Lee JH, Jang S, Ko K, Jeon SY, Lee YS, Kim YK, Lee DY, Mook-Jung I (2019) Plasma tau/amyloid-β1-42 ratio predicts brain tau deposition and neurodegeneration in Alzheimer’s disease. *Brain* 142, 771-786.

[95] Schindler SE, Bollinger JG, Ovod V, Mawuenyega KG, Li Y, Gordon BA, Holtzman DM, Morris JC, Benzingier TLS, Xiong C, Fagan AM, Bateman RJ (2019) High-precision plasma β-amyloid 42/40 predicts current and future brain amyloidosis. *Neurology* 93, e1647-e1659.

[96] Palmqvist S, Janelidze S, Stromrud E, Zetterberg H, Karl J, Zink K, Bittner T, Mattsson N, Eichenlaub U, Blennow K, Hansson O (2019) Performance of fully automated plasma assays as screening tests for Alzheimer disease-related β-Amyloid status. *JAMA Neurol* 76, 1060-1069.

[97] Florbetapir F 18 Injection (Amyvid™). http://www.accessdata.fda.gov/drugsatfda_docs/label/2012/202008s001lbl.pdf. Accessed January 8, 2020.

[98] Florbetaben F 18 Injection (Vizamyl™). http://www.accessdata.fda.gov/drugsatfda_docs/label/2017/203137s008lbl.pdf. Accessed January 8, 2020.

[99] Florbetaben F 18 Injection (NeuraCity™). http://www.accessdata.fda.gov/drugsatfda_docs/label/2014/204677s001blb.pdf. Accessed January 8, 2020.

[100] Clark CM, Schneider JA, Bedell BJ, Beach TG, Bilker WB, Mintun MA, Pontecorvo MJ, Hefti F, Carpenter AP, Flitter ML, Kraukramer MJ, Kung HF, Coleman RE, Doraiswamy PM, Fleisher AS, Reiman EM, Sabbagh MN, Sadowsky CH, Schneider JA, Arora A, Carpenter AP, Flitter ML, Joshi AD, Kraukramer MJ, Lu M, Mintun MA, Skovronsky DM; AV-45-A16 Study Group (2012) Cerebral PET with florbetapir compared with neuropathology at autopsy for detection of neuritic amyloid-β plaques: A prospective cohort study. *Lancet Neurol* 11, 669-678.

[101] Curtis C, Gamez JE, Singh U, Sadowsky CH, Villena T, Sabbagh MN, Beach TG, Duara R, Fleisher AS, Frey KA, Walker Z, Hunjan A, Holmes C, Escobar YM, Vera CX, Agronin ME, Ross J, Bozoki A, Akinola M, Shi J, Van-denberghe R, Ikonomovic MD, Sherwin PF, Grachev ID, Farrar G, Smith AP, Buckley CJ, McLain R, Salloway S (2015) Phase 3 trial of flutemetamol labeled with radioactive fluorine 18 imaging and neuritic plaque density. *JAMA Neurol* 72, 287-294.

[102] Sabri O, Sabbagh MN, Seibyl J, Barthel H, Akatsu H, Onuchi Y, Senda K, Murayama S, Ishii K, Takao M, Beach TG, Rowe CC, Leverenz JB, Ghetti B, Ironside JW, Catarfau AM, Stephens AW, Mueller A, Koglin N, Hoffmann A, Roth K, Reininger C, Schulz-Schaeffer WJ; Florbetaben Phase 3 Study Group (2015) Florbetaben PET imaging to detect amyloid beta plaques in Alzheimer’s disease: Phase 3 study. *Alzheimers Dement* 11, 964-974.

[103] Brier MR, Gordon B, Friedenreich K, McCarthy J, Stern A, Christensen J, Owen C, Aleida P, Su Y, Hassenstab J, Cairns NJ, Holtzman DM, Fagan AM, Morris JC, Benzingier TL, Ances BM (2016) Tau and Aβ imaging, CSF measures, and cognition in Alzheimer’s disease. *Sci Transl Med* 8, 1-10.

[104] Avila J (2010) Intracellular and extracellular tau. *Front Neurosci* 4, 1-10.

[105] Pike VW (2009) PET radiotracers: Crossing the blood-brain barrier and surviving metabolism. *Trends Pharmacol Sci* 30, 431-440.

[106] Martin L, Latypova X, Terro F (2011) Post-translational modifications of tau protein: Implications for Alzheimer’s disease. *Neurochem Int* 58, 458-471.

[107] Buée L, Büssière T, Buée-Scherrer V, Delacourte A, Hof NJ, Holtzman DM, Fagan AM, Morris JC, Benzingier TL, Ances BM (2016) Tau and Aβ imaging, CSF measures, and cognition in Alzheimer disease. *Neurology* 92, e601-e612.

[108] Avila J (2010) Intracellular and extracellular tau. *Front Neurosci* 4, 1-10.

[109] Villemagne VL, Fodero-Tavoletti MT, Masters CL, Rowe CC (2015) Tau imaging: Early progress and future directions. *Lancet Neurol* 14, 114-124.
[144] http://investors.biogen.com/news-releases/news-release-details/biogen-plans-regulatory-filing-aducanumab-alzheimers-disease. Accessed January 8, 2020.

[145] Wiessner C, Wiederhold KH, Tissot AC, Frey P, Danner S, Jacobson LH, Jennings GT, Lüönd R, Ortmann R, Reichwald J, Zurini M, Mir A, Bachmann MF, Staufenbiel M (2011) The second-generation active Aβ immunotherapy CAD106 reduces amyloid accumulation in APP transgenic mice while minimizing potential side effects. J Neurosci 31, 9323-9331.

[146] Neumann U, Ufer M, Jacobson LH, Rouzade-Dominguez ML, Huledal G, Kolly C, Lüönd RM, Machauer R, Veenstra SJ, Hurth K, Rueeger H, Tintelnot-Blomley M, Staufenbiel M, Shimshek DR, Frieauff W, Dubost V, Schiller H, Vogg B, Beltz K, Avrameas A, Kretz S, Pezous N, Rondeau JM, Beckmann N, Hartmann A, Vormfelde S, David OJ, Galli B, Ramos R, Graf A, Lopez Lopez C (2018) The BACE-1 inhibitor CNP520 for prevention trials in Alzheimer’s disease. EMBO Mol Med 10, e9316.

[147] U.S. National Library of Medicine. A study of CAD106 and CNP520 versus placebo in participants at risk for the onset of clinical symptoms of Alzheimer’s disease (Generation S1). https://clinicaltrials.gov/ct2/show/NCT02565511. Accessed January 8, 2020.

[148] U.S. National Library of Medicine. A study of CNP520 versus placebo in participants at risk for the onset of clinical symptoms of Alzheimer’s disease (Generation S2). https://clinicaltrials.gov/ct2/show/NCT03131453. Accessed January 8, 2020.

[149] PR Newswire. https://www.prnewswire.com/news-releases/amgen-novartis-and-banner-alzheimers-institute-discontinue-clinical-research-program-with-bace-inhibitor-cnp520-for-alzheimers-prevention-300883758.html. Accessed January 8, 2020.