Journal of Alzheimer's Disease Reports 4 (2020) 21–37
DOI 10.3233/ADR-190143
IOS Press

Review

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

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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 ($A\beta_{42}$) and tau are used in the clinical diagnosis of AD; nevertheless, blood biomarkers ($A\beta_{42}$ 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

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ISSN 2542-4823/20/$35.00

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 $\geq$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 $\geq$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 predementia 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 $A\beta_{42}$), "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/–: 61.3%, 95% confidence interval [CI] 55.9–66.7; ε4/4 : 14.1%, 95%CI 12.2–16.0) and the lowest regional estimates were in Asia (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/–: 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 $A\beta_{1-42}$ 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 $A\beta_{1-42}$ 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 ($\beta = -0.095$, $p = 0.003$), verbal fluency ($\beta = 0.075$, $p = 0.023$), and digit symbol tests ($\beta = -0.087$, $p = 0.004$) 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, $A\beta_{42}$, 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 $A\beta_{42}$ is predictive of AD. Studies have demonstrated that high levels of CSF tau and low CSF $A\beta_{42}$ 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 $A\beta_{42}$ (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 $A\beta_{42}$ 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 $A\beta_{42}$ (mean [SD] 324 [101] [MCI-AD] versus 700 [181] [controls] or 551 [188] ng/l [stable MCI], both $p < 0.0001$) and CSF $A\beta_{42}$/p-Tau_{181} 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-Tau_{181} (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 $A\beta_{42}$ of 17.7 (5.33–58.9; $p < 0.0001$), CSF p-Tau_{181} and $A\beta_{42}$ of 16.8 (5.02–56.5; $p < 0.0001$), and t-Tau and $A\beta_{42}$/p-Tau_{181} 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 $A\beta_{42}$ 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 $A\beta_{42}$; 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 $A\beta_{42}$, 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 $A\beta_{42}$ 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 $A\beta_{42}$–t-Tau (median [interquartile range] 0.442 [0.650] versus 3.12 [1.96]; $p < 0.001$) and $A\beta_{42}$–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 $A\beta_{42}/A\beta_{40}$ ratio [78] showed that patients with MCI at baseline who developed AD (MCI-AD, $n = 57$) had a significant decrease in CSF $A\beta_{42}/A\beta_{40}$ ratio and $A\beta_{42}$ concentration than those with stable MCI or controls ($A\beta_{42}/A\beta_{40}$ ratio: mean ± SD 0.78 ± 0.19 [MCI-

AD] versus 1.3 ± 0.66 [stable MCI] or 1.5 ± 1.1 [control], both $p < 0.001$; $A\beta_{42}$: 0.46 ± 0.12 [MCI-AD] versus 0.67 ± 0.26 [stable MCI] or 0.87 ± 0.36 [control] ng/ml, both $p < 0.001$). The AUC was significantly larger for the $A\beta_{42}/A\beta_{40}$ ratio compared with the $A\beta_{42}$ (0.87; 95%CI 0.80–0.92 versus 0.77; 95%CI 0.69–0.84; $p < 0.05$). In a study (76 patients with AD dementia) of CSF biomarkers for AD in South Korea [79] the $A\beta_{42}$ levels were significantly lower in patients with AD dementia 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/$A\beta_{42}$ and pTau/$A\beta_{42}$ 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 $A\beta_{42}$ 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 $A\beta_{42}$ 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 $A\beta_{42}$ (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 $A\beta_{42}$ levels in patients with AD (N = 11,277) versus controls (N = 8315) and lower baseline CSF $A\beta_{42}$ 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 $A\beta_{42}$ 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 $A\beta_{42}$ 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 $A\beta_{42}$ 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 $A\beta_{42}$ 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 $A\beta_{42}$ in patients with AD (N = 2336) versus controls (N = 4452) and did not differ in baseline plasma $A\beta_{42}$ 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/$A\beta_{1-42}$ and $A\beta_{1-40}/A\beta_{1-42}$ 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 $pTau_{181}$ may be used as a biomarker of AD pathology. A study of two-step immunoassay that measured concentration of $A\beta_{38}$, $A\beta_{40}$, and $A\beta_{42}$ in the human blood plasma showed that $A\beta_{42}/A\beta_{40}$ ratio is promising biomarker candidate of AD [93]. The areas under the ROC curves were 0.87 and 0.80 for the $A\beta_{42}/A\beta_{40}$ ratio and $A\beta_{42}/A\beta_{38}$ ratio, respectively. A study quantified plasma t-tau, p-tau, and $A\beta_{1-42}$ 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/$A\beta_{1-42}$ ratio was highly predictive of brain tau deposition, with high t-tau/amyloid-β_{1-42} 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/$A\beta_{1-42}$ (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 $A\beta_{42}/A\beta_{40}$ in cognitively normal individuals (N = 158) [95]. The study provided class II evidence that plasma $A\beta_{42}/A\beta_{40}$ was predictive of the brain amyloidosis, with area under the ROC curves of 0.88 and high correspondence with CSF p-tau181/$A\beta_{42}$ (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 $A\beta_{42}$ and $A\beta_{40}$ 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^{TM}) was the first approved radioactive diagnostic agent followed by Flutemetamol [98] (Vizamyl^{TM}) and Florbetaben [99] (NeuraCeq^{TM}) 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 florbetapir-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 florbetapir 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 [^{18}F] flutemetamol. Florbetaben 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 florbetaben 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 [^{18}F] 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 showed that the [^{18}F] flortaucipir PET had distinguish 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 [^{18}F] 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 [^{18}F] 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 [^{18}F] flortaucipir PET imaging and tau pathology in AD at autopsy. Of 156 patients who underwent [^{18}F] flortaucipir PET imaging, 67 were evaluated postmortem. In this study [^{18}F] 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

Table 1
CSF biomarkers – sensitivity and specificity

Studies	Sample size	Follow-up	Biomarker(s)	Cut-off definition	Sensitivity	Specificity
Wallin et al. [74]	N = 50	5- and 6-year	↓ CSF $A\beta_{42}$	Cut-off in healthy controls	CSF $A\beta_{42}$: 86% (18/21)	CSF $A\beta_{42}$: 88% (21/24)
	21 probable AD patients		↑ CSF t-Tau	CSF $A\beta_{42}$: <427 ng/l	CSF t-Tau: 86% (18/21)	CSF t-Tau: 88% (21/24)
	24 controls		↑ CSF p-Tau	CSF t-Tau: <445 ng/l	CSF p-Tau: 60% (12/20)	CSF p-Tau: 88% (21/24)
				CSF p-Tau: <74 ng/l		
Hansson et al. [75]	180 MCI patients	4.0–6.8 years	↓ CSF $A\beta_{42}$–p-Tau_{181}	Cut-off for pathological CSF:	CSF $A\beta_{42}$ and t-Tau: 95%	CSF $A\beta_{42}$ and t-Tau: 83%
	137 CSF was collected		↓ CSF $A\beta_{42}$	t-Tau: >350 ng/l		
	(56 stable MCI		↑ CSF t-Tau	$A\beta_{42}$: <530 ng/l		
	57 MCI-AD		↑ CSF p-Tau_{181}	p-Tau_{181}: >60 ng/l		
	21 MCI other			$A\beta_{42}$–p-Tau_{181}: <6.5		
	3 died before 4 years follow-up)					
Hansson et al. [78]	137 MCI patients CSF was collected	4.0–6.8 years	↓ CSF $A\beta_{42}$	Cut-off values for pathological CSF:	CSF $A\beta_{42}$: 93% (95%CI 82–98)	CSF $A\beta_{42}$: 53% (95%CI 41–64)
			↓ CSF $A\beta_{42}/A\beta_{40}$	$A\beta_{42}$: ≤0.64 ng/ml	$A\beta_{42}$–$A\beta_{40}$ ratio: 87% (95%CI 76–95)	CSF $A\beta_{42}$–$A\beta_{40}$: 78% (95%CI 67–86)
				$A\beta_{42}$–$A\beta_{40}$: ≤0.95		
Mattsson et al. [76]	750 patients with MCI	2–11 years	Incipient AD	Incipient AD	Incipient AD	Incipient AD
	529 with AD		↓ CSF $A\beta_{42}$	CSF $A\beta_{42}$: ≤482 ng/l	CSF $A\beta_{42}$: 79% (215 of 271; 95%CI 74–84)	CSF $A\beta_{42}$: 65% (321 of 479; 95%CI, 61–69)
	304 controls		↑ CSF t-Tau	CSF t-Tau: ≥320 ng/l	CSF p-Tau: 84% (227 of 270; 95%CI, 80–88)	CSF p-Tau: 47% (225 of 479; 95%CI, 42–52)
	420 stable MCI		↑ CSF p-Tau	CSF p-Tau: ≥52 ng/l	CSF t-Tau: 86% (232 of 271; 95%CI 82–90)	CSF t-Tau: 56% (268 of 479, 95%CI 51–61)
	271 incipient AD					
Shea et al. [77]	N = 48		↓ CSF $A\beta_{42}$	Tau: >325.7 pg/ml	t-Tau: 83%	CSF t-Tau: 91%
	24 AD patients		↑ CSF tau	p-Tau: >44.25 pg/ml	p-Tau: 79%	CSF p-Tau: 92%
	12 non-demented control		↑ CSF p-Tau_{181}	$A\beta_{42}$: ≤357.1 pg/ml	$A\beta_{42}$: 75%	CSF $A\beta_{42}$: 83%
	12 Non-AD dementia		↓ $A\beta_{42}$–t-Tau	$A\beta_{40}$: >331.2 pg/ml	$A\beta_{40}$: 46%	$A\beta_{40}$: 83%
			↓ $A\beta_{42}$–p-Tau	$A\beta_{42}$–t-Tau: ≤1.54	$A\beta_{42}$–t-Tau: 96%	CSF $A\beta_{42}$–t-Tau: 83%
				$A\beta_{42}$–p-Tau: ≤9.84	$A\beta_{42}$–p-Tau: 92%	CSF $A\beta_{42}$–p-Tau: 83%
Park et al. [79]	71 controls		↓ CSF $A\beta_{42}$	AD dementia versus control	AD dementia versus control	AD dementia versus control
	76 patients with AD dementia		↑ CSF t-Tau	$A\beta_{42}$: <481 pg/ml	$A\beta_{42}$: 94%	$A\beta_{42}$: 87%
	47 OND with cognitive decline		↑ CSF p-Tau	t-Tau: >326 pg/ml	t-Tau: 84%	t-Tau: 96%
				p-Tau: >57 pg/ml	p-Tau: 72%	p-Tau: 90%
				t-Tau/$A\beta_{42}$: >0.55	t-Tau/$A\beta_{42}$: 99%	t-Tau/$A\beta_{42}$: 95%
				p-Tau/$A\beta_{42}$: >0.10	p-Tau/$A\beta_{42}$:96%	p-Tau/$A\beta_{42}$: 96%
				AD dementia versus OND	AD dementia versus OND	AD dementia versus OND
				$A\beta_{42}$: 478 pg/ml	$A\beta_{42}$: 93%	$A\beta_{42}$: 70%
				tTau: 327 pg/ml	t-Tau: 83%	t-Tau: 85%
				p-Tau: 48 pg/ml	p-Tau: 86%	p-Tau: 85%
				t-Tau/$A\beta_{42}$: 0.76	tTau/$A\beta_{42}$: 93%	tTau/$A\beta_{42}$: 92%
				p-Tau/$A\beta_{42}$: 0.12	pTau/$A\beta_{42}$: 95%	pTau/$A\beta_{42}$: 89%

AD, Alzheimer's disease; CI, confidence interval; CSF, cerebrospinal fluid; MCI, mild cognitive impairment; OND, other neurological disorders.

prevent 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 intend for disease modification in AD clinical trials.

Aducanumab (BIIB037) is 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 $A\beta_{1–6}$ 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 helps in better understanding of early pathological changes in AD brain. This review paper identified $A\beta_{42}$ 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 $A\beta_{42}$ 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 ($A\beta_{42}$ 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.

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