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Review

Alzheimer's Disease Cerebrospinal Fluid and Neuroimaging Biomarkers: Diagnostic Accuracy and Relationship to Drug Efficacy

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Abstract. Widely researched Alzheimer's disease (AD) biomarkers include *in vivo* brain imaging with PET and MRI, imaging of amyloid plaques, and biochemical assays of $A\beta_{1\text{-}42}$, total tau, and phosphorylated tau (p-tau-181) in cerebrospinal fluid (CSF). In this review, we critically evaluate these biomarkers and discuss their clinical utility for the differential diagnosis of AD. Current AD biomarker tests are either highly invasive (requiring CSF collection) or expensive and labor-intensive (neuroimaging), making them unsuitable for use in the primary care, clinical office-based setting, or to assess drug efficacy in clinical trials. In addition, CSF and neuroimaging biomarkers continue to face challenges in achieving required sensitivity and specificity and minimizing center-to-center variability (for CSF-$A\beta_{1\text{-}42}$ biomarkers CV = 26.5%; http://www.alzforum.org/news/conference-coverage/paris-standardization-hurdle-spinal-fluid-imaging-markers). Although potentially useful for selecting patient populations for inclusion in AD clinical trials, the utility of CSF biomarkers and neuroimaging techniques as surrogate endpoints of drug efficacy needs to be validated. Recent trials of β- and γ-secretase inhibitors and Aβ immunization-based therapies in AD showed no significant cognitive improvements, despite changes in CSF and neuroimaging biomarkers. As we learn more about the dysfunctional cellular and molecular signaling processes that occur in AD, and how these processes are manifested in tissues outside of the brain, new peripheral biomarkers may also be validated as non-invasive tests to diagnose preclinical and clinical AD.

Keywords: Amyloid-β, cerebrospinal fluid biomarkers, ^{18}FDG-PET, MRI, neuroimaging, PiB-PET, SPECT, surrogate biomarkers, tau

The clinical diagnosis of Alzheimer's disease (AD) is based on neuropsychological tests and exclusion of other age-related dementias. Disease progression and increasing severity of symptoms can support a diagnosis of AD, but definitive diagnosis is only possible at autopsy, with the identification of characteristic AD pathologic brain lesions, amyloid plaques, and neurofibrillary tangles. AD progresses to its advanced stages through multiple prodromal stages over a period of approximately two decades. In addition, AD can develop in combination with other neurological disorders of old age, including age-related decline in cognitive function or mild neurocognitive disorder, making antemortem definitive diagnosis of AD very difficult. Although early treatment of AD may eventually slow disease progression, the ability to diagnose AD in its earliest stages (preclinical stage) is currently limited. This clinical need has fueled the search for AD biomarkers that can not only accurately diagnose early-stage AD, but also differentiate AD from non-AD dementias [frontotemporal dementia (FTP), Lewy body dementia (LBD), vascular dementia (VaD), transactive response DNA-binding protein pathology (TDP-43), tauopathy, etc.], assess risk of AD in combination with other known risk factors, facilitate identification and screening of potential therapeutic

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Table 1
Standard biomarkers of Alzheimer's disease

Method	CNS Biomarkers	Criteria for Alzheimer's disease
Brain tissue (at autopsy)	Neurofibrillary tangles	Higher Braak Stages
	Amyloid plaques	Higher amyloid plaque score
	Brain atrophy/decreased brain volume	Decreased volume of brain
CSF	$A\beta_{1\text{-}42}$*	Low CSF $A\beta_{1\text{-}42}$
	Total tau*	High CSF total tau
	p-tau-181*	High CSF p-tau-181
Neuronal imaging	MRI*	Medial temporal atrophy
	fMRI	Disrupted default-mode neural network
	^{11}C-PiB PET*	Increased amyloid plaques
	^{18}FDG PET*	Decreased glucose uptake
	^{99m}Tc-HMPAO SPECT	Disrupted regional cerebral blood flow

Aβ, amyloid-β; CNS, central nervous system; CSF, cerebrospinal fluid; p-tau-181, phosphorylated tau at threonine 181; MRI, magnetic resonance imaging; fMRI, functional MRI; PET, positron emission tomography; ^{11}C-PiB, [^{11}C]-Pittsburgh Compound;^{18}FDG, [^{18}F]-fluoro-2-deoxy-D-glucose; SPECT, single-photon emission computed tomography; ^{99m}Tc, metastable nuclear isomer of technetium-99; HMPAO, hexamethylpropyleneamine Oxime. *This biomarkers are included in National Institute on Aging-Alzheimer's Association 2011 AD criteria for research.

agents, track prodromal stages of AD, guide therapeutic decision-making, and monitor therapeutic efficacy.

Despite substantial investment by governments, the pharmaceutical industry, and private donors, accurate biomarker of AD remain elusive. Simplified clinical criteria for the diagnosis of AD were first established three decades ago by the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) and the Alzheimer's Disease and Related Disorders Association (ADRDA) [1]. More recently, the International working group (IWG) introduced a set of revised and updated criteria for the clinical diagnosis of AD that re-conceptualized the disease as a clinico-biological entity with a specific clinical phenotype that could be confirmed *in vivo* based on pathophysiologic evidence of disease [2, 3]. After further modification by IWG-2, the simplified AD criteria are clinical AD phenotype (typical or atypical), plus a pathophysiological AD biomarker consistent with the presence of AD pathology [4]. Such AD biomarkers are cerebrospinal fluid (CSF) biomarkers [decreased amyloid-β ($A\beta_{1\text{-}42}$), increased tau and phosphorylated tau at threonine 181 (p-tau-181)], increased tracer retention on amyloid positron emission tomography (PET), and volumetric magnetic resonance imaging (MRI). Criteria for atypical AD include a specific clinical phenotype plus *in vivo* evidence of one of the following AD pathologies: 1) decreased $A\beta_{1\text{–}42}$ together with increased tau or p-tau-131 in CSF; 2) increased tracer retention on amyloid PET; or 3) presence of an AD autosomal dominant mutation (in PSEN1, PSEN2, or AβPP) [4]. IWG-2 also introduced criteria for preclinical AD, pre-symptomatic AD, and mixed AD presenting with other non-AD dementias [4]. The Alzheimer's Association (AA) and the National Institute on Aging (NIA) have recommended that neuroimaging and CSF biomarkers should be incorporated into the diagnosis of AD for research purposes and that updated clinical criteria should be used in clinical practice [5]. Both IWG and NIA-AA criteria have expanded coverage of the full range of disease stages, from preclinical asymptomatic AD to most severe stages of AD [2–5]. The three distinct phases of AD are preclinical asymptomatic AD, symptomatic pre-dementia, or mild cognitive impairment (MCI) due to AD, and dementia due to AD [6]. Neuroimaging and CSF biomarkers of AD include (i) increased ^{11}C-PiB ([11C]-Pittsburgh Compound) binding to amyloid plaques, (ii) decreased ^{18}FDG ([18F]-fluoro-2-deoxy-D-glucose) uptake, (iii) low CSF $A\beta_{1\text{-}42}$, (iv) elevated CSF tau and phosphorylated tau, and (v) brain atrophy measured by MRI (Table 1). In this review, we provide a critical discussion of the clinical diagnostic performance and the utility in assessing drug efficacy of current CSF and neuroimaging biomarkers of AD, as reported in the literature. Despite decades of expensive research into their diagnostic utility, these costly and invasive AD bioassays have yet to be standardized, though some are already being used as part of standard protocols in the clinical research setting.

CSF BIOMARKERS

The dominant hypothesis regarding the pathogenesis of AD involves an increase in Aβ production and accumulation (due to low clearance from the brain), leading to the deposition of amyloid plaques

that ultimately disrupt cognitive function. Aβ plaques are aggregates of Aβ peptides (mostly $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$) formed upon enzymatic cleavage of Aβ by amyloid-β protein precursor (AβPP). AβPP is subject to post-translational processing by three major enzyme systems (α-, β-, and γ-secretase), and activation of α-secretase or inhibition of β- and/or γ-secretase decreases Aβ production *in vitro* and *in vivo*. In the normal non-amyloidogenic pathway, AβPP is cleaved by α-secretase (a member of the ADAM family of proteases), releasing non-toxic, neuro-protective, soluble sAβPPα into the extracellular fluid [7]. In the abnormal amyloidogenic pathway, AβPP is cleaved by β-secretase (β-site AβPP-cleaving enzyme 1, BACE-1 [8]), which releases sAβPPβ into the extracellular fluid and eventually into the CSF [9–11]. The γ-secretase complex (consisting of four components: presenilin, nicastrin, PEN2, APH1 [12]) acts on the remaining extracellular carboxy-terminated fragment (CTFβ) in the plasma membrane and generates $A\beta_{1\text{-}42}$ and several carboxy terminal truncated Aβ peptides ($A\beta_{1\text{-}40,}$ $A\beta_{1\text{-}17}$, and others) [9]. In an alternative pathway, cleavage by β-secretase is followed by α-secretase to produce several short forms of Aβ peptides ($A\beta_{1\text{-}16}$ to $A\beta_{1\text{-}13}$) [9]. Pathologically elevated Aβ has been found to be neurotoxic and well correlated with cognitive dysfunction [13], eliciting abnormal patterns of activity in neuronal network circuits in mouse models of AD [14]. Individuals with early-onset, or familial, AD have an overproduction of Aβ, whereas those with late-onset, or sporadic, AD show a dysregulation of Aβ clearance [15, 16]. Several studies have shown that accumulation of Aβ occurs early in AD progression, whereas tau-related pathology occurs later [17]. Neurofibrillary tangles are formed after abnormal phosphorylation of tau protein, which disrupts microtubule organization. However, loss of synapses has been found to occur prior to deposition of plaques and tangles in MCI and early stage AD [18].

In recent years, we have seen an explosive increase in the discovery, validation, and application of CSF AD biomarkers for disease diagnosis, prognosis, therapy, and clinical trials [19–21]. During the last two decades, several groups have reported that CSF from patients with AD has decreased $A\beta_{1\text{-}42}$, increased tau, and increased p-tau-181 compared with patients without AD [22–26]. Once a CSF AD biomarker is identified that reflects AD pathology in preclinical research, development and validation of analytical methods are needed to ensure the high sensitivity, specificity, and accuracy of the biomarker in the clinical setting. According to the Alzheimer's Disease Neuroimaging Initiative (ADNI), the cut-off values of CSF biomarkers for a diagnosis of AD are: $A\beta_{1\text{-}42}$ <192 pg/mL; total tau >93 pg/mL; and p-tau-181 >23 pg/mL; with threshold values of tau/$A\beta_{1\text{-}42}$ = 0.39 and p-tau-181/$A\beta_{1\text{-}42}$ = 0.1. Poorly developed and validated analytical methods lead to reduced sensitivity and specificity of the biomarker and increase the number of false positive and false negative results. The specificity and sensitivity of CSF biomarkers are reasonably good in single-site cohort studies, but are lower in multisite studies because of variability in assay materials and techniques, including collection tube materials, sample handling and storage, dilution and buffer composition, heat treatment, plasma contamination, and immunoassay procedures. In one multi-center study conducted at 12 sites in Europe and the US, in a total of 750 individuals with MCI, 529 with AD, and 304 control cases, and using all three CSF markers, diagnostic sensitivity for AD was 83%, specificity 72%, positive predictive value 62%, and negative predictive value 88% [27]. Similar levels of sensitivity, specificity, and accuracy were obtained for CSF biomarkers in several autopsy registry studies [28, 29] that included AD and non-demented control cases (but not non-AD dementia patients; Table 2). A combination CSF biomarkers and multimodal neuroimaging techniques may achieve higher sensitivity and specificity [30]; however, such combination testing approaches are more expensive, time consuming, and may not be suitable in all clinical settings. Conversely, peripheral biomarkers may achieve similar levels of sensitivity and specificity in some cases [31].

The scientific rationale for using CSF biomarkers to diagnose AD is based on the direct contact between CSF and interstitial brain fluid; its consistency with the dominant AD pathophysiologic hypothesis; and the ability of CSF biomarkers to predict the conversion of MCI to AD. It has been postulated that the reason CSF levels of $A\beta_{1\text{-}42}$ are low in patients with AD is because $A\beta_{1\text{-}42}$ aggregation is considerably high. Patients with MCI who show a decrease in $A\beta_{1\text{-}42}$ are more likely to develop AD later, suggesting that CSF $A\beta_{1\text{-}42}$ may be predictive of early AD. Furthermore, CSF biomarker levels also vary by age; in a study, older non-AD patients had lower $A\beta_{1\text{-}42}$ and higher p-tau-181 levels compared with younger non-AD patients [32]. These suggest that older individuals may have evidence of molecular pathophysiology of AD even in the absence of cognitive impairment, which supports the use of CSF biomarkers in the early diagnosis of AD or assessment of AD risk.

Table 2
Sensitivity, specificity, and accuracy of CSF biomarkers in autopsy-confirmed AD patients* and control cases

Study	$A\beta_{1-42}$(%)	Total tau (%)	p-tau-181 (%)	References
INNTEST®	SN = 94	SN = 69	SN = 75	Struyfs et al., 2014 [29]
AD (n = 51), autopsy-confirmed	SP = 88	SP = 94	SP = 79	
Control (n = 95), clinically confirmed	ACU = 90	ACU = 90	ACU = 77	
INNOBIO	SN = 88	SN = 82	SN = 69	Struyfs et al., 2014 [29]
AD (n = 51), autopsy-confirmed	SP = 92	SP = 87	SP = 91	
Control (n = 95), clinically confirmed	ACU = 90	ACU = 86	ACU = 83	
ADNI	SN = 96.4,	SN = 69.6,	SN = 67.9,	Shaw et al., 2009 [28]
AD (n = 56), autopsy-confirmed	SP = 76.9,	SP = 92.3,	SP = 73.1,	
Control (n = 52), clinically confirmed	ACU = 87	ACU = 80.6	ACU = 70.3	

*Not including non-AD dementia patients. AD, Alzheimer's disease; p-tau-181, tau phosphorylated on threonine 181; SN, sensitivity; SP, specificity; ACU, accuracy; ADNI, Alzheimer's Disease Neuroimaging Initiative.

Nevertheless, several issues must be addressed before CSF biomarkers can be used clinically to diagnose AD. In reported studies, levels of CSF $A\beta_{1-42}$ and p-tau-181 are not consistently different between control, other non-AD dementia, and AD groups, and several other studies have reported inconsistent differences in CSF biomarkers in patients with familial AD and sporadic AD [33–37].

Discrimination between AD and non-AD dementias by CSF biomarkers

Most published studies reported that CSF concentrations of $A\beta_{1-42}$ are not significantly different in AD and non-AD dementia (VaD, FTP, and LBD) cases, making it difficult to distinguish between AD and non-AD dementias (Table 3). There were no significant differences in CSF levels of $A\beta_{1-42}$ and total-tau concentrations in patients with AD and amyotrophic lateral sclerosis or amyloid angiopathy [38, 39]. Lower $A\beta_{1-42}$ and elevated tau have also been reported in the CSF of patients with Creutzfeldt-Jakob disease [39–41]. Again, most studies found that CSF concentrations of total-tau are not significantly different in AD and non-AD dementia cases, and reported low sensitivity, making it difficult to distinguish between AD and non-AD dementias (Table 3). Measurement of p-tau-181 may be used for differential diagnosis of AD and non-AD dementia; however, the existing data are inconsistent (Table 3) [42–44]. Several studies showed that CSF tau concentrations are at intermediate levels in control groups and AD patient groups [39, 45–51]. As a result, sensitivity and specificity are low, with poor accuracy when trying to distinguish between AD and VaD. Subcortical VaD (SVaD) is a very common type of vascular dementia. Many of the neuropsychological deficits are similar in AD and SVaD, making them difficult to distinguish by neuropsychological tests. Deep lacunuae infarcts in white matter, accumulative white matter destruction, and extensive diffusive demyelination of white matter in periventricular regions are the characteristics of SVaD. Small blood vessels in deep brain become stiff and twisted in aging and infarcts by strokes cause SVaD. Reduced blood flow through these types of vessels cause damage of nerve fibers and neuronal signaling. Unlike AD, the pathophysiology of SVaD is not related to elevated tau and hyperphosphorylated tau. In SVaD, there is no evidence of increased CSF-tau compared to controls; therefore, MCI-SVaD patients could be differentiated from patients with MCI-AD tests based on CSF tau levels [52]. Using multivariate analysis and a combination of CSF total tau, p-tau, $A\beta_{1-42}$, matrix metalloproteinases, and tissue inhibitors of metalloproteinasescan separate AD from SVaD with high sensitivity, specificity, and accuracy [53]. A meta-analysis of published articles that included VaD, AD, and control groups found statistically significant differences in CSF tau between VaD and control groups ($p < 0.01$) [54]. The same study also estimated sensitivity to be 70% (60%–86%) and specificity 86% (80%–94%) for detecting VaD versus AD. Several other studies showed that there is no correlation of CSF p-tau-181 with Braak neurofibrillary tangles and neuritic plaques, the gold standard for autopsy diagnosis of AD [55]. By reviewing most of the articles related to CSF biomarkers of non-AD dementia, the conclusion is that the tau concentrations are moderately elevated in LBD, FTD, and VaD; in contrast, p-tau-181 concentrations are only slightly elevated in LBD but not in FTD and VaD compare to age-matched control [42–44, 56].

Limitations of lumbar puncture in AD diagnostic testing

While the lumbar puncture procedure is fairly routine and consistent across centers, there are some risks and a few side effects that may limit its use in repeated

Table 3
CSF biomarker levels (average ± standard deviation) in patients with AD and non-AD dementia

AD versus non-AD dementia	CSF Biomarker	Comments	Reference
AD versus VaD	$A\beta_{1\text{-}42}$ (pg/ml):	No significant change	Le Bastard et al., 2007 [43]
AD ($n=64$);	318 ± 135 (AD)		
VaD ($n=21$)	492 ± 273 (VaD)		
76 of 85 autopsy-confirmed	p-tau-181 (pg/ml):	SN: 97–50%	
	103 ± 94 (AD)	SP: 38–81%	
	91 ± 139 (VaD)	SN and SP varied with cutoff value	
AD versus VaD	$A\beta_{1\text{-}42}$ (pg/ml):	SN: Not determined	Andreasen et al., 2001 [151]
Probable AD ($n=105$)	523 ± 180 (probable AD)	SP: 48%	
Possible AD ($n=58$)	572 ± 225 (possible AD)		
VaD ($n=23$)	704 ± 321 (VaD)		
	p-tau-181 (pg/ml):	$p=0.247$	
	759 ± 417 (probable AD)		
	699 ± 275 (possible AD)		
	461 ± 280 (VaD)		
AD versus VaD	$A\beta_{1\text{-}42}$ (pg/ml):	No significant change	Kaerst et al., 2013 [152]
AD ($n=47$)	580 ± 211 (AD)		
VaD ($n=44$)	701 ± 341 (VaD)		
	Total-tau (pg/ml):	$p=0.579$;	
	391 ± 232 (AD)	No significant	
	302 ± 252 (VaD)	Change	
AD versus LBD	$A\beta_{1\text{-}42}$ (pg/ml):	No significant change	Andreasen et al., 2001 [151]
Probable AD ($n=105$)	523 ± 180 (probable AD)		
Possible AD ($n=58$)	572 ± 225 (possible AD)	SN: Not determined	
LBD ($n=9$)	568 ± 183 (LBD)	SP: 67%	
AD versus Non-AD dementia	Total-tau (pg/ml):	Early-stage AD versus non-AD dementia:	Shoji et al., 2002 [39]
Severe AD ($n=123$)	460 ± 263 (severe AD)		
Moderate AD ($n=145$)	508 ± 268 (moderate AD)		
Early-stage AD ($n=98$)	463 ± 273 (early-stage AD)	SN = 59.1%	
Non-AD dementia ($n=33$)	271 ± 203 (non-AD dementia)	SP = 80.4%	
AD versus CJD, AA, ALS, FTD, LBD	213 ± 172 (VaD)		
	Total-tau (pg/ml):	SN and SP were not determined	Shoji et al., 2002 [39]
Severe AD ($n=123$)	460 ± 263 (severe AD)		
Moderate AD ($n=145$)	508 ± 268(moderate AD)		
Early-stage AD ($n=98$)	463 ± 273 (early-stage AD)		
CJD ($n=6$)	410 ± 400 (CJD)		
AA ($n=2$)	493 ± 441 (AA)		
ALS ($n=8$)	410 ± 147 (ALS)		
FTD ($n=14$)	331 ± 124 (FTD)		
LBD ($n=14$)	330 ± 204 (LBD)		
AD versus Non-AD dementia (autopsy confirmed)	$A\beta_{1\text{-}42}$ (pg/ml):	$p=0.409$;	Le Bastard et al., 2010 [44]
	304 (137–557) (AD, $n=14$)*	No significant change	
	519 (327–581) (non-AD* dementia, $n=6$)		
	Total-tau (pg/ml)	$p=0.94$;	
	532 (219–1094) (AD, $n=16$)*	No significant change	
	489 (198–1071) (non-AD dementia, $n=6$)*		
	p-tau-181 (pg/ml)	$p=0.029$**	
	66.2 (40.7–102.5) (AD, $n=15$)*		
	36.9 (25.4–49.2) (non-AD dementia, $n=6$)*		

AD, Alzheimer's disease; CSF, cerebrospinal fluid; VaD, vascular dementia; SN, sensitivity; SP, specificity; LBD, Lewy body disease; CJD, Creutzfeldt-Jackob disease; AA, amyloid angiopathy; ALS, amyotrophic lateral sclerosis; FTD, frontotemporal dementia; p-tau-181, tau phosphorylated on threonine 181. *Data range (standard deviation was not reported). **Significant (although the number of non-AD dementia patients was small).

diagnostic AD testing, particularly for elderly patients. Post-lumbar puncture headache is one of the minor side effects of the lumbar puncture procedure, due to inadvertent rupture of blood vessels [57–59]. One study found that only 2.6% of patients ($n = 1,089$; aged 23–89 years) reported post-lumbar puncture headache without other local or general complications [60]. This study included patients from a wide range of age groups. The amount of CSF removed at a single lumbar puncture did not influence the occurrence of headache [59]. A multicenter, 13-week study of CSF cholinesterase activity of AD patients reported a favorable safety profile of lumbar puncture procedures and that <2% of patients experienced a headache due to lumbar puncture [61]. In addition, low CSF pressure/volume in elderly patients may increase the possibility of an unsuccessful spinal trap [62]. Finally, performing the lumbar puncture procedure multiple times to track disease progression or treatment efficacy presents considerable logistical challenges.

Instability of baseline Aβ in CSF

Fluctuations in Aβ levels are a major concern that may limit the use of CSF Aβ as a diagnostic biomarker for AD. Aβ levels vary due to circadian fluctuations and the activity of patients. One study reported that CSF Aβ levels fluctuate 1.5- to 4-fold over a period of 12 to 36 hours, and appear to be dependent on the time of day or activity level [63]. A later *in vivo* microdialysis study in mice described that Aβ levels in brain interstitial fluid correlated with wakefulness, and that Aβ levels significantly increased during acute sleep deprivation [64]. The study also found clear evidence of diurnal fluctuations in Aβ in the CSF of young healthy male volunteers over a 33-hour period ($n = 10$); Aβ levels increased throughout the first day and peaked in the evening, then decreased at overnight, and increased throughout the second day.

Inter-laboratory variations in CSF analysis

The accuracy of CSF Aβ measurements can be confounded by inter-laboratory variations in the immunoassay materials and methods, including the type of sample and assay tubes used, the number of freeze/thaw cycles, storage and incubation temperatures, sample preparation protocols, and antibody selection. Between studies, there is considerable variation in the reported levels of CSF $A\beta_{1\text{-}42}$, total tau, and p-tau-181. The variation among laboratories ranges from 13% to 36% (for CSF $A\beta_{1\text{-}42}$ CV (co-efficient of variation) = 26.5%; Paris: Standardization a Hurdle for Spinal Fluid, Imaging Markers; http://www.alzforum.org/news/conference-coverage/paris-standardization-hurdle-spinal-fluid-imaging-markers). An internatio nal quality control survey of 14 laboratories in Germany, Austria, and Switzerland to assess variation in CSF biomarkers found a higher CV for each CSF biomarker (CV of CSF $A\beta_{1\text{-}42} = 29\%$, total tau = 26%, and p-tau-181 = 27%) [65]. Substantial inter-laboratory variations of CSF biomarker levels make assessments and comparisons of data from different laboratories problematic. To address this issue, international scientists working on CSF biomarkers have established a working group called the Alzheimer's Biomarkers Standardization Initiative (ABSI) [66]. To reduce inter-laboratory variability, the ABSI has reached a consensus on various pre-analytical issues such as the effect of fasting, CSF collection and storage tubes, storage temperature, length of storage time, centrifugation speed, and storage concentrations of CSF $A\beta_{1\text{-}42}$, total tau, and p-tau-181. A standard protocol for CSF preparation and immunoassay, internationally recognized reference standards, cut-off values, and a mechanism to evaluate assay performance are still needed. Ongoing standardization efforts have been introduced to harmonize good laboratory practice, standard operating procedures, defined procedures on CSF collection and handling, and assay calibration for different technology platforms, with the ultimate goal of reducing inter-laboratory variability in CSF biomarker assays [67–70].

Contamination of CSF samples

Because the blood-brain barrier becomes dysfunctional in AD, there is a greater likelihood of blood contamination of CSF samples during lumbar puncture [71]. Proteins in blood plasma such as albumin, α2-macroglobulin, and low-density lipoprotein receptor-related proteins can bind to Aβ, which may lead to an underestimation of CSF Aβ levels. By evaluating the positive and negative aspects CSF biomarkers, it has been concluded that the CSF biomarkers for AD can be used for clinical trials but not as clinical practice [72].

Diagnostic accuracy of CSF biomarkers with respect to autopsy validation

Most of the CSF biomarker study cohorts were validated using clinical confirmation of an AD diagnosis.

Based on autopsy confirmation, clinical diagnoses show high accuracy for diagnosing AD in patients after the first 4 years of the onset of dementia symptoms [73]. By contrast, clinical diagnostic markers, when validated by subsequent autopsy diagnosis, were not as accurate within the first few years of the onset of symptoms of dementia [73, 74]. Sensitivity, specificity, and accuracy of several autopsy-confirmed CSF biomarker studies showed moderate results, specifically for CSF p-tau-181 (Table 2). These studies included AD and age-matched control cases, but no non-AD dementia cases. Several studies have claimed that the CSF p-tau-181 biomarker can be used to distinguish AD cases from non-AD dementia [75] (Table 3). Some of the peripheral biomarker studies showed similar levels of accuracy with respect to autopsy confirmation [31]. Furthermore, CSF levels of $A\beta_{1\text{-}42}$, total tau, and p-tau-181 were not associated with ApoE4 (widely regarded as one of the main risk factors of sporadic AD), tangle, or plaque burden in 50 autopsy-confirmed AD patients [44].

NEUROIMAGING BIOMARKERS

Neuroimaging of the brain enables the measurement of various structural and functional biomarkers of AD, including atrophy, changes in metabolism, inflammation, blood flow and perfusion, and neuronal network activity. One of the exciting applications of non-invasive neuroimaging techniques is the ability to quantitatively assess discrete alterations in AD-specific brain anatomical structures and pathophysiological functions. The best studied neuroimaging biomarkers of AD are detected and monitored using structural MRI (sMRI), functional MRI (fMRI), magnetic resonance spectroscopy (^{1}H-MRS), PET, and single-photon emission computed tomography (SPECT) (Table 4). Longitudinal brain imaging biomarkers enable measurement of subtle structural transitions as patients move from preclinical disease to MCI to definitive AD. In cross-sectional studies, neuroimaging biomarkers have been proven to be excellent tools to support clinical diagnosis for AD investigators. Neuroimaging biomarkers are still the main non-invasive method used for recruiting patients for clinical trials.

Magnetic resonance imaging

Structural MRI

Brain atrophy measured by sMRI correlates with cognitive impairment in AD and is the most widely used neuroimaging biomarker. Advances in scanner technology, image acquisition protocols, experimental design, and analysis methods promise to move sMRI from a mere brain imaging technique to a method for the quantitative measurement of AD non-invasive biomarkers. Brain atrophy measured by sMRI is considered to be one of the most investigated AD biomarkers. High-resolution sMRI can assess atrophy of critical brain areas such as the parahippocampal gyrus, hippocampus, amygdala, posterior association cortex, and subcortical region [76–79]. In addition to visual assessment of scans, several techniques for quantitative assessment have been introduced, such as the quantitative region of interest-based volumetric technique, quantitative voxel-based technique, tensor-based morphometric technique, and global atrophy quantification technique. There are several potential applications of sMRI in the detection of biomarkers, including early diagnosis of AD, distinguishing AD from MCI [4, 80], evaluation of disease progression [81, 82], differentiating AD from other non-AD dementias [83–85], predicting the risk of progression of MCI to AD [86, 87], screening patients, and measuring drug efficacy [78, 88–92]. Atrophy measured by sMRI has been incorporated into the 2011 AD criteria as one of the 5 AD biomarkers [5] and the Dubois criteria [2–4]. Memory impairment in early AD occurs predominantly in the medial temporal lobe area, hippocampus, and dentate gyrus. Brain atrophy determined by sMRI was correlated with CSF biomarkers and levels of cognitive impairment and the combination provided better discrimination of AD from age-matched normal control cases [93–95]. Gray matter atrophy in AD is a reflection of change of brain morphometry and is related to loss of neurons, synapses, and dendritic structures. White matter changes are related to loss of structural integrity of the brain such as demyelination and dying axonal processes due to AD. Areas affected by white matter loss due to AD pathology are the posterior portion of the corpus callosum, cingulum, and temporoparietal regions [96–99]. White matter damage measured using sMRI and sophisticated analysis methods like voxel-based morphometric analysis can distinguish early-onset AD from late-onset AD [100, 101]. Most studies of sMRI to quantify medial temporal atrophy reported reasonably good diagnostic sensitivity for detecting AD compared with control cases. However, the sensitivity and specificity of brain atrophy by MRI are very low for non-AD dementia cases, such as VaD and LBD [84]. MRI-based measurements of whole-brain atrophy showed

Table 4
Neuroimaging biomarkers of Alzheimer's disease

Modality	Imaging	Imaging Biomarker	Comments	References
MRI	Various areas of the brain	• Brain volume • Brain atrophy	• Useful for longitudinal studies, but low specificity for AD versus non-AD dementias • No radiation exposure	Jack et al., 1999 [153] Visser et al., 1999 [154] Fox et al., 1999 [155]
fMRI	Blood flow in areas of the	• Paramagnetic properties of brain related to memory processing	• No radiation exposure oxy-hemoglobin/ deoxy-hemoglobin in blood flow	Machulda et al., 2003 [103] Pihlajamäki et al., 2009 [104]
PET	*In-vivo*, radiotracer binding/uptake by specific brain targets	• Aβ using ^{11}C-PiB; ^{18}F-Florbetapir • Glucose uptake using ^{18}FDG • Tau by ^{18}FDDNP • Activated microglia by ^{11}C-PK11195	• Radiation exposure • Unlikely to be useful for population screening or longitudinal monitoring	Klunk et al., 2004 [105] Choi et al. 2012 [111] Scheinin et al., 2009 [106] Jagust et al., 2009 [124] Shin et al., 2011 [156] Kropholler et al., 2007 [114]
SPECT	Brain perfusion as an indicator of brain metabolism	• Blood flow using ^{99m}Tc-HMPAO	• Low resolution compared with MRI • Radiotracers have longer half-lives than PET tracers • Radiation exposure • Unlikely to be useful for population screening or longitudinal monitoring	Dougall et al, 2004 [126] Bonte et al., 2004 [125]
^{1}H-MRS	Proton magnetic resonance spectroscopy	• Brain N-acetyl aspartate, creatine, choline, myoinositol.	• Differential ratios of metabolites	Bates et al., 1996 [128] Zhu et al., 2006 [129]

MRI, magnetic resonance imaging; fMRI, functional MRI; PET, positron emission tomography; Aβ, amyloid-β protein; SPECT, single-photon emission computed tomography; ^{11}C-PiB, [^{11}C]-Pittsburgh Compound; ^{18}F-Florbetapir, [^{18}F]-Florbetapir; ^{18}FDG, [^{18}F]-fluoro-2-deoxy-D-glucose; FDDNP, 2-(1-{6-[(2-[^{18}F]fluoroethyl)(methyl)amino]-2-naphthyl}-ethylidene)malononitrile; ^{11}C-PK11195, [^{11}C]-isoquinoline carboxamide; ^{99m}Tc, metastable nuclear isomer of technetium-99; HMPAO, hexamethylpropylene amine oxime.

a modest correlation with CSF biomarker levels in patients with AD [102], but a stronger correlation with clinical progression of AD, measured by changes in the Mini Mental Score Examination (MMSE) score.

With functional MRI (fMRI), it is possible to measure neuronal activity in specific brain regions by imaging the paramagnetic properties of oxy-hemoglobin/deoxy-hemoglobin in blood flowing through the brain. Whereas sMRI provides structural information, fMRI provides both structural and functional information [103, 104]. Combined with neuropsychologic and behavioral tests, fMRI brain imaging can identify preclinical structural changes in the posteromedial cortical, frontotemporal and parietal lobes and functional changes in neuronal activity associated with AD.

Positron emission tomography

Unlike MRI, PET uses radiolabeled tracers that either bind target proteins or are taken up by target tissues and reconstructs tomographic images of protein levels or brain metabolism based on the tracer emission patterns.

Amyloid imaging by PET

PET radiotracers include amyloid binding agents to detect Aβ-aggregates and radiolabeled glucose to measure brain metabolism. As amyloid plaque deposition is a hallmark of AD brain pathology at autopsy, PET imaging of the brain to detect Aβ aggregates was considered to be a promising antemortem diagnostic approach. Uptake of ^{11}C-PiB in the brain was developed as a potential neuroimaging biomarker for [105], and extensive studies have been conducted to validate it. Unfortunately, researchers from the Turku PET Center in Turku, Finland found that the rate of ^{11}C-PiB uptake did not correlate with either brain atrophy or cognitive impairment in a group of patients with AD [106]. In another multicenter comparative study conducted by the ADNI (supported by the NIH, pharmaceutical companies, and non-profit funding), there was no relationship between CSF biomarkers ($A\beta_{1-42}$, t-tau, and p-tau-181), PET neuroimaging of amyloid

plaques, and cognitive impairment as measured by MMSE score. Moreover, the brains of aged patients without clinical dementia may have a considerable number of amyloid plaques, which increases the rate of false positive rate of ^{11}C-PiB PET neuroimaging. In a study on co-twins, both the cognitively impaired subjects (monozygotic and dizygotic) showed typical Alzheimer-like patterns of ^{11}C-PiB uptake [107]. In a study conducted by the Klunk laboratory (which developed ^{11}C-PiB PET), amyloid plaques were detected in 22% of healthy, age-matched controls (without any cognitive impairment) by ^{11}C-PiB PET [108]. Another issue is that soluble Aβ, which is neurotoxic, cannot be detected by ^{11}C-PiB PET. In a transgenic mouse model of AD, amyloid plaque formation is not always associated with memory impairment, but elevated soluble Aβ is [109]. Another study found that 10 out of 63 patients with probable AD (clinically confirmed by NINCDS-ADRDA criteria, not autopsy) cases were ^{11}C-PiB PET negative [110]. Recently, several ^{18}F-labeled PET ligands such as ^{18}F-florbetapir (Amyvid^{TM}), ^{18}F-flutemetamol (Vizamyl^{TM}), and ^{18}F-florbetaben (Neuraceq^{TM}) have been approved in US and EU for amyloid imaging. Studies with ^{18}F-florbetapir showed good correlation with amyloid load in AD patients at autopsy [111]. Recently, an ADNI comparative study found that ^{18}F-florbetapir showed greater specificity than CSF $A\beta_{1\text{-}42,}$ although overall diagnostic accuracies were the same [112]. The positron-emitting isotope ^{18}F (half-life of 109.8 min) has a longer half-life than the ^{11}C (half-life of 20.4 min), providing a longer window for conducting an imaging study. This is significant, as the cyclotron facility for ^{18}F PET radioisotope production may not necessarily be in close proximity to the PET imaging center.

AD brain inflammation imaging by PET

Neuro-inflammation caused by activated microglia has been identified as one of the early events in AD pathophysiology [113]. PET imaging compounds like ^{11}C-PK11195 have been developed to measure brain inflammation levels and may be useful in the early diagnosis of AD or MCI [114].

Tau imaging by PET

Hyperphosphorylation of tau in AD leads to accumulation of insoluble paired helical filaments (PHF) that form neurofibrillary tangles, one of the 'gold standards' of AD diagnosis at autopsy. Some studies found better correlation of disease severity with neurofibrillary tangles than with amyloid plaques in postmortem AD brains [115, 116]. Tau imaging by PET was first reported using a radiofluorinated derivative of 2-(1-[6-(dimethylamino)-2-naphthyl] ethylidene)malononitrile (DDNP) (^{18}FDDNP), which showed higher retention times in the brains of AD and MCI patients than those of healthy control cases [117]. ^{11}C-phenyl/pyridinyl-butadienyl-benzothiazoles/benzothiazoliums (^{11}C-PBB3) retention was also found in AD and non-AD tauopathy cases [118]. Tau-binding novel quinolone derivatives (^{18}F-THK-523, ^{18}F-THK-5105, and ^{18}F-THK-5117) detected by PET were similarly retained in AD brains [119–121]. Among these, ^{18}F-THK-5117 was found to be superior in terms of signal-to-background ratio and the ability to distinguish between mild, moderate, and severe AD cases [122]. Recent studies have shown that tau imaging with PET detects tau pathology in brain areas of AD and MCI cases; however, it is less able to distinguish between AD and other tau-related non-AD dementias such as FTD, corticobasal degeneration, and progressive supranuclear palsy.

Glucose metabolism measurement by PET

The human brain consumes approximately 20% of the body's total energy requirement. Glucose is the sole source of energy for the brain; proteins and fatty acids are bound to albumin, and cannot cross the blood-brain barrier. Using [18F]-fluoro-2-deoxy-D-glucose (^{18}FDG) PET neuroimaging, it was found that glucose metabolism was impaired in the brains of AD patients [123]. In a comparative study of CSF biomarkers and neuroimaging biomarkers, ^{11}C-PiB PET correlated well with CSF biomarkers but not with cognitive impairment. However, ^{18}FDG PET was more strongly associated with MMSE score but not with CSF biomarkers [124]. It is important to point out that some ^{18}F-labeled PET ligands can accumulate in bone and interfere in PET imaging results.

Single-photon emission computed tomography

Cerebral blood flow can be measured by SPECT. The blood flow through the brain can be imaged with SPECT using either intravenously injected ^{99m}Tc-HMPAO (Hexamethylpropylene amine oxime) or inhaled Xe-133, a gamma ray emitter. The uptake of ^{99m}Tc-HMPAO by brain tissue is proportional to the rate of blood flow in the brain, which is tightly coupled to local brain metabolism; therefore, differences in blood flow in various areas of the brain correlate with differences in brain metabolism in those areas. In patients with AD, brain metabolism is impaired. Though some studies have shown that SPECT has

higher sensitivity for diagnosing advanced AD [125], SPECT is able to distinguish between AD and non-AD dementias [126]. Both SPECT and ^{18}FDG PET neuroimaging provide information about the metabolic state of the brain, and have comparable diagnostic sensitivity and specificity for AD. Of the two modalities, however, SPECT is more widely available and less expensive than PET, and also uses an isotope (^{99m}Tc) with a longer half-life and less complicated imaging protocols.

Magnetic resonance spectroscopy (^{1}H-MRS)

In ^{1}H-MRS, a small volume of tissue (voxel) is selectively excited in a magnetic field and the free induction decay is recorded to produce an MR spectrum [127]. A variety of brain metabolites can be measured in a single session. In the AD brain, typical metabolites measured include choline, creatine, N-acetylaspartate (NAA), and myoinositol. Among these specific metabolites, NAA is a neuronal marker seen only in nervous system tissue, choline is an indicator of membrane integrity, creatine is thought to be a marker of energetic status of cells, and myoinositol levels reflect the glial response in the brain. For a quantitative measurement of these metabolites, levels are normalized to an internal standard of creatine concentration [128, 129].

Discrimination between AD and non-AD dementias by neuroimaging biomarkers

In various studies, the levels of MRI biomarkers were not found to be consistently different between AD and non-AD dementia cases, making it difficult to distinguish between AD and non-AD dementias (Table 5). Reported high sensitivities and specificities for differential diagnosis of AD versus MCI and age-matched control cases by MRI were not observed in AD versus non-AD dementia cases (Table 5). There was no significant difference in hippocampal atrophy between FTD versus AD cases [130] or VaD versus AD cases [131] measured by structural MRI. Two structural MRI studies did find differences between AD and LBD in terms of brain atrophy [131, 132], but sensitivities and specificities were not reported. Significantly higher sensitivities and specificities for differential diagnosis of AD versus non-AD dementia by PET imaging have been reported (Table 6). ^{18}F-labeled Aβ tracers showed higher nonspecific white matter binding and, in some cases, lower cortical binding in AD that could be misleading scanned data [133].

LIMITATION OF NEUROIMAGING BIOMARKERS

A. Sophisticated and expensive technology

The main limitation to using neuroimaging of AD biomarkers modality is technical sophistication. Only very specialized centers with highly technically trained expert teams of neuroscientists, radiologists, and bioinformatics specialists and that meet all infrastructure and regulatory compliance requirements can perform this type of imaging. In addition, the imaging equipment and its maintenance is expensive. For these reasons, neuroimaging of AD biomarkers is more costly and geographically limited compared to other testing approaches.

B. Radioactivity exposure

Both PET and SPECT neuroimaging techniques require the use of radioactive tracers, which raises issues regarding radiation exposure safety. In addition, the radiotracer ^{11}C-PiB has very short half-life (∼20 min), which requires ready access to a cyclotron.

C. Non-specific PET tracer binding

The most widely studied PET radiotracers used to detect AD biomarkers are ^{11}C-PiB for amyloid plaques and ^{18}FDG for glucose uptake. Rowe et al. found that PiB binding increases from less than 10% in patients <70 years age to 40% in those aged 80 years, suggesting some nonspecific binding activity that may obscure test results [134]. In addition, 22% of healthy age-matched controls (without any cognitive impairment) were considered to be AD-positive based on their biomarker value with ^{11}C-PiB PET [135]. While ^{18}FDG PET imaging might be able to distinguish between FTD and AD, ^{18}F compounds (flutemetamol, flornetapir) have a high affinity to brain white matter that may increase non-specific binding [133].

Performance of CSF biomarkers in assessing drug efficacy in AD clinical trials

The purpose of incorporation of biomarkers into AD clinical trials is to measure the homogeneity of the recruited patient population, assess drug response, provide surrogate endpoints for drug efficacy, and give insights into the mechanisms of drug action. Despite promising preclinical results with anti-Aβ immunotherapies, as well as β- and γ-secretase inhibitors, all of these approaches have failed in recent AD clinical trials (Table 6) [135–141]. Along with neuropsychological

Table 5
Diagnosis of Alzheimer's disease (AD) versus control, mild cognitive impairment (MCI), and non-AD dementia using MRI

AD versus MCI/Control/ Non-AD Dementia MCI/Control/ Non-AD Dementia	MRI Biomarker	Comments	Reference
AD versus MCI Follow-up of MCI cases ($n = 282$) Data were obtained from the ADNI study	Structural MRI, hippocampal and entorhinal cortex volume: SN: 80% SP: 56% ACU: 74%	• MRI of hippocampal and had limited added predictive utility above memory and functional measures	Devanand et al., 2012 [157]
AD versus Control Follow-up of: MCI ($n = 173$) Converted to AD ($n = 112$) Control ($n = 61$) dNeuroMed consortium and ADNI	Structural MRI, hippocampal and entorhinal cortex volume: SN: 86.1% SP: 90.4% ACU: 95%	• Combination of multiple MRI features in the form of a severity index improved SN, SP, and ACU	Spulber et al., 2013 [158]
AD versus Control AD ($n = 15$) Control ($n = 16$)	Functional MRI, default-mode network: SN: 73.3–86.7% SP: 75–93.7% ACU: not determined Ventral attention network: SN: <70–73.3% SP: <70–81.2% ACU: not determined Dorsal attention network: SN: 85.7–100% SP: <81.2–100%	• Small sample size	Li et al., 2012 [159]
AD versus FTD Clinically confirmed AD ($n = 103$) Control ($n = 73$) FTLD: FTD ($n = 17$) Semantic dementia ($n = 13$) Progressive non-fluent aphasia ($n = 12$)	ACU: not determined Structural MRI, hippocampal atrophy: SN, SP, and ACU not determined	• No significant difference in hippocampal atrophy between FTLD and AD	van de Pol et al., 2006 [160]
AD versus LBD and VaD Clinically confirmed AD ($n = 25$) LBD ($n = 27$) VaD ($n = 24$) Control ($n = 26$)	Structural MRI, hippocampal atrophy: LBD had significantly larger temporal lobe, hippocampus, and amygdala volumes than those with AD SN, SP, and ACU not determined	• Significant difference in brain atrophy between LBD and AD; no significant difference in brain atrophy between VaD and AD	Barber et al., 2000 [131]
AD versus LBD Autopsy-confirmed AD ($n = 30$) LBD ($n = 20$) Mixed LBD/AD ($n = 22$) Control ($n = 15$)	Pure LBD were characterized by lower global and regional rates of atrophy, similar to control SN, SP, and ACU not determined	• Significant difference in brain atrophy between LBD and AD	Nedelska et al., 2015 [132]

AD, Alzheimer's disease; MCI, mild cognitive impairment; ADNI, Alzheimer's Disease Neuroimaging Initiative; MRI, magnetic resonance imaging; SN, sensitivity; SP, specificity; ACU, accuracy; FTD, frontotemporal dementia; FTLD, Frontotemporal lobar degeneration; LBD, Lewy body disease; VaD, vascular dementia.

tests, CSF biomarkers and MRI volumetric measurements were included for patient selection in most of these clinical trials. In general, CSF biomarkers of AD include elevation of CSF total tau and phospho-tau-181 (due to neuronal injuries), and reduction in $A\beta_{1\text{-}42}$ (reduced Aβ due to amyloid plaques deposition in brain areas). Drug treatment efficacy in trials would be detected as decreased tau and increased $A\beta_{1\text{-}42}$. The performance of CSF biomarkers in longitudinal studies to track AD progression has encouraged their use in selecting patients for inclusion in clinical trials, and changes in biomarker data with respect to trial dose/time may ultimately lead to correlation of biomarkers to clinical benefits such as reduced neurodegeneration [142].

Table 6
Differential diagnosis of Alzheimer's disease (AD) and non-AD dementia patients using PET

AD versus non-AD dementia	PET Biomarker	Comments	Reference
AD versus FTD Clinically confirmed AD ($n=62$) FTD ($n=45$) Control ($n=25$)	^{18}F-FDG: SN: 73% SP: 98% ACU: not determined ^{11}C-PiB: SN: 89% SP: 83% ACU: not determined	• Threshold value was estimated from control cases • ^{11}C-PiB had higher sensitivity (89% versus 73%) while FDG had higher specificity (83% versus 98%)	Rabinovici et al., 2011 [161]
AD versus non-AD dementia Autopsy confirmed AD ($n=20$) AD mixed with other dementia ($n=4$) Normal control ($n=9$) FTD ($n=1$) LBD ($n=3$) VaD ($n=1$) Unknown dementia ($n=6$)	^{18}F-FDG: SN: 84% SP:74% ACU: 80%	• Addition of ^{18}F-FDG • data improved clinical diagnosis	Jagust et al., 2007 [162]
AD versus FTD Autopsy confirmed AD ($n=31$) FTD ($n=14$)	^{18}F-FDG; SN: 97% SP: 86% ACU: 93%	• Addition of ^{18}F-FDG • data improved clinical diagnosis	Foster et al., 2007 [163]
AD versus LBD Autopsy confirmed AD ($n=10$) LBD ($n=11$)	^{18}F-FDG SN: 90%, SP: 82% ACU: 86%	• Addition of ^{18}F-FDG • data improved clinical diagnosis	Minoshima et al., 2001 [164]
Clinically confirmed AD ($n=40$) LBD ($n=13$)			
AD versus non-AD dementia AD ($n=199$) Control ($n=110$) MCI ($n=114$) FTD ($n=98$) LBD ($n=27$)	^{18}F-FDG: AD versus FTD SN = 90% SP = 65%; AD versus LBD SN = 99% SP = 71% AD versus control SN = 99% SP = 98%	• This multicenter study validated differential diagnosis of AD versus non-AD dementias	Mosconi et al., 2008 [165]

AD, Alzheimer's disease; PET, positron emission tomography; FTD, frontotemporal dementia; ^{18}FDG, [^{18}F]-fluoro-2-deoxy-D-glucose; SN, sensitivity; SP, specificity; ACU, accuracy; ^{11}C-PiB, [^{11}C]-Pittsburgh compound; LBD, Lewy body disease; VaD, vascular dementia; MCI, mild cognitive impairment.

Most of the Aβ immunization clinical trials resulted in clearance of plaques in AD. However, no improvement in neurodegeneration was seen (Table 6). Furthermore, changes in CSF biomarkers were not correlated with cognitive test results (Table 6). In addition, other studies have shown that changes in CSF biomarker levels were not related to changes in either MMSE or atrophy rate [102, 143]. An ideal biomarker would predict clinical trial benefits and acts as surrogate endpoint marker of neurodegeneration. CSF biomarkers cannot be used as a bio-signature of clinical endpoints in AD clinical trials and thus cannot be considered as surrogate endpoints of drug efficacy [144]. There are several conflicting reports regarding CSF biomarkers. For example, a patient with clinically and CSF-positive AD was negative for plaque burden by ^{13}PiB-PET neuroimaging [145], whereas in another study, normal individuals with cortical amyloid deposition had higher CSF levels of tau and p-tau [146]. Very recently, an AD autopsy report found only neurofibrillary tangles, but no amyloid plaques [147].

Performance of neuroimaging biomarkers in assessing drug efficacy in AD clinical trials

Volumetric MRI (vMRI) of the hippocampus, retention of ^{11}C-PiB by amyloid plaques in PET imaging (^{11}C-PiBPET), assessment of brain glucose metabolism by ^{18}FDG PET imaging, and cerebral

Table 7
Use of CSF and neuroimaging biomarkers of Alzheimer's disease in assessing drug efficacy in clinical trials

Modality	Cognitive effect	Biomarkers	Comments	References
Phase III trial: Aβ-immunotherapy with bapineuzumab	No significant improvement in cognitive function	• CSF p-tau low in APOE ε4 group • Slight decrease in plaques by ^{11}C-PiB-PET in APOE ε4 group	• No clinical improvement with treatment • Differences in CSF and neuroimaging biomarkers in APOE ε4 carriers	Salloway et al., 2014 [137]
Phase III trial: Aβ-immunotherapy with solanezumab (a humanized monoclonal antibody that binds Aβ)	No significant improvement in cognitive or functional ability	• Total CSF $A\beta_{1\text{-}42}$ was significantly higher after treatment	• No significant clinical improvement with treatment • CSF biomarker results were opposite to the trial results	Doody et al., 2014 [138]
Phase III trial of tramiprosate (ALZHEMED^{TM})	Cognitive improvement was lower than anticipated	• Hippocampus volume change measured by MRI (vMRI).	• No significant clinical improvement • vMRI results were opposite to the clinical results	Saumier et al., 2009 [139]
Phase II trial of TAI (tau aggregation inhibitor)	No clinical decline for 24 weeks treatment	• Changes in blood flow using ^{99m}Tc-HMPAO	• Brain perfusion as an indicator of brain metabolism • SPECT imaging showed response to treatment	Wischik and Staff, 2009 [140]
Phase II trial of γ-secretase inhibitor	No significant changes in cognitive and functional measures	• No significant reduction of CSF Aβ	• Lowered plasma Aβ consistent with the action of γ-secretase activity	Fleisher et al., 2008 [141]

MRI, magnetic resonance imaging; vMRI, volumetric MRI; PET, positron emission tomography; SPECT, single-photon emission computed tomography; Aβ, amyloid-β protein; ^{11}C-PiB, [^{11}C]-Pittsburgh Compound; ^{99m}Tc, metastable nuclear isomer of technetium-99; HMPAO, hexamethylpropylene amine oxime.

blood flow measured by ^{99m}Tc-HMPAO SPECT have been tested as potential AD biomarkers to assess the efficacy of AD therapies in clinical trials (Table 7). As a surrogate endpoint in clinical trials, vMRI and SPECT imaging results showed response to treatment that were opposite to the clinical results (Table 7). A phase 3 trial of tramiprosate (ALZHEMED^{TM}) showed positive vMRI biomarker results with no significant clinical improvement [139]. Lassere has proposed a qualitative scheme for evaluation of AD biomarkers as surrogate endpoints of drug efficacy [148], based on the character and performance of the biomarker in the context of specific targets, study design, statistical strength, and conflicting results. According to this scheme, neuroimaging biomarkers have not yet reached a level of accuracy to be considered as surrogate endpoint for AD clinical trials.

Early diagnosis of AD using CSF and neuroimaging biomarkers

Therapeutic interventions for AD are likely to have the greatest effect if initiated in the early, preclinical stages of the disease, before synaptic loss and neuronal death occur. The NIA-AA working group defined preclinical AD as a prodromal phase consisting of three stages. In the first stage, a patient is positive for amyloid plaques by PET imaging or has low CSF $A\beta_{1\text{-}42}$, but there is no sign of neurodegeneration by MRI and CSF tau values are normal. In the second stage, the patient has evidence of elevated CSF tau, neuronal injury, and amyloid plaques on imaging. In the third stage, the patient begins to experience subtle cognitive deficits that are less severe than those seen in MCI [5]. IWC includes two criteria: (a) clinical AD phenotype criterion manifested by episodic memory profile, and (b) the presence of biomarker evidences as a supportive of AD. Such biomarkers are (1) volumetric MRI; (2) PET imaging (^{18}FDG PET or PiB PET); or (3) CSF $A\beta_{1\text{-}42}$ or tau protein (total tau and phosphorylated tau concentrations [3]. According to the IWG-2 criteria that are the same as NIA-AA criteria a patient with what has been called "pre-clinical Alzheimer's disease" has no clinical signs or symptoms but has one of the following: a) decreased $A\beta_{1\text{-}42}$, together with increased tau or p-tau in CSF; or b) increased fibrillary amyloid on PET [4]. According to both working groups, CSF biomarkers may provide valuable information when combined with neuroimaging biomarkers for identifying the preclinical stages of AD.

CONCLUSION

The new guidelines for diagnosis of AD set by a joint NIA-AA panel of lead scientists recommend the assessment of: (A) dementia due to AD, (B) dementia due to MCI, (C) pathology for AD autopsy, and the need for (D) biomarker development for what has been called "pre-clinical Alzheimer's disease". According to the NIA-AA working group, biomarkers are appropriate for research purposes only and are not ready to be applied in the clinical setting. Once validation and standardization efforts have proven that these biomarkers are sufficiently accurate, they can be applied in the clinical setting. In contrast, the IWG-2 working group already recognizes the use of biomarkers as integral to the diagnosis of AD, as stated in the IGW-2 diagnostic criteria. The IWG-2 working group proposes to integrate biomarkers into the diagnostic scheme as a biological complement to the current assessment of AD. Despite decades of expensive research on CSF and neuroimaging biomarkers for AD, the conclusion remains that they are costly and invasive and have yet to be standardized in a clinical setting. Existing AD biomarkers based on neuroimaging and CSF biomarkers are insufficiently accurate for diagnosing preclinical dementia due to AD. CSF biomarkers continue to face center-to-center variability (for CSF-A$\beta_{1\text{-}42}$ biomarkers CV = 26.5%; http://www.alzforum.org/news/conference-coverage/paris-standardization-hurdle-spinal-fluid-imaging-markers) and different cutoff values for distinguishing AD from non-AD dementia cases, and p-tau-181 in particular has low sensitivity, specificity, and accuracy for distinguishing AD from non-AD dementia cases. A number of cellular and molecular signaling abnormalities occur decades before the clinical symptoms of AD manifest, such as cognitive dysfunction, and before AD-related neuropathology—Aβ accumulation and plaque formation—occurs [31, 149, 150]. Therefore, diagnostic tests that can detect bio-signatures in peripheral systems that are associated with early AD-related cellular signaling abnormalities may more accurately diagnose what has been called "pre-clinical Alzheimer's disease" in the future.

According to the predominant Aβ-hypothesis of AD, defective clearance of toxic Aβ from the brain and the resulting neurodegeneration leads to late-onset AD. Toxic Aβ accumulated over years to decades causes progressive neuronal injury and synaptic loss. Therefore, early defects in the signaling pathways involved in Aβ-clearance are ideal targets for diagnostic tests and therapeutics. Biomarkers of late-onset AD, such as CSF biomarkers and neuroimaging techniques, may detect events downstream of early defects in Aβ clearance, when the disease has reached an advanced stage. As a result, CSF biomarkers and neuroimaging techniques may not be the ideal biomarkers to assess drug efficacy in AD clinical trials. The current body of literature suggests that CSF biomarkers and neuroimaging techniques eventually may be useful for selecting patient populations for inclusion in AD clinical trials; however, the utility of these biomarkers as surrogate endpoints of drug efficacy needs to be validated.

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