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The critical need for defining preclinical biomarkers in Alzheimer's disease☆

Massimo S. Fiandaca[a,b], Mark E. Mapstone[c], Amrita K. Cheema[d,e], Howard J. Federoff[a,b,*]

[a]Department of Neurology, Georgetown University Medical Center, Washington, DC, USA
[b]Department of Neuroscience, Georgetown University Medical Center, Washington, DC, USA
[c]Department of Neurology, University of Rochester School of Medicine, Rochester, NY, USA
[d]Department of Oncology, Georgetown University Medical Center, Washington, DC, USA
[e]Department of Biochemistry, Georgetown University Medical Center, Washington, DC, USA

Abstract The increasing number of afflicted individuals with late-onset Alzheimer's disease (AD) poses significant emotional and financial burden on the world's population. Therapeutics designed to treat symptoms or alter the disease course have failed to make an impact, despite substantial investments by governments, pharmaceutical industry, and private donors. These failures in treatment efficacy have led many to believe that symptomatic disease, including both mild cognitive impairment (MCI) and AD, may be refractory to therapeutic intervention. The recent focus on biomarkers for defining the preclinical state of MCI/AD is in the hope of defining a therapeutic window in which the neural substrate remains responsive to treatment. The ability of biomarkers to adequately define the at-risk state may ultimately allow novel or repurposed therapeutic agents to finally achieve the disease-modifying status for AD. In this review, we examine current preclinical AD biomarkers and suggest how to generalize their use going forward.

Keywords: Alzheimer's disease; Alzheimer's Disease Neuroimaging Initiative; Amyloid-β; Apolipoprotein E ε4; Asymptomatic; Cerebrospinal fluid biomarkers; Epigenomics; Metabolomics; Mild cognitive impairment; Neuroimaging biomarkers; Peripheral blood biomarkers; Preclinical; Proteomics; Tau; Transcriptomics

1. Introduction

Alzheimer's disease (AD) is the most common form of dementia in the elderly, making up between 50% and 60% of all cases, with dementia with Lewy bodies combined with frontotemporal dementia (FTD) making up the other large segment (15%–25%) [1]. AD is a neurodegenerative disease that features loss of memory and impairment of cognitive function but is often difficult to differentiate from other forms of dementia, especially in the early clinical stages. Two major forms of AD have been recognized, a familial (genetic), early-onset AD (EOAD) form comprising a small percentage of those afflicted, and a sporadic late-onset AD (LOAD) variety affecting most AD patients. Although EOAD has a genetic basis and has been closely tied to the amyloid hypothesis [2] of the disease, LOAD has genetic associations and probably results from a combination of environmental factors, genetic susceptibilities, and yet to be determined influences. Some of these environmental factors are particularly relevant to the military. The growing military population exposed to significant stressors, especially over the last 15 years of multiple deployments, provides evidence that unique combat-related environmental factors can influence the risk of developing AD, possibly via shared and yet to be defined mechanisms associated with traumatic brain injury (TBI) and posttraumatic stress disorder (PTSD) [3].

There are clear signs that both military and civilian populations have increasing risks of AD going forward. A recent study by the Department of Veterans Affairs showed that their subject groups with PTSD had double the risk of

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*Corresponding author. Tel.: +1-202-687-4600; Fax: +1-202-687-1110.
E-mail address: hjf8@georgetown.edu

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developing dementia [4], whereas veterans with moderate or severe TBI had two to four times the risk of developing AD or other dementias as they age [5]. The increased risk of AD conferred by TBI is a growing concern not only in the military but also in the civilian arena, particularly as it relates to sport-related concussive injury. The World Health Organization currently estimates that approximately 35.6 million people are afflicted by AD worldwide. In the United States, approximately 7 million people older than 65 years are known to suffer from AD, and this number is expected to triple by 2050. According to the 2013 facts and figures from the Alzheimer's Association (AA) [6], although the number of deaths from major diseases such as cancer and cardiovascular disease has declined in the past decade, the number of deaths related to AD has increased 68% during the 2000 to 2010 period (Fig. 1). Major advances in treatment for the various diseases, except AD, are reflected in these statistics, as well as the increasing longevity of our population. In addition, the increasing numbers of AD-related deaths reflect an augmented precision by the medical community in diagnosing clinical dementia and documenting the suspected cause of demise. In an era of decreasing autopsy confirmation of clinical dementia diagnoses, the absolute numbers may be uncertain, but the trend is irrefutable.

The social and psychological burden associated with caring for those afflicted with AD remains difficult to quantify. The health-care costs associated with managing these individuals are staggering and threaten to bankrupt not only the United States but also the rest of the world economies if left unchecked. In the United States alone, AD-related health-care costs were estimated in 2010 to surpass $170 billion and projected to exceed $1 trillion by 2050 [7]. Such a societal need and cost have driven significant research funding by governments, pharmaceutical companies, and private organizations toward developing successful remedies for AD. Unfortunately, the progressive course of this illness has yet to be significantly impacted by any of the developed therapeutic strategies to date. Projected delays in disease onset by as little as 5 years, resulting from a successful therapeutic strategy, have the potential to reduce the Medicare costs of AD nearly in half [8].

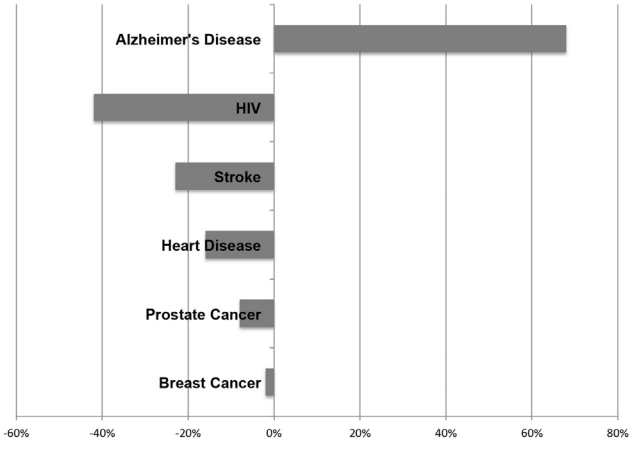

Fig. 1. Bar chart representation of estimated percent changes in reported deaths related to specific diseases during the 2000 to 2010 period, based on World Health Organization statistics [6]. HIV, human immunodeficiency virus.

Patient selection for therapeutic AD trials has been predicated on the presence of symptomatic disease, either mild cognitive impairment (MCI) [9] or AD [10], based on recently updated clinical criteria. There has not been a clear distinction, unfortunately, in the development and testing of therapeutic agents targeting the treatment of EOAD versus LOAD, despite their distinct etiologies and clinical trajectories and relatively rare occurrence of the former. Although certain transgenic animal models approach pathogenic modeling of human EOAD [11,12], no such models exist for LOAD, which would need to replicate both etiologic genetic predisposition and environmental factors. Although military blast-related TBI is associated with certain neuropathologic features similar to those of chronic traumatic encephalopathy [13], including overexpression of phosphorylated tau (p-tau), these changes remain distinct from those associated with LOAD [14]. Although drugs directed toward attenuating the amyloidogenic process may be supported in cases of EOAD [15], similar clinicopathologic evidence is lacking for LOAD. Unfortunately, the bulk of drugs tested so far in the clinic have been in LOAD (MCI or AD) subjects and focused toward modulating amyloid pathophysiology. Resultant efficacy measures in these investigations have either been unimpressive or lacking in late-stage clinical trials for the various therapeutic agents tested to date and with significant associated cost of these failures. An upcoming therapeutic clinical trial for genetically defined EOAD [16], the Alzheimer's Prevention Initiative, may have a higher likelihood of efficacy because of the improved definition of the afflicted pathobiologic networks in the proposed subjects and treatment during the preclinical stage of the disease. Unfortunately, EOAD subjects comprise only a small portion of the afflicted population, and therapeutic success in this group of subjects may not necessarily generalize to those with LOAD.

As a result of this lack of therapeutic efficacy in LOAD, many geriatricians and neurodegenerative disease specialists have postulated that the neural substrate in this disorder may not be responsive to currently used pharmacologic agents after the onset of clinical symptoms. Although possible that the right therapeutic agent has not been tested yet, the wide variety of drugs examined make this less likely. For many, the lack of therapeutic success may result from the decision to initiate treatment trials during the clinical stages of AD. The lack of efficacy documented within these well-financed and well-developed drug trials certainly supports this clinical observation. As a result, over the last 5 years, there has been a push to better understand the preclinical (asymptomatic) stages of AD and consider secondary prevention studies [8], where the neural substrate may remain more receptive to therapeutic intervention.

The preclinical stages of AD are based on documentation of the temporal neuropathologic changes in clinically

asymptomatic subjects. These neural substrate alterations are believed to include the clinical manifestations of AD after progressing beyond a "tipping point" of compensatory mechanisms [17,18]. Enhanced insights into the molecular neuropathology and neurochemistry have led investigators to devise methods for assessing the pathobiologic signatures of the various preclinical and clinical AD stages. Most biomarkers have been used to support the clinical diagnosis of AD, correlating findings in vivo with postmortem brain specimens and differentiating neuropathologic observations from those in normal individuals. Few, if any, have successfully defined preclinical signatures that accurately predict those destined to phenoconvert from normal to impaired cognition during the asymptomatic stages of AD.

Despite major advances in clinical chemistry, brain imaging, and genomic capabilities, there remains a need for the definition of accurate biosignatures for preclinical AD, which will differentiate subjects without risk of progression to dementia from those at risk for developing prodromal or actual AD. Such biomarkers are urgently needed. Until accurate preclinical indicators are defined and validated, the promise of disease-modifying therapeutic strategies for AD will remain elusive.

In this review, we update the reader on the current state of biomarker development for preclinical AD, concentrating on the status and continuing issues associated with cerebrospinal fluid (CSF), neuroimaging, and peripheral blood methodologies. We will stress the importance of consistently and strictly defining the clinical assessments correlated with the various putative preclinical biosignatures. We will make the case for consistency in clinical and biomarker data collection for all AD biomarker types, to allow more thorough and rapid comparisons between investigations. Such uniformity in data collection may provide rapid insight into novel biosignatures that may advance preclinical definition and treatment options for AD and further the hope of modifying disease outcome.

2. The recognition of preclinical AD

The major impetus behind the preclinical AD effort was the emerging realization that decades of concerted work in understanding the pathophysiology and mechanisms had led to no viable cures or disease-modifying therapeutics. Symptomatic treatments are available but with less than desirable efficacy. In addition, animal models of AD, even the highly touted triple transgenic mouse models, do not demonstrate the same pathology or the behavioral phenotype of the human disease. Finally, there is a greater realization that those forms that have strong genetic components (EOAD) may not represent most cases (LOAD), where there may be less genetic and more epigenetic contributions. There is also increasing recognition that AD can present with various behavioral manifestations, beyond the most common primary amnestic variant, including those principally involving language, visuospatial [19], and behavioral cognitive spheres. Thus, with the prodromal diversity of AD and variable genetic contributions, it is overwhelmingly likely that AD may represent more than nosologic entities, with multiple etiological substrates that include amyloid plaques and neurofibrillary tangles (NFTs).

In 2011, the National Institute on Aging (NIA) and the AA convened an international panel of scientists and thought leaders in dementia to discuss diagnostic guidelines for detection and diagnosis of AD. The panel's mandate was to review and consolidate the past several decades of work on AD and come to a consensus on a set of criteria for diagnosis of AD. Additionally, the requisite next steps in research strategy were proposed, which will lead to effective treatments, a cure, and/or primary and secondary prevention of AD. The new guidelines that emerged from the meeting added to and expanded on the existing diagnostic guidelines for dementia due to AD [10], MCI due to AD [9], and neuropathologic findings at autopsy that support a diagnosis of AD [20]. The fourth and perhaps most provocative guideline put forth by the group outlines the preclinical stage of AD [8]. This new guideline recognizes that the disease process, as evidenced by the underlying neuropathology of AD, likely begins years to decades before clinical symptoms such as memory loss manifest. The guideline stops short of advocating for diagnosis of preclinical AD or setting out diagnostic criteria but instead sets out subsequent steps for a research agenda that focuses on biomarker development for preclinical AD. The agenda was proposed to motivate researchers to develop longitudinal studies examining the relationships between specific biomarkers, initially focusing on amyloid-β (Aβ) pathology, and cognitive decline. The proposed framework was to facilitate future clinical trials of disease-modifying agents in the preclinical stages of AD, whose efficacy will be monitored via biomarker progression and/or the emergence of the clinical prodrome.

The NIA-AA guideline on preclinical AD adds a major theoretical context to our understanding of AD by recognizing that the pathobiology begins years to decades before the clinical picture is apparent. The asymptomatic (preclinical) state put forth by the working group consists of three distinct stages, representing the presumed evolution of AD pathobiology. Stage 1 is characterized by asymptomatic amyloidosis that may begin as early as young adulthood and evolve slowly through midlife and into old age. This Aβ pathology within the central nervous system (CNS) may be evident by increased positron emission tomography (PET) Aβ ligand binding and low CSF $A\beta_{1\text{-}42}$ levels. Stage 2 represents an evolution of stage 1 to include not only amyloidosis but also early neurodegeneration as evidenced by (1) neuronal dysfunction based on neuroimaging, such as PET or magnetic resonance imaging (MRI), (2) cellular indicators of neurodegeneration, including high CSF tau or p-tau concentrations, or (3) structural brain changes based on MRI, including cortical thinning and hippocampal atrophy (HA). Finally, stage 3 is evidenced by amyloidosis,

neurodegeneration, and subtle cognitive decline as evidenced by mild change from baseline cognitive function and poor performance on more challenging cognitive tests. The cognitive deficits in stage 3 fall short of frank impairment, loosely defined as between 1 and 1.5 standard deviations below the mean, and do not significantly affect functional capacities such as typical activities of daily living. Thus, stage 3 cognitive deficits do not meet criteria for MCI. In summary, the three preclinical stages are, by definition, without the cognitive impairment that defines MCI and the cognitive and functional impairments that define AD.

Recognition of a preclinical state is a major advancement in our thinking about AD because the preclinical state represents a window of opportunity for timely disease-modifying intervention or secondary prevention. Insights from understanding the early disease process manifest within the preclinical state may also lead to primary prevention strategies that may significantly alter the prevalence of AD. Analogies to other chronic diseases such as heart disease and diabetes can be made. In cardiovascular disease, for example, hypertension may be present for years before a positive stress test or cardiac catheterization indicates the presence of coronary artery disease. It is clear that in most preclinical cardiac patients, identification and management of hypertension can prevent or at least significantly delay the emergence of symptoms. In a similar fashion, the recognition of a preclinical AD state now requires a valid and reliable biomarker to help define the disease stage and to monitor progression of the underlying pathobiology. We do not currently have the equivalent of a blood pressure sphygmomanometer to detect or monitor preclinical AD. The need for such valid and accurate biomarkers and the means to measure them remains urgent.

The notion of a preclinical AD state provides a hierarchy for early pathology that is thought to proceed in a nearly linear fashion from asymptomatic amyloidosis to neurodegeneration, to presenting with appreciable cognitive deficits (see Fig. 2). We must be careful not to consider this hierarchy as suggesting that the pathologic process necessarily produces cumulative effects. That is, the amyloidosis of stage 1 is not necessarily more evident in stage 2 or 3, and the neuronal degeneration in stage 2 is not necessarily greater in stage 3. This lack of clearly incremental pathology allows for the possibility that a transient pathophysiology may exist, whose manifestations may or may not return to normal or near normal during the proximate period(s) to clinical disease. That is, the underlying pathobiology may present in a more punctuated and transient fashion within the preclinical state and may not represent a long slow accumulation of pathology. Significant environmental factors (e.g., combat-related TBI) could participate in influencing these pathobiologic fluctuations. For example, a blast-related TBI may be associated with time-limited upregulation of inflammatory pathways, which may act to precipitate a biological cascade that results in amyloid deposition, similar to what occurs in early AD. For biomarker discovery,

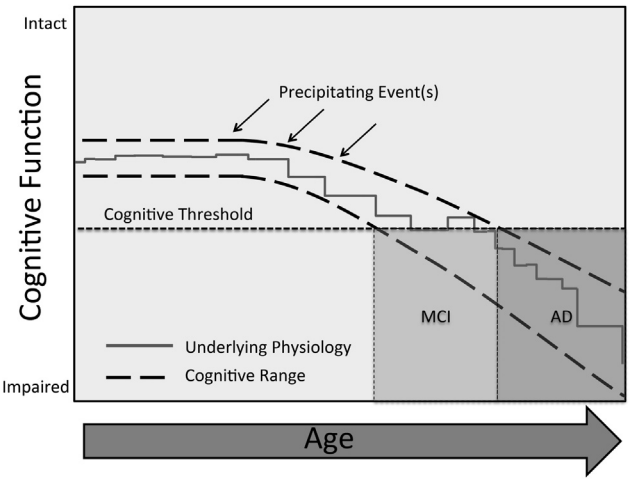

Fig. 2. A schematic representation of changes in cognitive function with advancing age, in the presence of Alzheimer's disease (AD). Cognitive function varies from the intact state to the impaired state. The cognitive threshold (small dashed black horizontal line) separates the two states of cognitive function and determines the onset of clinical progression (or phenoconversion) from preclinical to prodromal mild cognitive impairment (MCI) and eventual AD. Within the intact state, above the clinical threshold, are the preclinical stages described in the text (stages 1, 2, and 3). The cognitive range for an individual (area between large dashed black curves) determines the significant perceived variability of behavior and function during both intact and impaired states and increases with advancing age. The underlying physiology (gray line within cognitive range) is based on the underlying pathobiologic process and impacted by one or more precipitating events, eventually leading to AD. The underlying physiology fluctuates in a limited stuttering fashion, becoming progressively skewed toward cognitive impairment with advancing age, especially after crossing the cognitive threshold.

this means that an optimal biosignature for one stage of preclinical disease may not be best for other stages. This is an important point when considering the use of a single biomarker to predict an at-risk population or monitor disease-modifying consequences of an agent. A single biomarker may not be sensitive to the underlying pathology at multiple stages of the preclinical disease.

Another important consideration for preclinical biomarker development is that the cognitive state may lag significantly behind the underlying pathophysiologic changes. This is likely to impact the sensitivity of a biosignature for cognitive change. The behavioral and functional (cognitive) range is also likely to be coarse and nonlinear, compared with the underlying physiology (see Fig. 2). The cognitive decline related to the pathophysiologic alterations occurring during the preclinical stages, however, finally reaches a threshold level in which manifestations of clinical MCI or AD become evident. Thereafter, progression of cognitive and pathophysiologic decline is certain.

In the absence of valid and reliable biomarkers, neuropsychological testing remains the most accurate, standardized, and widely used premortem screening method to determine clinical MCI or AD [9,21]. Unfortunately, current approaches to neuropsychological assessment are likely to be less informative in the preclinical state as, by definition, cognition is expected to be normal or near normal during

this period. Furthermore, the most meaningful inferences from these assessments demand specificity of the cognitive data, and there are numerous factors such as perceptual deficits [22], mood disorders [23], and other systemic illness [24] that can confound behavioral output in older adults. Cognitive assessment is typically applied to detect deviation from a hypothesized or empirically derived "norm." However, the boundaries of "normal" are not always clear and are also often poorly operationalized in research. There is little standardization, for example, on what constitutes impairment in cognitive research studies. Does impairment mean 1, 1.5, or 2.0 standard deviation(s) below the mean? Which normative data are used to compare and what covariates need to be considered (age, education, and sex) are also important issues that lack a standard in the field. Furthermore, few studies rigorously apply cognitive inclusion and exclusion criteria for their normal control (NC) groups. There are also no specific guidelines related to the time of day (or year) that longitudinal evaluations are carried out and whether patients are assessed while fasting or not or before receiving any of their medications. The inconsistent use of these operationalized criteria in clinical studies creates major problems for interpretation of intrinsic results and greatly confounds comparison of results from one study with another.

Exactly when does an individual transition from no cognitive deficits in preclinical stage 2 to subtle cognitive deficits that characterize stage 3? This becomes a more problematic issue as we drive the limits of resolution for what our cognitive testing can provide. Precision of our cognitive measurements will need to be improved possibly through the use of computerized adaptive cognitive assessments. Such methods will allow for higher resolution information to be gathered and hopefully allow for more accurate definition of preclinical transition. In the short term, however, traditional cognitive assessment will most certainly play an ongoing and important role in the confirmation of a diagnosis in which cognitive impairment (MCI) and cognitive and functional impairment (AD) are required. The need for minimally invasive, clinically useful, and accurate biomarkers of preclinical AD, therefore, has never been greater.

3. Current clinical biomarkers for AD

3.1. Cerebrospinal fluid

Sampling CSF is the least invasive direct method for assessing pathologic alterations occurring within the CNS. Not only bathing the superficial portions of the brain, spinal cord, and portions of the cranial and spinal nerves, CSF also communicates directly with the cerebral ventricles and extracellular fluid of these structures. As such, many believe that CSF provides an optimal source of various biomarkers for diverse pathobiologic events occurring within the CNS, including AD [25]. The most consistent AD correlates within CSF have been related to concentrations of a proteolytic fragment of the amyloid precursor protein, $A\beta_{1\text{-}42}$, in addition to total tau (t-tau) and p-tau levels [26–28].

Diagnostic accuracy of CSF biomarkers continues to be defined but has usually been assessed in cross-sectional studies that typically compare results from asymptomatic subjects and those cognitively impaired (see Table 1) [29–32]. Although Table 1 is not an exhaustive review, it underscores the difficulty for individual and combined CSF biomarkers to accurately differentiate even between NCs and those with AD. From additional CSF analyses, an "AD signature" has been proposed, featuring low levels of $A\beta(A\beta_{1\text{-}42}$ or $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ ratio) and elevated quantities of t-tau and p-tau [25,28,33]. Differences in tau phosphorylation epitopes have been used to define stages of NFT development within the CNS in AD [34], documented in preclinical and clinical neuropathologic stages [35]. Specifically, elevated levels of p-tau S262 (serine 262) or p-tau T181 (threonine 181) are noted in earlier stages of NFT development, whereas p-tau S396 accumulates in later Braak stages when tau accumulations are extracellular [34]. The combination of three CSF biomarkers has been effective in differentiating NC subjects from those with symptomatic disease. The primary role of CSF signatures has been to help confirm suspected clinical diagnosis of AD. Serial CSF correlations within longitudinal preclinical to clinical AD studies are lacking, however, primarily due to difficulty justifying this relatively risky and invasive approach in asymptomatic individuals.

Despite reports of relatively low associated morbidity with CSF collections via lumbar puncture [36,37], subjects are exposed to special risks with this approach, ranging from spinal headache and local back or radiating leg pains to meningitis, epidural abscess, subdural hematoma, and death [38–41]. Significant clinical skill of the lumbar puncturist (MD, physician assistant, certified registered nurse anesthetist, or certified registered nurse practitioner) is required, as well as the use of a sterile technique, to minimize morbidity associated with accessing lumbar CSF [42]. Additionally, low CSF pressure/volume states [43,44], are prevalent in the elderly, predispose these subjects to unsuccessful taps (sometimes >20% of the time) [45] and to higher risk of other sequelae.

3.2. Neuroimaging

The regional and sequential pathophysiological brain alterations associated with AD are uniquely assessed through neuroimaging methods. The minimally invasive nature of MRI, PET, and single-photon emission computed tomography (SPECT) scanning protocols allows regional interrogation of the CNS, with the development of rich anatomic, chemical, and physiological data sets that can be followed temporally within individuals. Such capabilities have provided AD investigators with excellent tools for assessing transitions from the preclinical to the clinical stages of the illness. With some of the earliest substrate changes in AD thought to occur at the synaptic level within the earliest affected regions [46,47], neuroimaging has the capability to provide a premortem assessment of the altered

Table 1
Accuracy of cerebrospinal fluid biomarkers in selected AD studies

Comparison groups*	Clinical determinants	Biomarkers	Reported biomarker accuracy (Sens %/Spec %/Accu %)	Reference
AD vs. NC	MMSE	t-tau	55–100/86–94/NA	Multiple refs. reviewed in [29]
		$p\text{-tau}_{181+231}$	88/97/NA	
		$p\text{-tau}_{231+235}$	53/100/NA	
		$p\text{-tau}_{199}$	94/80/NA	
		$p\text{-tau}_{231}$	85/97/NA	
		$p\text{-tau}_{181}$	44/94/NA	
		$A\beta_{1\text{-}42}$	85/84/NA	
		$A\beta_{x\text{-}42}$	64–85/82–91/NA	
AD† vs. NC	MMSE+	t-tau	69.6/92.3/80.6	[30]
	ADAS-cog (11)	$A\beta_{1\text{-}42}$	96.4/76.9/87.0	
		$p\text{-tau}_{181}$	67.9/73.1/70.4	
		$t\text{-tau}/A\beta_{1\text{-}42}$	85.7/84.6/85.2	
		$p\text{-tau}_{181}/A\beta_{1\text{-}42}$	91.1/71.2/81.5	
		LR_{TAA} model	98.2/79.5/89.9	
MCI vs. AD	MMSE+	Combined	83/88/NA	[31]
MCI vs. NC	Others	$A\beta_{1\text{-}42}$, p-tau, t-tau		
AD vs. NC	MMSE+	Combined	85/80–95/NA	[32]
	Others	$A\beta_{1\text{-}42}$, p-tau, t-tau		

Abbreviations: AD, Alzheimer's disease; Sens %, sensitivity (%); Spec %, specificity (%); Accu %, accuracy (%); NC, normal control; MMSE, Mini-Mental Status Examination; t-tau, total tau; NA, not available; $A\beta_{1\text{-}42}$, amyloid-β 1-42 peptide; $A\beta_{x\text{-}42}$, amyloid β variable peptide; ADAS-Cog (11), Alzheimer's Disease Assessment Scale-cognitive subscale 11; $p\text{-tau}_{181}$, phosphorylated tau at amino acid 181 (or 199, 231, or 235); $p\text{-tau}_{181}/A\beta_{1\text{-}42}$, ratio of $p\text{-tau}_{181}$ to $A\beta_{1\text{-}42}$; $t\text{-tau}/A\beta_{1\text{-}42}$, ratio of t-tau to $A\beta_{1\text{-}42}$.

NOTE. LR_{TAA} model = $A\beta_{1\text{-}42}$ + t-tau + *APOE* ε4(1) + *APOE* ε4(2). Others include ADAS-Cog (11), Consortium to Establish a Registry for Alzheimer's Disease Assessment Scale-Cognitive Subscale, Wechsler Adult Intelligence Scale-Revised, National Institute for Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association criteria, Trail Making Test, verbal fluency test, learning trials, delayed recall tests, and clock drawing.

*Comparison groups featured in cross-sectional studies.

†Autopsy-diagnosed AD.

neurochemistry and physiology, before the focal anatomic alterations and the eventual diffuse neuropathology of the later disease stages. In the following sections, benefits and limitations of the major neuroimaging modalities currently used in the study of AD subjects will be presented.

Although the time commitment and discomfort involved with most neuroimaging investigations stress the limits of many participating subjects, especially the elderly and demented, cost and need of technical expertise remains the major hurdle preventing their widespread use in clinical medicine. Cost comparisons of neuroimaging with other biomarker methods will be covered in a separate section of this review. Clearly, imaging technologies (MRI and PET) require major structural facilities for regulatory compliant operation, which adds to their economic burden, separate from the expensive imaging technology hardware and software. Digital imaging data collected are massive because of the ever-increasing sophistication of the acquisition tools. Such rich data sets require significant computing capabilities, networking, and storage solutions to properly assess and compare. Neuroimaging is a labor-intensive option, often requiring several full-time skilled technicians to work with the radiologists and scientists in properly managing the subjects being scanned and effectively collecting data. In addition, physicians and scientists expend tremendous effort in post hoc data analyses, typically along with a team of bioinformatics and biostatistical experts. For specialized PET imaging related to AD, costly radioligands are required, often needing a local or on-site cyclotron for their development, along with the specialized technical, engineering, chemical, and physics expertise required for effective radiopharmaceutical production and safe operation.

The capability of the neuroimaging modalities continues to improve, and their role in defining the preclinical state of AD is evolving. Like other biomarkers, accuracy of diagnosis is critical to their continued clinical utility and potential use as part of novel investigative strategies (see Table 2) [48–67]. In the following sections, specific strengths and weaknesses of the current technologies are reviewed. Because of their minimally invasive nature and sensitivity to the earliest changes within the brain substrate, many of the following neuroimaging methods have been promoted as being able to identify "leveraged cohorts" of individuals with an elevated risk of developing clinical AD in the short term [68]. This notion is yet to be realized, but many are hopeful that some of the novel techniques recently developed will provide breakthroughs in AD and other diseases.

3.2.1. Structural MRI

Structural MRI (sMRI) continues to lead the commonly used neuroimaging modalities in the clinic for investigating clinical complaints referable to the CNS. Offering a variety of analytical options that continue to expand with improved hardware and software, sMRI is optimal for investigating

Table 2
Accuracy of neuroimaging biomarkers in selected AD studies

Method used (groups)	Biomarker	Reported biomarker accuracy (Sens %/Spec %/AUC)	Reference
sMRI			
MCI vs. AD	Hippocampal and entorhinal volumes	ADNI set: 80/56/0.74	[48]
		QD set: 80–90/68/0.82	[48]
AD vs. NC	OPLS modeling	86.1/90.4/0.95	[49]
fMRI			
AD vs. NC	DMN	73.3–86.7/75–93.7/NA	[50]
	DAN	85.7–100/81.2–100/NA	[50]
	VAN	<70–73.3/<70–81.2/NA	[50]
PET			
CX (AD vs. NC)	^{18}FDG	90–100/85–100/NA	[51–54]
Long. (MCI vs. AD+)	^{18}FDG	44–78/81–83/NA	[55,56]
Post* (AD vs. non-AD)	^{18}FDG	84–96.7/73–85.7/NA	[57–60]
AD vs. NC	^{11}C-PiB	100/85/NA	[61]
AD vs. NC	^{18}F-flutemetamol	93/93/NA	[62]
AD vs. NC	^{18}F-florbetaben	80/91/NA	[63]
SPECT			
AD vs. DLB	^{99m}Tc-exametazime	81/88/0.87	[64]
AD vs. FTD	^{99m}Tc-HMPAO	45–95/67–100/0.85	[65]
AD vs. NC	^{99m}Tc-HMPAO	43–100/63–100/91	[65]
^{1}H-MRS			
AD vs. NC	mI/NAA or NAA/mI	57–90/73–95/NA	[66]
PSEN+ vs. NC†	^{1}H-MRS	98/77.4/0.88	[67]

Abbreviations: AD, Alzheimer's disease; Sens %, sensitivity (%); Spec %, specificity (%); AUC, area under the curve; sMRI, structural magnetic resonance imaging; MCI, mild cognitive impairment; ADNI set, patient set from Alzheimer's Disease Neuroimaging Initiative; QD set, patient set from the Questionable dementia study; NC, normal control; OPLS, orthogonal projection to latent structures; fMRI, functional magnetic resonance imaging; DMN, default mode network; NA, not available; DAN, dorsal attention network; VAN, ventral attention network; PET, positron emission tomography; CX, cross-sectional study; Long., longitudinal study; ^{18}FDG, fluorine-18 fluorodeoxyglucose; ^{11}C-PiB, carbon-11 Pittsburgh compound B; AD+, AD with mixed diagnoses; SPECT, single-photon emission computed tomography; DLB, dementia of Lewy body type; FTD, frontotemporal dementia; HMPAO, hexamethylpropyleneamine oxime; ^{1}H-MRS, proton magnetic resonance spectroscopy; PSEN+, normal asymptomatic carriers of E280A mutation.

NOTE. Non-AD includes progressive supranuclear palsy, vascular dementia, Parkinson's disease, frontotemporal dementia, DLB, or mixed.

*Cohort postmortem.

†Normal non-E280A carriers.

parenchymal and vascular anatomic variability in health and disease. Enhanced processor speeds and software capabilities allow acquisition times of less than 15 minutes for routine structural details on most 1.5- or 3-Tesla (T) machines. The high-resolution capabilities of modern sMRI technology allow neuroimaging correlates in vivo of the previously described postmortem neuropathologic changes noted in preclinical AD stages [35], including parahippocampal, hippocampal, and amygdalar atrophies. HA has the strongest association with the diagnosis of AD and has been incorporated into the Dubois criteria [69] and the NIA-AA criteria [10] for AD. Generalized atrophic progression, as measured on sMRI, is also accepted as an individual biomarker for the transition from MCI to AD [70]. Although manual segmentation of the hippocampal volume to determine HA remains the standard, problems with too much variability in measurements and difficulty with reproducibility of results have prompted the neuroimaging community to develop automated segmentation algorithms [71]. Automating the volumetric assessments helps limit variability and enhances reproducibility of results and will augment sMRI's utility in clinical decision making and patient selection for clinical trials. As noted in Table 2, however, accuracy provided by sMRI alone, in most cases, has failed to reach the threshold of clinical utility [48], typically requiring a more complex and multimodal assessment to reach significant sensitivity and specificity [49].

Beyond the use of HA assessments alone or in combination with clinical measures [72], advanced sMRI techniques for AD now include the use of automated regional assessments of cortical thickness, loss of basal forebrain cholinergic neuronal populations [73], and analyses of alterations in structural connectivity. Although the former two methods provide a step beyond the sMRI determination of HA, the latter offers a look at consequences of early neuronal loss and alterations in white matter connectivity through the use of diffusion tensor imaging (DTI) and tractography [74–76]. Investigations using DTI-based methods have provided consensus that early white matter changes in MCI and AD affect the uncinate fasciculus, cingulum, and corpus callosum. Together with advances in functional MRI (fMRI; see the following), DTI-based white matter assessments provide increasing anatomic correlates to clinical complaints and subsequent physiologic alterations assessed via fMRI. Additional improvements in these techniques are required. Unfortunately, current sMRI methods continue to be

confounded by high variability in anatomic and DTI-derived results [77–79]. Novel protocols are being developed to improve the reliability of results and include diffusion kurtosis imaging [80], diffusion spectrum imaging [81], anomalous diffusion imaging [82], higher-order tensor modeling [83], and compartment modeling [84]. Most assessments using sMRI technologies and methods have been used to compare normal subjects with those clinically defined as having either prodromal or actual AD in cross-sectional studies. In an effort to improve diagnostic accuracy of sMRI in the future, investigations will require serial assessments of disease progression in at-risk subjects, to better define clinically relevant anatomic variations. In addition, as novel sMRI methods become more widely available and tested under clinical conditions, their potential utility in defining preclinical AD may be realized.

3.2.2. Functional MRI

fMRI is used to assess the physiological status of the resting brain state and complex neural responses to tasks. Resting-state fMRI has featured the analysis of spontaneous intrinsic brain activity under basal conditions (without stimulation or task-based activity), typically depicted as altered states of blood oxygen level–dependent signals [50], and provides insight into the functional capability of resting state networks, known as the default-mode network (DMN) [85]. AD features a disrupted DMN, probably resulting from the evolving pathologic anatomy of disease progression. These explicit DMN deviations have been suggested as biomarkers for AD [86,87]. In addition, dorsal and ventral activation networks have been defined and monitored for specific deviations related to the dementing process, in addition to DMNs [50], allowing assessment of a variety of interconnected brain regions. The use of episodic memory tasks on subjects undergoing fMRI allows visualization of hippocampal and medial temporal lobe memory-related activation and is thought to represent a physiologic correlate to information encoding. With the memory-associated deficits seen with prodromal and actual AD, task-based methods are gaining momentum in AD research. Decreased activation within the medial temporal lobe has been noted in MCI patients undergoing such task-based memory testing [88,89]. As can be seen in Table 2, monitoring any of these individual networks provides variable but potentially significant sensitivity and specificity in discriminating between preclinical and clinically manifest subjects. Further investigations and imaging protocol modifications will probably be required to limit variability of results using these techniques and for investigating the combined use of fMRI with other clinical and biomarker modalities to improve accuracy in defining the preclinical state of AD.

3.2.3. Positron emission tomography

For decades [90], PET imaging has provided focal differences in fluorine 18 fluorodeoxyglucose (^{18}FDG) allocation to provide rates of regional cerebral metabolic rate (rCMR) activity through glucose utilization and, thereby, helping define physiological brain changes seen as part of the normal aging process or a dementing condition. A recent review [91] has assessed the pertinent post-2000 literature related to diagnostic accuracy of ^{18}FDG PET in AD studies, primarily with clinically defined diagnoses in cross-sectional studies, but also in longitudinal investigations and those with pathologic correlations. These reviewed studies have been summarized in Table 2 and indicate variable sensitivity and specificity for modern ^{18}FDG PET methods. The differentiation between AD and FTD is where ^{18}FDG PET is gaining significant clinical utility [92].

Amyloid plaque pathology is frequently found in brains of individuals without clinical or pathologic evidence of AD. Amyloid PET imaging using the carbon 11–labeled Pittsburgh compound B (^{11}C-PiB) has found a similar incidence of amyloid binding in comparable subjects [93,94]. Although PET ligands labeled with ^{18}F have a half-life of 110 minutes, allowing production and distribution from a nearby cyclotron facility, early amyloid PET imaging with ^{11}C-PiB was limited to centers having their own cyclotron, radiochemistry, and pharmaceutical capabilities because of a half-life approaching 20 minutes. The Food and Drug Administration approved ^{18}F-florbetapir in 2012, a fluorinated ligand, opening the possibility of more widespread use of amyloid imaging options in clinical centers. Additional ^{18}F amyloid-binding compounds continue to be developed, including flutemetamol, florbetaben, and AZD4694. Although these ^{18}F compounds have shown some problematic nonspecific white matter binding, except the latter [95], they all show similar amyloid binding results to ^{11}C-PiB when correlated to pre- and postmortem analyses [93,96]. Risk of using these ligands is extremely low, especially in the dementia population age groups.

Information related to in vivo amyloid plaque presence in elderly subjects remains to be adequately correlated with preclinical (asymptomatic) stages of AD and those at risk for phenoconversion. Some cognitively normal seniors show increased ^{11}C-PiB binding compared with other normals in their age group. In most cognitively normal subjects, ^{11}C-PiB binding increases from less than 10%, below the age of 70 years, up to 40% in those aged 80 years [97]. As noted in Table 2, PET amyloid radioligands provide significant single-method sensitivity and specificity in differentiating between those with AD and NCs, as well as other conditions.

3.2.4. Single-photon emission computed tomography

SPECT imaging methods have typically used technetium 99m compounds to assess regional cerebral blood flow (rCBF), with alterations in perfusion noted in various dementing conditions [98]. With rCBF tightly coupled to rCMR, many investigations have shown the close correlation between SPECT and PET in relation to regional assessments. Although this physiologic linkage is indeed present, SPECT images suffer from a lack of spatial resolution compared with PET images. These resolution performance differences

may lead to the greater variability and reduced accuracy when comparing SPECT with PET diagnostic accuracy (see Table 2). Although the major additional advantage of SPECT over PET had previously been related to cost differences and availability, the advent of ^{18}F compounds is allowing a greater number of PET imaging facilities to be justified and bringing down their imaging costs toward levels that compete with SPECT.

Differentiation of various dementing conditions with SPECT imaging (see Table 2) has been reported useful but typically with less accuracy than with PET imaging modalities. The advent of improved image analytics for both PET and SPECT make obvious discrepancies between the modalities more difficult to discern. Ultimately, the visual resolution provided might be the deciding factor as to whether PET or SPECT is selected by investigators for use in their analyses.

3.2.5. Magnetic resonance spectroscopy

Proton magnetic resonance spectroscopy (^{1}H-MRS) is a neuroimaging method that allows sampling and measurement of a variety of different brain metabolites in a single session. Each metabolite has the ability to provide information that is sensitive to pathologic changes at the cellular and even molecular levels. Typically monitored metabolites include N-acetylaspartate (NAA), myoinositol (mI), and choline (Cho), with creatine (Cr) typically used as an internal reference standard. Quantitative measures are often presented as ratios with the internal standard or other metabolites (e.g., NAA/Cr, NAA/mI, mI/Cr, Cho/Cr). NAA's intraneuronal localization allows its ^{1}H-MRS signature to represent neuronal density and/or viability. Generation of NAA is thought to occur within mitochondria [99], so it has also been used as a marker of mitochondrial integrity and function. In contrast, most brain mI is within glial cells [100], and increased levels have been correlated with glial proliferation responses in inflammation or gliosis [101,102].

Voxel volumes determine the regions of interest, within which metabolite signals are quantified. The larger the voxel volumes used, the greater the partial volume averaging seen with the surrounding tissues. With greater partial volume averaging, there is reduced localization specificity and metabolite signals. The smaller voxel volumes ($\leq$8 cm^3) used in ^{1}H-MRS to reduce partial volumetric effects require higher signal-to-noise ratios (SNRs), which can be augmented by increasing magnetic field strength (Tesla or T). With ^{1}H-MRS, field strength is a major determinant of SNR and, therefore, of the quality of the data obtained. Three-Tesla scanners, for example, provide SNRs at smaller voxel volumes than associated comparable SNRs in 1.5-T scanners. High-resolution spectroscopic methods combined with improved sMRI resolutions may ultimately provide a metabolic imaging method [103].

Ratios of NAA to mI are reduced in parietal cortex of AD subjects compared with frontal cortex [104], corresponding to previously reported regional neuropathologic differences [35]. Table 2 depicts findings from ^{1}H-MRS studies comparing clinical AD to NC subjects and from genetically defined preclinical EOAD subjects and controls not harboring the dominant mutation associated with EOAD. The LOAD review [66] investigates findings from subjects with either AD or normal cognition, defined on a clinical basis. The five investigations assessed a total of 150 AD subjects and 138 controls in cross-sectional studies. Calculated ^{1}H-MRS mean sensitivity and specificity for these combined groups were 78.8 and 84.8, respectively, in differentiating those with AD from controls, and with the sensitivity and specificity ranges depicted in Table 2. As has been previously noted with other biomarkers, combining ^{1}H-MRS with other biomarkers increases diagnostic accuracy.

The EOAD study [67] summarized in Table 2 is of particular interest to the discussion of preclinical biomarkers in this review because ^{1}H-MRS is used to differentiate known at-risk subjects, harboring an autosomal dominant and fully penetrant presenilin-1 gene mutation, from subjects without this mutation. In comparing these two distinct groups, brain metabolite differences in the posterior cingulate and precuneus appeared to be optimally sensitive and specific in predicting those harboring the mutation from those who did not. Similar investigations in LOAD patients will await the development of an accurate preclinical biomarker of eventual clinical disease.

3.3. Peripheral blood

Currently, there is a paucity of easily executed inexpensive methods that provide reliable biomarkers able to predict the at-risk phenotype of AD, despite the previously described and commonly used CSF and neuroimaging modalities. New techniques are required to provide novel insight and means to support preventive strategies during the asymptomatic stages of AD. Individuals selected via these methods could then be placed on therapeutic regimens that may alter disease trajectory [105]. Biomarkers that have been characterized using CSF and neuroimaging are highly accurate, but they remain difficult to generalize into clinical practice because of the invasive nature of the former and cost-prohibitive technology associated with the latter. By definition, a biomarker should expedite early detection and diagnosis with high degree of accuracy, should be cost-effective, and easily translated to general use [106]. In recent years, there has been a paradigm shift toward interrogating peripheral blood because it is accessible by minimally invasive means, offers a relatively inexpensive substrate for analysis, and provides biosignatures that may be predictive, diagnostic, and prognostic for AD and other neurologic diseases [107,108].

3.3.1. Issues with brain biomarkers in the blood

Historically, the sensitivity and specificity of blood biomarkers have been lower than those from CSF. Reasons for this include the fact that the concentration of the CNS analytes

in peripheral blood is very low because of the inability to exit the brain owing to the blood-brain barrier (BBB), challenging the ability of analytical methods to identify CNS-specific signals of illness. Results from most studies reporting putative blood biomarkers for AD have been difficult to reproduce and validate with adequate specificity and sensitivity. There is a growing body of evidence, however, that preclinical AD may have a biosignature that can be deduced from peripheral blood (see Table 3) [109–116]. For example, Kiddle et al. [114] replicated blood-based proteomic biomarkers of AD for a total of 163 candidate entities reported by 21 different discovery-based studies. Using the SOMAscan proteomics technology (SomaLogic, Inc, Boulder, CO, USA), they were able to replicate 94 proteins. Nine of these proteins were directly related to the AD phenotype [114]. Although more validation studies with heterogeneous populations are needed to further refine the biomarker panel, these findings suggest consistent alteration of the blood proteome in AD patients.

Blood comprises a liquid component (serum or plasma) and different types of cells, including the mononuclear leukocytes, which contain the deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), erythrocytes, and platelets. Each blood component has its advantages for delineation of different biomarker species. The serum and plasma can be effectively used to identify proteins and small molecule metabolites or lipid profiles. These same biomarkers can also be used for following disease progression or response to treatment. RNA can be obtained from leukocytes but is also present in the plasma (or serum) exosomes and, hence, represents a unique opportunity for biomarker discovery and development. With the advent of newer and sophisticated technological platforms, there has been an aggressive effort toward developing bioinformatics tools for analyses of high throughput and high dimensional "omics" approaches to build classifier models that predict different physiological states. Another aim of bioinformatics is also to integrate different "omics" data using computational modeling approaches [117]. A potential issue, however, is the variability in results depending on the statistical or computational methods used to identify these biomarkers. It is now believed that the use of univariate methods to delineate blood biomarkers is not sufficiently sensitive or specific for the diagnosis of complex, multifactorial, and heterogeneous physiologies as AD. To circumvent these limitations, development of "biomarker panels" assessing multiple molecular entities is likely to yield higher consistency and accuracy.

Finally, lack of standardization of procedures for blood collection, processing, and storage leads to preanalytical variability causing inconsistencies in downstream analysis and results [118]. Preanalytical variables include incubation times before separating plasma (or serum) from cells, type of blood collection tubes, temperature for collection and storage [119], time in storage, and the number of subsequent freeze-thaw cycles. Thus, the availability and use of standard protocols is imperative to ensure cross-platform and interlaboratory reproducibility [120–122].

3.3.2. *Novel blood-based biomarkers for AD*

Despite these challenges, a concerted effort toward identification and validation of blood-based biomarkers of AD using a wide array of technological platforms is available today. For example, circulating microRNAs, as biomarkers of AD, are gaining credence as more research efforts are made in this direction [115,116,123–125]. A blood-based validated transcriptomic signature was recently reported with a specificity of 67.1% and a sensitivity of 81.3% [112]. Whole transcriptome sequencing (RNAseq) technology is a promising next-generation approach that has advantages for the identification of blood biomarkers of AD. Specifically, RNAseq offers high efficiency, broad dynamic range, and deep scope of detection [126].

A number of independent studies have focused on discerning blood-based proteomic biomarkers of AD. Blood represents a rich mixture of molecules for biomarker discovery for risk and disease stratification. Blood affords measurements of proteins over a broad dynamic range, featuring multiple isoforms and splice variants, as well as entities resulting from different posttranslational modifications. Moreover, because serum and plasma contain high amounts of

Table 3
Accuracy of blood-based biomarkers in selected AD studies

Name of biomarker (groups)	Method used	Reported biomarker accuracy (Sens %/Spec %/Other)	Reference
Panel of 18 proteins (AD vs. NC)	Antibody array	NA/NA/accuracy ~90%	[109]
Plasma clusterin (AD vs. NC)	LC-MS/MS	NA/NA/P = .05, not effective alone	[110]
Panel of 25 proteins (AD vs. NC)	Immunoassay	80/91/ROC AUC = 0.91	[111]
Aclarus Dx (AD vs. NC)	Transcriptomics	81.3/67.1/NA	[112]
Desmosterol (AD vs. NC)	LC-MS	NA/NA/ROC AUC = 0.8	[113]
Panel of 9 proteins (AD vs. NC)	SOMAscan proteomic assay	NA/NA/FDR q values <0.1	[114]
miRNA 125-b (AD vs. NC)	miRNA profiling	80.8/68.3/NA	[115]
12 miRNA signature (AD vs. NC)	miRNA profiling	92/95/accuracy 93%	[116]

Abbreviations: AD, Alzheimer's disease; Spec %, specificity (%); Sens %, sensitivity (%); NC, normal control; NA, not available; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LC-MS, liquid chromatography-mass spectrometry; ROC, receiver operating characteristic; AUC, area under the curve; FDR, false discovery rate; miRNA, micro–ribonucleic acid.

NOTE. "Other" indicates additional accuracy measure presented. Aclarus Dx is manufactured by the Diaxonhit Group, Paris, France.

albumin and immunoglobulins, successful protein biomarker discovery requires enrichment techniques for low abundance proteins and sensitive technological platforms to facilitate detection and quantification of protein biomarkers. Mass spectrometry (MS) has emerged as the most popular platform for candidate biomarker development. Several studies have used two-dimensional electrophoresis in combination with MS for identification of protein biomarkers [127,128]. Ray et al. [109] have reported a panel of 18 signaling proteins in plasma capable of predicting AD with 90% accuracy, although these studies have not been replicated. Lundstrom et al. [129] have provided a novel plasma glycan signature to discriminate NC subjects from those with MCI and AD, with a sensitivity of 89.3% and a specificity of 79.1%. Recent technological advancements in analytical technologies such as nuclear magnetic resonance and ultra-performance liquid chromatography or gas chromatography coupled with high-resolution tandem MS have enabled accurate detection and quantitation of small-molecule metabolites for which high throughput, relatively inexpensive, clinical assays can be developed to facilitate early detection, while also providing prognostic information and monitoring capabilities. Additionally, identification of dysregulated pathways in AD is likely to facilitate the development of novel therapeutics. Several metabolomic studies in the recent years have focused on serum and plasma profiling using targeted and untargeted approaches. A recent combined cross-sectional and longitudinal study identified a panel of 18 plasma proteins (cytokines, chemokines, growth factors, and binding proteins), which discriminated symptomatic AD from cognitively NC subjects with nearly 90% accuracy and predicted conversion from symptomatic MCI to AD with 91% accuracy [109]. Unfortunately, several subsequent studies using this profile failed to replicate the accuracy of conversion from MCI to AD reporting lower accuracies of 60% to 70% [130,131]. Another promising cross-sectional study reported a panel of 18 biomarker species, many related to inflammation, which correctly classified symptomatic AD from cognitively NC subjects with a sensitivity and specificity of 85% and an area under the curve (AUC) of 93% [132]. A reduced panel of eight metabolites including cortisol and pancreatic polypeptide showed a sensitivity and specificity of correct classification of 83%. The biomarker panel was validated in an independent cohort of healthy controls and symptomatic AD from the Alzheimer's Disease Neuroimaging Initiative sample set and provided sensitivity and specificity of 80% with an AUC of 85%. Sato et al. [113] have reported a decrease in plasma desmosterol levels in AD patients compared with control subjects and a significant decrease in desmosterol/cholesterol levels. Another study reported a decrease in plasma levels of lysophospholipid 18:1 of AD patients, which correlated with severity of the disease [113]. Trushina et al. have conveyed a ultra performance liquid chromatography-time of flight-mass spectrometry (UPLC-TOF-MS)–based identification of a multimetabolite panel that includes the amino acid phenylalanine, lysine, leucine, serotonin, cholesterol, and phospholipids that could discriminate between NC and individuals with MCI [133–135], whereas Han et al. [136] reported differences in plasma ceramide levels as a potent discriminator of AD. The Framingham Heart Study, meanwhile, disclosed a decrease in phosphatidylcholine (PC) levels of AD patients [137]. Studies have shown deficits in brain structural glycerophospholipids and sphingolipids, as well as alterations in metabolites of these complex structural lipids, which act as signaling molecules [138]. These alterations may well be reflected in blood profiles and hence are an interesting class of metabolites to pursue as biomarkers of AD. In summary, there has been a concerted research effort toward the development of biomarker panels using blood as a discrimination matrix. More longitudinal and cross-validation studies with larger and more diverse ethnic cohorts, however, are required to develop biomarker panels that could be used effectively in the clinic in conjunction with the current testing methods.

4. Novel methods to define preclinical fluid-based biomarkers for AD

As noted in the CSF and blood-based biomarker sections, novel approaches using biofluids include the use of specific p-tau isoforms to determine disease stage and a variety of specific "omic" analyses for blood components (see Table 3). Blood appears to offer a distinctly rich environment compared with CSF for collecting novel information regarding ongoing disruptions in pathobiologic networks. The lack of significant cellular components within CSF limits the ability to access DNA and RNA for investigations and primarily focuses on assays using metabolomic or proteomic methods for differentiation. In contrast, peripheral blood's cellular and liquid components provide a rich source of both genetic materials for interrogation in addition to proteomic and metabolomic species.

The major advantage offered by CSF over blood is the unique direct contact that the former has with the CNS environment, whereas the latter's interactions are indirect and limited by the BBB. These facts, related to compartmentalization of assayable analytes, have made it difficult for some to accept blood-derived findings as germane to AD-related processes within the CNS because of the apparently small flux of putative biomarkers from the CNS to peripheral blood. It remains vital to understand the communication between these two compartments and discern the implications for disease detection and progression. Although many CNS metabolites or other entities are not in established steady-state relationships with blood, injury to or disease within the CNS clearly drives a temporal evolution of peripherally detectable signatures [139]. A recent abstract presented at the Sixth Annual Clinical Trials on Alzheimer's Disease Conference [140], and subsequent manuscript publication [141], underscores the potential for such observations and correlations in a longitudinal study assessing preclinical and clinical AD.

Investigators reported evidence of significant alterations in specific plasma lipid species (Fig. 3) present in cognitively normal subjects who phenoconvert to either amnestic MCI (aMCI) or AD within 2 to 3 years compared with those remaining cognitively intact [141]. The panel of plasma lipids discovered was validated in an independent group of subjects within the same study cohort, with greater than 90% sensitivity and 90% specificity. Although the identified lipid species are not currently known to freely equilibrate between the blood and CNS, reductions in certain plasma lipids [142] and parenchymal brain lipids [143] have been previously reported in MCI and AD. Although further validation of these lipidomic findings is required, as well as application to larger and more diverse populations, this investigation [141] supports the use of peripheral blood for unbiased screening to determine alterations in pathobiologic networks within the CNS of preclinical AD patients that are reflected in the periphery.

5. Biomarker epidemiology, costs, and benefits

With the increasing pressure to develop accurate biomarkers of preclinical AD, many have started to examine the costs and benefits associated with the various biosignatures. Ultimately, these analyses will be germane to the overall cost of care for AD-afflicted individuals. The costs are not insignificant for accessing various biomarkers discussed in this review (see Table 4). What is clearly noted, however, is the increased risk associated with obtaining CSF for biomarker analysis compared with both neuroimaging and blood-based methods. Additionally, there is a major cost differential between obtaining blood for biomarker analysis versus both CSF and neuroimaging methods. Based on available information, calculated costs for blood collection for biomarker analysis are less than 10% the cost of obtaining biomarkers via CSF or neuroimaging. As noted earlier in the discussion of diagnostic accuracy of each of

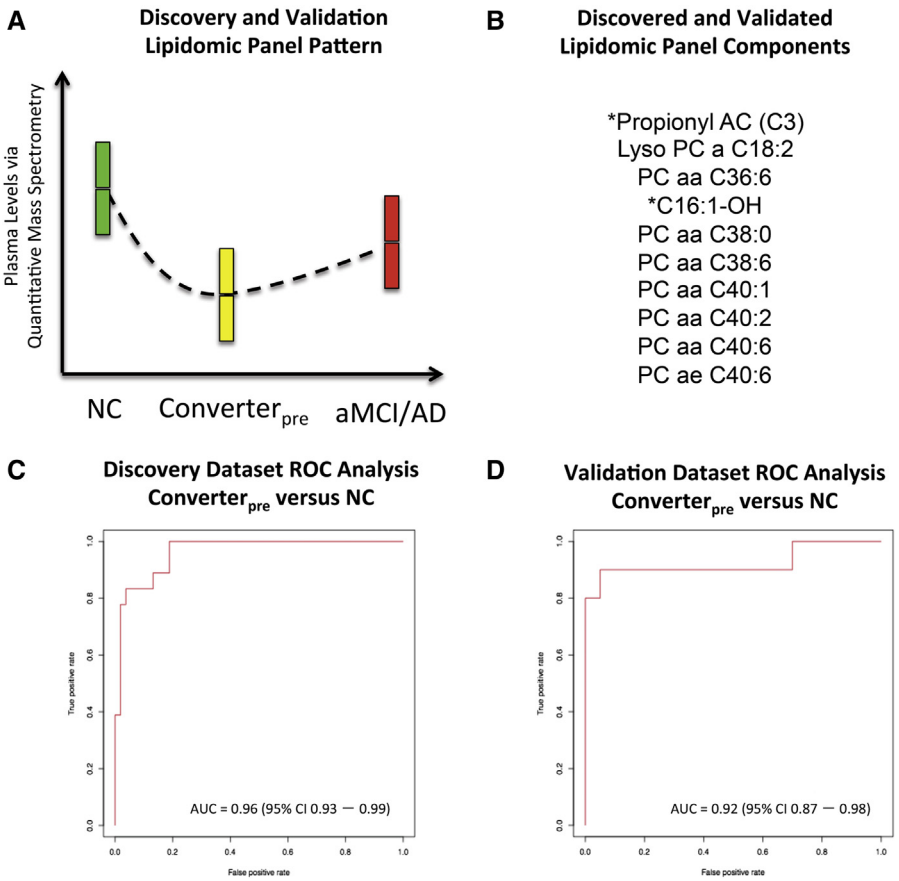

Fig. 3. A representation of lipidomic results from the study of Mapstone et al. [141]. (A) Schematic depiction of the relative quantitative lipidomic differences seen, for each member of the lipidomic set, between cognitively normal subjects who remain normal (NC), those cognitively unimpaired destined to phenoconvert to either amnestic MCI (aMCI) or Alzheimer's disease (AD) (Converter$_{pre}$), and those with presenting with aMCI or AD (aMCI/AD). The box sizes are not to scale. The dotted line portrays the reduction in select lipid levels seen with Converter$_{pre}$ subjects compared with NC and aMCI/AD. (B) The list of the 10 lipid species identified and validated using MS-based metabolomic analyses that accurately differentiate between cognitively unimpaired Converter$_{pre}$ from NC subjects. (C) Representation of receiver operating characteristic (ROC) curve analysis of Converter$_{pre}$ versus NC for the discovery set of subjects using the 10–lipid biomarker panel. Area under the curve (AUC) = 0.96. (D) Representation of ROC curve analysis of Converter$_{pre}$ versus NC for the validation set of subjects using the 10–lipid biomarker panel. AUC = 0.92. The asterisk (*) in panel B indicates $P < .005$, whereas for all other analytes in panel B, $P \leq .05$. AC, acylcarnitine; PC, phosphatidylcholine; CI, confidence interval.

Table 4
Alzheimer's disease biomarker costs*

Biomarker method	Patient discomfort	Risk	Est. cost per 1000 subjects	Additional considerations
Cerebrospinal fluid	Significant	Moderate to high	$350,000 to >$1,000,000	Risks include significant headache (in 40%), back or leg pain (in 11%), and rare meningitis, epidural abscess, or subdural hematoma. Requisite: skill of staff performing procedure.
Neuroimaging	Mild to moderate	Low		Claustrophobia, need for lying still for long periods of time, expensive facility and imaging equipment, specialized staff, significant time for post hoc analysis, and variability between facilities
sMRI			$400,000 to >$800,000	
fMRI			$600,000 to >$900,000	
PET			$1,000,000 to $2,000,000	
SPECT			$1,000,000 to $2,000,000	
MRS			$700,000 to $1,000,000	
Blood based	Minimal	Low	$40,000 to $100,000	Possible bruising at the site of venipuncture, and vasovagal reaction

Abbreviations: Est., estimated; sMRI, structural magnetic resonance imaging; fMRI, functional magnetic resonance imaging; PET, positron emission tomography; SPECT, single-photon emission computed tomography; MRS, magnetic resonance spectroscopy.

*Cost calculations based on available online information regarding estimated individual testing charges. These are procedural charges only and do not include the costs of assays performed using cerebrospinal fluid or blood-based analyses or the personnel charges for time spent in association with imaging or fluid-based bioinformatic analyses.

these biomarker methods (see Tables 1–3), current studies support CSF and neuroimaging biomarker results over blood-based methods. Most of these cited studies have been cross-sectional in nature, comparing clinically normal subjects with those having clinical AD (either MCI or AD). If additional investigations using blood-based biomarkers provide comparable or greater diagnostic accuracy than CSF and neuroimaging modalities, there is no question about which will become the preferred screening method in preclinical investigations and perhaps subsequently in clinical practice. Because of the minimally-invasive nature, low risk, and low cost to access blood for biomarker analyses (see Table 4), asymptomatic subjects and their physicians will have fewer issues moving forward with diagnostic screening. This development of preclinical data will be important in further definition of the underlying pathobiologic networks that characterize high-risk individuals, and through analysis of network nodes, additional targets for therapeutic development will emerge. This financial calculus suggests that biomarker modalities applicable to screening large populations of preclinical subjects require low costs and high accuracy. It is through systematic application of these methods that investigations are expected to yield clinical prescription of disease-modifying therapeutics.

What is much more difficult to estimate is the importance of refinements in study design and biomarker specimen/data handling in future investigations. The ability to compare different study results is predicated on uniform application of reliable clinical instruments to assess cognitive and other functions. In the clinical AD research field, there is a need for further consensus on the optimal clinical approach for cognitive assessments that will yield consistency among individuals from different studies. In addition, the rigorous collection and processing of CSF and blood for biosignature measurements must be standardized to minimize technical and biologic variance that is unrelated to disease or at-risk status.

6. Conclusions

After the initial success of CSF biomarkers in defining differences between symptomatic AD subjects from asymptomatic controls, the biomarker momentum has shifted toward minimally invasive modalities to define the preclinical stages. Advances in neuroimaging, with the attendant low risk associated with these investigations and significant accuracy, have indicated the validity of preclinical biomarkers for AD. Although the definition of differences between manifest disease and normals has been an important early step in the genesis of these biosignatures, longitudinal investigations of subjects at risk, who subsequently phenoconvert to clinical AD, will be required for an improved definition of the preclinical phenotype. Blood-based biomarkers may offer such a potential in screening asymptomatic subjects while providing significant insight into the ongoing pathobiology. Once the at-risk preclinical AD group is defined using this relatively inexpensive low-risk solution, the addition of other biomarker methods to further refine the understanding of the operational network aberrations may be considered. A clear definition of the preclinical phenotype, however, is necessary in leading to eventual secondary prevention trials and the potential to modify the course of AD.

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