Current Neuropharmacology, 2022, *20*, 713-737

REVIEW ARTICLE

Biomarkers and Tools for Predicting Alzheimer's Disease in the Preclinical Stage

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Abstract: Alzheimer's disease (AD) is the only leading cause of death for which no disease-modifying therapy is currently available. Over the past decade, a string of disappointing clinical trial results has forced us to shift our focus to the preclinical stage of AD, which represents the most promising therapeutic window. However, the accurate diagnosis of preclinical AD requires the presence of brain β-amyloid deposition determined by cerebrospinal fluid or amyloid-positron emission tomography, significantly limiting routine screening and diagnosis in non-tertiary hospital settings. Thus, an easily accessible marker or tool with high sensitivity and specificity is highly needed. Recently, it has been discovered that individuals in the late stage of preclinical AD may not be truly "asymptomatic" in that they may have already developed subtle or subjective cognitive decline. In addition, advances in blood-derived biomarker studies have also allowed the detection of pathologic changes in preclinical AD. Exosomes, as cell-to-cell communication messengers, can reflect the functional changes of their source cell. Methodological advances have made it possible to extract brain-derived exosomes from peripheral blood, making exosomes an emerging biomarker carrier and liquid biopsy tool for preclinical AD. The eye and its associated structures have rich sensory-motor innervation. In this regard, studies have indicated that they may also provide reliable markers. Here, our report covers the current state of knowledge of neuropsychological and eye tests as screening tools for preclinical AD and assesses the value of blood and brain-derived exosomes as carriers of biomarkers in conjunction with the current diagnostic paradigm.

ARTICLE HISTORY

Received: January 23, 2021
Revised: April 27, 2021
Accepted: May 08, 2021

DOI:
10.2174/1570159X19666210524153901

Keywords: Alzheimer's disease, preclinical AD, biomarker, neuropsychological test, eye test, blood, exosomes, brain-derived exosomes.

1. INTRODUCTION

Alzheimer's disease (AD) is an evolving challenge that places an enormous burden on families and societies [1, 2]. At present, AD has been recognized as a distinct entity and is defined pathologically by the presence of a specific neuropathological profile: extracellular deposition of β-amyloid (Aβ) in the form of senile plaques and intraneuronal existence of neurofibrillary tangles (NFTs) that are composed of aggregated hyperphosphorylated tau proteins, while cognitive impairment in an elderly individual is an external clinical syndrome that can be caused by multiple pathological insults, including AD [3]. Advances in biomarker technology have made it possible to visualize AD neuropathology *in vivo*. According to the A-T-(N) research framework recently proposed by the National Institute on Ageing-Alzheimer's Association (NIA-AA), an individual is thought to be in the Alzheimer's continuum as long as the "A" (Aβ) biomarker is positive (A+), and a diagnosis of definite AD requires an additional positivity of the "T" (NFTs) biomarker (T+), both regardless of the state of the "(N)" (neurodegeneration) biomarker or cognitive status [4]. Given the failures over the past two decades in developing therapeutics to reverse the course of neurodegeneration in patients with symptomatic stages of AD [1], and given the fact that AD-induced cognitive decline lags far behind its pathological changes for decades [3], research interests have shifted from the late stages to the preclinical stage of the disease [5]. Patients in the preclinical stage of AD are those individuals who are cognitively healthy but are considered to be in the Alzheimer's continuum [4, 6]. This stage is probably the most promising stage for developing AD-modifying therapies.

Currently, the established Aβ detection relies on cerebrospinal fluid (CSF) analysis or amyloid-positron emission tomography (PET) examination; both methods show a high degree of consistency with autopsy results [4]. However,

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1875-6190/22 $65.00+.00

their shortcomings are also notable, as they are time-consuming, invasive, expensive, and may have side effects and limited availability, especially in primary care clinical settings. Therefore, there still exists an urgently unmet need for convenient and cost-effective biomarkers for Aβ detection.

There are many comprehensive reviews describing the biomarkers of AD, but these studies mainly focused on symptomatic patients [7-10], and studies specifically assessing biomarkers and (or) tools of individuals with preclinical AD are still lacking. Comparatively, biomarkers with predictive properties of future cognition may be more valuable than biomarkers purely specialized for identifying symptomatic patients, as the former could provide a promising opportunity for early detection during the preclinical stage and spur secondary prevention trials targeting precise or programmatic interventions to minimize cognitive decline in asymptomatic but high-risk individuals. Since the concept of preclinical AD was first proposed in international research criteria [11], exploration of its biomarkers has never stopped. To date, we have clearly realized that individuals with preclinical AD, especially those at the later or transition stage to the clinical phase, are not necessarily "asymptomatic", as they may have already developed several clinical signs such as subtle or subjective cognitive decline [12]. In addition, blood-derived biomarkers also have been intensively investigated in this stage, and brain-derived exosomes extracted from blood further enable us to accurately acquire the functional state of the brain, which plays an important role in the identification of preclinical AD [13]. Furthermore, studies centering on the eye and its associated structures indicate that they may also contribute to the diagnosis of preclinical AD [14].

The current A-T-(N) research scheme is expandable to incorporate new biomarkers [4]. Before clinical application, it is indispensable to evaluate new biomarkers in the preclinical stage of AD and to associate them with existing biomarkers. In this study, we aimed to review the biomarkers and (or) tools for studying preclinical AD from the perspective of neuropsychological tests, the blood, brain-derived exosomes, and eye-associated tests, as well as assess their value as emerging tools in conjunction with the clinical diagnostic paradigm. Although some novel CSF biomarkers or multiparametric magnetic resonance imaging hold enormous promise for identifying preclinical AD [8, 15, 16], they are not within the scope of our discussion due to inherent hurdles in their acquisition.

2. IS THERE DETECTABLE COGNITIVE DECLINE IN THE PRECLINICAL STAGE OF AD?

In the cognitive continuum, individuals with mild cognitive impairment (MCI) have undoubtedly suffered objective and measurable cognitive decline. More specifically, the mean performances of their single or multiple cognitive subdomains obtained by neuropsychological examinations are 1.0 or 1.5 standard deviation below their age and education-matched mates [17-20]. For individuals in the preclinical stage of AD, our understanding of their cognition is gradually deepening. While the NIA-AA 2011 diagnostic criteria required third stage individuals to have evidence of amyloidosis, neuronal injury, and subtle cognitive decline concurrently [21], the latest 2018 version described a transitional stage (stage 2) between the cognitively intact state and MCI along the AD trajectory, in which individuals may be documented by evidence of subtle decline on longitudinal cognitive testing [4]. However, both criteria do not operationalize the term "subtle". In a previous study that modeled the curve of cognitive changes versus time, the preclinical trajectory suggested not only a long and slow change pace but also a period of acceleration of cognition decrement that may begin several years before MCI onset [22]. Although the damage extent of MCI has been confirmed, the process on how to quantify the impending "subtle decline", as well as whether it can be embodied in neuropsychological scales at the individual level, remains to be determined.

Compared with amyloid-negative cognitively normal or MCI subjects, considerable studies have shown that the cognitive function of positive subjects declines faster, suggesting that Aβ is a direct factor influencing cognitive decline [23-32], but these studies often ignored the impact of NFTs and/or neurodegeneration. According to recent clinical and animal studies [33-44], the current mainstream view indicates that elevated brain Aβ deposition alone is probably insufficient to produce serious cognitive changes, while the NFTs and/or neurodegeneration are the real culprits. Nevertheless, controversy still exists; after all, Aβ is the actual initiating factor of AD downstream pathological changes based on the "amyloid cascade hypothesis" or real-world studies [3, 36, 45, 46]. Accordingly, Harten *et al.* found that the extra positivity of NFTs and/or neurodegeneration biomarkers did not increase the rate of cognitive decline in amyloid-positive subjects [47], and the mere existence of NFTs or neurodegeneration cannot cause subsequent cognitive decline, the Aβ was an essential [35, 37, 48]. Similar conclusions were obtained in the study of primary age-related tauopathy; that is, the dual effects of Aβ and tau aggravated the deterioration of cognition more than tau alone [49]. Furthermore, it should be noted that it was the levels of CSF Aβ significantly correlated with global cognition, not the levels of NFTs deposition or neurodegeneration [50]. The above findings illustrate the interaction complexity of Aβ, NFTs, and neurodegeneration with or without other pathological changes in the preclinical stage, and lead to the exploration of whether neuropsychological tests can be used to distinguish between preclinical patients and healthy controls.

Uncertainty also remains in cross-sectional studies. Several groups have found that Aβ has negative effects on global cognition or cognitive subdomains [28-30, 34, 51-53], and is related to the ability discrepancy of subdomains [54, 55]. Importantly, Baker *et al.* performed a meta-analysis that included 5005 participants from 30 studies and concluded that Aβ could induce cognitive impairment at the global level, as well as affect visuospatial function, processing speed, episodic memory, and executive function [56]. In addition to cognition, neuropsychiatric symptoms such as loneliness and mood disturbances may also be relevant to the deposition of Aβ in the preclinical stage of AD [57, 58]. In contrast, other reports have reported different results, stating that the score discrepancies of clinical scales were caused by neuronal

damage [35, 36, 40, 41, 47]. Furthermore, the neuropsychological differences between A-(N)+ individuals (suspected non-Alzheimer pathway) and A-(N)- healthy controls also caused confusion in terms of the identification of preclinical AD [34, 40, 41, 51]. Note that the associations between Aβ and neuropsychological changes obtained by the above observational studies were all at the group level and cannot be reflected at the individual level, which is currently the biggest drawback.

Subjective cognitive decline (SCD) is a state in which subjects have self-experienced persistent cognitive decline in the absence of objective impairment [59]. SCD represents a high-risk state of AD and is considered an independent risk factor for cognitive deterioration [59], with approximately 22.0%-64.3% of individuals with SCD on the Alzheimer's continuum [60-65]. A recent study suggested that SCD was not completely subjective; these individuals have already developed impairments in memory, executive function, and language abilities, and the extent of the deficits was associated with CSF biomarkers. However, these deficits were also minor and were only seen at the group level [12]. Studies that focused on identifying the quantitative and qualitative aspects of SCD specifically related to the underlying AD pathology have described several "plus features" such as low cognitive awareness [66] and worries [59, 67], recognizing these characteristics may increase the likelihood of preclinical AD compared to pure neuropsychological examinations.

Some individuals with preclinical AD indeed appeared very slight cognitive deficit, but they probably did not include those who are purely amyloid-positive [35, 36, 40, 41, 47]. The neuropsychological scales were not unified and have not been verified across ethnic and cultural backgrounds, and current evidence suggests that preclinical AD cannot be distinguished at the individual level based on neuropsychological tests; it is likely that measured cognitive changes over time will be more sensitive than any one-time measure [68]. SCD is a potential marker that can be used to detect preclinical AD, and future research should focus on standardizing the diagnosis across different centers [65], clarifying as well as quantifying more "plus features" in order to more accurately detect potential pathological changes.

3. BLOOD BIOMARKERS FOR PRECLINICAL AD

Since blood samples are easier to obtain than CSF, finding reliable blood biomarkers for preclinical AD is advisable. Some inherent difficulties need to be noted: the existence of the blood-brain barrier; the lower concentrations of target brain-derived proteins in blood compared to CSF; the significant increase in liquid capacity and confounding factors; and the presence of degrading proteases, which all make this task a challenge [69]. Although blood biomarkers are difficult to find, with the recent emergence of novel highly sensitive technologies and advancements in *in vivo* pathological diagnosis, some recent studies have presented encouraging results.

3.1. Aβ

The plasma concentration of Aβ is the most promising surrogate biomarker for brain amyloid deposition and has been continuously and intensively investigated in the symptomatic stages of AD, but results have been contradictory [7]. A great number of studies have found that plasma Aβ, especially $A\beta_{42}$ and the $A\beta_{42}/A\beta_{40}$ ratio, were reduced in patients with dementia or MCI [70-73], and that their levels can predict clinical conversion [74, 75]. In contrast, other studies have shown that higher levels were associated with AD or conversion [76-78] or found no relationship between plasma Aβ and AD [79, 80]. It may be more difficult to study plasma Aβ in cognitively healthy subjects, because in theory, Aβ levels were limited to a smaller range, making it difficult to obtain statistically significant correlations with AD pathology and acquire differences between groups. Several studies have shown that levels of plasma Aβ were significantly correlated with brain amyloid deposition [70, 71, 76, 77, 81] and that it was differentially expressed between amyloid positive and negative groups [46, 81-84], but these relationships were both obtained in a mixed sample of different cognitive levels, which may be driven primarily by symptomatic patients. For example, Devanand *et al.* found that the associations between amyloid deposition and plasma $A\beta_{42}/A\beta_{40}$ ratio were strongest in amyloid-positive subjects and only within the MCI group [72]. Similar conclusions were obtained in studies where the above correlation was significant in a mixed sample but could not be repeated in cognitively intact individuals [70, 71, 85]. Furthermore, some researchers found that there were no differences between preclinical patients and controls in terms of plasma Aβ [79, 86, 87]. However, it should be noted that these negative results were derived from the same kit-based detection method, and previous contradictory findings of symptomatic patients have already reflected the complexity of measuring Aβ and preanalytical and analytical differences between quantitative methods [71, 87-89].

The single-molecular array (Simoa) immunoassay and immunoprecipitation-mass spectrometry (IP-MS) method both greatly improve the sensitivity of Aβ detection and have achieved consistent and relevant results in the diagnosis of preclinical AD. More specifically, Verberk *et al.* analyzed the plasma Aβ levels of 248 subjects with SCD using Simoa technology, and found that the levels of $A\beta_{42}$ and the $A\beta_{42}/A\beta_{40}$ ratio were both reduced in CSF-based amyloid-positive individuals when compared with negative individuals, and were positively associated with CSF $A\beta_{42}$ levels, furthermore, they also found that when combining the plasma $A\beta_{42}/A\beta_{40}$ ratio with age and apolipoprotein E (APOE) ε4 status, the accuracy of identifying CSF/PET-based amyloid abnormality reached a value of 0.79-0.83 of area under the curve (AUC) [90]. These results were confirmed in another cohort, which included 276 subjects with SCD, who were dichotomized according to amyloid-PET results, and found that the levels of $A\beta_{42}$ and the $A\beta_{42}/A\beta_{40}$ ratio were also reduced in amyloid-positive individuals, and that there was a significant negative association between global/regional amyloid deposition and plasma $A\beta_{42}$. The accuracy of using the $A\beta_{42}/A\beta_{40}$ ratio to predict PET status reached about 0.80 [AUC] [91]. In a relatively small sample (n=95), researchers observed that the differences in plasma $A\beta_{42}/A\beta_{40}$ ratio between amyloid positive and negative groups were only found in SCD subjects but not in non-SCD

healthy controls [92], which needs to be further verified. *Nakamura* and colleagues established the important position of IP-MS and studied the ability of Aβ precursor protein$_{669-711}$/Aβ$_{42}$ ratio, Aβ$_{40}$/Aβ$_{42}$ ratio, and their composites to predict individual brain amyloid status as determined by amyloid-PET imaging, and found that all test indicators were significantly correlated with both PET burden and CSF Aβ$_{42}$ levels in two independent cohorts with mixed cognitive levels. This demonstrates that the three different types of Aβ-related biomarkers (plasma, CSF, and PET) are highly correlated with each other, convincingly suggesting the potential utility of plasma biomarkers by using this method [81, 83]. Recently, these researchers further indicated that applying the composite score to cognitively healthy individuals is also recommendable [93], after dividing these subjects into two groups based on the blood index, there appeared statistical differences in PET-based amyloid positive rates and amyloid burdens between groups, and both continuous and categorical measures of the index were significantly related to decline in episodic memory and executive function. Using the same method, another research group reported that cognitively healthy individuals with a positive amyloid-PET had a lower plasma Aβ$_{40}$/Aβ$_{42}$ ratio compared to the negative individuals. More importantly, the plasma ratio was significantly correlated with brain amyloidosis that was determined by PET or CSF Aβ$_{40}$/Aβ$_{42}$ and could predict PET status with an accuracy of 0.88 [AUC]. When age and APOE ε4 status was added to the model, the value improved to 0.94 [64]. Other studies that specialized in or mentioned individuals with preclinical AD and used plasma Aβ as biomarkers are summarized in Table **1** [94-96]. High-sensitivity enzyme-linked immunosorbent assay methods are also very promising [74, 88, 97, 98].

Although novel detection technologies, such as Simoa or IP-MS, have acquired a very high correspondence between the current "gold standard" (amyloid-PET or CSF) and plasma Aβ, brought dawn to the early diagnosis of preclinical AD, however, it should be noted that both methods may be difficult to implement in most clinical settings due to the stringent requirements of equipment platforms and technical complexity. Future research should recognize the inter-assay variability and inconsistency of plasma Aβ measurements among centers [70, 99], focus on integrating resources, standardizing experimental procedures, employing multicenter cooperation, and combining pathological biomarkers for verification. In addition, APOE ε4 status should also be considered because it may influence the relationship between plasma Aβ analyte levels and other outcomes [80, 100]. Importantly, the Aβ detected in peripheral blood is not necessarily brain-derived, because platelets and fibroblasts can also theoretically secrete Aβ [101-103], which may result in less pronounced alterations in plasma compared with CSF. Thus, plasma Aβ is unlikely to be more specific than CSF or amyloid-PET in evaluating brain amyloidosis, while it may be more valuable as a screening tool. For example, by using this biomarker, primary clinics could identify amyloid abnormalities among SCD subjects who actively seek medical help with a sensitivity of up to 0.76-0.83 [90, 91, 97]. This value can be further increased when combining demographic information and APOE ε4 status. Therefore, performing selective referral of SCD subjects with abnormal plasma Aβ levels can greatly reduce medical burden and optimize medical resources.

3.2. Total-Tau (t-tau) and Phosphorylated-Tau (p-tau)

In addition to Aβ deposition, the presence of intraneuronal NFTs and neuropil threads within dystrophic neurites consisting of aggregated hyperphosphorylated tau protein is another hallmark of AD [3]. The "T" biomarker of the A-T-(N) scheme of the NIA-AA diagnostic framework is elevated CSF p-tau and cortical tau-PET ligand binding, and the diagnosis of AD is required for "T" positivity [4]. Comparatively, elevated CSF t-tau, as one type of "N" biomarker, reflects only the severity of neuronal damage and is not specific for AD. In recent years, blood-derived t-tau and p-tau have drawn increasing attention owing to their exceptional advantages.

In four small-sample studies, researchers have shown that there are significant differences in plasma t-tau between AD-dementia patients and healthy controls [73, 77, 104, 105]. A study based on the large Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort also confirmed an increase in plasma t-tau in patients with AD-dementia, but with a substantial overlap with levels found in cognitively unimpaired subjects [106]. It should also be noted that expression differences may only be detected by specific epitope-related antibodies [105], and the results obtained in the ADNI cohort cannot be repeated in the separate Swedish "Biomarkers For Identifying Neurodegenerative Disorders Early and Reliably (BioFINDER)" cohort [106]. On the other hand, the relationships between plasma t-tau and specific neuropathological changes in AD are not that clear. Although higher plasma t-tau levels were positively associated with amyloid-PET and tau-PET (entorhinal cortex) across the total population, including different cognitive levels, the results cannot be acquired in subgroups, and the predictive accuracy of amyloid-PET abnormality was also less than 0.60 using this biomarker [107]. In another study, researchers verified that plasma t-tau was strongly and positively associated with the degree of brain tau deposition observed on tau-PET, and even showed a significantly high performance in discriminating between positive and negative tau-PET subjects (AUC 0.80) [108]. Interestingly, in the above-mentioned study that contained two separate cohorts [106], higher plasma t-tau levels were found to be weakly correlated with lower CSF Aβ$_{42}$ levels in one cohort (ADNI) and with higher CSF t-tau and p-tau levels in another cohort (BioFINDER), however, neither correlation could be repeated in the opposite cohort. In a recent study of the "tau Stable Isotope Labeling Kinetics (SILK)" cohort, plasma t-tau also showed no relationship with brain amyloidosis determined by CSF or amyloid-PET [109]. In contrast, plasma t-tau was found to be significantly associated with cortical thickness [107], and longitudinal evaluations showed correlations between high baseline levels of plasma t-tau and future cognitive decline, increased

Table 1. Summary of studies that specialized in or mentioned individuals with preclinical AD and used plasma Aβ as biomarkers.

Samples	Diagnosis of Preclinical AD	Blood Biomarkers	Methods Used	Significance	Refs.
Converters, 339, with 570 plasma samples available; non-converters, 339	9.4±4.0 (range 0.2-20.7) years before converting to dementia; mostly pure clinical diagnosis, part was further supported by CSF analysis	$A\beta_{42}$, $A\beta_{40}$, $A\beta_{42}/A\beta_{40}$	INNO-BIA plasma Aβ forms kit, xMAP technology	No differences in the full sample or in subgroups defined according to sex and age	[86]
Converters, 53 (37 converted to AD-dementia, 11 to vascular dementia, 5 to other dementia); non-converters, 677	5-year follow-up, pure clinical diagnosis	$A\beta_{42}$, $A\beta_{40}$, $A\beta_{42}/A\beta_{40}$	INNO-BIA plasma Aβ forms kit, xMAP technology	No differences between converters (to dementia or AD-dementia) and non-converters; baseline $A\beta_{40}$ levels above the median had an increased risk of developing AD-dementia during the follow-up, even after adjustment for covariates (OR: 2.2)	[79]
Converters (HC to MCI/dementia, MCI to dementia), 26; non-converters (HC to HC), 119	Base line and 36 months follow-up; clinical transition and supported by amyloid-PET	$A\beta_{42}$, $A\beta_{40}$, $A\beta_{42}/A\beta_{40}$, $A\beta_{x\text{-}42}$, $A\beta_{x\text{-}40}$, $A\beta_{x\text{-}42}/A\beta_{x\text{-}40}$	INNO-BIA plasma Aβ forms kit, xMAP technology	$A\beta_{42}$ and $A\beta_{42}/A\beta_{40}$ were decreased at baseline and at 18 months in the conversion group	[75]
HC, 189	Amyloid-PET	$A\beta_{42}$, $A\beta_{40}$, $A\beta_{x\text{-}42}$, $A\beta_{x\text{-}40}$	INNO-BIA plasma Aβ forms kit, xMAP technology	No relationships were observed between mean SUVR and Aβ	[85]
HC, numbers not clear	CSF analysis	$A\beta_{42}$, $A\beta_{40}$, $A\beta_{42}/A\beta_{40}$	INNO-BIA plasma Aβ forms kit, xMAP technology	No differences between HC+ and HC- when stratified by CSF biomarkers	[87]
HC+, 28; HC-, 187	Amyloid-PET	MPP-$A\beta_{42}$, MPP-$A\beta_{40}$, MPP-$A\beta_{42}/A\beta_{40}$	INNO-BIA plasma Aβ forms kit, xMAP technology	MPP-$A\beta_{42}/A\beta_{40}$ was decreased in the HC+; MPP-$A\beta_{40}$ was correlated with PET SUVR	[70]
HC, 167	Amyloid-PET	$A\beta_{42}$, $A\beta_{40}$, $A\beta_{42}/A\beta_{40}$	INNO-BIA plasma Aβ forms kit, xMAP technology	No relationships were observed between mean SUVR and Aβ (Perth site); only $A\beta_{42}/A\beta_{40}$ (Melbourne site) was correlated with SUVR	[71]
HC, 189	CSF analysis	$A\beta_{42}/A\beta_{40}$	Not mentioned	Pure $A\beta_{42}/A\beta_{40}$ predicted abnormal amyloid status with SEN 30.8%, SPE 71.0%; added $A\beta_{42}/A\beta_{40}$ not extra increased AUC of the base model (age plus APOE ε4) (from 0.74 to 0.76)	[99]
Converters, 64; non-converters, 394	14.8±4.9 (range 4.1-23.5) years before converting to dementia; pure clinical diagnosis	$A\beta_{38}$, $A\beta_{40}$, $A\beta_{42}$, $A\beta_{40}/A\beta_{42}$	ELISA	Lower levels of $A\beta_{38}$ and $A\beta_{42}$ were associated with increased risk of AD-dementia	[74]
HC+, 20-36; HC-, 42-52	Base line and 18, 36, 54, 72 months follow-up; by amyloid-PET at the 18, 36, 54 months follow-up time point	Total $A\beta_{42}/A\beta_{40}$, free $A\beta_{42}/A\beta_{40}$, $A\beta_{42}/A\beta_{40}$ bound to plasma components	ELISA	Inverse associations of $A\beta_{42}/A\beta_{40}$ and PET SUVR both in cross-sectional and longitudinal analyses; total $A\beta_{42}/A\beta_{40}$ plus demographic covariates provided a median 0.81 positive predictive value of abnormal amyloid status	[88]

(Table 1) contd….

Samples	Diagnosis of Preclinical AD	Blood Biomarkers	Methods Used	Significance	Refs.
SCD, 200	Amyloid-PET	Total and free plasma $A\beta_{42}$, $A\beta_{40}$, $A\beta_{42}/A\beta_{40}$	ELISA	Total plasma $A\beta_{42}/A\beta_{40}$ was correlated with PET SUVR after controlling for age and APOE ε4 status; when cut-off at 0.08, SEN=83.3% and SPE=51.9% in identifying SCD+	[97]
HC+, 5; HC-, 13	Amyloid-PET	Total and free $A\beta_{42}/A\beta_{40}$	ELISA	Total $A\beta_{42}/A\beta_{40}$ can predict abnormal amyloid status with 1.00 accuracy in the logistic regression model and had an AUC of 0.91 in the ROC curve model	[98]
HC-, 18; SCD-, 25	CSF analysis	$A\beta_{42}$	Immunomagnetic reduction	Plasma $A\beta_{42}$ was weakly positive correlated with CSF $A\beta_{42}$ (R=0.186)	[94]
HC, 513 (SCD, 195; non-SCD, 318, or, HC+, 147; HC-, 366)	CSF analysis	$A\beta_{42}$, $A\beta_{40}$, $A\beta_{42}/A\beta_{40}$	Elecsys immunoassays	$A\beta_{42}$ and $A\beta_{42}/A\beta_{40}$ were lower in HC+; plasma $A\beta_{42}$ and $A\beta_{40}$ model can predict amyloid status with AUC about 0.77	[96]
HC+, 74; HC-, 200; SCD+, 60; SCD-, 114	CSF analysis; 125 HC and 103 SCD had amyloid-PET	$A\beta_{42}$, $A\beta_{40}$, $A\beta_{42}/A\beta_{40}$	SIMOA	No statistical differences between HC and SCD; $A\beta_{42}$ and $A\beta_{42}/A\beta_{40}$ were both correlated with their corresponding CSF Aβ isoforms in HC or SCD group; $A\beta_{42}/A\beta_{40}$ was correlated with SUVR in SCD group; $A\beta_{42}$ and $A\beta_{42}/A\beta_{40}$ were both lower in HC+ or SCD+ group compared with HC-	[95]
SCD+, 73; SCD-, 203	Amyloid-PET	$A\beta_{42}$, $A\beta_{40}$, $A\beta_{42}/A\beta_{40}$	SIMOA	$A\beta_{42}$ and $A\beta_{42}/A\beta_{40}$ were lower in SCD+; $A\beta_{42}$ was negatively correlated with global/regional PET SUVR, predicted amyloid status with AUC 0.68, AUC of $A\beta_{42}/A\beta_{40}$ was 0.79	[91]
SCD, 248	CSF analysis; 69 had amyloid-PET	$A\beta_{42}$, $A\beta_{40}$, $A\beta_{42}/A\beta_{40}$, total-tau	SIMOA	$A\beta_{42}$ and $A\beta_{42}/A\beta_{40}$ were both lower in SCD+, and positively associated with CSF $A\beta_{42}$ levels and negatively associated with CSF total-tau and p-tau181; pure $A\beta_{42}/A\beta_{40}$ and $A\beta_{42}$ both can predict CSF/PET-based abnormal amyloid status (AUC 66%-77%) and clinical progression (HR 2.31 and 1.74)	[90]
HC+, 32 (SCD, 23; non-SCD, 9); HC-, 63 (SCD, 49; non-SCD,14)	Amyloid-PET	$A\beta_{42}$, $A\beta_{40}$, $A\beta_{42}/A\beta_{40}$	SIMOA	$A\beta_{42}/A\beta_{40}$ was lower in HC+ of both whole samples or SCD samples, not in non-SCD samples; $A\beta_{42}/A\beta_{40}$ additionally increased the predictive accuracy of base model (age plus APOE ε4) from 0.75 to 0.78	[92]
Cohort 1: HC+, 66; HC-, 90. Cohort 2: HC+, 9; HC-, 48	Amyloid-PET	$APP_{669-711}/A\beta_{42}$, $A\beta_{42}/A\beta_{40}$, Aβ composite score	Immunoprecipitation-mass spectrometry	Dichotomy by Aβ composite scores, the PET SUVR and PET status were both different between HC+ and HC-	[93]

(Table 1) contd….

Samples	Diagnosis of Preclinical AD	Blood Biomarkers	Methods Used	Significance	Refs.
HC+, 43; HC-, 115. A sub-cohort of 100 had at least 1 amyloid-PET >1.5 years following their baseline plasma sample	Amyloid-PET; mostly also supported by CSF analysis	$A\beta_{42}$, $A\beta_{40}$, $A\beta_{42}/A\beta_{40}$	Immunoprecipitation-mass spectrometry	$A\beta_{42}/A\beta_{40}$ was lower in HC+, correlated with PET SUVR and CSF $A\beta_{42}/A\beta_{40}$, and can predict PET-based abnormal amyloid status at baseline (AUC 0.88); $A\beta_{42}/A\beta_{40}$ was lower in PET status converters to non-converters, individuals<0.1218 had a 15-fold increased risk of conversion	[64]

Note: The number of participants and contents do not represent the total number and content of the original study. **Abbreviations:** AD, Alzheimer's disease; Aβ, β-amyloid; CSF, cerebrospinal fluid; OR, odds ratio; HC, cognitively healthy controls; MCI, mild cognitive impairment; PET, positron emission tomography; SUVR, standardized uptake value ratio; MPP, mixture of protease inhibitors and phosphatase inhibitors; SEN, sensibility; SPE, specificity; AUC, area under curve; APOE, apolipoprotein E; ELISA, enzyme linked immunosorbent assay; ROC, receiver operating characteristic; SCD, subjective cognitive decline; SIMOA, single molecule array; HR, hazard ratio; p-tau181, tau phosphorylated at threonine 181; APP, Aβ precursor protein; '+' means amyloid positive; '-' means amyloid negative.

atrophy rates and hypometabolism [106, 110]. In studies involving patients with the preclinical stage of AD, it is disappointing that plasma t-tau cannot distinguish them from amyloid-negative cognitively healthy individuals (Table **2**) [91, 106, 107, 109]. These results do not support plasma t-tau as a reliable symptomatic AD biomarker in individuals; furthermore, its relationships with AD neuropathology are uncertain, which were affected by study participants and analysis methods to some extent [106-109], and the biomarker is also less likely to be used in the preclinical stage. Instead, like CSF-derived t-tau, blood-derived t-tau is probably a biomarker reflecting neuronal damage [106, 107], suggesting its potential application as a non-disease-specific screening tool.

Tau can be phosphorylated at 85 different residues [3]. Traditional detection methods are not sufficiently sensitive to assay p-tau in human blood, assaying it requires an extremely sensitive assay [104]. In a recent small exploratory study, researchers have used a novel ultrasensitive immunoassay (Simoa) to quantify plasma tau phosphorylated at threonine 181 (p-tau181), and the results showed that its levels were significantly higher in clinically diagnosed AD-dementia patients than in healthy controls [111]. Identical conclusions were obtained in a cohort established by pure clinical diagnosis in the primary care setting, wherein plasma p-tau181 could distinguish AD-dementia patients from controls with an AUC of 0.84 [112]. These were also seen in other studies using the immunomagnetic reduction method [104] or Meso Scale Discovery platform [84, 107]. Recent in-depth studies have included multiple cohorts from different medical centers and autopsy-confirmed subjects, with these studies viewing AD as a clinical-pathologic entity and not just a clinical symptom. They have yielded near perfect results, proving that plasma p-tau181 can reflect AD neuropathology regardless of the clinical presentation and other comorbid pathological changes, has strong correlations with brain Aβ and tau depositions determined by CSF or PET [84, 107, 108, 112-114], can predict brain tau and Aβ pathology status with an AUC of 0.73-0.98 and 0.70-0.94, respectively, across the Alzheimer's associated cognitive continuum [84, 107, 108, 112, 113], and can distinguish AD from other neurodegenerative diseases, with an AUC of more than 0.80 [84, 112-114]. In addition, its baseline levels were correlated with one-year cognitive decline and hippocampal atrophy [112], and each one standard deviation increment in the log of its baseline levels were associated with greater risk of future AD dementia in both cognitively unimpaired and MCI subjects (hazard ratio more than 3.5) [113]. In terms of the preclinical stage, the results were still encouraging. More specifically, plasma p-tau181 was increased in individuals in the preclinical stage of AD when compared with amyloid-negative healthy controls, which was achieved in different cohort studies, including the BioFINDER-2 study [112], the "Translational Biomarkers in Aging and Dementia (TRIAD)" study (AUC 0.77) [112], the study mentioned by Thijssen *et al.* [84], and two cohorts of the BioFINDER study [113]. Although there was no statistical difference between the two groups in the "Mayo Clinic Study of Aging (MCSA)" study, plasma p-tau181 also showed a certain ability in distinguishing patients with preclinical AD from controls with an AUC of 0.70, which was significantly higher than the 0.57 AUC value of plasma t-tau [107]. Importantly, in cognitively intact subjects, plasma p-tau181 showed a clear correlation with amyloid-PET [107, 112] and tau-PET [113]. It was also correlated with CSF p-tau181, but this association was only significant in individuals in the preclinical stage, and not in amyloid-negative individuals [113]. These relationships suggest that plasma P-tau181 reflects brain amyloid deposition and changes in hyperphosphorylated tau that occur in the preclinical stage of AD to some extent; thus, it is not surprising that it can distinguish between the preclinical stage of AD and other neurodegenerative diseases [112, 113]. Compared with p-tau181, emerging evidence indicates that CSF tau phosphorylated at threonine 217 (p-tau217) better reflects AD-related specific pathologies [115, 116]. Interestingly, some studies have suggested that plasma levels of p-tau217 start to change almost at the same time as CSF levels [46, 117], which then naturally inspired subsequent studies to focus on blood, demonstrating that plasma p-tau217 is another promising biomarker of AD. Plasma p-tau217 was also strongly correlated with AD-specific neuropathological changes [109, 117, 118]. In terms of identifying amyloid abnormalities, tau abnormalities, and distinguishing them from other neurodegenerative diseases, the AUC of plasma p-tau217 reached an amazing 0.87, 0.93, and 0.96, respectively, which were even more favorable than plasma p-tau181 AUC values of 0.76, 0.83, and 0.81,

Table 2. Summary of studies that specialized in or mentioned individuals with preclinical AD and used plasma tau as biomarkers.

Samples	Diagnosis of Preclinical AD	Blood Biomarkers	Methods Used	Significance	Refs.
SCD+, 73; SCD-, 203	Amyloid-PET	total-tau	SIMOA	No statistical differences between SCD+ and SCD-	[91]
ADNI cohort: HC, 189. BioFINDER cohort: HC, 274; SCD,174	CSF analysis	total-tau	SIMOA	No statistical differences between HC+ and HC- in ADNI cohort, and probably no statistical differences between HC+ and HC-, SCD+ and SCD- in BioFINDER cohort	[106]
HC+, 72; HC-, 100	Amyloid-PET	total-tau, p-tau181	SIMOA for total-tau, MSD platform for p-tau181	No statistical differences between HC+ and HC- of both total-tau and p-tau181; p-tau181, but not total-tau, was associated with amyloid-PET in total HC; p-tau181 can predict PET-based abnormal amyloid status (AUC 0.70), total-tau was 0.57	[107]
HC+, 11; HC-, 29	Amyloid-PET	p-tau181	MSD platform	p-tau181 can differentiate between HC+ and HC- with AUC of 0.86 (p<0.0001)	[84]
TRIAD cohort: HC, 113; FTD, 8. BioFINDER-2 cohort: HC, 337	Amyloid: CSF $A\beta_{42}$ and (or) amyloid-PET; also had CSF p-tau181, total-tau and tau-PET	p-tau181	SIMOA	TRIAD cohort: p-tau181 was higher in HC+, compared with HC- or FTD; correlated with tau-PET (R=0.143, p=0.09) and amyloid-PET (R=0.405, p=0.0001); can predict PET-based abnormal amyloid status (AUC 0.77). BioFINDER-2 cohort: p-tau181 was higher in HC+ compared with HC-	[112]
BioFINDER cohort 1: HC-, 26; HC+, 38; non-AD disease, 52. Cohort 2: HC-, 126; HC+, 93. Notably, the HC included SCD in both cohorts	Amyloid: CSF $A\beta_{42}$ and (or) amyloid-PET; cohort 1 also had tau-PET; cohort 2 was followed for 4.9±1.3 (up to 8) years	p-tau181	MSD platform	Cohort 1: p-tau181 was higher in HC+ compared with HC- or non-AD; associated with CSF p-tau181 in HC+ (p=0.035), and with tau-PET in total HC. Cohort 2: p-tau181 was higher in HC+ compared with HC-; associated with CSF p-tau181 in HC+ (p=2.0*10^{-6}); can predict AD-dementia conversion (HR=2.48 after adjustment for age, sex and education, HR=2.37 when plus other plasma biomarkers)	[113]
BioFINDER-2 cohort: HC+, 77; HC-, 224. Colombian autosomal-dominant AD registry cohort: *PSEN1* mutation healthy noncarriers, 257; healthy carriers, 259; cognitively impaired carriers, 106	BioFINDER-2 cohort: CSF $A\beta_{42}$ and (or) amyloid-PET for amyloid status; also had CSF p-tau and tau-PET. Colombian cohort: *PSEN1* mutation and cognitively healthy	p-tau181, p-tau217, NFL	SIMOA for p-tau181 and NFL, MSD platform for p-tau217	BioFINDER-2 cohort: p-tau217 was higher in amyloid and tau positive HC (n=58), compared with HC- (AUC 0.90; while p-tau181 was 0.78, NFL 0.71) and preclinical AD (HC+, n=19); p-tau217 was higher in preclinical AD, compared with HC-; associated with tau-PET in HC+, with CSF p-tau217 in total HC; can predict PET-based abnormal tau status (AUC 0.93), better than p-tau181 (0.83) and NFL (0.67), and predict PET-based abnormal amyloid status (0.87), better than p-tau181 (0.76) and NFL (0.69). Colombian cohort: p-tau217 was gradually increased in noncarriers, healthy carriers and cognitively impaired carriers; mutation carriers showed a significant difference from noncarriers at 24.9 years old, about 20 years before MCI onset	[118]

(Table 2) contd….

Samples	Diagnosis of Preclinical AD	Blood Biomarkers	Methods Used	Significance	Refs.
SILK discovery cohort: HC+, 5; HC-, 8; young HC-, 9. SILK validation cohort: HC+, 20; HC-, 31	Discovery and validation cohorts: CSF analysis and (or) amyloid-PET; discovery cohort also had tau-PET	p-tau217, p-tau217/T217 ratio, p-tau181, p-tau181/T181 ratio, p-S202-tau/S202 ratio and total-tau	Immuno-purification nano liquid chromatog-raphy coupled to tandem mass spec-trometry	Discovery cohort: p-tau217, p-tau217/T217 ratio was higher in HC+, compared with HC-, while not the other four biomarkers. Validation cohort: p-tau217, p-tau217/T217 ratio was higher in HC+, compared with HC- (AUC 0.86 and 0.86, respectively), while not the other three biomarkers (except total-tau, 0.61-0.67)	[109]
BioFINDER-2 cohort: HC, 314. Notably, the HC included SCD	Amyloid-PET	p-tau217	MSD platform	Plasma p-tau217 was correlated with CSF p-tau217 in both HC+ and HC- groups; its levels was different among the Aβ-PET negative/tau-PET negative (n=252), Aβ-PET positive/tau-PET negative (n=47) and Aβ-PET positive/tau-PET positive (n=14) groups, increased in the Aβ-PET posi-tive/tau-PET negative group compared with double negative group, with the AUC of 0.83; a high agreement (87.9%) be-tween binarized plasma p-tau217 and entorhinal tau-PET data; 111 tau-PET negative individuals had 2 or 3 scans, the yearly rate of increase in entorhinal tau retention was higher in the group with high baseline levels of p-tau217 compared with the group with low levels	[117]

Note: The number of participants and contents do not represent the total number and content of the original study. **Abbreviations:** AD, Alzheimer's disease; SCD, subjective cogni-tive decline; SIMOA, single molecule array; HC, cognitively healthy controls; CSF, cerebrospinal fluid; PET, positron emission tomography; MSD, meso scale discovery; AUC, area under curve; Aβ, β-amyloid; FTD, frontotemporal dementia; HR, hazard ratio; NFL, neurofilament light chain; MCI, mild cognitive impairment; p-tau181, tau phosphorylated at threonine 181; p-tau217, tau phosphorylated at threonine 217; p-S202-tau, tau phosphorylated at serine 202; '+' means amyloid positive; '-' means amyloid negative.

respectively [118]. Notably, plasma p-tau217 also had the ability to distinguish preclinical patients from amyloid-negative cognitively intact individuals [109, 117, 118], and among gene mutation patients who inevitably develop clini-cally manifested AD, it has already significantly increased 20 years before the onset of symptoms [118]. Furthermore, in cognitively intact individuals, the correlation between plasma p-tau217 and AD-specific pathologies can still be maintained, and the yearly rate of increase in entorhinal tau pathology was higher in the group with high baseline levels of plasma p-tau217 compared with the group with low base-line levels [117]. Detailed results of studies that specialized in or mentioned individuals with preclinical AD and other biomarkers are summarized in Table **2**.

The findings of current research on blood-derived p-tau are encouraging. Their diagnostic effects have been verified in the AD-associated cognitive continuum and for other easi-ly confused neurodegenerative diseases from multiple per-spectives, including CSF, PET, autopsy, and clinical follow-ups, as well as from multiple medical centers. Further re-search is needed to optimize and standardize the assay, vali-date the results in diverse and unselected populations, and determine the potential role in clinical care.

3.3. Other Biomarkers

In addition to the deposition of Aβ and NFTs, accumulat-ing evidence underlines the importance of other interacting molecular pathophysiological cascades, such as neuronal damage and synaptic degeneration [119], innate immune responses and neuroinflammation [120], impaired iron mobi-lization [121], abnormity of the ubiquitin-proteasome and autophagic-lysosomal systems [122], insulin resistance [123], and others [3]. Whether blood-derived biomarkers that reflect these pathological events have changed in the preclin-ical stage of AD is worth exploring.

The neurofilament light chain (NfL) represents a typical biomarker of neuronal damage that has been widely studied, and any pathological process that leads to axonal damage or neuronal death should release NfL proteins into the sur-rounding extracellular fluid, the CSF, and then the blood [124, 125]. Notably, advancements in measurements of NfL have revealed strong correlations between CSF NfL and blood NfL, and they have similar capacities in diagnosing symptomatic patients with AD [125-129]. In familial AD, *Weston et al.* found that serum NfL concentration was in-creased prior to symptom onset [130], and estimated differ-ences showed that NfL concentration was significantly in-creased in mutation carriers compared with non-carriers 6.8-15 years before expected symptom onset [131, 132]. When using the yearly change rate, the difference was evident at least a decade earlier [132]. These results suggest that blood NfL may be a feasible biomarker of ultra-early AD-related neurodegeneration. However, this conclusion was not sup-ported by another study, which observed no differences be-

tween asymptomatic mutation carriers and non-carriers [133]. Inconsistent results were also obtained in patients in the preclinical stage of sporadic AD, with only one study proving that there was a slight increase in plasma NfL when compared to controls [126], while other studies found no changes in blood or CSF NfL [127, 134-138]. By comparison, it is relatively clear that its levels increased faster in patients with preclinical AD [78, 134, 139]. Specifically, trajectory analyses revealed that mean plasma NfL levels increased 3.4 times faster in participants who developed AD versus those who remained healthy, and that these changes were detectable 9.6 years before diagnosis [78]. In addition, the correlation between blood NfL and Aβ deposition was not identified [126, 127, 129, 140, 141]. Comparatively, its levels are more likely to reflect CSF t-tau concentration, cognitive level, brain atrophy rate, metabolic function, and white matter alterations [126, 129, 130, 134, 140]. These findings suggest that blood-derived NfL is not a reliable biomarker for diagnosing preclinical AD. Visinin-like protein-1 (VILIP-1) is another biomarker of neuronal damage, which can reflect the dysregulation of calcium homeostasis caused by Aβ deposition [142]. Much work so far has focused on VILIP-1 expression in CSF, proving that its levels are significantly higher in patients with AD-dementia than in controls [143, 144]. In contrast, there are also significant differences in plasma VILIP-1 that exist between the two groups, but these are smaller than that of CSF [143]. Currently, there are no studies focusing on plasma VILIP-1 in the preclinical stage of AD. It should be noted that there was no difference in CSF VILIP-1 expression between individuals with preclinical AD and amyloid-negative healthy controls [138]; therefore, plasma VILIP-1 is unlikely to have diagnostic value at this stage.

The triggering receptor expressed on myeloid 2 (TREM2) is a transmembrane glycoprotein innate immune phagocytic receptor expressed on brain microglia that can be activated by both pathogen- and danger-associated molecular patterns in the innate immune response, it releases its ectodomain as a soluble form (sTREM2) into the extracellular space upon protease-mediated shedding [145]. In a recent meta-analysis, CSF sTREM2 levels changed throughout the whole continuum of AD, suggesting continuous microglial activation induced by Aβ deposition [146]. However, other studies have verified that the effect of Aβ deposition on CSF sTREM2 is very limited [147, 148], and when participants were classified following the A-T-(N) scheme, researchers have found that the Aβ pathology in the absence of downstream tau-related neurodegeneration is associated with a decrease in CSF sTREM2 [149]. To the best of our knowledge, no studies have focused on blood sTREM2 in the preclinical stage of AD, and studies on its expression in patients with symptomatic stages of AD or Parkinson's disease revealed no significant differences from healthy controls [146, 150-152]. Regarding TREM2, some studies reported that its peripheral levels were higher in the symptomatic AD group than in the control group [153-155], while Guven *et al.* suggested that the differential expression of *TREM2* mRNA levels between patients and controls might be independent of AD disease status and merely results from an age-related increase in *TREM2* expression [156]. Together, it is challenging to use blood-derived TREM2 or sTREM2, and even CSF-derived sTREM2, to diagnose preclinical AD. Chitinase 3-like 1 (YKL-40) is another robust neuroinflammatory candidate biomarker [157]. Accumulating evidence supports the use of CSF YKL-40 concentration to distinguish patients with AD-dementia from cognitively healthy controls [158, 159], while there were no changes between individuals with preclinical AD and amyloid-negative healthy controls [138, 160, 161]. Plasma YKL-40 was correlated with CSF YKL-40, it was also higher in the symptomatic stages of AD but was not correlated with Aβ deposition as determined by CSF or amyloid-PET, indicating its limited value in diagnosing preclinical AD [159].

Exposure to Aβ deposition also leads to deterioration of cholinergic neurons and dysfunction of the cholinergic system [162]. In a cohort of 241 cognitively healthy individuals, lower levels of plasma acetylcholinesterase (AChE) and its enzymatic activity were detected in amyloid-positive subjects than in negative subjects, and both were negatively correlated with the degree of Aβ deposition, however, AChE inhibitor treatment can affect the plasma levels of AChE protein, which may cause uncertainty in its clinical application [163]. Lipocalin-2 is an acute-phase protein with pleiotropic functions, with circulating lipocalin-2 proposed as a biomarker for several diseases, including AD. However, its relationship with AD is not very clear, and it may be required for Aβ to exert its toxicity to astrocytes [164]. A recent study found that plasma lipocalin-2 levels were higher in preclinical AD than in control subjects and were associated with CSF Aβ levels, thus raising the possibility that circulating lipocalin-2 is involved early in AD pathogenesis, thus being a potential biomarker of preclinical AD [165]. There is mounting evidence of a disruption in brain iron homeostasis in AD pathogenesis [121], and elevated blood ferritin levels have been observed in patients with AD-dementia [166]. In individuals in the preclinical stage, *Goozee et al.* found that the blood ferritin was significantly elevated compared with healthy controls and was positively associated with neocortical Aβ load; furthermore, it increased the predictive accuracy of Aβ status of the "base model" (age, APOE ε4 status, and gender) from 0.77 to 0.81 [167]. Other studies have tried to explore the preclinical biomarkers from the perspective of lipid metabolism, fatty acid composition, and gene expression, and found that blood-derived apolipoprotein B [168], apolipoprotein J [169], arachidonic acid [170], docosapentaenoic acid [170], as well as the *CDKN2A* gene [171], have also already changed in the preclinical stage.

In conclusion, except for Aβ and p-tau, there are currently no reliable biomarkers directly derived from blood that can be used in the diagnosis of preclinical AD. This result is understandable, as the biomarkers of neuronal damage represented by t-tau, NfL, and VILIP-1; and the biomarkers of the innate immune response and neuroinflammation represented by TREM2, sTREM2 and YKL-40; as well as other unmentioned biomarkers [172], such as ubiquitin, that relate to the clearance of neurotoxic proteins, they are all downstream events caused by Aβ deposition [3]. In the preclinical stage, the secretory cells of these biomarkers, such as neurons and microglia, may still be able to tolerate the insults of Aβ and have not yet triggered the "breaking point" of the Aβ and tau pathology containment system, where they can keep up with

plaques and tangles. Therefore, it is difficult to detect changes in these biomarkers directly from blood, which is full of confounding factors. It is undeniable that the biomarker AChE, which reflects another downstream event, that is, the imbalance of brain neurotransmitter system; and other biomarkers, such as lipocalin-2, ferritin, apolipoprotein B, apolipoprotein J, arachidonic acid, docosapentaenoic acid, and the *CDKN2A* gene, which are independent of the "amyloid cascade hypothesis" to some extent, can indeed identify the preclinical stage of AD. However, they were all based on solitary evidence and need to be further verified in a larger sample. In addition, none of these above biomarkers were AD-specific. For example, in mouse models, NfL levels in the CSF and blood increased in association with the existence of α-synucleinopathy, tauopathy, and β-amyloidosis [125], while the elderly often has multiple comorbidities, which in turn may influence their levels in the blood. And although some studies have found differences between groups, but there existed considerable overlaps. Furthermore, it should be noted that some biomarkers in blood most likely have a peripheral rather than a central nervous system origin, such as retinol binding protein 4 [173], sTREM2 [150], AChE [163], lipocalin-2 [164], and ferritin [167], and some biomarkers are strongly influenced by age, such as NfL [129, 135, 174] and sTREM2 [156]. All of these factors can lead to uncertainties and failures in diagnosing preclinical AD, as well as severely limit their clinical application. Nevertheless, the lack of diagnostic prowess is not necessarily an undesirable thing as there are many uses for biomarkers, and no one biomarker will fit all needs. The identification of blood-based nonspecific biomarkers is critical for following disease progression from the preclinical stage through the clinical stages, as well as for assessing the rate of progression or therapeutic utility.

4. BRAIN-DERIVED EXOSOMES AS EMERGING CARRIERS OF BIOMARKERS

Extracellular vesicles (EVs) refer to nanoscale particles that are comprised of a lipid bilayer membrane and variable "cargos" of proteins, DNA, and RNA [175]. Exosomes, as an important subpopulation of EVs, are characterized by their spherical shape structure and small size ranging from 30 to 150 nm. Several features determine that exosomes are promising to be excellent carriers of diseases biomarkers: their wide-ranging cell sources and abundant "cargos" [176], as well as their ability to pass through the blood-brain barrier [177], resist the peripheral enzyme degradation of substances that respond to pathological events [178], and reflect the state of the cell of origin [175]. Of utmost importance, with recent methodological advances, *Goetzl* and colleagues harvested brain-derived exosomes (or still termed EVs [179], not unified), such as neuron-derived exosomes (NDEs) or astrocyte-derived exosomes (ADEs), from peripheral blood by using immuno-precipitation technology with antibodies to membrane proteins specific for the cells of origin [9]. These types of exosomes have great advantages over blood or non-enriched total plasma exosomes, as they can more directly reflect the functional state of nerve cells. Essentially, exosomes are messengers of cell-to-cell communication [175, 176], and they indeed play roles in Aβ propagation and tau seeding as well as the accumulation and spread of other AD-associated toxic or antitoxic proteins [13]. Therefore, this process is likely to leave clues, and researchers tried to grasp these clues through the use of plasma brain-derived exosomes in detecting changes in target proteins. Several excellent reviews have described the diagnostic application of exosomes in AD [9, 10]; however, there is a paucity of published literature assessing their role in the preclinical stage of AD.

Since the successful enrichment of brain-derived exosomes, Aβ and tau have always been ranked as exosomal biomarkers that researchers are most concerned about. To the best of our knowledge, 11 studies investigated Aβ and (or) tau levels in plasma/serum-isolated brain-derived exosomes [73, 179-188]. Specifically, all these studies have focused on NDEs, while only one focused on ADEs [183], and five studies were performed by a single group or in collaboration with a member of the group [179-181, 183, 187]. Although some studies have failed to obtain differences between groups with regard to $A\beta_{42}$ [73], p-tau181 [73, 184], and t-tau [184, 185], most studies have found that levels of $A\beta_{42}$ [180-183, 187, 188], p-tau181 [180-183], t-tau [73, 180, 182], and tau phosphorylated at serine 396 (p-S396-tau) [180, 181, 183] in NDEs were all highly expressed in individuals in the symptomatic stages of AD than in healthy controls. A recent study by Jia *et al.* suggested that $A\beta_{42}$, p-tau181, and t-tau levels in NDEs have the same capacities as those in CSF for the diagnosis of AD-dementia and MCI [182]. No statistical differences in the levels of full-length tau [184], mid-region tau [184], or tau fragments ending at amino acids 123 and 224 [186] in NDEs were found between AD-dementia patients and healthy controls, while there were differences in tau phosphorylated at serine 202 (p-S202-tau) in one study [73]. ADEs have also been reported to cargo p-tau181, p-S396-tau, and $A\beta_{42}$, but only the levels of $A\beta_{42}$ were significantly lower in patients with symptomatic AD than in controls [183]. Among these 11 studies, only two involved patients in the preclinical stage [179, 180] (Table **3**). By establishing a longitudinal cohort, Fiandaca *et al.* demonstrated increased (relative to healthy individuals) levels of p-tau181, p-S396-tau, and $A\beta_{42}$ in plasma-isolated NDEs as early as 10 years before the diagnosis of AD-related cognitive impairment [180]. *Kapogiannis et al.* recently performed a longitudinal study with the largest number of case-control samples to date [179]. They collected 887 longitudinal plasma samples from 350 cognitively healthy participants, including 128 individuals who ultimately developed AD, and 222 matched controls who remained cognitively healthy at the end of the follow-up. They found that participants with future AD had a higher level of p-tau181 and tau phosphorylated at threonine 231 (p-tau231) in neuronal-enriched EVs (identical to NDEs) than controls; however, the levels of $A\beta_{42}$ did not change in the preclinical stage, nor did the t-tau. In addition, elevated levels of $A\beta_{42}$ [181, 188] and p-tau181 [181, 188] in NDEs, but not p-S396-tau [181], played roles in predicting cognitive deterioration in MCI patients within 3 years, suggesting that they can be used as predictive biomarkers of AD to some extent.

Table 3. Summary of studies that specialized in or mentioned individuals with preclinical AD and used brain-derived exosomes as carriers of biomarkers.

Samples	Diagnosis of Preclinical AD	Blood Biomarkers	Methods Used	Significance	Refs.
Converters, 24, with 24 blood samples at AP, 24 at the time of initial AD diagnosis (aMCI, 13; dementia, 11); HC, 24	1-10 years before converting to AD; all patients were further supported by CSF Aβ analysis (emailed to the corresponding author)	Plasma NDEs: total-tau, p-tau181, p-S396-tau, and $A\beta_{42}$	ELISA	Total-tau: AP=AD=HC; p-tau181 and p-S396-tau: AP=AD>HC; $A\beta_{42}$: AD>AP>HC	[180]
Converters, 128, with 304 blood samples at AP; non-converters, 222, with 583 samples before the deadline of follow-up (HC)	3.5±2.3 (range 0-9.7) years before converting to symptomatic AD; diagnosis based on all available clinical and neuropsychological data, not detected biomarkers	Plasma neuronal-enriched EVs (identical to NDEs): TSG101, total-tau, p-tau231, p-tau181, p-panY-IRS-1, p-S312-IRS-1, $A\beta_{42}$, concentration and diameter	MSD platform for TSG101, total-tau, p-tau231, p-tau181, p-panY-IRS-1, and p-S312-IRS-1, SIMOA for $A\beta_{42}$, NTA for concentration and diameter	p-tau231, p-tau181, p-panY-IRS-1, p-S312-IRS-1, and particle diameter: AP>HC; TSG101, total-tau, $A\beta_{42}$, and particle concentration: AP=HC	[179]
Converters, 9, with 9 blood samples at AP, 9 at the time of initial AD-dementia diagnosis; HC, 9	1-10 years before converting to AD; all patients were further supported by CSF Aβ and p-tau analysis	Plasma NDEs: synaptotagmin 2, synaptopodin, synaptophysin, neurogranin, GAP43, synapsin 1, and P-S9-synapsin 1	ELISA	Synaptotagmin 2, synaptophysin, and neurogranin: AP=AD<HC; synaptopodin and GAP43: AD<AP<HC; not mentioned synapsin1 and P-S9-synapsin 1 in the preclinical stage	[189]
Converters, 18, with 18 blood samples at AP, 18 at the time of initial AD-dementia diagnosis; HC, 9	6-11 years before converting to AD; not mentioned biomarkers, clinical diagnosis	Plasma NDEs: NPTX2, AMPA4, NLGN1, NRXN2α	ELISA	AMPA4, NLGN1, NRXN2α: AD<AP<HC; NPTX2: AD<AP=HC	[190]
Cohort 1: converters, 160, with 160 blood samples at AP; HC, 160. Cohort 2: mutation carriers, 59; non-mutation carriers, 62	Cohort 1: 1-10 years before converting to AD; all patients were further supported by CSF analysis. Cohort 2: inevitable AD-dementia in the future	Plasma NDEs: GAP43, neurogranin, SNAP25, and synaptotagmin 1	ELISA	In cohort 1, GAP43, neurogranin, SNAP25, and synaptotagmin 1 were all AP<HC (p=0.045, 0.050, 0.046, and 0.046, respectively), but with great overlaps; a single index was not effective to detect AP, with AUC of 0.56-0.60, while the composite model (all indexes plus APOE ε4 status) produced the AUC up to 0.89. Cohort 2 acquired similar results, all indexes were AP<HC (p=0.11, 0.13, 0.084, and 0.046, respectively), with great overlaps; AUC of single index was 0.56-0.60, composite model was 0.87	[191]
Converters, 22, with 22 blood samples at AP, 22 at the time of initial AD diagnosis (aMCI, 11; dementia, 11); HC, 22	1-10 years before converting to AD; all patients were further supported by CSF Aβ analysis	Plasma NDEs: IRS-1, p-S312-IRS-1, p-panY-IRS-1	ELISA	P-S312-IRS-1 and p-S312-IRS-1/p-panY-IRS-1: AP=AD>HC; p-panY-IRS-1: AP=AD<HC; IRS-1: AP=AD=HC	[193]

(Table 3) contd….

Samples	Diagnosis of Preclinical AD	Blood Biomarkers	Methods Used	Significance	Refs.
Converters, 20, with 20 blood samples at AP, 20 at the time of initial AD-MCI or dementia diagnosis; HC, 20	1-10 years before converting to AD; some patients were further supported by CSF Aβ and p-tau analysis	Plasma NDEs: Ubiquitin, LAMP-1, HSF70, cathepsin D	ELISA	Ubiquitin, LAMP-1 and cathepsin D: AP=AD>HC; HSF70: AP=AD<HC	[194]
Converters, 16, with 16 blood samples at AP, 16 at the time of initial AD-dementia diagnosis; HC, 16	5-12 years before converting to AD; not mentioned biomarkers, clinical diagnosis	Plasma ADEs: IL-6, TNF-α, IL-1β, C1q, C4b, factor B, factor D, CD46, CD59, DAF, and other factors	ELISA	CD59 and DAF: AD<AP<HC	[196]
Converters, 16, with 16 blood samples at AP, 16 at the time of initial AD diagnosis (aMCI, 7; dementia, 9); HC, 16	2-10 years before converting to AD; all patients were further supported by CSF Aβ analysis (emailed to the corresponding author)	Plasma NDEs: REST, LRP6, HSF1	ELISA	REST, LRP6, HSF1: AD<AP<HC	[195]
Converters, 15, with 15 blood samples at AP, 15 at the time of initial AD-dementia diagnosis; HC, 15	3-8 years before converting to AD; not mentioned biomarkers, clinical diagnosis	Plasma CSPG4Es: HGF, FGFs 2 and 13, IGF-1	ELISA	HGF, FGFs 2 and 13, IGF-1: AD=AP<HC	[197]

Note: The number of participants and contents do not represent the total number and content of the original study. **Abbreviations:** AD, Alzheimer's disease; AP, preclinical stage of AD; aMCI, amnestic mild cognitive impairment; HC, cognitively healthy controls; CSF, cerebrospinal fluid; Aβ, β-amyloid; NDEs, neuron-derived exosomes; ADEs, astrocyte-derived exosomes; p-tau181, tau phosphorylated at threonine 181; p-tau231, tau phosphorylated at threonine 231; p-S396-tau, tau phosphorylated at serine 396; ELISA, enzyme linked immunosorbent assay; EVs, extracellular vesicles; TSG101, tumor susceptibility gene 101; IRS-1, insulin receptor substrate-1; p-S312-IRS-1, phospho-serine 312-insulin receptor substrate-1; p-panY-IRS-1, phospho-pan-tyrosine-insulin receptor substrate-1; MSD, meso scale discovery; NTA, nanoparticle tracking analysis; GAP43, growth associated protein 43; p-S9-synapsin 1, phosphorylation of serine 9 in synapsin 1; NPTX2, neuronal pentraxin 2; AMPA4, GluA4-containing glutamate; NLGN1, neuroligin 1; NRXN2α, neurexins 2α; SNAP25, synaptosomal-associated protein 25; AUC, area under curve; APOE, apolipoprotein E; LAMP-1, lysosomal-associated membrane protein 1; HSF 1 and 70, heat shock factor 1 and 70; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; DAF, decay-accelerating factor; REST, repressor element 1-silencing transcription factor; LRP6, low-density lipoprotein receptor-related protein 6; CSPG4Es, chondroitin sulfate proteoglycan 4 type neural precursor cells; HGF, hepatocyte growth factor; FGFs 2 and 13, fibroblast growth factors 2 and 13; IGF-1, type 1 insulin-like growth factor.

As mentioned in the above section, AD is an extremely complex pathological aggregation, involving not only Aβ and tau. To date, six studies have concentrated on synaptic dysfunction and investigated synaptic proteins in plasma/serum isolated NDEs [181, 187, 189-192], with four of them performed by members of the same group [181, 187, 189, 190]. A total of 13 proteins were mentioned in these studies, including synaptotagmin 1 and 2, synaptopodin, synaptophysin, neurogranin, growth associated protein 43 (GAP43), synapsin 1, phosphorylation of serine 9 in synapsin 1, synaptosomal-associated protein 25 (SNAP25), neuronal pentraxin 2, GluA4-containing glutamate, neuroligin 1, and neurexins 2α. Their exosomal levels were all reported to be reduced in individuals in the symptomatic stages of AD relative to healthy controls. Notably, in two independent cohorts, *Jia et al.* proved that the exosomal levels of synaptotagmin 1, neurogranin, GAP43, and SNAP25 were highly correlated with those in the CSF, and that these biomarkers in NDEs showed similar abilities in distinguishing patients with AD-dementia or MCI from controls, even showing better abilities in distinguishing AD-dementia from MCI than biomarkers in the CSF [191]. Among these six studies, only three involved patients in the preclinical stage [189-191] (Table **3**). More specifically, a recent research group established a longitudinal cohort of 160 patients with preclinical AD and 160 matched controls, as well as another separate cohort of 59 asymptomatic mutation carriers, who would inevitably develop AD-dementia, and 62 non-mutation healthy carriers. The authors measured NDEs concentrations of synaptotagmin 1, neurogranin, GAP43, and SNAP25 and found that each biomarker was slightly reduced in the preclinical stage, overlapped hugely with the matched control group, and had a poor diagnostic efficiency (AUC 0.56-0.60). However, when these four exosomal targets and APOE ε4 status were combined together, the efficiency improved up to 0.87-0.89 [191]. The other two studies were performed by Goetzl and colleagues [189, 190], and they observed lower NDEs levels of synaptotagmin 2, synaptophysin, neurogranin, synaptopodin, GAP43, GluA4-containing glutamate, neuroligin 1, and neurexins 2α in the preclinical period (up to 10 years before dementia onset) when compared with healthy controls, however, it should be noted that the sample size for both comparisons was relatively low (9 or 18 individuals per group). These results revealed that exosomal synaptic proteins may help predict AD before the onset of cognitive impairment.

Meanwhile, Goetzl and colleagues also focused on other pathological mechanisms and explored exosomal biomarkers from different perspectives by establishing longitudinal cohorts following the above-mentioned research paradigm of Aβ, tau, and synaptic proteins [179, 180, 189, 190]; that is, the researchers retrospectively analyzed blood samples taken from the preclinical stage of AD-induced symptomatic pa-

tients (about 1-10 years before diagnosis), and then compared them with current samples of these patients as well as samples of cognitively healthy controls. Eventually, they proved that levels of phospho-serine 312-insulin receptor substrate-1, phospho-pan-tyrosine-insulin receptor substrate-1, and their ratio, as biomarkers of insulin resistance [193], and ubiquitin, lysosomal-associated membrane protein 1, and lysosomal proteolytic enzyme cathepsin D, as biomarkers of ubiquitin-proteasome and autophagic-lysosomal systems [194], and other biomarkers, including repressor element 1-silencing transcription factor [195], low-density lipoprotein receptor-related protein 6 [195], heat shock factor 1 [195] and 70 [194], in NDEs, and levels of CD59 and decay-accelerating factor, as biomarkers of complement system [196], in ADEs, and levels of hepatocyte growth factor, fibroblast growth factors 2 and 13, and type 1 insulin-like growth factor, as biomarkers of neuronal growth factors, in chondroitin sulfate proteoglycan 4 type neural precursor cells-derived exosomes [197], they were all differentially expressed in individuals with preclinical stage of AD compared with healthy controls; details are presented in Table **3**. Notably, another study performed by the same research group found an opposite result for phospho-pan-tyrosine-insulin receptor substrate-1 [179], finding that its levels in NDEs were increased in the preclinical stage rather than decreased [193]. The authors attributed this surprising result to a difference in the tyrosine epitopes recognized by the detection antibody in different methods. Details of the study were introduced in the preceding paragraph regarding Aβ and tau.

Goetzl and colleagues were highly admirable for their outstanding contributions in this field [179, 180, 189, 190, 193-197], but at the same time, there are some issues that should be considered: the enrolled participants were relatively fewer and the sources were relatively limited, despite their efforts to recruit participants from different medical centers; the results were not validated in other more laboratories, and even the same research group arrived at different conclusion ($A\beta_{42}$ [179, 180] and phospho-pan-tyrosine-insulin receptor substrate-1 [179, 193]) by using different experimental methods involving patients from different centers. Theoretically, all these biomarkers mentioned in the preceding paragraph reflect the downstream pathological events of the Aβ cascade reaction, but the current available evidence indeed support their value in diagnosing preclinical AD. On the one hand, it illustrates the complexity of AD, while on the other, it also shows the superiority of brain-derived exosomes extracted from peripheral blood. Nevertheless, there are more biomarkers to be explored. The development of techniques for the isolation of brain-derived exosomes from peripheral blood has opened new avenues for detecting and monitoring neuropathological processes in living individuals, and we suppose that the exosome-based diagnostic paradigm can make more at-risk or preclinical AD individuals acquire early intervention, thus reducing the incidence of dementia. Furthermore, the serious failure in developing sole-mechanism anti-AD drugs has forced us to focus on multi-target drugs, and the brain-derived exosomes may be novel candidates because of their ability to cross the blood-brain barrier in both directions [177], as well as their involvement in a variety of intricate and interrelated pathophysiological mechanisms in as early as the preclinical stage, which is an excellent therapeutic window.

5. PRECLINICAL AD DIAGNOSIS THROUGH THE EYE

The eye and its associated structures own a rich sensory-motor innervation. In particular, as an extension of the brain, the retina is the sole part of the central nervous system that can be accessed non-invasively and has been described as a "window". Previous studies have shown that Aβ plaques are deposited in the retina, and that its amount is significantly correlated with plaques loaded in the brain at an even earlier stage than intracranial deposition [198-200]. Similarly, these plaques can also cause a series of downstream damages, including changes at structural, functional, and vascular levels, making the retina a novel biomarker for AD [201-203]. Although current research of its use in the preclinical stage is premature, it is encouraging. By using curcumin, which binds to the plaques, a research group noninvasively observed individual retinal plaques directly in AD patients and animal models with a new imaging eye-test technology [199, 200]. Theoretically, this method is also suitable for the preclinical stage, but the requirement of oral administration of curcumin may limit its application. Optical coherence tomography (OCT) provides an *in vivo* cross-sectional view, direct microstructural analysis, and live imaging of the retina [202]. Several research groups have tried to replicate the positive OCT results of patients with symptomatic stages of AD in the preclinical stage [14, 203-206]. More specifically, *Synder et al.* reported a larger volume of the inner plexiform layer in preclinical AD patients compared with healthy controls [205], but the results were not verified in another two studies [203, 204], which suggested that there were no differences in retinal layer thickness in both the macular and peripapillary regions between preclinical AD patients and healthy controls. In addition, a recent study proved that the inner foveal thickness was smaller, and that the foveal avascular zone was larger in preclinical AD [14]. From the perspective of retinal vascular imaging, researchers further found that the amplitude of retinal arterial pulsations and venous pulsations both changed in the preclinical stage [206]. The other results are summarized in Table **4** [207, 208]. It should be noted that the quality retinal images that are suitable for analysis are limited by several factors, including pupil size, formation of senile cataracts, and media opacities. Moreover, the retina is prone to be affected by systemic diseases, such as diabetes and age-related degenerative conditions. In previous studies, many participants were excluded due to many common senile eye diseases, which means that the specificity of these biomarkers needs to be further verified. In comparison, anterior eye structures, such as the lens, cornea, and aqueous humor, are more accessible for imaging and less affected by confounding factors; however, it is currently controversial whether there is Aβ aggregation in the lens [209]. And to the best of our knowledge, studies on the use of anterior eye components for the diagnosis of preclinical AD are still lacking.

Table 4. Summary of studies that specialized in or mentioned individuals with preclinical AD and used "eyes" as biomarkers.

Samples	Diagnosis of Preclinical AD	Eyes Biomarkers	Methods Used	Significance	Refs.
HC+, 18; HC-, 147. Notably, including 75 monozygotic twin pairs and 15 twins from incomplete pairs	Amyloid-PET	Thickness of total RT inner/outer ring, RNFL inner/outer ring, GCL inner/outer ring, IPL inner/outer ring and pRNFL (average, nasal superior, nasal, nasal inferior, temporal inferior, temporal, temporal superior)	Spectral domain OCT	No statistical differences between HC+ and HC-; no relationships were observed with mean SUVR	[204]
SCD+, 15; SCD-, 41. Notably, all self-reported first-degree family history of AD	Amyloid-PET	mRNFL, pRNFL, GCL, IPL, OPL, INL and ONL. All included total volume (except pRNFL), average thickness, inferior, nasal, superior and temporal thickness	Spectral domain OCT	No statistical differences between SCD+ and SCD-; SCD+ showed a larger reduction in volume of mRNFL, ONL and IPL, and a larger reduction in thickness of inferior quadrant of ONL and IPL, and a larger increase in thickness of temporal quadrant of OPL, over 27 months, compared to the SCD-; mRNFL volume change was correlated with mean SUVR (R^2=0.106)	[203]
SCD+, 10; SCD-, 53. Notably, all self-reported first-degree family history of AD	Amyloid-PET	Volume of RNFL, GCL, INL, IPL, ONL, OPL; inclusion bodies volume	Spectral domain OCT; blue-peak autofluorescence imaging	SCD+ group had a higher volume of IPL than SCD-, but the difference was not significant after controlling for multiple comparisons using FDR correction; inclusion bodies volume was correlated with mean SUVR (R=0.46), and IPL volume (R=0.41)	[205]
SCD+, 23; SCD-, 50	Amyloid-PET	Amplitude of RAP and RVP, flicker light-induced retinal vasodilation, RNFL thickness, GCL thickness	Spectral domain OCT; retinal vascular imaging	SCD+ group had an increased amplitude of RAP and a decreased amplitude of RVP than SCD- group; the former was positively correlated with mean SUVR (R=0.33), the latter was negatively correlated with mean SUVR (R=0.4)	[206]
HC+, 24; HC-, 33	CSF analysis (HC+, 16; HC-, 38), and (or) amyloid-PET (HC+, 17; HC-, 33)	A variety of pupil flash response parameters	NeurOptics PLR-200 Pupillometer	None of the pupillary parameters showed a significant difference between groups; no relationships were observed between any parameters of pupillometer and CSF Aβ levels	[207]
HC+, 14; HC-, 16	CSF analysis (HC+, 10; HC-, 18), and (or) amyloid-PET (HC+, 7; HC-, 20)	Total and temporal RNFL thickness; GCL thickness; macular volume; inner, outer, and total foveal thickness; total macular, foveal, and parafoveal vascular density; and foveal avascular zone	OCT angiography	Based on CSF, outer and total foveal thickness were both thinner in HC+ than in HC-; based on PET, foveal avascular zone was larger in HC+ than in HC-; based on both CSF and PET, inner foveal thickness was smaller, and foveal avascular zone was larger in HC+ than in HC-	[14]
HC+, 13; MCI+, 2; HC-, 27	CSF analysis and (or) amyloid-PET	cataract type and grade, lens light scattering	Scheimpflug camera	No statistical differences between biomarker positive and biomarker negative individuals	[208]

Note: The number of participants and contents do not represent the total number and content of the original study. **Abbreviations:** AD, Alzheimer's disease; HC, cognitively healthy controls; PET, positron emission tomography; RT, retinal thickness; RNFL, retinal nerve fiber layer; GCL, ganglion cell layer; IPL, inner plexiform layer; pRNFL, peripapillary RNFL; OCT, optical coherence tomography; SUVR, standardized uptake value ratio; SCD, subjective cognitive decline; mRNFL, macular RNFL; OPL, outer plexiform layer; INL, inner nuclear layer; ONL, outer nuclear layer; FDR, false discovery rate; RAP, retinal arterial pulsations; RVP, retinal venous pulsations; CSF, cerebrospinal fluid; '+' means amyloid positive; '-' means amyloid negative.

CONCLUSION

AD is an extremely complex aggregate of a series of pathological changes, and the need to obtain a comprehensive picture of AD pathogenesis is strong as ever. The ultimate goal of in-depth research is always to find an effective treatment. In the actual clinical practice, the biggest dilemma faced by doctors is that patients already have pathological changes in the preclinical stage, but they cannot be diagnosed until they progress to the symptomatic stages, wherein certain irreversible changes have taken place in the brain structures. This severely limits the development of anti-AD therapies that can intercept the disease before its clinical presentation. Reliable biomarkers that target ultra-early AD pathogenesis can bridge the gap between diagnosis and effective treatment. As the population ages, future screening for individuals with preclinical AD is likely to be done on a large-scale basis; consequently, such screening must be non-invasive, convenient, rapid, and affordable for the general populace. In this review, we evaluated the clinical application potentials of clinical scales, biomarkers directly derived from the blood, biomarkers derived from the brain-derived exosomes, and ophthalmic examinations, in the preclinical stage of AD. In addition, we assessed their value as emerging tools in conjunction with the current "gold standard" for diagnosing AD. Note that we subjectively ignored promising biomarkers in the CSF and the medical imaging data, because they do not meet the above requirements; some biomarkers in saliva and urine, as well as other indicators, such as body mass index and olfactory sensation, also have the potential to diagnosis and predict AD, but due to their secondary status, we also did not include them in the current study. In addition, the limitations of this study should be acknowledged. First, there may be omissions in literature screening. Second, our selective analysis of the preclinical stage may lead to bias.

In summary, the progress of AD diagnosis is based on the development of detection technologies, although preclinical subjects are not likely to be identified by clinical scales, however, the Aβ and p-tau proteins in blood, as well as the abundant "cargos" in brain-derived exosomes, all showed remarkable discrimination power, and the retina also showed subtle changes. Some problems, such as standardization of the detection process, determination of the diagnostic thresholds, multi-center cooperation, inter-ethnic verification, specificity verification, and screening ability verification in the general population, need to be addressed in the future. Importantly, given the special nature of the preclinical stage, a group of biomarkers, rather than a single one, may be the best choice for ultra-early diagnosis; however, relevant studies are still lacking.

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

This work was supported by grants from the National Key Research and Development Program of China (2016YFC1306300, 2018YFC1312001), and the National Natural Science Foundation of China (61633018, 82020108013).

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Feng-Qi Liu (Peking University People's Hospital, Beijing, China), for her help in consulting and collating the literatures, and Dr. Di-Yang Lyu (Xuanwu Hospital of Capital Medical University, Beijing, China), for his free sharing of a self-developed literature management tool.

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