Opinion Paper

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Blood-based biomarkers in Alzheimer's disease – moving towards a new era of diagnostics

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Abstract: Alzheimer's disease (AD), a primary cause of dementia globally, is traditionally diagnosed via cerebrospinal fluid (CSF) measures and positron emission tomography (PET). The invasiveness, cost, and limited accessibility of these methods have led to exploring blood-based biomarkers as a promising alternative for AD diagnosis and monitoring. Recent advancements in sensitive immunoassays have identified potential blood-based biomarkers, such as Aβ42/Aβ40 ratios and phosphorylated tau (p-tau) species. This paper briefly evaluates the clinical utility and reliability of these biomarkers across various AD stages, highlighting challenges like refining plasma Aβ42/Aβ40 assays and enhancing the precision of p-tau, particularly p-tau181, p-tau217, and p-tau231. The discussion also covers other plasma biomarkers like neurofilament light (NfL), glial fibrillary acidic protein (GFAP), and synaptic biomarkers, assessing their significance in AD diagnostics. The need for ongoing research and development of robust assays to match the performance of CSF and PET biomarkers is underscored. In summary, blood-based biomarkers are increasingly crucial in AD diagnosis, follow-up, prognostication, treatment response evaluation, and population screening, particularly in primary care settings. These developments are set to revolutionize AD diagnostics, offering earlier and more accessible detection and management options.

Keywords: Alzheimer's disease; amyloid; blood; biomarker; CSF; tau

Introduction

Alzheimer's disease (AD) is a chronic, neurodegenerative disease and the leading cause of dementia that affects more than 50 million people worldwide [1]. This number is projected to be greater than 150 million by 2050 [1]. The pathological hallmarks of AD are extracellular amyloid-β (Aβ) plaques, intracellular tau tangles, and neurodegeneration, which are needed to make a definitive diagnosis *post-mortem*. However, considerable progress has been made to detect and quantify these pathologies *in vivo* using biomarkers. Cerebrospinal (CSF) fluid measures of Aβ42, Aβ42/Aβ40, phosphorylated tau (p-tau), total tau, and neurofilament light (NfL) chain are routinely examined as accessible measures of Aβ, tau and neurodegeneration [2]. CSF Aβ42 has been validated analytically and clinically; assays to measure the marker are now also fully standardized through the development of certified reference methods and materials [3, 4]. This work is well underway for Aβ40 and has just started for p-tau and NfL. Positron emission tomography (PET) allows for regional visualization of Aβ and tau pathologies, which have a high concordance with CSF measures. Glucose metabolism (fluorodeoxyglucose [FDG]-PET) and temporal atrophy by magnetic resonance imaging (MRI) provide imaging measures of neurodegeneration [5]. These biomarkers are now defined in the context of AD research frame ATN (amyloid/tau/neurodegeneration)

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which proposes a shift in the definition of the disease from a clinical syndrome to a biological construct [3]. This definition is of immediate clinical importance given that disease-modifying therapies that effectively remove Aβ pathology from the brain have received regulatory approval in the US, and will likely become widely available globally in the coming years. The ATN criteria are currently undergoing revision to describe the progression of biomarker changes over time and to encourage their clinical use for diagnosis and staging of the disease [6]. The perceived invasiveness or reluctance of a lumbar puncture and the relatively high cost of PET examination restrict their use for large-scale ATN classification of patients–especially in primary care settings or population screening. Therefore, current efforts have been made to develop blood-based biomarkers to replace or complement the currently available CSF and PET biomarkers. With the advent of highly sensitive immunoassays, the development of blood-based biomarkers has accelerated; mounting evidence now support their clinical usefulness and reliability in both prodromal and dementia stages of the disease.

Blood-based biomarkers in AD

In the context of AD, the most used blood-based biomarkers are Aβ42/Aβ40 and phosphorylated tau (p-tau) species (e.g., p-tau181, p-tau217, p-tau231). A detailed summary of key biomarkers relevant to Alzheimer's disease is presented in Table 1.

Plasma amyloid beta biomarkers

Initially, plasma Aβ42 concentration was measured using enzyme-linked immunosorbent assays (ELISA); there was, however, not much hope for its usefulness due to the low performance of the assay and inconsistent findings in different cohorts [18]. This changed with the advent of Single molecule array (Simoa) technology that demonstrated significant reduction of Aβ42/Aβ40 in the AD *continuum* despite weak correlations with CSF and PET [19, 20]. Other assays (e.g., Elecsys using electrochemiluminescence [ECL] [21],

Table 1: Comprehensive overview of the blood-based biomarkers in Alzheimer's disease: methods, correlations, and implications.

Biomarker	Methods and process	Remarks
Beta amyloid 1–42/beta amyloid 1–40 (Aβ42/Aβ40) ratio	Mass spectrometry assays, immunoassays;	A decline in Aβ42 levels is indicative of amyloid accumulation in the brain [2, 7, 8]
	Cerebral Aβ pathology	A lower Aβ42/Aβ40 ratio aligns closely with amyloid deposition as identified by amyloid PET imaging [9, 10]
		There is a high correlation between cerebrospinal fluid (CSF) and blood Aβ42/Aβ40 levels [4, 9]
Phosphorylated tau protein (P-tau)	Mass spectrometry assays, immunoassays;	P-tau levels are increased in both manifest and asymptomatic stages of Alzheimer's disease [9]
	Neuronal tau phosphorylation and secretion	There is a significant correlation between CSF and blood levels of P-tau [4, 9]
Subtypes of P-tau (P-tau 181, P-tau-217, P-tau-205, P-tau231)	Mass spectrometry assays, immunoassays; neuronal tau phosphorylation and secretion	Variants of phosphorylated tau protein, including P-tau 181, P-tau 217, P-tau 205, and P-tau 231, show increased levels in both symptomatic and asymptomatic [11–14] Alzheimer's disease.
		These subtypes of P-tau are highly sensitive and specific markers for Alzheimer's disease [7, 9]
Neurofilament light (NfL) protein	Immunoassays, neurodegeneration	NfL mirrors the pathology of neurofibrillary tangles and the overall severity of Alzheimer's disease [15, 16]
		There is a close correlation between CSF concentrations of NfL and its levels in Alzheimer's disease [16]
Glial fibrillary acidic protein (GFAP)	Immunoassays, astrocyte activation	GFAP indicates the presence of neuroinflammation and neurodegeneration [17]
		The levels of GFAP in the plasma are indicative of the extent of astrogliosis [9, 17]

This Table provides a detailed summary of key biomarkers relevant to Alzheimer's disease, including Aβ42/Aβ40 ratio, phosphorylated tau protein variants, neurofilament light protein, and glial fibrillary acidic protein. Each biomarker is accompanied by its detection methods, primarily mass spectrometry and immunoassays, and remarks on its significance in Alzheimer's pathology.

Lumipulse using chemiluminescent enzyme immunoassay [CLEIA] [22], and immunoprecipitation mass-spectrometry [IP-MS] [23, 24]) independently confirmed the decrease in plasma Aβ42 and Aβ42/Aβ40 ratio in those with confirmed cerebral amyloid pathology. In a head-to-head study to detect brain Aβ pathology via blood-based measurements, mass spectrometry-based methods (IP-MS) have been shown to perform slightly better than immunoassays [25], but the latter are catching up [26]. Previous research has indicated that reductions in both CSF and plasma Aβ42/Aβ40 ratios occur early in the Alzheimer's *continuum* and predict the transition from amyloid-negative to amyloid-positive PET status [24, 27]. Nevertheless, the main confounding factor with plasma Aβ measurements that must be overcome is the small-fold change between Aβ-positive and -negative individuals (only 8–15 % in plasma compared with 40–60 % in CSF [9]). In addition, multiple pharmacodynamic effects on plasma Aβ by common drugs (e.g., neprilysin inhibitors) may further restrict the use of Aβ peptides in older adults (Brum et al., [28]). Overall, the low robustness of plasma Aβ42/Aβ40 is a significant challenge to making use of the biomarker in clinical settings [10] – small drifts in assay performance or variation in pre-analytical factors could cause individuals to be misclassified. Plasma Aβ could be highly important for establishing novel disease-modifying drugs. Gamma-secretase modulators (GSMs), shifting Aβ production from the amyloidogenic Aβ42 to the less pathogenic Aβ38 and maintaining overall Aβ levels, hold promise for primary prevention of AD pathology, making Aβ38 an important biomarker in blood [29].

Plasma tau biomarkers

So far, several plasma p-tau assays have been developed and used to show AD-related changes. Immunoassay and mass spectrometric measures of p-tau181, p-tau217 and p-tau231 discriminate Aβ-positive from -negative individuals with high performance in many studies [11, 30, 31]. High-performing p-tau blood tests exhibit a substantial increase in AD patients with increases occurring concurrently with extracellular Aβ plaque deposition. This relationship is observed across the AD *continuum*, including the presymptomatic phase in sporadic and familial AD [7]. This includes individuals with Down syndrome who have a genetically determined form of AD [32]. P-tau species (especially p-tau217 and p-tau231) start to increase just after the drop in CSF Aβ42/Aβ40 ratio and prior to the cut-point for Aβ-PET positivity has been reached. This is also a promising finding for including people in the pre-dementia stage in clinical trials, which is of great importance for disease-modifying treatments. Certain p-tau species [12], however, are associated with neurofibrillary tangle pathology [33, 34], as indexed by tau-PET imaging and neuropathological examination. Unlike plasma Aβ42/40, plasma p-tau is reliably measured using immunoassays with large fold-changes, which is an encouraging finding to implement this biomarker in routine clinical practice widely. There is much debate as to which p-tau biomarker has the most potential as a clinical biomarker. While all p-tau tests have high discriminative accuracy, p-tau217 has the largest fold-change between AD and non-AD disorders [8] and is more related to AD progression [7]. All in all, plasma p-tau is a promising biomarker to show AD-type brain pathologies, detect individuals with pre-clinical AD, stage them as they progress, and, potentially, track treatment efficacy [35].

Building on the research of p-tau, the role of the microtubule-binding region (MTBR) of tau in AD becomes particularly pertinent [36, 37]. This region of tau is contains the building blocks of neurofibrillary tangles and could thus be a very different biomarker compared with the N-terminal p-tau fragments discussed above, which mostly reflect Aβ pathology.

Plasma neurodegeneration biomarkers

In the ATN criteria, total tau is classified as biomarker for neurodegeneration in CSF. However, this relationship has not translated well to blood [38]. Plasma total tau levels are not well correlated with its CSF levels, possibly because of peripheral production of total tau, and are not specific for AD [4]. Thus, NfL has been used as a neuroaxonal injury marker research settings and specialized clinical routine laboratories where NfL has already been implemented [15, 39]. As a general neurodegeneration marker, NfL plasma levels are also elevated in amyotrophic lateral sclerosis, frontotemporal dementia, traumatic brain injury, and peripheral neuropathy, which should be considered while assessing possible AD cases [16]. NfL is also an excellent prognostic marker for neurological outcome acutely after cardiac arrest [40]. In AD cases, plasma NfL levels are found to be increased in relation to Aβ and tau positivity [41], but the lack of specificity to AD, minimal longitudinal change associated to atrophy [7], cognitive decline and amyloid clearance [42] may limit NfL in the context of AD. To accurately detect and track AD-specific brain-derived tau levels in the blood, a novel brain-derived tau assay has recently been developed, and it discriminated autopsy-confirmed AD and non-AD tauopathies, which was better than NfL performance [43]. To

overcome the lack of robustness of plasma total tau assays currently used, and distinguish AD-type neurodegeneration, brain-derived total tau has great promise for its future implementation in AD management [43]. In addition, recent studies highlight the significance of plasma N-terminal tau (NTA) in AD, demonstrating its ability to predict future cognitive decline and neurodegeneration even in pre-symptomatic stages of AD, with higher baseline levels of NTA strongly associated with the progression to mild cognitive impairment (MCI) to AD dementia, and effectively differentiating between normal, mildly-impaired, and AD dementia populations, thereby underlining the potential of NTA as a non-invasive, sensitive biomarker for early detection and monitoring of AD pathology [44, 45].

Plasma astrocytic activation biomarker

Glial fibrillary acidic protein (GFAP) is one of the most studied glial marker, which is likely released as a quick response to Aβ pathology in the context of AD. Interestingly, it has been shown that plasma GFAP levels are more related to brain Aβ pathology than CSF levels of GFAP, which may be due to instability of GFAP in CSF [17, 46, 47]. Although GFAP is not an AD-specific marker, the magnitude of GFAP change is relatively large in AD compared with those of non-AD neurodegenerative diseases such as FTD [47]. Furthermore, along with the plasma markers of amyloid, tau, and neurodegeneration, plasma GFAP levels were also observed to decrease after amyloid-removal therapy (lecanemab) in a phase 3 clinical trial [35].

In addition to GFAP, the role of S100B in AD appears multifaceted and warrants careful interpretation. While various studies suggest its potential as a biomarker due to its correlation with brain atrophy [48] and inverse relationship with MMSE scores [49] in AD patients, the expression of S100B in other cell types beyond the brain necessitates caution in interpreting serum levels, as they might reflect peripheral changes rather than central nervous system pathology [50]. Moreover, the involvement of S100B in AD pathophysiology is further supported by its astrocytic over-expression driven by IL-1 from activated microglia, indicating its potential as a marker of underlying pathological processes in AD [51]. However, considering the complexity of its expression and the factors influencing its levels, the utility of S100B utility in AD diagnosis and monitoring is uncertain.

YKL-40, chitinase 3-L1, CHI3L1, shows promise as a biomarker for early detection and progression monitoring of MCI-AD and dementia due to AD, with its levels correlating with disease severity [52, 53]. However, its effectiveness is enhanced when combined with other AD biomarkers, due to its non-specificity to AD dementia alone [54]. Given its lack of specificity to AD, the diagnostic potential of YKL-40 could be enhanced when used in conjunction with other biomarkers like Aβ42/40, total tau, and p-tau, offering a more comprehensive approach to AD diagnosis and monitoring.

Synaptic biomarkers

The loss of synapses is generally considered the most accurate indicator of the deterioration in cognitive abilities associated with AD [55]. In relation to this, the dendritic protein neurogranin (Ng) has been the subject of thorough research. Studies have shown that the level of Ng in the CSF is elevated in individuals with AD. This elevation correlates with the concentrations of t-tau and p-tau proteins. Furthermore, the increased levels of Ng are linked to the gradual deterioration of cognitive abilities as time progresses. The impact of this increase is further influenced by the presence of Aβ pathology [56]. However, plasma Ng levels do not correlate with those in the CSF. This lack of correlation is likely due to the production of the protein outside the brain [57]. Hence, it is improbable that Ng levels in the plasma will serve as a useful blood-based biomarker for AD. However, β-synuclein and synaptosomal-associated protein 25 (SNAP-25) are promising blood biomarkers for synaptic damage in AD. β-Synuclein is elevated in both CSF and blood of AD patients [58]. This increase is notably associated with brain atrophy in AD, distinguishing it from other conditions like frontotemporal lobar degeneration [59]. However, further research is needed to understand its role in preclinical stages of AD and its relationship with amyloid and tau pathologies [60]. SNAP-25 is a crucial presynaptic protein that belongs to the soluble N-ethylmaleimide-sensitive factor attachment receptor (SNARE) family. It plays a vital role in cognitive processes because of its involvement in the mechanism of vesicular exocytosis [61]. In the past, the levels of these proteins could only be determined in CSF, and they have been found to be elevated in individuals with AD [61]. A recent study has demonstrated increased SNAP-25 levels in the plasma of AD patients, showing a significant correlation with cognitive function and cortical atrophy [62]. Further studies are required to confirm these findings in separate groups and to investigate how plasma SNAP-25 levels relate to other biomarkers across a wide spectrum of neurodegenerative diseases.

Conclusions

Blood-based biomarkers of AD have great promise in AD diagnosis, follow-up, prognostication, tracking treatment response, and population screening. Using highly sensitive assays, the levels of blood-based biomarkers are easily determined and may reliably be used in routine clinical settings. Much more effort should be on developing more robust assays and achieving as high performance as core CSF and PET biomarkers of AD. If individuals with pre-clinical AD can be caught via blood-based biomarkers, especially in primary care settings, more promising treatment options would appear on the horizon. Simplified protocols for blood biomarker assessment through finger prick testing are currently being developed [63].

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