

REVIEW

A Review of Fluid Biomarkers for Alzheimer's Disease: Moving from CSF to Blood

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ABSTRACT

A set of core cerebrospinal fluid (CSF) biomarkers for Alzheimer's disease (AD) includes total tau (T-tau), phosphorylated tau (P-tau) and β -amyloid 42 (A β 42). These biomarkers reflect some of the key aspects of AD pathophysiology, including neuronal degeneration, tau phosphorylation with tangle formation, and A β aggregation with deposition of the peptide into plaques. The core AD CSF biomarkers have been validated clinically in numerous studies, and found to have a very high diagnostic performance to identify AD, both in the dementia and in the mild cognitive impairment stages of the disease. CSF A β 42 has also been found to show very high concordance with amyloid PET to identify brain amyloid deposition. The synaptic protein neurogranin is a novel candidate CSF biomarker for AD and prodromal AD. High CSF

neurogranin predicts future cognitive decline and seems to be more specific for AD than, for example, T-tau. Importantly, technical developments have given ultrasensitive measurement techniques that allow measurement of brain-specific proteins such as tau and neurofilament light (NFL) in blood samples. Both plasma tau and NFL are increased in AD, and a recent study showed that plasma NFL has a diagnostic performance comparable to the core AD CSF biomarkers, and predicted future cognitive decline. Future large longitudinal clinical studies are warranted to determine the potential for plasma tau and NFL to serve as first-in-line screening tools for neurodegeneration in primary care.

Keywords: Alzheimer's disease; Biomarkers; Cerebrospinal fluid; β -Amyloid; Tau protein; Neurofilament light; Neurogranin

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INTRODUCTION

Biomarkers for chronic neurodegenerative disorders such as Alzheimer's disease (AD) are of great importance, since the cognitive symptoms often are diffuse and overlap with other disorders; the clinical progression is slow and variable even between patients with the same disease. Biomarkers reflecting different types of pathophysiology in the brain can be used for

clinical diagnosis, especially in the early stages of the disease, to predict progression, to monitor effects of novel drug candidates in clinical trials, and lastly also in clinical research to deepen our understanding of the pathogenesis of the disease [1]. In this context, the AD arena is in a good situation such that a set of highly validated and specific biomarkers are at hand. Imaging biomarkers for AD include amyloid and tau positron emission tomography (PET) to measure the amount of these protein deposits in the brain and magnetic resonance imaging (MRI) measurements of brain volume and neuronal connectivity. In addition, a set of cerebrospinal fluid (CSF) tests reflecting key aspects of disease pathology (neurodegeneration, tau pathology and amyloid deposits) are available. Laboratory medicine tests influence up to 70% of clinical decisions and thus have a central position in clinical medicine [2].

This paper comments on some caveats on the road to develop and validate these CSF biomarkers and some recent developments on novel biochemical tests.

Compliance with Ethics Guidelines

This article is based on previously conducted studies and does not involve any new studies of human or animal subjects performed by the author.

THE CORE CSF BIOMARKERS FOR ALZHEIMER'S DISEASE

The core AD CSF biomarkers include total tau (T-tau), phosphorylated tau (P-tau) and the 42 amino acid isoform (A β 42) of β -amyloid (Table 1). These analytes reflect key aspects of disease pathogenesis, i.e., neuronal and axonal degeneration, phosphorylation of tau with tangle formation, and oligomerization, aggregation and deposition of the A β 42 peptide into plaques [3].

The most commonly used technique to measure these AD biomarkers is the enzyme-linked immunosorbent assay (ELISA), including an assay for T-tau that measures all tau isoforms irrespective of phosphorylation state [4], for tau

phosphorylated at threonine 181 (P-tau181) [5], and for the 42 amino acid forms of β -amyloid (A β 1-42) [6]. Numerous studies have very consistently found a marked increase in both T-tau and P-tau in AD, accompanied by a decrease in CSF A β 1-42, as reviewed in a recent meta-analysis [7]. A high diagnostic performance of the core AD CSF biomarkers has also been validated in studies that confirmed diagnosis by autopsy. These studies showed better diagnostic performance of the CSF biomarkers than studies based on pure clinical diagnoses [8, 9].

Although the AD core CSF biomarkers have been well-validated clinically, a problem has been the variability in measurements between clinical laboratories, and over time between batches of reagents, which are more pronounced for A β 42 than for T-tau or P-tau [10]. This problem stems from differences in analytical procedures for the manual ELISA methods between laboratories and from variability in reagent quality and manufacturing procedures for the kits. Standardization efforts to control for and resolve these issues include development of a fully validated mass spectrometry-based Reference Measurement Procedures (RMP) for CSF A β 42 to serve as the “gold standard” for A β 42 measurement [11], and Certified Reference Materials (CRM) for the core AD CSF biomarkers [12]. In addition, novel assays have been developed on fully automated laboratory analyzers, resulting in very precise and stable measurements, also between many reagents and laboratories [13]. These developments will provide the basis for validated and stable measurements of the AD CSF biomarker, and enable the introduction of uniform cut-off levels and a more general use of the CSF diagnostic in the routine clinical evaluation of patients with cognitive impairment and suspected AD.

CSF Biomarkers for Early Diagnosis

Mild cognitive impairment (MCI) is regarded as the earliest clinical phase of AD, typically characterized by disturbances in episodic memory, although also disturbances in other cognitive domains may present early. However, MCI is a heterogeneous syndrome that may be caused by many disorders (only around half of

Table 1 Cerebrospinal fluid and blood biomarkers for Alzheimer's disease

Application	Fluid	Biomarker	Change in AD	Stage of development	Interpretation
Core biomarkers for clinical diagnostics	CSF	T-tau	High T-tau is found in AD and prodromal AD	Several validated commercially available immunoassays	High T-tau reflects intensity of neurodegeneration and probably reflects disease progression
			Mean change in AD is to around 250% of age-matched controls	Fully automated methods are soon to be launched	High T-tau is also found in some other neurodegenerative (e.g., CJD) and acute (e.g., stroke) brain disorders
			Sensitivity for AD and prodromal AD >90%		
	CSF	P-tau	High P-tau is found in AD and prodromal AD	Several validated commercially available immunoassays	High P-tau reflects phosphorylation state of tau and thus probably tau pathology in AD
			Mean change in AD is to around 200% of age-matched controls	Fully automated methods are soon to be launched	P-tau is specific for AD than for T-tau; high CSF levels have not been found in other neurodegenerative disorders and is not found in acute brain disorders
			Sensitivity for AD and prodromal AD >90%		
CSF	A β 42	Low A β 42 is found in AD and prodromal AD	Several validated commercially available immunoassays	Low A β 42 reflects brain amyloid deposition, and show very high concordance with amyloid PET	
		Mean change in AD is to around 50% of age-matched controls	Two fully validated mass spectrometry Reference Measurement Procedures (RMP) approved	Low A β 42 may be earlier during the course of AD than amyloid PET	
		Sensitivity for AD and prodromal AD >90%	Fully automated methods are soon to be launched		
Candidate diagnostic biomarker	CSF	A β 42/A β 40	Low A β 42/A β 40 ratio is found in AD and prodromal AD	Several validated commercially available immunoassays	The A β 42/A β 40 ratio is thought to compensate for between-individual variations in "total" A β production
			Mean change in AD is to around 50% of age-matched controls	Fully automated methods are soon to be launched	
			Sensitivity and specificity is higher than for A β 42 alone		
Candidate screening tools	CSF	Neurogranin	High neurogranin is found in AD and prodromal AD	Several research grade assays have been published	High neurogranin reflects synaptic dysfunction or degeneration Increased neurogranin may be specific for AD
	Blood	Tau	High plasma tau is found in AD and prodromal AD, but with overlap between groups	Several research grade assays published One commercially available immunoassay	High plasma tau is a general biomarker for neurodegeneration, and not specific for AD
	Blood	NFL	High plasma NFL is found in AD and prodromal AD, but with overlap between groups	One validated research grade assay published One commercially available immunoassay	High plasma NFL is a general biomarker for neurodegeneration, and not specific for AD

A β amyloid- β , AD Alzheimer disease, CJD Creutzfeldt–Jakob disease, CSF cerebrospinal fluid, NFL neurofilament light, PET positron emission tomography, P-tau phosphorylated tau, T-tau total tau

cases have AD), which introduces obvious diagnostic difficulties. At the same time, treatment with disease-modifying agents will likely be most effective in this stage of AD, or even pre-clinically, before neurodegeneration is too severe and widespread. Thus, studies examining the potential of the AD CSF biomarkers for early diagnosis were warranted. The first was published in 1999, by Andreasen and co-workers, and showed that MCI patients who progress to AD with dementia during clinical follow-up have the typical AD CSF biomarker profile (decreased CSF A β 42 together with increased T-tau and P-tau) already in this stage of the disease [14]. Importantly, the CSF biomarkers were stable at follow-up when patients had reached the dementia stage, indicating that these biomarkers do not change during the clinical stages of the disease [14].

An extended clinical follow-up period is needed to ascertain which MCI will not progress to dementia, or even will improve so that they no longer have memory problems. The first study with such extended clinical follow-up was published in 2006 by Hansson and co-workers [15]. This study showed cognitively stable MCI patients do not have the AD biomarker profile, while progressive MCI patients (with prodromal AD) could be identified with 95% sensitivity and 92% specificity against elderly controls and 83% specificity against stable MCI cases [15]. This very high diagnostic accuracy for the core AD CSF biomarkers for prodromal AD was later verified in several large multi-center studies, including the DESCRIPA study in Europe [16], the American ADNI study [9], and the Swedish Brain Power study [17]. Taken together, these, and several subsequent studies, support that the AD core CSF biomarker profile has diagnostic value to identify MCI prodromal AD cases in unselected MCI populations.

BIOMARKER-BASED DIAGNOSTIC RESEARCH CRITERIA FOR AD

Recent reports show promise that we may have disease-modifying drugs for AD in the near future. For example, β -secretase (BACE1) inhibitors show a marked, dose-dependent,

reduction in brain and CSF A β levels [18], which may translate into effects on brain A β aggregation, plaque formation and a beneficial effect on clinical disease progression. Furthermore, the Phase1b aducanumab trial showed a dose- and time-dependent reduction in brain amyloid plaques as measured by florbetapir PET, but also a high frequency of amyloid-related imaging abnormalities (ARIA) [19].

Thus, given that we may soon have effective, but most likely also expensive, drugs that may give side-effects such as ARIA, and that mild cognitive impairment (MCI) is a heterogeneous syndrome caused by many disorders (only around half of cases have AD), there is a large need for biomarker-based diagnostic criteria that allow an accurate diagnosis of AD already during the MCI stage, for review see [20]. The first such criteria were published in 2007, Dubois and co-workers presented novel research criteria for the diagnosis of prodromal AD, i.e., before the dementia stage, which are based on clinical core of early and significant episodic memory impairment together with at least one or more abnormal biomarkers among structural neuroimaging with MRI, molecular neuroimaging with PET, and CSF analyses of amyloid β and tau protein [21]. A revised and updated version of these criteria was published in 2014, the main difference being that downstream topographical biomarkers (volumetric MRI and FDG-PET), were not included among core diagnostic biomarkers (now restricted to amyloid PET and the CSF biomarkers) but only used for monitoring disease course or stage [22].

Since two amyloid biomarkers are included in the updated International Working Group (IWG-2) criteria [22], there is a need to understand if they give complementary information, or if they can be used interchangeably. A meta-analysis show very high concordance between CSF A β 42 concentrations (measured using different assay formats) and amyloid PET (several different ligands) for classifying cases as being either brain amyloid positive or negative [23]. For example, a study using CSF A β 42 measurements in clinical routine during two years compared concordance with ^{18}F -flutemetamol amyloid PET in an unselected cohort of patients with memory complaints,

and found a concordance (both CSF A β 42 and amyloid PET being either positive or negative) of 92–97% [24]. Furthermore, a study based on the ADNI-2 cohort showed CSF A β 42 alone gives the same diagnostic accuracy for prodromal AD or AD dementia as either global amyloid PET scores or regional (temporal, parietal, frontal or cingulate) SUV_r evaluations [25]. These findings suggest that CSF A β 42 and amyloid PET may be used interchangeably, and that regional PET assessments do not add to diagnostic accuracy.

However, the most common variant of discordance is cases being positive for CSF A β 42 but have negative amyloid PET scans [23]. One study examining the reason for this in more detail showed that discordancy is much more common in cognitively normal elderly and early MCI cases than in late MCI and AD dementia cases [26]. Furthermore, a recent study showed that non-demented elderly who are CSF A β 42 positive but amyloid PET negative builds up amyloid deposits in the brain to the same degree as cases who are positive for both biomarkers [27]. Taken together, these data suggest that CSF A β 42 detects cortical amyloid accumulation earlier than amyloid PET. This knowledge may be important in the clinical decision whether to perform CSF analysis or amyloid PET in patients with mild memory complaints and suspected early AD pathology. Lastly, while amyloid PET gives a static estimate of the amount of brain amyloid deposits, the addition of CSF T-tau and P-tau (to CSF A β 42 measurements) will provide an estimation on the rate of future cognitive decline and thus prognosis [28, 29].

NOVEL BIOMARKERS FOR SYNAPTIC DYSFUNCTION AND DEGENERATION

While we have well-validated CSF biomarkers for neurodegeneration as well as plaque and tangle pathology, there is no established or validated biomarker test for synaptic dysfunction or degeneration that is a key feature of AD pathophysiology. Synaptic degeneration and loss is one of the best pathoanatomical

correlates of cognitive deficits in AD and predicts disease better than A β plaque load [30, 31]. Several potential pathogenic mechanisms have been proposed to cause synaptic dysfunction with dendritic spine loss in AD, including A β fibrils [32], diffusible A β oligomers [33], intracellular A β accumulation [34], but also tau hyperphosphorylation and activation of microglia [35, 36].

It is clear that reliable biomarkers to monitor synaptic and dendritic function and loss directly in AD patients and cognitively normal elderly would be a very valuable addition to the AD diagnostic biomarker toolbox, but also in clinical trials to monitor pharmacodynamic effects of novel drug candidates on synaptic dysfunction and degeneration.

Neurogranin is a dendritic protein expressed in the cortex and hippocampus by excitatory neurons [37, 38], and has a key role in synaptic plasticity and induction of long-term potentiation (LTP) in the hippocampus [39, 40]. In the normal human brain, Ng expression is highest in associative cortical areas, while in AD, neurogranin levels are in the cortex and hippocampus [41, 42] reflecting the synaptic loss. Thus, measurement of neurogranin in CSF may give a biomarker reflecting dendritic instability and degeneration and thereby serve as the direct link to clinical symptoms in AD (Table 1).

In 1999, we showed that a number of synaptic proteins, including neurogranin, are secreted to the CSF [41]. A first pilot study using immunoprecipitation combined with Western blotting to evaluate CSF neurogranin as an AD biomarker showed a marked increase in AD as compared with controls [43]. After developing novel monoclonal antibodies to measure neurogranin by ELISA, high CSF levels were found to predict prodromal AD in MCI [44]. High CSF neurogranin in AD dementia and prodromal AD has been confirmed in several subsequent papers [45, 46], including in the ADNI study [47]. High CSF neurogranin also correlates with the future rate of hippocampal atrophy measured by MRI and rate of metabolic reductions on FDG-PET [47]. Interestingly, a recent study suggests that high CSF neurogranin may be specific for AD, and not found in other neurodegenerative disorders such as

frontotemporal dementia, Lewy body dementia, Parkinson disease, progressive supranuclear palsy, or multiple system atrophy [48].

Mass spectrometry characterization of neurogranin in CSF suggests that it is present in CSF as a series of C-terminal peptides [44], while a study combining immunoprecipitation and Western blotting identify a band in CSF corresponding to the full-length protein [43]. A study using an immunoassay based on combining N- and C-terminal neurogranin antibodies in a sandwich format, which in theory measures full-length neurogranin, also found high CSF levels in AD and MCI as compared with controls [49]. Thus, we need further studies on in which way neurogranin is processed before being released to the CSF, including studies comparing the diagnostic potential of full-length versus C-terminal neurogranin peptides.

BLOOD BIOMARKERS: THE HOLY GRAIL IN THE AD BIOMARKER ARENA

Both imaging biomarkers (PET imaging of amyloid and tau aggregates) and CSF measurement of amyloid and tau work well to identify AD pathophysiology. However, PET imaging is very expensive and only accessible in specialized centers, and thus unlikely to be a routine diagnostic tool in the assessment of the very high number of patients seeking medical advice for cognitive symptoms. Further, even if CSF collection is routine in clinical neurology, and the cost for the AD CSF biomarker tests are much lower than for EPT scans, lumbar puncture may be regarded as complicated, time-consuming and invasive by many clinicians. Thus, measurement of biomarkers in blood samples would be a more practical approach.

However, a major challenge in developing blood biomarkers is that brain-specific proteins reflect AD molecular mechanisms at much lower concentrations in blood than in CSF. As an example, the CSF level of the neuronal protein tau is around 2–300 pg/mL [50], while the plasma level is 100 times lower, around 5 pg/mL [51]. Except for requirements regarding analytical sensitivity, these very low levels must be

quantified within a matrix of other proteins (e.g., albumin and immunoglobulins) that are 10 billion times higher [52]. Importantly, technological developments have given ultrasensitive measurement techniques, such as the immuno-magnetic reduction (IMR) and Single-molecule array (Simoa) methods, that allow accurate analyses of biomarkers in blood samples [53].

Increased tau levels in plasma in AD has been found using both the IMR [54], and Simoa [51], methods. A recent study on the large ADNI and BIOFINDER cohorts confirmed an increase in plasma tau in AD dementia, although with a large overlap in levels with controls [55]. Further, longitudinal data from the analysis in ADNI showed significant associations between plasma tau and worse cognition, more atrophy, and more hypometabolism during follow-up. Longitudinal analyses in ADNI showed that higher plasma tau predicts future rate of cognitive decline, increases in brain atrophy measured by MRI, and reductions of more cortical glucose metabolism examined by FDG-PET [55].

While plasma and CSF tau levels correlate poorly [55], the correlation is very tight between plasma and CSF neurofilament light (NFL) protein [56], another axonal neuron-specific protein. Blood (serum or plasma) NFL can be accurately measured by an immunoassay developed on the Simoa platform [57]. The Simoa assay has 25- to 125-fold better analytical sensitivity than when the same anti-NFL antibodies are used in immunoassays based on the electrochemiluminescence (ECL) Meso Scale Diagnostics (MSD) technique or standard ELISA [57], meaning that NFL can be measured also in blood samples from normal individuals, which are below the level for accurate quantification when using ECL-MSD or ELISA methods.

Using the Simoa NFL assay, a recent study on the large ADNI cohort showed a marked increase in plasma NFL in AD and MCI patients as compared with controls, with a diagnostic performance comparable to the core AD CSF biomarkers [58]. Further, plasma NFL was highest in AD and MCI cases with positive amyloid PET scans, and correlated with poorer cognition and higher rate of future brain atrophy (measured by MRI) and hypometabolism as

measured by FDG-PET [58]. However, high plasma levels of NFL are also found in several other neurodegenerative disorders such as FTD and PSP [59, 60].

A possible future application for plasma tau and NFL is in the clinical evaluation of patients with cognitive disturbances in primary care. Here, plasma tau and NFL may serve as simple, non-invasive, and cheap screening tools to identify, or rule out, neurodegeneration. However, future large longitudinal studies are needed to determine the cut-off for positivity and the sensitivity and specificity to identify AD.

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Compliance with Ethics Guidelines. This article is based on previously conducted studies and does not involve any new studies of human or animal subjects performed by the author.

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