Applying fluid biomarkers to Alzheimer's disease

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Henrik Zetterberg is co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Venture-based platform company at the University of Gothenburg, and has served at advisory boards for Roche Diagnostics, Eli Lilly and Pharmasum Therapeutics.

Abstract

Alzheimer's disease (AD) is a common neurodegenerative disease that starts with a clinically silent phase of a decade or more during which brain pathologies accumulate predominantly in the medial temporal lobe but also elsewhere in the brain. Network dysfunction and clinical symptoms typically appear when senile plaque (amyloid β) and neurofibrillary tangle (tau) pathologies meet in the brain parenchyma, producing synapse and neuronal loss. For plaque and tangle pathologies, reliable fluid biomarkers have been developed. These require sampling of cerebrospinal fluid. Reliable blood tests for plaque and tangle pathologies are currently lacking, but blood tests for general neurodegeneration have recently been developed. In AD, plaques and tangles often co-exist with other pathologies, including Lewy bodies, and to what extent these contribute to symptoms, is currently unknown. There are also important differential diagnoses that may be possible to distinguish from AD with the aid of biomarkers. The scope of this review is fluid biomarkers for AD and related pathologies. The purpose is to provide the reader with an updated account of currently available fluid biomarkers for AD and clinically relevant differential diagnoses.

Introduction

Neurodegenerative dementias constitute a broad category of brain diseases that cause a long term and often gradual decrease in the ability to think and remember that is great enough to affect a person's daily functioning. The most common type of dementia is Alzheimer's disease (AD) that makes up 50% to 70% of the cases (98). AD causes a progressive loss of cognitive abilities with short-term memory impairment being the most typical initial symptom. However, there are also atypical clinical presentations of AD, *e.g.*, primary progressive aphasia or posterior cortical atrophy (52), and there are many other dementia-causing diseases that may be important differential diagnoses (70).

A dementia diagnosis is usually based on the history of the illness, the pattern of cognitive deficits, with investigations including, *e.g.*, blood work used to rule out other possible (non-cerebral) causes, and imaging both to rule out alternative diagnoses and to provide positive evidence for a given diagnosis. Specific dementia diagnoses can be made using clinical criteria that may be supplemented by information from biomarkers (20), but a definite diagnosis requires autopsy confirmation, based on the fact that each of the degenerative dementia-causing brain disorders is characterised by more or less distinct neuropathology (35). A striking feature is that most neurodegenerative dementias show aggregates or inclusions of specific proteins in the brain extracellular matrix or within neurons or other cell types of the brain (43). Some researchers have even classified them as "proteopathies" (90).

Neuropathologically, AD is characterized by neuronal loss in specific brain regions, intraneuronal neurofibrillary tangles composed of aggregated and often hyperphosphorylated tau protein, and extracellular neuritic plaques that are deposits of amyloid β (Aβ) peptides, mainly ending at amino acid 42 (7). Additionally, synapse loss (71) and microglial activation (89) have been suggested as integral, albeit non-specific, parts of AD pathology. Other neurodegenerative diseases that may cause AD-like symptoms include frontotemporal dementia (FTD), where tau and/or TDP-43 may form inclusions, Parkinson's disease dementia (PDD) and dementia with Lewy bodies (DLB), where α-synuclein inclusions are important parts of the pathology, and cerebral small vessel disease, where demyelination of subcortical brain regions is prominent. There is often also a considerable degree of multi-morbidity in neurodegenerative pathologies, suggesting that pathologically deposited proteins may interact and are influenced by other factors to promote cognitive decline and other clinical symptoms. Here, I discuss how biomarkers for different neuropathological changes

may help inform clinical decision-making and potentially also in the future help to personalize treatment. Table 1 summarizes replicated fluid biomarker findings in this context.

In regards to the biomarkers discussed, CSF indicates lumbar CSF collected according to published standard operating procedures (8); biomarker results derived from ventricular CSF may be quite different. Further, it is important how samples are collected, processed and stored, which is also detailed in published protocols (8). Regarding blood-based biomarkers, the sample matrix (plasma or serum) is specified wherever important. It should also be mentioned that the potential clinical context of use of the biomarkers discussed below is in a memory or neurology clinic. It is important to ensure that the patient has not had any acute CNS disease at least 3-6 months before sampling of the fluid, as for example a stroke, head trauma or meningitis may affect biomarker concentrations for this time window.

Fluid biomarkers for plaque pathology

CSF

The 42 amino acid isoform of amyloid β (Aβ42) is a major component of senile plaques in AD (51). It is a breakdown product of unclear physiological function, which is released from neurons when the type I transmembrane protein amyloid precursor protein (APP) is metabolized by β- and γ-secretases in synaptic vesicles (APP is metabolized by many cell types but Aβ42 secretion is by far the highest from neurons and seems to depend on synaptic activity (14)). Aβ42 can be measured in cerebrospinal fluid (CSF) by antibody-dependent techniques such as enzyme-linked immunosorbent assay (ELISA), as well as by antibody-independent techniques such as mass spectrometry (44). AD patients have decreased CSF concentrations of Aβ42, a finding that has been replicated and verified in hundreds of papers (62). This decrease reflects Aβ42 sequestration in senile plaques in the brain, as evidenced by both autopsy and *in vivo* amyloid positron emission tomography (PET) imaging studies (9). CSF Aβ42 concentration is fully altered already in mild cognitive impairment (MCI) as well as pre-clinical stages of AD (4, 62). A plaque pathology-associated decrease in CSF Aβ42 concentration is also seen in DLB, another disease characterized by cerebral Aβ aggregation (1).

Blood

It has been much more difficult to establish robust blood biomarkers for plaque pathology. Aβ proteins can be measured in plasma but the correlation with cerebral β-amyloidosis is absent or weak (statistically significant but clinically meaningless) (38, 61), and plasma Aβ concentrations are probably influenced by production in platelets and other extra-cerebral tissues (103). Pilot data suggest associations of the concentrations of a number of plasma proteins (*e.g.*, pancreatic polypeptide Y, IgM, chemokine ligand 13, interleukin 17, vascular cell adhesion protein 1, α2-macroglobulin, apolipoprotein A1 and complement proteins) with amyloid burden in the brain (12, 97, 100). However, these data should be interpreted with some caution, as they are derived from multi-marker panels and as a mechanistic understanding of the associations is currently lacking.

Fluid biomarkers for tangle pathology

CSF

Abnormally phosphorylated and truncated tau proteins are the major components of neurofibrillary tangles in AD and other so called tauopathies (26). The normal function of tau is to bind to and stabilize tubulin multimers in neuronal axons. Tangle-marked neurons release phosphorylated tau that can be measured in CSF by ELISA using antibody combinations specific against mid-domain phospho-tau epitopes. AD patients have increased CSF P-tau concentrations (62). CSF P-tau concentration correlates weakly with neurofibrillary tangle pathology in AD (11, 72); a finding that has been replicated in recent tau PET imaging studies (13), although the results are less clear than for CSF Aβ42. A major outstanding research question is why other tauopathies, including some forms of FTD and progressive supranuclear palsy, do not show P-tau increase, at least not as systematically as seen in AD. It is possible that these disorders show disease-specific tau phosphorylation, or that tau is processed or truncated in a way that is not recognized by available assays. Another potential explanation for the AD specificity of CSF P-tau is if the amount of pathology were simply greater in AD than in other tauopathies (there is to the best of my knowledge no published data addressing this hypothesis). CSF P-tau is currently considered the most specific biomarker for AD; except for herpes encephalitis (25) and superficial CNS siderosis (36, 42), no other condition shows systematic increase in this biomarker (104).

Blood

There are so far no reliable blood biomarkers for neurofibrillary tangle pathology, although there is an emerging literature on P-tau concentrations in neuronally derived blood exosomes with contrasting results in regards to association with AD (75, 99).

Fluid biomarkers for neuroaxonal degeneration

CSF

Total tau (T-tau), measured using assays with antibodies against mid-domain tau amino acid sequences that are not phosphorylated, can be used as a general marker of neuroaxonal degeneration/injury in AD. AD patients have increased CSF T-tau concentrations (62), and the higher the increase, the more intense neurodegenerative process (92). However, CSF T-tau increase is not specific for AD; it is also seen in, *e.g.*, Creutzfeldt-Jakob disease (CJD) (67) and following stroke (33). Similar results have been reported using CSF visinin-like protein 1 (VLP-1) and fatty acid-binding protein (FABP) that are enriched in neurons, but the associations with AD are less strong than for CSF T-tau (62). Neuron-specific enolase (NSE) has been proposed as another candidate biomarker for neuronal loss in AD, but the association with AD is variable (62) and the results are easily confounded by blood contamination, as NSE (in contrast to what its name implies) is highly expressed in erythrocytes (66).

Another CSF biomarker for axonal degeneration is neurofilament light (NF-L), which is a structural protein in long axons (102). CSF NF-L concentration is increased in AD and especially so in patients with rapid disease progress (105), but among the dementias, the highest concentrations are seen in FTD and vascular dementia (VaD) (18, 47, 76); a result that was recently confirmed in a large retrospective analysis of data from the Swedish Dementia Registry (77), as well as in atypical parkinsonian disorders (28, 49). As for T-tau, the highest CSF concentrations of NF-L are seen in CJD (80, 93).

Blood

CSF assays for T-tau and NF-L were recently developed into ultrasensitive blood tests using Single molecule array (Simoa) technology (2). Serum or plasma NF-L concentration (either sample matrix works well) correlates with CSF (correlation coefficients of 0.75 to 0.97) and

most CSF findings (increased NF-L concentrations in AD, FTD, VaD and atypical parkinsonian disorders) have been replicated in blood (102). For tau, the situation is promising but less clear. Firstly, for unknown reasons, tau concentrations are higher in plasma than in serum (unpublished observation). Secondly, the correlation with the corresponding CSF concentration is absent (106) or weak (54). Plasma T-tau concentration in AD is increased but less so than in CSF and there is no detectable increase in the MCI stage of the disease (54, 106).

Fluid biomarkers for synaptic pathology

CSF

Neurogranin (Ng) is a neural-enriched dendritic protein involved in long-term potentiation of synapses, particularly so in the hippocampus and basal forebrain. Recently, several independent studies have shown that the CSF concentration of Ng is increased in AD (31, 41, 45, 46, 85), but not in other neurodegenerative disorders (95), and that the marker predicts future cognitive decline, brain atrophy and reduction in glucose metabolism in prodromal disease stages (65, 83). Currently, CSF Ng is the best established CSF biomarker for synapse loss or dysfunction in AD, although there are other promising markers, including SNAP-25 and Rab3A, in development (5, 10).

Blood

There are so far no reliable blood biomarkers for synaptic pathology. Ng concentration in plasma is unchanged in AD (19).

Fluid biomarkers for microglial activation

CSF

Recent reports suggest that the CSF concentration of the secreted ectodomain of triggering receptor expressed on myeloid cells 2 (Trem2), a molecule that is selectively expressed on microglia in the CNS (48, 82) and genetically linked to AD (27, 39), is increased in AD in a disease-specific manner and correlates with CSF T-tau and P-tau (32, 64, 81). These results are backed by an abundant literature showing increased CSF concentrations of several other

microglia- and/or macrophage-derived proteins, including chitotriosidase (53, 94), CD14 (101) and YKL-40 (16, 60). Another microglial marker, the C-C chemokine receptor 2, is expressed on monocytes and one of its ligands, C-C chemokine ligand 2 (CCL2), that can be produced by microglia, is present at increased concentration in AD CSF (15, 23, 24). Most studies suggest that these increases are modest with large overlaps between cases and controls, if compared to the more prominent changes seen in traditional neuroinflammatory conditions, such as multiple sclerosis (58) or HIV-associated neurocognitive dysfunction (63). It should also be noted that most proteins mentioned above may also be released from activated astrocytes; microglial and astrocytic activation are difficult to tease apart using fluid biomarkers.

Blood

When measured in plasma or serum, the concentrations of most of the microglia-related proteins mentioned above are higher than in CSF and probably reflect release from monocytes and macrophages in peripheral blood rather than CNS-related changes. However, a few studies suggest a slightly increased plasma concentration of YKL-40 in blood from AD patients (61).

Fluid biomarkers for Lewy body pathology

CSF

α-Synuclein is the major component of Lewy bodies that are characteristic inclusions of Parkinson's disease (PD) and DLB (55) but often also seen in AD (69). In PD and other synucleinopathies, CSF α-synuclein concentrations are typically lower than in controls (29, 56), whilst in AD and CJD, the concentrations are increased and correlate with T-tau, suggesting that α-synuclein may also be an non-specific marker of neurodegeneration (56, 59, 79, 84, 96). This has been reported not only in AD and CJD, but also in DLB, where there may be a competition between aggregation of α-synuclein into Lewy bodies and release of the protein from degenerating synapses, making the data complex to interpret (40). Currently available assays for α-synuclein measure total amounts of the protein and not Lewy body-specific isoforms; sensitive and specific assays for the latter would resolve this issue. However, there are some preliminary reports on increased CSF concentrations of α-synuclein

oligomers in CSF from PD patients (30, 87) and very recently a sensitive assay that detects and amplifies the biochemical signal of seeds of α-synuclein oligomers in CSF was published, giving positive test results in 67 out of 76 PD patients, 10 out of 10 DLB patients and in eight out of 10 people with MSA (73). Additionally, 12 out of 97 non-PD controls tested positive, most of whom had AD (73), which might indicate concomitant AD and Lewy body pathologies.

Blood

α-Synuclein is highly expressed in red blood cells, a reason why blood contamination during CSF collection may limit the diagnostic value (3, 34). For the very same reason, blood tests for α-synuclein pathology in the brain may prove hard to develop. Nevertheless, as peripheral Lewy body pathology, *e.g.*, in the salivary gland and gut, has been reported in PD (88), blood or salivary tests for α-synuclein seeds may be something to explore in the future.

Fluid biomarkers for TDP-43 pathology

CSF

Hyperphosphorylated transactive response DNA-binding protein 43 (TDP-43) proteinopathy accounts for about 50% of FTD patients and has recently been described in aging and in association with cognitive impairment, especially in the context of AD pathology (37). TDP-43 can be measured in CSF but, unfortunately, most of the protein appears to be blood-derived and its CSF concentration does not reflect TDP-43 pathology and is unaltered in FTD (22).

Blood

No reliable blood test for TDP-43 pathology in the CNS exists.

Fluid biomarkers for blood-brain barrier (BBB) integrity

CSF

The BBB is the interface between the blood and the brain, regulating the transport of molecules between the blood and the central nervous system. Its primary function is to maintain the tightly controlled microenvironment of the brain, which is a critical part in sustaining a healthy nervous system. The most commonly used measure of BBB function in clinical laboratory practice is the CSF/serum albumin ratio (86). Proteins cross the BBB at different rates, depending on their hydrodynamic radii, with passage of larger proteins being more restricted than that of smaller proteins (21). As albumin is not produced in the CNS, CSF/serum albumin ratio can be used to assess the integrity of the BBB. A large number of studies have examined the CSF/serum albumin ratio in AD without finding any clear increase (61). In contrast, CSF/serum albumin ratio is increased in VaD, suggesting that cerebrovascular changes are associated with a leakier barrier (78, 91).

Blood

There are no established blood tests for BBB function, although a number of candidates do exist. One such protein is occludin, a 65-kDa integral membrane protein that contributes to tight junction stabilization at barriers (17). However, this protein is not specific to the brain, but also expressed at high levels in testis, kidney, liver and lung (68), which may explain why this marker, at least when examined in traumatic brain injury, has failed to produce interpretable results (74).

Increasing the interpretability of fluid biomarker test results by physiological studies in cell and animal models

When we try to relate concentrations of different proteins in human-derived biofluids to cellular and/or pathological changes in the CNS, we struggle to know if what we measure is a breakdown product of dying cells, a cellular reaction to a pathogenic exposure, what cell type is responsible for the biomarker signal and to what extent the measured change reflects increased production or decreased clearance. For example, we assume that increased T-tau concentration in lumbar CSF reflects neuroaxonal breakdown, but are currently failing to give an answer to why this increase appears rather AD-specific and is absent in most other neurodegenerative diseases. One potential answer comes from recent studies in disease

models, where it appears like neurons may respond to Aβ exposure by increasing their *secretion* of tau in the absence of frank neuronal death (50). Thus, extracellular T-tau concentration may be more of an Aβ response marker than a direct marker of neuroaxonal injury (the temporal disconnect of 5 years or more between onset of amyloid deposition and CSF T-tau increase could hypothetically be an indicator of differences between an inert build-up and a toxic breakdown/diffusion/leakage phase of Aβ pathology). Similar studies could potentially shed light on mechanisms by which concentrations of other biomarkers discussed in this review change in different diseases. Here, recent advances in the generation of neuronal cell models from stem cells may prove important (6, 57). Such models could easily be used to test the effects of exposure of neurons to disease-promoting agents and the release and concentration of biomarkers could be monitored over time and related to cellular markers of disease.

Concluding remarks

Three CSF biomarkers reflecting the core pathological features of AD have been established and are in common use in clinical neurochemistry laboratories worldwide: T-tau (broadly, but not exclusively, reflecting neurodegeneration), P-tau (reflecting tau phosphorylation and tangle formation) and Aβ42 (which inversely correlates with plaque pathology). According to revised clinical criteria, these markers may help diagnose AD more accurately and open up the possibility of detecting pre-dementia stages of the disease. A number of additional biomarkers for other pathologies common in AD and other neurodegenerative proteopathies do exist. In the future, such biomarker tests could be applied in longitudinal studies to sort out the temporal appearance of different pathologies during disease progression and assess how they may interact to produce clinical symptoms. As multi-morbidity appears common, one potential future scenario is that the biomarkers may be used to sub-classify clinical syndromes in individual patients according to their pathological signature and, hopefully, individualize treatment.

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762 **Table 1.** Replicated fluid biomarker candidates that correlate with AD-related
763 pathologies

Pathology	Biomarker	Biofluid	Direction of Change	Context of use
Plaque pathology	Aβ42	CSF	Decrease in AD	Clinical and research
Neurofibrillary tangle pathology	P-tau	CSF	Increase in AD	Clinical and research
Neurodegeneration	T-tau	CSF	Increase in AD	Clinical and research
		Plasma	Slight increase in AD	Research
	NF-L	CSF	Increase in AD	Clinical and research
		Plasma/serum	Increase in AD	Research
	VLP-1	CSF	Increase in AD	Research
	FABP	CSF	Increase in AD	Research
Synaptic pathology	Ng	CSF	Increase in AD	Research
Astroglial activation	sTREM2	CSF	Slight increase in AD	Research
	YKL-40	CSF	Slight increase in AD	Research
Blood-brain (blood-CSF) barrier impairment	CSF/serum albumin ratio	CSF/serum	Normal to slight increase in AD	Clinical and research

764 Abbreviations: AD, Alzheimer's disease; Aβ42, the 42 amino acid form of amyloid β;
765 P-tau, phosphorylated tau; T-tau, total tau; NF-L, neurofilament light; VLP-1, visinin-
766 like protein 1; FABP, fatty acid-binding protein; Ng, neurogranin; sTREM2, secreted
767 triggering receptor expressed on myeloid cells 2; CSF, cerebrospinal fluid.
768