# FLUID-BASED BIOMARKERS FOR NEURODEGENERATIVE DISEASES

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## INTRODUCTION

The prevalence of neurodegenerative diseases is expected to rise alongside the increase in life expectancy in most of the world. According to a publication from The Alzheimer's Disease International, the estimated global prevalence of dementia was 36 million in 2010 which rose to 43.8 million by 2016 and is estimated increase to 130 million by 2050.[1] The most common neurodegenerative disease is Alzheimer's disease (AD), accounting for 60-70% of dementia cases.[2,3] This is followed by vascular dementias which account for up to 20% of dementia patients.[3] Less common causes of dementia include frontotemporal dementias (FTD) and dementia with Lewy Bodies (LBD). The second most common neurodegenerative disease is Parkinson's disease (PD), with an estimated global prevalence of over six million.[2]

The diagnosis and management of patients with dementia presents challenges as the underlying cause of disease can clinically present in a manner that masks its true nature or reflect influence of confounding comorbidities.[3] Moreover, misdiagnosis can lead to suboptimal care and treatment. Therefore, it is important to have reliable biomarkers that increase the accuracy of clinical diagnosis. Additionally, accurate and timely diagnosis of neurodegenerative diseases in the clinical setting provides the ability to optimize treatment strategies and allows patients the opportunity to join appropriate clinical treatment trials.

It is becoming increasingly common for patients to seek earlier medical attention with the onset of mild cognitive impairment (MCI) - characterized by impairment in a single cognitive domain (usually memory), or moderate impairment in several cognitive domains, but without functional impairment sufficient to meet the criteria for dementia.[4] Nearly 30 -50% of patients presenting with MCI have incipient AD, however the underlying etiology is difficult to determine without biomarkers.[2] In regard to PD, while the accuracy of a clinical diagnosis is around 80%, correctly identifying other parkinsonian disorders is more difficult.[2,5,6] Therefore, specific biomarkers for neurodegenerative indications are needed to improve the diagnostic workup of these diseases and allow for the treatment effect monitoring and repeated longitudinal tracking of disease progression over the course of clinical trials. The following biomarker sections detail current fluid-based biomarkers for various neurodegenerative disease pathologies.

## CURRENT BLOOD AND CSF-BASED BIOMARKERS FOR NEURODEGENERATIVE DISEASES

### Biomarkers of Amyloid Beta Pathology

Amyloid beta (Aß) plaques formed within the extracellular space of telencephalic structures are one of the major neuropathological features in In AD.[7] Studies using positron emission tomography (PET) have shown that Aß accumulation starts ~ 20 years before the onset of AD dementia.[7,8]

Different Aß species can be reliably measured in cerebrospinal fluid, including Aß40 and Aß42. Before Aß -PET shows abnormal findings, CSF levels of AB42 are decreased (~50%) in AD.[9] Using Aß42 to Aß40 ratios (Aß42/ Aß40) results in high concordance with

Aß -PET (primarily above 90%).[10] Moreover, CSF Aß42 measurements when used in conjunction with Aß40 or phosphorylated tau, (P-tau), can be used to predict the development of AD dementia in patients with mild cognitive impairment with high accuracy.[11]

Current clinical routine Aß measurement procedures are based on enzyme linked immunosorbent assays (ELISA) or immunoassays on other technology platforms. Recently, reference materials based on human CSF that are certified for the mass concentration of Aß42 became available and are used to ensure equivalence of results across methods and platforms.[12] Additionally, an international consensus protocol for the collection and handling of CSF has been established to avoid false abnormal values due to confounding preanalytical factors.[13]

However, while Aß biomarkers in CSF are already in clinical use, the collection procedure (lumbar puncture) is invasive and is not widely available outside of specialist centers. Therefore, considerable effort has been made to develop blood-based assays for measuring cerebral Aß pathologies.[2] As the levels of these markers in plasma/serum are expectedly lower than those found in CSF, ultra-sensitive detection methodologies are required for quantification in this matrix. In cerebral Aß pathology, plasma levels of Aß42/ Aß40 are decreased by only 10–20% while CSF levels are decreased by 40–60%.[14-16] This is most likely due to the production of Aß outside of the brain that may affect plasma levels. As such, CSF Aß42/ Aß40 ratios show higher diagnostic accuracy as compared to plasma levels. However, combinations of plasma Aß42/Aß40 with other plasma CNS markers such as P-tau and GFAP, have shown strong associations with cerebral Aß pathology.[17,18]

## Biomarkers of Tau Pathology

In addition to Aß accumulation, neurofibrillary tangles of tau protein are well-known neuropathologic characteristic of AD. Several different soluble species of tau can be quantified in CSF.[2] Some of these include total tau, tau phosphorylated at threonine 181 (p-tau181), and tau phosphorylated at threonine 217. While total tau (T-tau) concentration is increased in AD patients, T-tau is not AD specific as it is also found markedly increased in Creutzfeldt-Jacobs disease (CJD) and temporarily increased with brain trauma or stroke.[19]

On the other hand, p-tau (p-tau181 and p-tau217) has been shown to be selectively increased in AD 20 and correlated with the formation of neurofibrillary tangles in the brain.[21,22] When combined with CSF Aß42, CSF levels of T-tau and p-tau181 increase the diagnostic validity for AD with a combined sensitivity and specificity of 85–90% 23 and predict the cognitive decline in patients with MCI.[24] Specifically, CSF p-tau181 has been found to be notably useful at differentiating AD from other dementias, including Lewy body disease (LBD), frontotemporal dementia (FTD), and vascular dementia.[25] Recently, it has been shown that CSF p-tau217 displays a moderately stronger association with tau-tangle load and disease severity and may distinguish AD dementia from other dementias with even higher accuracy than P-tau181.[26,27]

While tau biomarkers are commonly assessed in CSF, ultrasensitive assays that can reliably quantify T-tau and p-tau in blood have recently been developed. In addition to the minimally invasive collection process for plasma/serum samples (as opposed to CSF), clinic-based cohort studies have shown that plasma p-tau (181 and 217) can differentiate AD dementia from other neurodegenerative diseases with high accuracy.[2] Plasma p-tau217 performed alongside CSF AD biomarkers and tau-PET imaging exhibits a diagnostic accuracy of around 90%, 28 while increased plasma p-tau181 levels have been associated with ensuing cognitive decline and conversion to AD dementia in patients with MCI.[29,30] Moreover, longitudinal studies have demonstrated that plasma p-tau217 increases during early AD and continues to increase over time in both preclinical and prodromal stages of AD, indicating its potential use in the objective monitoring of AD progression.[31] Finally, plasma levels of P-tau181 and P-tau217 were shown to be associated with the amount of Aß plaques (early AD stages) and tau-tangles (later AD stages) in recent neuropathology-based and PET-based studies.[32] Additionally, plasma p-Tau and p-Tau/ Aß42 ratios have been used to predict cognitive and executive function decline in levodopa treated Parkinson's disease patients.[33]

## Biomarkers of Alpha Synuclein Pathology

CSF oligomeric α-synuclein is found to be consistently higher in PD patients when compared with healthy controls.[35] Additionally, several studies have found that phosphorylated a-synuclein is increased in CSF from PD patients when compared to healthy controls. However, the diagnostic accuracy for these α-synuclein species when used alone was found to be unsatisfactory for potential use in clinal practice.[34] An increase in diagnostic accuracy was observed when combined with other neurodegenerative markers (ratio of oligomeric α-synuclein to total α-synuclein, together with phosphorylated α-synuclein and phosphorylated p-tau).[36] Due to the very low concentrations of phosphorylated α-synuclein in CSF, mass spectrometry remains one of the primary methods for quantifying this biomarker.[34]

Seeding assays such as Protein-Misfolding Cyclic Amplification (PMCA) and Real-Time Quaking Induced Conversion (RT-QuIC) that have been developed to indirectly reveal misfolded prion protein and other prion-like proteins – can be used to detect pathogenic a-synuclein aggregates in CSF.[2,34]

Recent studies have evaluated the clinical use of quantifying α-synuclein in peripheral blood samples from PD patients using an ultrasensitive single molecule array technology (SIMOA by Quanterix)[37] and an immunomagnetic reduction (IMR) assay,[38] with both studies concluding that peripheral blood α-synuclein levels may differentiate between healthy controls and patients with PD. However, given the considerable overlap with controls and other neurodegenerative diseases, the use of α-synuclein alone as a single biomarker is not presently sufficient as a diagnostic marker in clinical practice and trials and therefore must be used in conjunction with other documented biomarkers.[34]

## Biomarkers of Neurodegeneration

Several biofluid markers for neurodegeneration have come to light. Of note, neurofilaments have been studied extensively in neurodegenerative indications and are useful markers of acute and chronic neuronal injury.[39,40] Neurofilament light (NfL) and Phosphorylated neurofilament heavy chain (pNFH) proteins have been extensively studied as diagnostic and prognostic indicators of ALS. The protein pNFH is released in the CSF and subsequently into blood as a result of axonal damage and has been found to be advantageous over other biomarkers for ALS disease identification due it its high specificity for neuronal cell damage.[40]

Neurofilament light chain (NfL) can also be measured in CSF and blood and reflects axonal degradation, regardless of cause - with elevated levels observed in ALS, FTD, and atypical parkinsonian disorders.[2,40] Additionally, NfL levels are also elevated in AD with studies demonstrating that NfL levels in CSF and serum correlate with one another and are increased during presymptomatic stages of autosomal dominant AD.[41] Notably, NfL can serve as an indicator of the intensity of ongoing neurodegeneration as increased levels of NfL are associated with faster disease progression and higher brain atrophy rates in most neurodegenerative indications.[41,42] Recent studies have supported a role for plasma and CSF NfL levels as a treatment response biomarker for multiple sclerosis and spinal muscular atrophy, respectively, with reductions associated with the clinical effectiveness of the drug.[43,44]

CSF levels of the postsynaptic neurogranin protein serve as a potential biomarker of neurodegenerative disease as this analyte has been shown to be selectively increased in AD patients and can predict future cognitive decline.[45-47] Synaptosomal associated protein (SNAP25) has also been labeled as a potential biomarker of synaptic dysfunction and neurodegeneration. Increased CSF levels of SNAP25 were found in AD patients versus healthy controls[48] and may predict progression to AD in patients with MCI.[49] Finally, CSF Visinin-like protein-1 (VILIP-1) is also found elevated in AD patients as compared to healthy controls[50,51] and has demonstrated potential utility as a marker of neuronal injury. CSF VILIP-1 and the ratio to Aß42 (VILIP-1/Aß42) were found to predict rates of global cognitive decline similarly to tau and the tau/Aß42 ratio.[52]

## Biomarkers of Oxidative Stress

Oxidative stress due to increased levels of reactive oxygen and nitrogen species, together with compromised antioxidant defense mechanisms, is prominent in many neurodegenerative diseases[53]. Elevated concentrations of MDA and NME have been reported in the plasma and cerebrospinal fluid (CSF) of PD patients[54] and increased levels of 8-OHdG and nitrotyrosine have been observed in the serum and leukocytes of HD patients.[53,55,56]

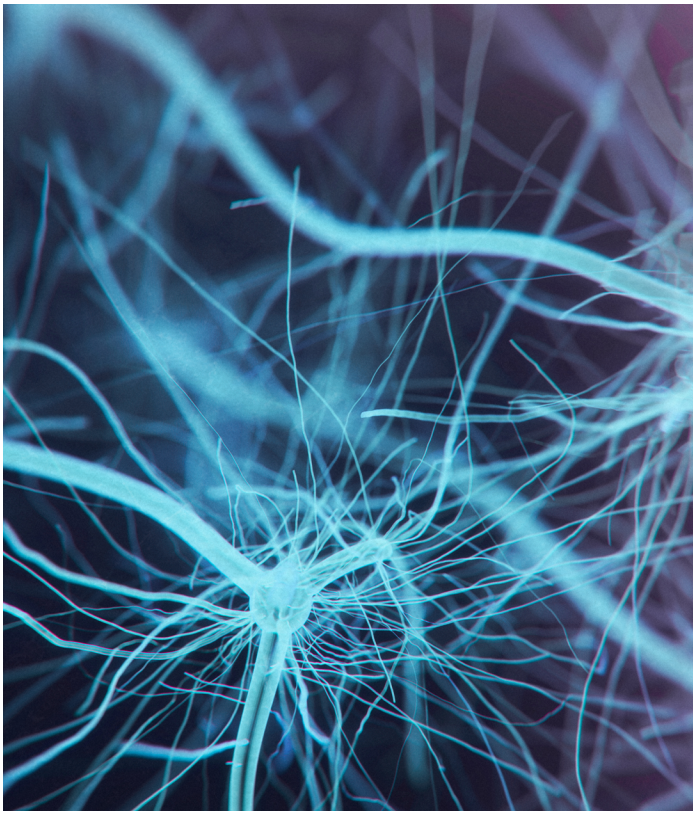
Additionally, about 20% of familial ALS patients carry mutations in the superoxide dismutase (SOD) gene, resulting in the production of ROS.[53,57] The overall level of oxidative damage can be evaluated by monitoring the oxidative stress marker, MDA, in blood and CSF using the TBARS (thiobarbituric acid-reacting substances) assay.[53]

DJ-1 is a multifunctional protein associated with several cellular processes. Loss of function PARK7 gene mutations, the gene that encodes DJ-1, are linked to familial forms of PD and lead to oxidative stress.[2,53] As such, plasma levels of DJ-1 have been evaluated in PD patients in addition to other oxidative stress-related biomarkers such as uric acid.[58] Urate is a strong antioxidant that may exert protective activity towards the development of PD as elevated levels of serum uric acid conferred a lower risk of developing PD.[58-60]

## Biomarkers of Microglia Activation, Neuroinflammation, and Blood-Brain-Barrier Dysfunction

Neuroinflammation is a pathological feature of a wide range of central nervous system (CNS) diseases including neurodegenerative diseases.[61] Microglia and astrocytes are primary cellular drivers and regulators of neuroinflammation. Different blood and CSF molecules have been proposed as reliable markers for glial cell activation within CNS. These include markers of reactive astrocytes (glial fibrillary acidic protein (GFAP)), microglia activation (soluble triggering receptor expressed on myeloid cell 2 (sTREM2)), microglial mobilization (YKL-40, chitinase-3-like protein 1), and astrocytic activation (monocyte chemoattractant protein 1 (MCP-1)).[2,61]

Plasma levels of GFAP were found to be increased in FTD caused by progranulin mutations[62] and individuals with Aß pathology.[17] Moreover, plasma GFAP levels served as a predictive factor for future cognitive decline and conversion to AD dementia in cognitively unimpaired individuals.[63] Additionally, it has been shown that microglia are activated during the preclinical stage of AD as indicated by increased levels of CSF sTREM2, with increased levels of MCP-1 and YKL-40 observed in the later phase of the disease.[64] Finally, neuroinflammation panels capable of providing multiplex analysis of cytokines and chemokines in CSF are available on multiplexing platforms offered by Mesoscale Discovery (MSD®) and Luminex®.



Disruption of blood-brain barrier integrity is observed in several neurodegenerative diseases. This disruption is largely observed through specific MRI techniques that depend on the leakage of gadolinium-based contrast reagents into the brain.[65] However, measurement of fluid biomarkers has also been utilized. The ratio of CSF albumin to plasma albumin has been shown to be elevated in non-AD dementia disorders and used to demonstrate the permeability of the blood-brain barrier.[66] More recently, CSF levels of soluble PDGFRß (sPDGFRß) has been proposed as a marker of capillary pericyte damage and BBB leakiness, with elevated levels associated with cognitive decline and AD pathology.[67]

## Genetic Biomarkers

AD is classified into two subtypes based on the age of disease onset: early age onset which accounts for approximately 1-5% of AD cases (EOAD, before the age of 65 years) and late onset (LOAD, after the age of 65 years).[58] EOAD is frequently caused by mutations in the amyloid precursor protein (APP), Presenilin 1 (PSEN1), or Presenilin 2 (PSEN2).[68] In the majority of LOAD cases, AD is assumed to be developed due to a combination of genetic variants contributing to

disease formation, in addition to other factors such as environmental exposure, lifestyle, and aging. Currently, the E4 allele of the apolipoprotein E gene (APOE) is the most characterized risk factor for LOAD.[58] Specifically, of the three APOE alleles (E2, E3, and E4) one copy of the E4 allele increases the risk of AD by four-fold while two copies increase the risk by 12-fold.[69] APOE mutation detection and genotyping can be routinely performed in central and reference laboratories on DNA extracted from EDTA-anticoagulated whole blood via sanger sequencing.

In PD, approximately 15% of patients have a family history of the disorder caused by mutations in the following genes: LRRK2, SNCA, PARK2, PARK7, GBA, or PINK1. More recently, RNA-based biomarkers (miRNAs) have been found as potential markers of PD. These include the miRNAs, miR-7 and miR-153, which were found to modulate α-synuclein mRNA.[58]

## CONCLUSION

Over the past two decades considerable advancement has been made regarding fluid-based biomarker research in the field of neurodegeneration. As clinical trials of potential disease-modifying therapeutics targeting neurodegenerative disorders have shifted focus towards the preclinical and early stages of these diseases, accessibility to sensitive and specific biomarkers that could be used for initial screening phases within a multistage diagnostic process for disease differentiation, or could aid in earlier diagnosis, subject selection, and disease tracking has become of paramount importance.[72]

Most progress has been made with CSF biomarkers, with lumbar puncture considered to be a safe and generally well-tolerated procedure, and an international consensus protocol for the collection and handling of CSF has been established.[13] However, CSF use may be limited by contraindications such as the use of anticoagulants, subject non-compliance, and the availability of this procedure at sites outside of specialist centers.[72,73] As such, measurement of CNS biomarkers in more accessible biofluids, such as serum and plasma, would provide a less invasive means for sample material collection and would facilitate access to repeated longitudinal samples for tracking disease progression.[72] Additionally, collection of serum/plasma is possible for sites outside of specialist centers.

As a result of the selectivity of the BBB which prevents the free passage of molecules between the CNS and blood compartments and affects the blood to CSF volume ratio,[50] the concentration of CNS-derived proteins in blood is much lower than that found in CSF. This concentration difference has made it difficult to detect CNS proteins in blood samples using standard ELISA assays. Fortunately, in recent years several ultrasensitive immunoassays with superior analytical sensitivity have been developed, such as the automated single molecule array (SIMOA) by Quanterix, which has allowed for the reliable detection of CNS biomarkers in peripheral blood samples (biomedicines paper). At the present time the following analytes Aß40, Aß42, BDNF, GFAP, NfL, α-synuclein, P-tau 231, P-tau 181, total tau and SNAP-25 can be measured by the SIMOA technology in peripheral blood samples. Recently, the ultrasensitive SIMOA technology was utilized by Eli Lilly to measure p-tau 217 using their proprietary antibodies and a licensing agreement was made to provide Quanterix access to Lilly's P-tau 217 antibody technology.[74] Medpace Reference Laboratories can support these biomarker assays and help further the clinical development of disease-modifying neurodegenerative disease pharmaceuticals.

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