

Biomarkers for Neurodegenerative Diseases

Hai Ha Nguyen

Ph.D. candidate in Neuroscience Program, Faculty of Medicine, Khon Kane University

Abstract

Neurodegenerative disorders, especially Alzheimer's disease (AD) and Parkinson's disease (PD), are global challenges recently, with an increasing incidence and high expenses for treatment. The key pathophysiological characteristics are the accumulation of amyloid beta ($A\beta$), tau protein in AD and α -synuclein (α -syn) in PD. Pre-clinical stages of neurodegenerative diseases have been progressing for a long period prior to the appearance of specific symptoms. It is crucial to develop accurate and reliable biomarkers to improve early diagnosis and treatment that can extend the preclinical stage of patients. Because magnetic resonance imaging (MRI), positron emission tomography (PET) imaging, and cerebrospinal fluid (CSF) biomarkers are invasive or expensive, blood and salivary biomarkers that are widely available and reasonable in cost have been developed recently. This article advances our understanding of the current biomarkers for neurodegenerative disorders and their promising application in clinical practice and trials.

Introduction

Neurodegenerative diseases are a dominant etiology of cognitive decline in the elderly, and their prevalence is increasing in most countries. In 2015, around 50 million individuals globally reported having dementia, and this figure is predicted to increase to 131.5 million in 2050, which significantly raises the burden of disability, illness, and medical expenses¹. AD is

the most prevalent kind of dementia globally, around 60-80% of dementia patients². People with AD often experience memory impairment, which is followed by additional cognitive symptoms such as language difficulties and executive and visuospatial function impairments. Although acetylcholinesterase inhibitor therapy can enhance cognitive function, it cannot stop the disease's progression.

Extracellular amyloid beta ($A\beta$) plaque accumulation and intracellular Tau fibril-based neurofibrillary tangles are primarily pathophysiological characteristics of AD³. $A\beta$ plaques initially form in the basal isocortex (frontal, temporal, and occipital), then extend to the association cortices, subcortical regions (striatum, thalamus, and hypothalamus), and ultimately the primary sensorimotor areas. Tau fibrils are firstly found in the entorhinal cortex, then spread to the hippocampus and other paralimbic regions (amygdala, basal forebrain nuclei, anterodorsal thalamic nuclei) as well as mesial temporal and parietal/retrosplenial isocortex, eventually reaching the prefrontal areas³. In AD, medial temporal lobe pathologic alterations first appear, then extend to the neocortex^{4,5}, with the changes occurring decades prior to clinical symptom onset^{6,7}. AD progression is classified into 3 stages: pre-clinical stage, prodromal stage or mild cognitive impairment (MCI stage), and clinical stage or dementia⁸. 10-20% of estimated patients with MCI develop AD each year⁹. In other neurodegenerative

Received: September 9th, 2022, Revised: September 16th, 2022, Accepted: September 17th, 2022.

disorders, such as progressive supranuclear palsy (PSP), frontotemporal dementia (FTD) and corticobasal degeneration (CBD), Tau concentrates in different structures, and is not associated with A β pathology¹⁰.

With an annual incidence of 5-35/100,000 new cases, PD is the 2nd most prevalent neurodegenerative disorder following AD¹¹. Bradykinesia, resting tremor, muscle rigidity, postural and gait abnormalities are main motor-symptoms of this movement disorder. Dopaminergic neuronal degeneration in the pars compacta of the substantia nigra is related to symptoms of PD. Non-motor symptoms are hyposmia, rapid eye movement sleep behavior disorder (RBD) and constipation, which generally occur 5-10 years before motor symptoms and are not related to dopamine loss¹². The primary pathological characteristic of PD is the accumulation of α -syn in Lewy bodies and Lewy neuritis¹³. Similar to the classification of AD, there are three stages in the progression of PD: preclinical stage (from onset to appearance of nonmotor symptoms), prodromal stage (non-motor symptoms), and clinical stage (motor symptoms)¹⁴. Dementia with Lewy bodies (DLB) and multiple system atrophy (MSA) are synucleinopathies similar to PD, while PSP and CBS are categorized as tauopathies¹⁵.

The necessity of biomarkers for neurodegenerative diseases

Because most specific neurons have degraded and brain neuroplasticity has been diminished by the manifestation of specific symptoms, diagnosis of neurodegenerative disorders is carried out a long time after the onset^{16, 17}. Early diagnosis (preclinical stage) and preventa-

tive therapy are able to extend the preclinical stage of patients¹⁸. The rate of misdiagnosed AD is high at specialized dementia clinics (25–30%), and this rate is higher for other dementias, such as vascular dementia, FTD, DLB¹⁹⁻²¹. In primary care, misdiagnosis occurs much more frequently²². In the case of PD, because of clinical overlap with other etiologies of parkinsonism, the rate of misdiagnosis in PD is also high, up to 20%²³. Dopaminergic neuronal degeneration in the substantia nigra reaches nearly 50% before the first motor symptoms, so the preclinical and prodromal stages provide the best therapeutic window^{24,25}. The benefits of optimal therapeutic interventions are diminished by delayed diagnosis and misdiagnosis. As a result, it is crucial to find and recognize precise and valid biomarkers for the early diagnosis of neurodegenerative disorders.

A biomarker is defined as “a measurable indicator of some biological state or condition that is objectively measured and evaluated to examine normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention”²⁶. Based on their functional features, biomarkers are categorized into: pathophysiological biomarkers representing the correlation with the pathology of diseases; diagnostic biomarkers including clinical diagnosis and differential diagnosis; and prognostic biomarkers showing the prediction of development from preclinical or prodromal to clinical stage^{27,28}. Overall, biomarkers are crucial for the diagnosis of neurodegenerative disorders, particularly during the early stages. Early and accurate diagnosis is important in supporting the optimization of therapeutic treatment.

Biomarkers that are easily accessible, inexpensive, and precise have the promise of being widely used in clinics, especially in primary care²⁹.

Imaging biomarkers

Structural MRI is an inexpensive and extensively utilized neuroimaging technique for supporting the diagnosis of AD³⁰. MRI is thought to be a marker that can identify abnormalities in the later stages of neurodegenerative disorders³¹. Patients with AD mostly have hippocampal atrophy (sensitivity-Sn 95%; specificity-Sp 92%) as well as entorhinal cortex atrophy (Sn 90%; Sp 94%) compared to the normal cognitive elderly. When AD is distinguished from non-AD dementias, these figures reduce to 79 and 81 %, respectively³².

FDG-PET (18F-fluorodeoxyglucose-PET) is a method of functional neuroimaging that measures the brain's glucose metabolism rate, which frequently decreases before structural brain alterations are visible³³. Neurodegeneration can be observed as decreased glucose absorption in the temporoparietal and posterior cingulate cortex in AD or in amnesic MCI who later developed AD³⁴. When distinguishing AD from healthy elderly people, FDG-PET has a high sensitivity and specificity (> 90%). When it is used for distinguishing between AD and non-AD dementias, specificity drops to 78%³². FDG-PET has also been shown to be superior than structural MRI in predicting the transformation to AD in people with MCI³⁵.

In PD, neurochemical alterations of the dopamine system are evaluated by dopamine transporter single-photon emission computed tomography (DAT-SPECT) and fluorodopa-PET

(F-DOPA PET)³⁶. Dopamine in the basal ganglia decreases in PET scans of patients with PD, MSA, PSP and CBD³⁷. Many studies have reported that DAT-SPECT has good diagnosis accuracy for PD subjects (Sn 79-100% and Sp 80-100%)^{36,38}. PET can be utilized to diagnose PD in the prodromal period by demonstrating the decrease of dopamine production, absorption, vesicular storage, and secretion in the nigrostriatal dopaminergic pathway³⁹⁻⁴¹.

CSF biomarkers

Because of its direct interaction and close relationship with the brain, CSF is thought to be a reliable biomarker for the evaluation of people with AD^{42,43}. Moreover, lumbar puncture is a common and well-tolerated technique with few complications⁴⁴. Although CSF collection is an invasive procedure and can cause anxiety and fear in the patient, it can reveal crucial information reflecting the biochemical alterations in the preclinical stages of AD brain⁴⁵.

CSF amyloid

A major constituent of extracellular plaques is A β peptides, formed by the sequential cutting of β -amyloid precursor protein (APP) by β -site amyloid precursor protein-cleaving enzyme 1 (BACE1) and γ -secretase^{46,47}. Among the forms of A β peptides released, A β 42 is a main element of the plaques found in the AD brain⁴⁸. According to research, A β 42 levels in the CSF correlate inversely with plaque accumulation in autopsy or PET^{49,50}.

The abnormality of A β 42 is first recognized in the CSF during the earliest stages of neurodegeneration, prior to the amyloid PET⁵¹. This implies that A β 42 in CSF is a more sensitive

biomarker for AD detection at very early stages and that amyloid-PET could be utilized to grade early AD stages more accurately²⁸. While cut-off values fluctuate among laboratories, a 50% reduction in A β 42 concentration in the CSF reveals its accumulation in the brain parenchyma of AD patients^{42, 52}. Hulstaert et al. reported that using A β 42 alone to distinguish AD patients from elderly controls had a Sn of 78% and a Sp of 81%^{53, 54}. However, these figures decrease to Sn 73% and Sp 67% when distinguished from non-AD dementias³². In MCI patients, decreased CSF A β 42 has a low predictive accuracy for AD progression (Sn 81% and Sp 64%). Therefore, it is not suggested to use CSF A β 42 alone to diagnose AD pathology in MCI patients⁵⁵. Additionally, in other neurodegenerative disorders, A β 42 is also reduced in DLB and PD dementia but is normal in FTD patients⁵⁶.

In the case of differences between CSF biomarker characteristics, the CSF A β 42/A β 40 ratio has been considered as a potential differentiating biomarker for AD. Lewczuk et al. reported that the CSF A β 42/40 ratio had a stronger correlation with amyloid-PET than A β 42, (area under the ROC curve - AUC 0.936 and 0.814, respectively)⁵⁰. In addition, the CSF A β 42/A β 40 ratio was also found to distinguish AD from other types of dementias, with a Sn and Sp of 85% and 82%, respectively⁵⁷. The effectiveness of the CSF A β 42/A β 40 ratio could be related to its ability to modulate differences among individuals in: total A β synthesis or release from neurons; CSF synthesis and clearance; and pre-analytical processing of CSF that influenced A β 40, A β 42⁵⁰.

CSF tau

Tau proteins are members of the microtubule-related molecule family, which can be detected in both neuronal and non-neuronal cells^{58, 59}. Phosphorylation of tau can take place at more than 40 different sites⁶⁰. Many studies have been conducted on CSF total tau (t-tau), tau phosphorylated at threonine 181 (p-tau181) and at threonine 217 (p-tau217)^{60, 61}. According to a meta-analysis, all research demonstrated that CSF t-tau and p-tau elevated in AD⁶¹, with cut-off values of 300% and 200% elevated levels of t-tau and p-tau respectively^{42, 52}. Moreover, t-tau or p-tau increases are not only found in primary tauopathies⁶² but also reflect tau metabolism changes induced or mediated by A β ⁶³. CSF p-tau concentrations associate with brain tau accumulation on tau-PET as well as with A β deposition measured on PET or CSF^{60, 64}. CSF p-tau217 is reported to have a higher association with tau level and severity of disease than p-tau181⁶⁰.

CSF t-tau increases in AD but also in other disorders like stroke and Creutzfeldt–Jakob disease, so t-tau is non-specific for AD. In contrast, p-tau is demonstrated to be a more specific CSF biomarker than t-tau for the AD pathological process^{65, 66}. Bloudek et al. investigated using CSF to differentiate AD from healthy people and found results that sensitivity of 82% and specificity of 90% for t-tau, and sensitivity of 80% and specificity of 83% for p-tau. Nevertheless, when it comes to distinguish AD from non-AD dementias, these sensitivity and specificity reduce to 79% and 80% for p-tau, 78% and 75% for t-tau, respectively³². Many studies have revealed that CSF p-tau181 concentration

is significantly higher in AD than in FTD, DLB, PD, or MSA patients, and can be a biomarker to discriminate AD from these diseases⁶⁷⁻⁶⁹. Additionally, CSF p-tau217 may be used to differentiate AD from non-AD dementias with greater precision than p-tau181^{60,70}.

Early diagnosis of AD in the prodromal stage (MCI stage) is crucial because novel disease-modifying medications are most effective when started in the early stage. Buchhave carried out a study to assess the prediction of CSF p-tau in the progression of AD in MCI patients after 9-10 years. Patients who transformed to AD had lower CSF A β 42 at baseline and higher p-tau concentrations than non-converters. In addition, early converters (0-5 years) had significantly higher CSF p-tau concentrations than late converters (5-10 years). The A β 42/p-tau ratio at baseline predicted the conversion to AD in 9.2 years (Sn 88% and Sp 90%)⁷¹. Therefore, some authors suggest that using CSF p-tau combined with A β 42 instead of CSF A β 42 alone for predicting the conversion of AD in MCI patients^{71,72}.

CSF α -synuclein

The pathological characteristics of PD are thought to include α -syn misfolding and accumulation in Lewy bodies, which are related to dopaminergic neuron loss⁷³. Because of the prominent role of α -syn in PD pathology, CSF α -syn concentration has received the most concern as a potential biomarker. In comparison with healthy controls, many studies consistently reported decreased CSF total- α -syn (t- α -syn) concentrations in PD patients⁷⁴⁻⁷⁶. Other α -synuclein forms, including oligomeric α -synuclein folding Cyclic Amplification (PMCA) and the

(o- α -syn) and phosphorylated α -synuclein (p- α -syn), have been investigated as promising biomarkers for PD^{77,78}. CSF o- α -syn and o- α -syn/t- α -syn ratio have been evaluated and revealed to be increased in PD subjects. Moreover, the increase in o- α -syn/t- α -syn was related to motor impairment, especially in PD with gait disorders and postural instability subgroups⁷⁹. In the BioFIND cohort, Protein MisReal-Time Quaking-Induced Conversion (RT-QuIC) assay were utilized to evaluate o- α -syn in discriminating PD from controls with AUC 0.93, Sn 95.2%, Sp 89.9% for PMCA and AUC 0.89, Sn 96.2%, Sp 82.3% for RT-QuIC⁸⁰. Other research applied the RT-QuIC assay for detecting α -syn to discriminate Lewy body related disorders with a Sn of 95.3% and a Sp of 98%⁸¹. Singer et al. reported that applying PMCA to o- α -syn had a high separation of MSA from PD/DLB patients⁸². However, neither PMCA nor RT-QuIC can discriminate between different synucleinopathies. Although α -syn biomarker is crucial in PD pathogenesis, it does not significantly influence in the early diagnosis of PD. The current methods for the improvement of early diagnosis are based on the identification of pre-motor symptoms and nigrostriatal dopaminergic system failure on PET during the prodromal phase, which corresponds to 10 years before motor symptom onset⁸³⁻⁸⁵. Non-motor symptoms of PD, which are constipation, hyposmia and REM sleep behavior disorder, are considered potential biomarkers in the identification of prodromal PD⁸⁶. A study found that RBD is significantly related to PD, with a 45% risk of PD development after 5 years and a 76% risk after 10 years⁸⁷. In the RBD

cohort, the prodromal criteria had a Sn of 81.3% and a Sp of 67.9% for the development of PD after 4 years⁸⁸. Because non-motor symptoms have insufficient specificity for early diagnosis, imaging biomarkers combined with non-motor symptoms might enhance the predictive value of biomarkers in PD. The combination of hyposmia and DAT-SPECT was demonstrated to detect the risk of PD development, with a 5% decrease in dopamine each year⁸⁹.

Blood biomarkers

Blood biomarkers are very popular biological technologies in research and clinical settings, especially in primary care. Blood biomarkers have several significant advantages over CSF procedures, including non-invasive, fast, convenient, inexpensive, easily repeatable and simple to measure. Thus, it can be carried out in a variety of places, like primary care, hospitals, and the patient's home, as well as through longitudinal research⁹⁰⁻⁹². It is difficult to detect AD biomarkers in the blood because of the low concentrations of Tau proteins and A β in peripheral blood, which requires the development of a highly sensitive technique for diagnostic biomarkers in AD⁹⁰. As a result, some of the advanced technologies that enhance the transference of blood biomarkers from CSF have emerged, including immunoprecipitation-mass spectrometry (IP-MS), electrochemiluminescence (ECL), Simoa (single molecule array), immunomagnetic reduction (IMR), and Meso Scale Discovery (MSD)⁹³.

Plasma amyloid

Most efforts failed until a 2016 research publication found that an ultra-sensitive technique for measuring plasma A β 42/40

could predict abnormal amyloid-PET with moderate accuracy⁹⁴. Subsequently, the IP-MS method was established for detecting A β in plasma and identifying cerebral A β pathology with significantly better accuracy than most methods before⁹⁵⁻⁹⁷. Whereas the A β 42/40 ratio in the CSF is reduced to around 50% to reflect the A β 42 accumulation in the brain, it is reduced to 14.3% in the plasma of amyloid (+) people compared to amyloid (-) subjects⁹⁵. The plasma A β 42/40 ratio has been demonstrated to detect A β plaques in order to support the diagnosis of AD with high diagnostic accuracy (AUC 0.89)⁹⁵.

Plasma tau

While there are limitations to assessing the total tau and A β 42/40 ratio in plasma because of the minor changes in AD compared to in CSF, plasma p-tau has been proven to be a diagnostic, pathophysiological, and prognostic biomarker for AD²⁷. According to neuropathology and PET studies, plasma p-tau181 and p-tau217 levels are related to both A β plaques (early stages) and tau-tangles (later stages)⁹⁸. In sporadic AD, p-tau217 and p-tau181 in CSF/plasma increase in the pre-clinical stage when A β plaques appear and even before detection of tau-tangles by tau-PET⁹⁹⁻¹⁰². Mielke reported that plasma p-tau181 was associated with tau/amyloid PET and CSF p-tau181, suggesting plasma p-tau181 can be utilized as a strong predictor of AD pathophysiology in the brain¹⁰³. Plasma p-tau181 increases significantly after A β 42 in the CSF and plasma but before amyloid PET¹⁰⁰. After that, Palmqvist demonstrated that plasma p-tau217 was mildly superior to p-tau181 for AD diagnosis and association with

tau/amyloid-PET¹⁰⁴. Therefore, plasma p-tau181 and p-tau217 may be effective biomarkers for diagnosing and determining the stage of AD in primary care, specialized centers, and clinical trials¹⁰⁵.

Plasma p-tau, similar to CSF p-tau, could distinguish AD from non-AD dementias and other neurodegenerative disorders¹⁰⁴. In recent studies, plasma p-tau181 has the ability to differentiate AD from healthy people by Simoa¹⁰⁶, IMR¹⁰⁷, and MSD technologies¹⁰³. Moreover, p-tau181 was revealed to distinguish AD from non-AD dementias with high accuracy (AUC = 0.93)⁹⁹. Plasma p-tau217 also had a high accuracy (AUC = 0.96) in distinguishing AD dementia from other neurodegenerative disorders, which was similar to p-tau181 and p-tau217 in the CSF, but better than plasma p-tau181¹⁰⁴. Similar to plasma p-tau181, plasma p-tau231 can distinguish AD from other dementias with high accuracy (AUC = 0.93)¹⁰⁸. In summary, p-tau217, p-tau181 and p-tau231 have high accuracy in differentiating AD patients from healthy people as well as from other dementias.

In MCI patients, plasma p-tau has prognostic value and its prediction of progression to AD has accuracy as well as CSF p-tau^{109, 110}. Palmqvist et al. demonstrated the prediction of transformation to AD dementia at 4 years in MCI or cognitive impairment patients had a high AUC of 0.9 for p-tau181 (ADNI study) and 0.83 for p-tau217 (BioFINDER study)¹⁰⁹. In addition, longitudinal research has demonstrated that p-tau in the plasma (especially p-tau217) rises over time in AD pre-clinical and prodromal stages, suggesting that it can be utilized to identify pharmacodynamic effects of medications¹¹¹.

It is still too early to widely utilize these plasma biomarkers to screen the general population without cognitive symptoms¹¹². P-tau can be applied in clinical trials for patient recruitment, stratification, and monitoring therapeutic effectiveness⁹¹. Although plasma p-tau has a potential biomarker in clinical practice, p-tau181 or p-tau217 combined with A β 42/A β 40 ratio may provide a better evaluation of AD pathology in pre-clinical or prodromal stages, as well as diagnostic and prognostic value¹¹³.

Blood α -synuclein

Because of its high degree of expression and synthesis by erythrocytes, α -syn could be identified in the blood⁷³. Nevertheless, many studies have found inconsistent results when comparing the blood α -syn concentrations of PD patients to normal controls¹¹⁴⁻¹¹⁶. This could be related to the fact that red blood cells can be contaminated easily, as well as that different research utilizes different methods for sample collection and analysis⁸⁴.

Salivary biomarkers

Saliva is considered a promising biomarker because of several advantages including non-invasive, stress-free, easy and repeatable. Moreover, they do not require special storage conditions or clinician training¹¹⁷. Additionally, saliva has minimal to no danger of pathogen exposure or cross-contamination, is carried out easier than blood, does not coagulate, and is stable over time¹¹⁸.

Most studies reveal higher levels of salivary A β 42 in AD patients than in healthy controls, which is in contrast to CSF data, suggesting that salivary A β 42 may be a specific and important biomarker for the diagnosis of AD^{119, 120}.

Nevertheless, other studies found a decrease in salivary concentrations^{121, 122} or were unable to identify the presence of A β 42 in saliva¹²³. A variety of saliva sample collection, storage, processing, and analysis techniques, such as ELISA kit or an antibody-based magnetic nanoparticle immunoassay, as well as variations in the demographic and inclusion criteria, could all be contributing factors to the inconsistent results^{119, 123}.

The majority of research found that AD patients had higher salivary levels of p-tau as well as p-tau/t-tau ratio^{121, 124}. Whereas, salivary t-tau appears to decline or remain constant in patients when compared to healthy controls^{121, 125}. However, salivary levels of t-tau and p-tau are broadly variable, and the p-tau/t-tau ratio did not significantly correlate with the CSF value in AD patients, which makes it difficult to consider salivary tau as a diagnostic biomarker¹²⁴.

Similar to CSF results, salivary t- α -syn levels decreased in PD patients compared to healthy subjects. Additionally, salivary o- α -syn and o- α -syn/t- α -syn ratio were demonstrated to be significantly higher in PD patients compared to healthy controls¹²⁶⁻¹²⁸. These results suggested that salivary t- α -syn and o- α -syn/t- α -syn ratio might be used as a biomarker for PD diagnosis.

Novel candidate biomarkers for neurodegenerative diseases

A number of novel potential candidate biomarkers have been investigated, based on the corresponding pathophysiologic mechanisms, including synaptic dysfunction, neuronal injury, inflammation and glial activation. Nevertheless, the majority of these have yet to be validated and added to clinical guidelines.

Synaptic dysfunction

Synaptic dysfunction is defined as a disturbance in synaptic activity, and synapse loss is considered an early event in the pathophysiology of AD and other neurodegenerative disorders¹²⁹. Neurogranin is a postsynaptic protein that is related to synaptic plasticity and long-term potentiation¹³⁰. CSF neurogranin has been proposed as a biomarker of early synapse loss and degeneration in AD and may predict progression of disease¹³¹. The study revealed that CSF neurogranin levels increase in the early clinical stages of AD and are specific to AD¹³². Other emerging biomarkers reflecting synaptic degeneration comprise of synaptosomal-associated protein 25 (SNAP-25)¹³³, synaptotagmin-1 (SYT-1)¹³⁴ and growth-associated protein 43 (GAP-43)¹³⁵, which increased in AD when comparing to healthy controls. CSF GAP-43 tends to be specific to AD and could help distinguish AD from other neurodegenerative diseases like PD and FTD¹³⁵.

Inflammation and glial activation

The majority of neurodegenerative disorders have primary characteristics of neuroinflammation, activation of microglial cells, and astrocytes¹⁰⁵. YKL-40, also known as chitinase-3-like protein-1, is a glycoprotein that is produced in both astrocytes and microglia near A β plaques and is related to t-tau and p-tau, revealing an involvement in the inflammatory conditions of AD¹³⁶. Increased levels of CSF YKL-40 may be related to disease progression and may discriminate AD from DLB, PD, or vascular dementia¹³². Some findings reveal that plasma YKL-40 is elevated in people with early AD^{137, 138}, but additional research is required to confirm

these results. Another marker expressed in microglial cells is soluble triggering receptor expressed on myeloid cells 2 (sTREM-2). CSF sTREM-2 was positively related to key neurodegenerative biomarkers including A β 42, p-tau, and t-tau¹³⁹. Additionally, although CSF sTREM-2 levels were significantly higher in AD than controls, plasma sTREM-2 levels demonstrated no significant differences between groups¹⁴⁰. Besides that, MCP-1 (monocyte chemoattractant protein-1) is a proinflammatory signaling protein that regulates macrophage and monocyte recruitment, migration, and infiltration into inflammation locations¹⁴¹. CSF MCP-1 concentrations are detected in both the early and late stages of AD¹³². According to one study, increased plasma MCP-1 levels in AD patients are associated with the severity of disease and faster cognitive impairment¹⁴².

Neuronal injury

Neurofilament light (NFL) is a significant biomarker for neuronal degeneration that is elevated in neurodegenerative, inflammatory, traumatic, and vascular disorders¹⁴³. CSF NFL levels were highest in patients with AD, FTD, vascular dementia and could be used to evaluate disease progression in AD¹⁴⁴. Concentrations of plasma NFL also increased in patients with AD¹⁴⁵ and FTD¹⁴⁶ when compared with controls. However, NFL's diagnosis potential in CSF is not better than other CSF markers and does not sufficiently distinguish between AD and other non-AD dementias¹³². Another potential biomarker reflecting neuronal injury is the neuronal calcium sensor protein visinin-like protein 1 (VLP-1). CSF VLP-1 levels were higher in AD patients compared to healthy controls¹⁴⁷.

VLP-1 is also used as a predictor for progression from MCI to AD¹⁴⁸. Unlike NFL, VLP-1 can distinguish AD from non-AD dementias¹⁴⁹. In conclusion, VLP-1 may be utilized together with NFL as a biomarker for neurodegeneration and disease progression in AD¹⁰⁵.

Conclusion

Because neuron degeneration and neuroplasticity depletion have already taken place in many brain regions by neurodegenerative disorders that are clinically diagnosed, it is crucial to have early and reliable diagnostic biomarkers for detection and preventive measures. Biomarkers for neurodegenerative disorders are necessary not only to improve clinical diagnosis but also to support the evaluation of therapeutic effectiveness. A β 42, A β 42/A β 40 ratio, t-tau, p-tau181 or p-tau 217 biomarkers in CSF and PET can be utilized to better understand A β and tau pathology, increase clinical diagnostic accuracy of AD and predict the conversion of MCI to AD. CSF A β 42 is the earliest biomarker to become abnormal in the preclinical stage of AD, whereas p-tau is considered a highly specific biomarker to discriminate AD from healthy controls as well as other dementias. However, because CSF is invasive and PET imaging is expensive, blood and saliva are promising biomarkers widely used in primary care. Blood biomarkers for AD have the crucial role of significantly transferring from CSF to the diagnosis of AD. Plasma p-tau181 or p-tau 217 has been demonstrated as a non-invasive and inexpensive biomarker in the diagnosis and prognosis of AD, especially when combining with plasma A β 42/40 ratio in evaluating the progression in clinical trials. α -syn is a biomarker paid more

attention to in the diagnosis of PD. Unfortunately, all attempts to develop an early diagnosis for PD were unsuccessful. The current methods to improve early diagnosis are premotor symptoms combined with nigrostriatal dopaminergic system failure on PET in the prodromal stage. Future studies will be needed to validate salivary biomarkers and novel potential biomarkers based on other pathophysiologic mechanisms.

References

1. Prince MJ, Wimo A, Guerchet MM, et al. World Alzheimer Report 2015-The Global Impact of Dementia: An analysis of prevalence, incidence, cost and trends. 2015.
2. Reitz C, Brayne C, Mayeux R. Epidemiology of Alzheimer disease. *Nature Reviews Neurology* 2011;7:137-52.
3. Braak H, Braak E. Neuropathological staging of Alzheimer-related changes. *Acta Neuropathologica* 1991;82:239-59.
4. De Leon M, Golomb J, George A, et al. The radiologic prediction of Alzheimer disease: the atrophic hippocampal formation. *American Journal of Neuroradiology* 1993;14:897-906.
5. Blennow K, de Leon MJ, Zetterberg H. Alzheimer's disease. *Lancet* 2006;368:387-403.
6. 2021 Alzheimer's disease facts and figures. *Alzheimers Dement* 2021;17:327-406.
7. Braak H, Braak E. Frequency of stages of Alzheimer-related lesions in different age categories. *Neurobiology of Aging* 1997;18:351-7.
8. Dubois B, Feldman HH, Jacova C, et al. Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS-ADRDA criteria. *The Lancet Neurology* 2007;6:734-46.
9. Petersen RC, Aisen P, Beckett LA, et al. Alzheimer's disease neuroimaging initiative (ADNI): clinical characterization. *Neurology* 2010;74:201-9.
10. Bang J, Spina S, Miller BL. Frontotemporal dementia. *The Lancet* 2015;386:1672-82.
11. Twelves D, Perkins KS, Counsell C. Systematic review of incidence studies of Parkinson's disease. *Movement Disorders: official journal of the Movement Disorder Society* 2003;18:19-31.
12. Kalia LV, Lang AE. Parkinson's disease. *Lancet* 2015;386:896-912.
13. Kalia LV, Lang AE. Evolving basic, pathological and clinical concepts in PD. *Nature Reviews Neurology* 2016;12:65-6.
14. Ugrumov M. Development of early diagnosis of Parkinson's disease: Illusion or reality? *CNS Neuroscience Therapeutics* 2020;26:997-1009.
15. Kwon EH, Tennagels S, Gold R, et al. Update on CSF Biomarkers in Parkinson's Disease. *Biomolecules* 2022;12:329.
16. Blesa J, Trigo-Damas I, Dileone M, et al. Compensatory mechanisms in Parkinson's disease: circuits adaptations and role in disease modification. *Experimental Neurology* 2017;298:148-61.
17. Golpich M, Amini E, Mohamed Z, et al. Mitochondrial dysfunction and biogenesis in neurodegenerative diseases: pathogenesis and treatment. *CNS Neuroscience Therapeutics* 2017;23:5-22.
18. Sperling RA, Aisen PS, Beckett LA, et al. Toward defining the preclinical stages of Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's Dementia* 2011;7:280-92.
19. Knopman D, DeKosky ST, Cummings J, et al. Practice parameter: diagnosis of dementia

- (an evidence-based review): report of the Quality Standards Subcommittee of the American Academy of Neurology. *Neurology* 2001;56:1143-53.
20. Rizzo G, Arcuti S, Copetti M, et al. Accuracy of clinical diagnosis of dementia with Lewy bodies: a systematic review and meta-analysis. *Journal of Neurology, Neurosurgery Psychiatry* 2018;89:358-66.
 21. Beach TG, Monsell SE, Phillips LE, et al. Accuracy of the clinical diagnosis of Alzheimer disease at National Institute on Aging Alzheimer Disease Centers, 2005–2010. *Journal of Neuropathology Experimental Neurology* 2012;71:266-73.
 22. Bradford A, Kunik ME, Schulz P, et al. Missed and delayed diagnosis of dementia in primary care: prevalence and contributing factors. *Alzheimer Disease Associated Disorders* 2009;23:306.
 23. Rizzo G, Copetti M, Arcuti S, et al. Accuracy of clinical diagnosis of Parkinson disease: a systematic review and meta-analysis. *Neurology* 2016;86:566-76.
 24. Cheng HC, Ulane CM, Burke RE. Clinical progression in Parkinson disease and the neurobiology of axons. *Annals of Neurology* 2010;67:715-25.
 25. Mahlknecht P, Seppi K, Poewe W. The concept of prodromal Parkinson's disease. *Journal of Parkinson's Disease* 2015;5:681-97.
 26. Strimbu K, Tavel JA. What are biomarkers? *Current Opinion in HIV AIDS* 2010;5:463.
 27. Zetterberg H, Blennow K. Moving fluid biomarkers for Alzheimer's disease from research tools to routine clinical diagnostics. *Molecular Neurodegeneration* 2021;16:1-7.
 28. Lewczuk P, Łukaszewicz-Zajac M, Mroczko P, et al. Clinical significance of fluid biomarkers in Alzheimer's disease. *Pharmacological Reports* 2020;72:528-42.
 29. Hansson O. Biomarkers for neurodegenerative diseases. *Nature Medicine* 2021;27:954-63.
 30. Kehoe EG, McNulty JP, Mullins PG, et al. Advances in MRI biomarkers for the diagnosis of Alzheimer's disease. *Biomarkers in Medicine* 2014;8:1151-69.
 31. Jack Jr CR, Bennett DA, Blennow K, et al. NIA-AA research framework: toward a biological definition of Alzheimer's disease. *Alzheimer's Dementia* 2018;14:535-62.
 32. Bloudek LM, Spackman DE, Blankenburg M, et al. Review and meta-analysis of biomarkers and diagnostic imaging in Alzheimer's disease. *Journal of Alzheimer's Disease* 2011;26:627-45.
 33. Ou Y-N, Xu W, Li J-Q, et al. FDG-PET as an independent biomarker for Alzheimer's biological diagnosis: a longitudinal study. *Alzheimer's Research Therapy* 2019;11:1-11.
 34. Anchisi D, Borroni B, Franceschi M, et al. Heterogeneity of brain glucose metabolism in mild cognitive impairment and clinical progression to Alzheimer disease. *Archives of Neurology* 2005;62:1728-33.
 35. Yuan Y, Gu Z-X, Wei W-S. Fluorodeoxyglucose-positron-emission tomography, single-photon emission tomography, and structural MR imaging for prediction of rapid conversion to Alzheimer disease in patients with mild cognitive impairment: a meta-analysis. *American Journal of Neuroradiology* 2009;30:404-10.
 36. Suwijn SR, van Boheemen CJ, de Haan RJ, et al. The diagnostic accuracy of dopamine transporter SPECT imaging to detect nigrostriatal cell loss in patients with Parkinson's disease or

- clinically uncertain parkinsonism: a systematic review. *EJNMMI research* 2015;5:1-8.
37. Brooks DJ. Imaging approaches to Parkinson disease. *Journal of Nuclear Medicine* 2010;51:596-609.
38. Bajaj N, Hauser RA, Grachev ID. Clinical utility of dopamine transporter single photon emission CT (DaT-SPECT) with (123I) ioflupane in diagnosis of parkinsonian syndromes. *Journal of Neurology, Neurosurgery Psychiatry* 2013;84:1288-95.
39. Zou J, Weng RH, Chen ZY, et al. Position emission tomography/single-photon emission tomography neuroimaging for detection of premotor Parkinson's disease. *CNS Neuroscience Therapeutics* 2016;22:167-77.
40. Le W, Dong J, Li S, et al. Can biomarkers help the early diagnosis of Parkinson's disease? *Neuroscience Bulletin* 2017;33:535-42.
41. Mantri S, Morley JF, Siderowf AD. The importance of preclinical diagnostics in Parkinson disease. *Parkinsonism Related Disorders* 2019;64:20-8.
42. Blennow K, Dubois B, Fagan AM, et al. Clinical utility of cerebrospinal fluid biomarkers in the diagnosis of early Alzheimer's disease. *Alzheimer's Dementia* 2015;11:58-69.
43. Strittmatter WJ. Bathing the brain. *The Journal of Clinical Investigation* 2013;123:1013-5.
44. Alcolea D, Martínez-Lage P, Izaguirre A, et al. Feasibility of lumbar puncture in the study of cerebrospinal fluid biomarkers for Alzheimer's disease: a multicenter study in Spain. *Journal of Alzheimer's Disease* 2014;39:719-26.
45. Fishman RA. *Cerebrospinal fluid in diseases of the nervous system*: WB Saunders company; 1992.
46. Kang J, Lemaire H-G, Unterbeck A, et al. The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. *Nature* 1987;325:733-6.
47. Carroll CM, Li Y-M. Physiological and pathological roles of the γ -secretase complex. *Brain research bulletin* 2016;126:199-206.
48. Lewczuk P, Esselmann H, Meyer M, et al. The amyloid-beta (A β) peptide pattern in cerebrospinal fluid in Alzheimer's disease: evidence of a novel carboxyterminally elongated A β peptide. *Rapid Commun Mass Spectrom* 2003;17:1291-6.
49. Fagan AM, Mintun MA, Mach RH, et al. Inverse relation between in vivo amyloid imaging load and cerebrospinal fluid A β 42 in humans. *Annals of Neurology* 2006;59:512-9.
50. Lewczuk P, Matzen A, Blennow K, et al. Cerebrospinal fluid A β 42/40 corresponds better than A β 42 to amyloid PET in Alzheimer's Disease. *Journal of Alzheimer's Disease* 2017;55:813-22.
51. Palmqvist S, Mattsson N, Hansson O, et al. Cerebrospinal fluid analysis detects cerebral amyloid- β accumulation earlier than positron emission tomography. *Brain* 2016;139:1226-36.
52. Forlenza OV, Radanovic M, Talib LL, et al. Cerebrospinal fluid biomarkers in Alzheimer's disease: Diagnostic accuracy and prediction of dementia. *Alzheimer's Dementia: Diagnosis, Assessment Disease Monitoring* 2015;1:455-63.
53. Hulstaert F, Blennow K, Ivanoiu A, et al. Improved discrimination of AD patients using β -amyloid (1-42) and tau levels in CSF. *Neurology* 1999;52:1555.
54. Galasko D, Chang L, Motter R, et al. High cerebrospinal fluid tau and low amyloid β 42 levels in the clinical diagnosis of Alzheimer disease and relation to apolipoprotein E geno-

- type. *Archives of Neurology* 1998;55:937-45.
55. Ritchie C, Smailagic N, Noel-Storr AH, et al. Plasma and cerebrospinal fluid amyloid beta for the diagnosis of Alzheimer's disease dementia and other dementias in people with mild cognitive impairment (MCI). *Cochrane Database of Systematic Reviews* 2014(6).
56. Ewers M, Mattsson N, Minthon L, et al. CSF biomarkers for the differential diagnosis of Alzheimer's disease: a large-scale international multicenter study. *Alzheimer's Dementia* 2015;11:1306-15.
57. Biscetti L, Salvadori N, Farotti L, et al. The added value of A β 42/A β 40 in the CSF signature for routine diagnostics of Alzheimer's disease. *Clin Chim Acta* 2019;494:71-3.
58. Mandelkow E, Von Bergen M, Biernat J, et al. Structural principles of tau and the paired helical filaments of Alzheimer's disease. *Brain Pathology* 2007;17:83-90.
59. Buée L, Bussièrè T, Buée-Scherrer V, et al. Tau protein isoforms, phosphorylation and role in neurodegenerative disorders. *Brain Research Reviews* 2000;33:95-130.
60. Janelidze S, Stomrud E, Smith R, et al. Cerebrospinal fluid p-tau217 performs better than p-tau181 as a biomarker of Alzheimer's disease. *Nature Communications* 2020;11:1-12.
61. Blennow K, Zetterberg H. Biomarkers for Alzheimer's disease: current status and prospects for the future. *Journal of Internal Medicine* 2018;284:643-63.
62. Skillbäck T, Farahmand BY, Rosen C, et al. Cerebrospinal fluid tau and amyloid- β 1-42 in patients with dementia. *Brain* 2015;138:2716-31.
63. Maia LF, Kaeser SA, Reichwald J, et al. Changes in amyloid- β and Tau in the cerebrospinal fluid of transgenic mice overexpressing amyloid precursor protein. *Science Translational Medicine* 2013;5:194re2-re2.
64. La Joie R, Bejanin A, Fagan AM, et al. Associations between [18F] AV1451 tau PET and CSF measures of tau pathology in a clinical sample. *Neurology* 2018;90:e282-e90.
65. Hesse C, Rosengren L, Andreasen N, et al. Transient increase in total tau but not phospho-tau in human cerebrospinal fluid after acute stroke. *Neuroscience Letters* 2001;297:187-90.
66. Otto M, Wiltfang J, Cepek L, et al. Tau protein and 14-3-3 protein in the differential diagnosis of Creutzfeldt-Jakob disease. *Neurology* 2002;58:192-7.
67. Vanmechelen E, Van Kerschaver E, Blennow K, et al. CSF-Phospho-tau (181P) as a promising marker for discriminating Alzheimer's disease from dementia with Lewy Bodies. *Alzheimer's disease: Advances in Etiology, Pathogenesis Therapeutics* 2001:285-91.
68. Blennow K, Vanmechelen E, Hampel H. CSF total tau, A β 42 and phosphorylated tau protein as biomarkers for Alzheimer's disease. *Molecular Neurobiology* 2001;24:87-97.
69. Parnetti L, Lanari A, Amici S, et al. CSF phosphorylated tau is a possible marker for discriminating Alzheimer's disease from dementia with Lewy bodies. *Neurological Sciences* 2001;22:77-8.
70. Hanes J, Kovac A, Kvartsberg H, et al. Evaluation of a novel immunoassay to detect p-tau Thr217 in the CSF to distinguish Alzheimer disease from other dementias. *Neurology* 2020;95:e3026-e35.
71. Buchhave P, Minthon L, Zetterberg H, et al. Cerebrospinal fluid levels of β -amyloid 1-42, but not of tau, are fully changed already 5 to 10

- years before the onset of Alzheimer dementia. *Archives of General Psychiatry* 2012;69:98-106.
72. Blennow K, Shaw LM, Stomrud E, et al. Predicting clinical decline and conversion to Alzheimer's disease or dementia using novel Elecsys A β (1-42), pTau and tTau CSF immunoassays. *Scientific Reports* 2019;9:1-11.
73. Fayyad M, Salim S, Majbour N, et al. Parkinson's disease biomarkers based on α -synuclein. *Journal of neurochemistry* 2019;150:626-36.
74. F \ddot{o} rland MG, Tysnes OB, Aarsland D, et al. The value of cerebrospinal fluid α -synuclein and the tau/ α -synuclein ratio for diagnosis of neurodegenerative disorders with Lewy pathology. *European Journal of Neurology* 2020;27:43-50.
75. Shim KH, Kang MJ, Suh JW, et al. CSF total tau/ α -synuclein ratio improved the diagnostic performance for Alzheimer's disease as an indicator of tau phosphorylation. *Alzheimer's Research Therapy* 2020;12:1-12.
76. Chahine LM, Beach TG, Brumm MC, et al. In vivo distribution of α -synuclein in multiple tissues and biofluids in Parkinson disease. *Neurology* 2020;95:e1267-e84.
77. Majbour NK, Vaikath NN, van Dijk KD, et al. Oligomeric and phosphorylated alpha-synuclein as potential CSF biomarkers for Parkinson's disease. *Molecular Neurodegeneration* 2016;11:1-15.
78. Parnetti L, Farotti L, Eusebi P, et al. Differential role of CSF alpha-synuclein species, tau, and A β 42 in Parkinson's Disease. *Frontiers in Aging Neuroscience* 2014;6:53.
79. Majbour NK, Vaikath NN, Eusebi P, et al. Longitudinal changes in CSF alpha-synuclein species reflect Parkinson's disease progression. *Movement Disorders* 2016;31:1535-42.
80. Kang UJ, Boehme AK, Fairfoul G, et al. Comparative study of cerebrospinal fluid α -synuclein seeding aggregation assays for diagnosis of Parkinson's disease. *Mov Disord* 2019;34:536-44.
81. Rossi M, Candelise N, Baiardi S, et al. Ultrasensitive RT-QuIC assay with high sensitivity and specificity for Lewy body-associated synucleinopathies. *Acta Neuropathol* 2020;140:49-62.
82. Singer W, Schmeichel AM, Shah Nawaz M, et al. Alpha-Synuclein Oligomers and Neurofilament Light Chain in Spinal Fluid Differentiate Multiple System Atrophy from Lewy Body Synucleinopathies. *Ann Neurol* 2020;88:503-12.
83. Postuma RB, Berg D. Advances in markers of prodromal Parkinson disease. *Nature Reviews Neurology* 2016;12:622-34.
84. Li T, Le W. Biomarkers for Parkinson's disease: how good are they? *Neuroscience Bulletin* 2020;36:183-94.
85. Postuma RB, Berg D. Prodromal Parkinson's disease: the decade past, the decade to come. *Movement Disorders* 2019;34:665-75.
86. Fullard ME, Morley JF, Duda JE. Olfactory dysfunction as an early biomarker in Parkinson's disease. *Neuroscience Bulletin* 2017;33:515-25.
87. Iranzo A, Tolosa E, Gelpi E, et al. Neurodegenerative disease status and post-mortem pathology in idiopathic rapid-eye-movement sleep behaviour disorder: an observational cohort study. *The Lancet Neurology* 2013;12:443-53.
88. Fereshtehnejad SM, Montplaisir JY, Pelletier A, et al. Validation of the MDS research criteria for prodromal Parkinson's disease: longitudinal assessment in a REM sleep behavior disorder (RBD) cohort. *Movement Disorders* 2017;32:865-73.
89. Jennings D, Siderowf A, Stern M, et al. Conversion to Parkinson disease in the PARS hypos-

- mic and dopamine transporter–deficit prodromal cohort. *JAMA neurology* 2017;74:933-40.
90. Ausó E, Gómez-Vicente V, Esquiva G. Biomarkers for Alzheimer's disease early diagnosis. *Journal of Personalized Medicine* 2020;10:114.
91. Verde F. Tau proteins in blood as biomarkers of Alzheimer's disease and other proteinopathies. *Journal of Neural Transmission* 2022;129:239-59.
92. Parnetti L, Paoletti FP, Gaetani L. Blood biomarkers may distinguish among dementia disorders. *Journal of Neurology, Neurosurgery Psychiatry* 2022;93:571.
93. Chong JR, Ashton NJ, Karikari TK, et al. Blood-based high sensitivity measurements of beta-amyloid and phosphorylated tau as biomarkers of Alzheimer's disease: a focused review on recent advances. *Journal of Neurology, Neurosurgery Psychiatry* 2021;92:1231-41.
94. Janelidze S, Stomrud E, Palmqvist S, et al. Plasma β -amyloid in Alzheimer's disease and vascular disease. *Scientific Reports* 2016;6:1-11.
95. Ovod V, Ramsey KN, Mawuenyega KG, et al. Amyloid β concentrations and stable isotope labeling kinetics of human plasma specific to central nervous system amyloidosis. *Alzheimer's Dementia* 2017;13:841-9.
96. Schindler SE, Bollinger JG, Ovod V, et al. High-precision plasma β -amyloid 42/40 predicts current and future brain amyloidosis. *Neurology* 2019;93:e1647-e59.
97. Nakamura A, Kaneko N, Villemagne VL, et al. High performance plasma amyloid- β biomarkers for Alzheimer's disease. *Nature* 2018;554:249-54.
98. Mattsson-Carlgrén N, Janelidze S, Bateman RJ, et al. Soluble P-tau217 reflects amyloid and tau pathology and mediates the association of amyloid with tau. *EMBO Molecular Medicine* 2021;13:e14022.
99. Janelidze S, Mattsson N, Palmqvist S, et al. Plasma P-tau181 in Alzheimer's disease: relationship to other biomarkers, differential diagnosis, neuropathology and longitudinal progression to Alzheimer's dementia. *Nature Medicine* 2020;26:379-86.
100. Palmqvist S, Insel PS, Stomrud E, et al. Cerebrospinal fluid and plasma biomarker trajectories with increasing amyloid deposition in Alzheimer's disease. *EMBO Molecular Medicine* 2019;11:e11170.
101. Janelidze S, Berron D, Smith R, et al. Associations of plasma phospho-Tau217 levels with tau-positron emission tomography in early Alzheimer disease. *JAMA Neurology* 2021;78:149-56.
102. Barthélemy NR, Li Y, Joseph-Mathurin N, et al. A soluble phosphorylated tau signature links tau, amyloid and the evolution of stages of dominantly inherited Alzheimer's disease. *Nature Medicine* 2020;26:398-407.
103. Mielke MM, Hagen CE, Xu J, et al. Plasma phospho-tau181 increases with Alzheimer's disease clinical severity and is associated with tau-and amyloid-positron emission tomography. *Alzheimer's Dementia* 2018;14:989-97.
104. Palmqvist S, Janelidze S, Quiroz YT, et al. Discriminative accuracy of plasma phospho-tau217 for Alzheimer disease vs other neurodegenerative disorders. *JAMA* 2020;324:772-81.
105. Simrén J, Ashton NJ, Blennow K, et al. An update on fluid biomarkers for neurodegenerative diseases: recent success and challenges ahead. *Curr Opin Neurobiol* 2020;61:29-39.
106. Tatebe H, Kasai T, Ohmichi T, et al. Quantification of plasma phosphorylated tau to use

- as a biomarker for brain Alzheimer pathology: pilot case-control studies including patients with Alzheimer's disease and down syndrome. *Molecular Neurodegeneration* 2017;12:1-11.
107. Yang C-C, Chiu M-J, Chen T-F, et al. Assay of plasma phosphorylated tau protein (threonine 181) and total tau protein in early-stage Alzheimer's disease. *Journal of Alzheimer's Disease* 2018;61:1323-32.
108. Ashton NJ, Pascoal TA, Karikari TK, et al. Plasma p-tau231: a new biomarker for incipient Alzheimer's disease pathology. *Acta NeuroPathologica* 2021;141:709-24.
109. Palmqvist S, Tideman P, Cullen N, et al. Prediction of future Alzheimer's disease dementia using plasma phospho-tau combined with other accessible measures. *Nature Medicine* 2021;27:1034-42.
110. Cullen NC, Leuzy A, Palmqvist S, et al. Individualized prognosis of cognitive decline and dementia in mild cognitive impairment based on plasma biomarker combinations. *Nature Aging* 2021;1:114-23.
111. Mattsson-Carlgren N, Janelidze S, Palmqvist S, et al. Longitudinal plasma p-tau217 is increased in early stages of Alzheimer's disease. *Brain* 2020;143:3234-41.
112. Teunissen CE, Verberk IM, Thijssen EH, et al. Blood-based biomarkers for Alzheimer's disease: towards clinical implementation. *The Lancet Neurology* 2022;21:66-77.
113. Simrén J, Leuzy A, Karikari TK, et al. The diagnostic and prognostic capabilities of plasma biomarkers in Alzheimer's disease. *Alzheimer's Dementia* 2021;17:1145-56.
114. Lin C-H, Yang S-Y, Horng H-E, et al. Plasma α -synuclein predicts cognitive decline in Parkinson's disease. *Journal of Neurology, Neurosurgery Psychiatry* 2017;88:818-24.
115. Lee P, Lee G, Park H, et al. The plasma alpha-synuclein levels in patients with Parkinson's disease and multiple system atrophy. *Journal of Neural Transmission* 2006;113:1435-9.
116. Li Q-X, San Mok S, Laughton KM, et al. Plasma α -synuclein is decreased in subjects with Parkinson's disease. *Experimental Neurology* 2007;204:583-8.
117. Schepici G, Silvestro S, Trubiani O, et al. Salivary Biomarkers: Future Approaches for Early Diagnosis of Neurodegenerative Diseases. *Brain Sci* 2020;10:245.
118. Malon RS, Sadir S, Balakrishnan M, et al. Saliva-based biosensors: noninvasive monitoring tool for clinical diagnostics. *Biomed Res Int* 2014;2014:962903.
119. Lee M, Guo JP, Kennedy K, et al. A Method for Diagnosing Alzheimer's Disease Based on Salivary Amyloid- β Protein 42 Levels. *J Alzheimers Dis* 2017;55:1175-82.
120. Sabbagh MN, Shi J, Lee M, et al. Salivary beta amyloid protein levels are detectable and differentiate patients with Alzheimer's disease dementia from normal controls: preliminary findings. *BMC Neurol* 2018;18:155.
121. Lau HC, Lee IK, Ko PW, et al. Non-invasive screening for Alzheimer's disease by sensing salivary sugar using *Drosophila* cells expressing gustatory receptor (Gr5a) immobilized on an extended gate ion-sensitive field-effect transistor (EG-ISFET) biosensor. *PLoS One* 2015;10:e0117810.
122. Shi M, Sui YT, Peskind ER, et al. Salivary tau species are potential biomarkers of Alzheimer's disease. *J Alzheimers Dis* 2011;27:299-305.
123. Tvarijonaviciute A, Zamora C, Ceron JJ, et al.

- Salivary biomarkers in Alzheimer's disease. *Clin Oral Investig* 2020;24:3437-44.
124. Pekeles H, Qureshi HY, Paudel HK, et al. Development and validation of a salivary tau biomarker in Alzheimer's disease. *Alzheimers Dement (Amst)* 2019;11:53-60.
125. Ashton NJ, Ide M, Schöll M, et al. No association of salivary total tau concentration with Alzheimer's disease. *Neurobiology of Aging* 2018;70:125-7.
126. Shaheen H, Sobhy S, El Mously S, et al. Salivary alpha-synuclein (total and oligomeric form): potential biomarkers in Parkinson's disease. *The Egyptian Journal of Neurology, Psychiatry and Neurosurgery* 2020;56(1):22.
127. Vivacqua G, Suppa A, Mancinelli R, et al. Salivary alpha-synuclein in the diagnosis of Parkinson's disease and Progressive Supranuclear Palsy. *Parkinsonism Relat Disord* 2019;63:143-8.
128. Cao Z, Wu Y, Liu G, et al. α -Synuclein in salivary extracellular vesicles as a potential biomarker of Parkinson's disease. *Neurosci Lett* 2019;696:114-20.
129. Sheng M, Sabatini BL, Südhof TC. Synapses and Alzheimer's disease. *Cold Spring Harb Perspect Biol* 2012;4:005777.
130. Kvartsberg H, Portelius E, Andreasson U, et al. Characterization of the postsynaptic protein neurogranin in paired cerebrospinal fluid and plasma samples from Alzheimer's disease patients and healthy controls. *Alzheimers Res Ther* 2015;7:40.
131. Thorsell A, Bjerke M, Gobom J, et al. Neurogranin in cerebrospinal fluid as a marker of synaptic degeneration in Alzheimer's disease. *Brain Research* 2010;1362:13-22.
132. McGrowder DA, Miller F, Vaz K, et al. Cerebrospinal Fluid Biomarkers of Alzheimer's Disease: Current Evidence and Future Perspectives. *Brain Sci* 2021;11:215.
133. Zhang H, Therriault J, Kang MS, et al. Cerebrospinal fluid synaptosomal-associated protein 25 is a key player in synaptic degeneration in mild cognitive impairment and Alzheimer's disease. *Alzheimers Res Ther* 2018;10:80.
134. Öhrfelt A, Brinkmalm A, Dumurgier J, et al. The pre-synaptic vesicle protein synaptotagmin is a novel biomarker for Alzheimer's disease. *Alzheimers Res Ther* 2016;8:41.
135. Sandelius Å, Portelius E, Källén Å, et al. Elevated CSF GAP-43 is Alzheimer's disease specific and associated with tau and amyloid pathology. *Alzheimers Dement* 2019;15:55-64.
136. Querol-Vilaseca M, Colom-Cadena M, Pegueroles J, et al. YKL-40 (Chitinase 3-like I) is expressed in a subset of astrocytes in Alzheimer's disease and other tauopathies. *J Neuroinflammation* 2017;14:118.
137. Craig-Schapiro R, Perrin RJ, Roe CM, et al. YKL-40: A Novel Prognostic Fluid Biomarker for Preclinical Alzheimer's Disease. *Biological Psychiatry* 2010;68:903-12.
138. Choi J, Lee H-W, Suk K. Plasma level of chitinase 3-like 1 protein increases in patients with early Alzheimer's disease. *Journal of Neurology* 2011;258:2181-5.
139. Henjum K, Almdahl IS, Årskog V, et al. Cerebrospinal fluid soluble TREM2 in aging and Alzheimer's disease. *Alzheimer's Research & Therapy* 2016;8:17.
140. Liu D, Cao B, Zhao Y, et al. Soluble TREM2 changes during the clinical course of Alzheimer's disease: A meta-analysis. *Neuroscience Letters* 2018;686:10-6.

141. Semple BD, Kossmann T, Morganti-Kossmann MC. Role of chemokines in CNS health and pathology: a focus on the CCL2/CCR2 and CXCL8/CXCR2 networks. *J Cereb Blood Flow Metab* 2010;30:459-73.
142. Lee WJ, Liao YC, Wang YF, et al. Plasma MCP-1 and Cognitive Decline in Patients with Alzheimer's Disease and Mild Cognitive Impairment: A Two-year Follow-up Study. *Sci Rep* 2018;8:1280.
143. Gaetani L, Blennow K, Calabresi P, et al. Neurofilament light chain as a biomarker in neurological disorders. *J Neurol Neurosurg Psychiatry* 2019;90:870-81.
144. Skillbäck T, Farahmand B, Bartlett JW, et al. CSF neurofilament light differs in neurodegenerative diseases and predicts severity and survival. *Neurology* 2014;83:1945-53.
145. Lewczuk P, Ermann N, Andreasson U, et al. Plasma neurofilament light as a potential biomarker of neurodegeneration in Alzheimer's disease. *Alzheimers Res Ther* 2018;10:71.
146. Rohrer JD, Woollacott IO, Dick KM, et al. Serum neurofilament light chain protein is a measure of disease intensity in frontotemporal dementia. *Neurology* 2016;87:1329-36.
147. Olsson B, Lautner R, Andreasson U, et al. CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis. *The Lancet Neurology* 2016;15:673-84.
148. Zhang H, Ng KP, Therriault J, et al. Cerebrospinal fluid phosphorylated tau, visinin-like protein-1, and chitinase-3-like protein 1 in mild cognitive impairment and Alzheimer's disease. *Transl Neurodegener* 2018;7:23.
149. Babić Leko M, Borovečki F, Dejanović N, et al. Predictive value of cerebrospinal fluid Visinin-Like Protein-1 levels for Alzheimer's disease early detection and differential diagnosis in patients with mild cognitive impairment. *J Alzheimers Dis* 2016;50:765-78.