Expert Review of Neurotherapeutics

Cerebrospinal fluid in the differential diagnosis of Alzheimer's disease: an update of the literature

Abstract

Introduction: The importance of cerebrospinal fluid (CSF) biomarkers in Alzheimer's disease (AD) diagnosis is rapidly increasing, and there is a growing interest in the use of CSF biomarkers in monitoring the response to therapy, especially in the light of newly available approaches to the therapy of neurodegenerative diseases.

Areas covered: In this review we discuss the most relevant measures of neurodegeneration that are being used to distinguish patients with AD from healthy controls and individuals with mild cognitive impairment, in order to provide an overview of the latest information available in the scientific literature. We focus on markers related to amyloid processing, markers associated with neurofibrillary tangles, neuroinflammation, neuroaxonal injury and degeneration, synaptic loss and dysfunction, and markers of α-synuclein pathology.

Expert opinion: In addition to neuropsychological evaluation, core CSF biomarkers ($A\beta_{42}$, t-tau, and p-tau181) have been recommended for improvement of timely, accurate and differential diagnosis of AD, as well as to assess the risk and rate of disease progression. In addition to the core CSF biomarkers, various other markers related to synaptic dysfunction, neuroinflammation, and glial activation (neurogranin, SNAP-25, Nfl, YKL-40, TREM2) are now investigated and have yet to be validated for future potential clinical use in AD diagnosis.

Keywords: Alzheimer's disease; Amyloid-beta; Biomarkers; Cerebrospinal fluid; Diagnosis; Neuroaxonal injury; Neuroinflammation; α-Synuclein; Synaptic dysfunction; Tau

Article highlights

- Besides decreased levels of $A\beta_{42}$ in CSF, CSF $A\beta_{40}$, $A\beta_{37}$ and $A\beta_{38}$ may help in distinguishing AD from healthy controls and other dementias, such as FTD and LBD.
- CSF $A\beta_{42}/A\beta_{38}$ and $A\beta_{42}/A\beta_{40}$ ratios exhibit enhanced performance in distinguishing AD from other forms of dementia, including PD, LBD, subcortical VaD and FTD.
- Elevated CSF levels of p-tau (p-tau181 and p-tau231) are a valuable marker in differentiating AD from other types of dementia.
- The p-tau217 exhibits higher accuracy than p-tau181 and p-tau231 in distinguishing AD dementia from non-AD.
- CSF p-tau/$A\beta_{42}$ ratio could be accurate predictor of conversion from MCI to AD dementia.
- Nfl provides important information about the progress of neurodegeneration and should be used as a biomarker in AD, but not as a biomarker of AD.
- CSF VILIP-1 levels are significantly increased in AD and MCI patients compared to controls, and correlates well with the progression and pathology of AD.
- Elevated CSF Ng and SNAP-25 levels are found in patients diagnosed with AD, even in prodromal phase of the disease.
- Individuals with AD exhibit higher levels of glycoprotein YKL-40 in CSF and plasma compared to healthy controls.
- AD pathology is associated with elevated CSF sTREM2 levels, especially in the case of tau-related neurodegeneration.
- Increased CSF α-syn levels are present in patients with MCI and AD, and they correlate with disease progression and/or severity of cognitive decline.

1 Introduction

Alzheimer's disease (AD) is a chronic progressive neurodegenerative disease with an irreversible but slow time course, and it is the most frequent of all types of dementia (around 60-70% cases). It is characterized with cognitive deterioration, various neuropsychiatric symptoms, and behavioral problems, and in the later stage with inability to perform daily living activities [1]. The main neuropathological features in AD are the accumulation of extracellular amyloid β (Aβ) plaques and the intracellular accumulation of hyperphosphorylated tau proteins as neurofibrillary tangles, that result in progressive neurodegeneration and cerebral atrophy.
The detailed description about the AD pathophysiology was reviewed recently [2]. Pathophysiology of AD is described with different hypotheses: cholinergic hypothesis, amyloid cascade hypothesis and hyperphosphorylation of tau proteins in the brain [3]. All hypotheses are confirmed with some pathological or pharmacological findings: a decreased acetylcholine concentration in the brain is associated with neuronal loss and is responsible for the memory loss, cognitive deterioration and AD development, and this hypothesis is confirmed by the clinical efficacy of the acetylcholinesterase inhibitors. Amyloid cascade hypothesis is the most accepted, suggesting that the primary cause of AD is the extent accumulation of Aβ peptides and Aβ plaque deposition in the brain. After the enormous and long-term efforts to develop a treatment, some new drugs targeting Aβ have shown satisfactory results [4,5]. A common hypothesis is also the hyperphosphorylation of tau proteins, due to the excessive accumulation of the amyloid proteins in the brain tissue. The hypothesis is based on abnormal changes in tau proteins which result in tau dysfunction, and these hyperphosphorylated tau proteins shape aggregates (i.e. neurofibrillary tangles) that are deposited within the neurons, with consequent induction of neuronal damage and negative effects on neuronal function [3]. The full description of the AD pathophysiology regarding the Aβ plaques and neurofibrillary tangles is provided in the text in paragraphs 2.1. (markers of beta-amyloid accumulation and amyloid processing) and 2.2. (markers of tau pathology), while other pathophysiological features of AD are described further in the text in the paragraphs 2.3-2.6 (markers of neuroaxonal injury and degeneration, markers of synaptic dysfunction, markers of neuroinflammation, markers of α-synuclein pathology).
Currently there is no cure for AD, no disease-modifying treatment, and no therapeutic strategy exists to prevent AD or reverse disease progression, while few classes of medication (i.e. acetylcholinesterase inhibitors and non-competitive NMDA antagonist) can only slow AD progression, with modest benefit on cognition [1]. Medication used to treat symptoms in AD include donepezil (reversible non-competitive acetylcholinesterase inhibitor), galantamine (reversible, competitive acetylcholinesterase inhibitor and modulator of nicotinic acetylcholine receptor), rivastigmine (acetylcholinesterase and butyrylcholinesterase inhibitor) and memantine (non-competitive NMDA antagonist) [1]. However, recently two additional treatment strategies have been approved by the U.S. Food and Drug Administration (FDA) for the treatment of AD, aducanumab [4] and lecanemab [5]. Both drugs are based on a humanized IgG1 monoclonal antibody that targets aggregated Aβ and both approaches have been shown to reduce Aβ burden measured with PET [6]. Lecanemab exhibits stronger biding to Aβ protofibrils, while aducanumab has grater affinity for highly aggregated Aβ fibrils [6]. In contrast to aducanumab, the efficiency of lecanemab was found to be more consistent across different studies [7–9].
The National Institute on Aging–Alzheimer's Association (NIA-AA) in 2018 shifted its guidelines of the definition of AD from the syndromal entity to biologically based entity and described AD as "underlying pathologic processes that can be documented by postmortem

examination or in vivo by biomarkers" [10]. This definition should be used for the research framework, and not for clinical practice [11]. The "A/T/N" classification scheme was proposed in order to categorize AD biomarker findings into a format which is easy to understand and use [11]. The classification included 7 major AD biomarkers subdivided into 3 categories: "A" (Aβ plaques i.e. biomarkers detected with cortical amyloid PET ligand binding or decreased cerebrospinal fluid (CSF) $A\beta_{42}$),"T" (related to aggregated tau (neurofibrillary tangles), i.e. increased CSF phosphorylated tau (P-tau) and cortical tau PET ligand binding), and "N" (biomarkers of neurodegeneration or neuronal injury) [11]. The "N" biomarkers include CSF T-tau, FDG PET hypometabolism, and brain atrophy detected with MRI, but are open to other (novel) markers of neurodegeneration. This classification points out the importance of separating biomarkers related to neurofibrillary tangles from markers associated with neuronal injury/neurodegeneration since this approach might help differentiate neuronal AD from non-AD causes [11]. This shift in definition was suggested since these neuropathological changes (β-amyloid plaques and neurofibrillary tau deposits, evaluated as biomarkers) define AD, and might offer more precise characterization and improve the knowledge of the order of neurobiological events that cause AD, however, in addition, AD should be assessed also in different stages across its entire spectrum [11]. Therefore, the difference is that AD is not only defined by its clinical symptoms (cognitive deterioration that affects thinking, remembering, and reasoning, and behavioral changes that interfere with daily life and activities), but also with characteristic neuropathologic changes that can be assessed with biomarkers in vivo and postmortem examination. However, when evaluating biomarkers, besides sex-specific contributions to AD risk biomarkers [12], racial differences should also be considered. The African American individuals were suggested to have lower levels of CSF t-tau and p-tau181 compared to white individuals, suggesting that in these individuals ATN biomarkers must be controlled for race [13].

Regarding the clinical perspective, AD diagnosis is meet when all criteria defined by The American Psychiatric Association's Diagnostic and Statistical Manual (DSM-5) [14] for dementia are present, and the neuropsychiatric symptoms (cognitive decline associated with reduced ability for reasoning or thinking, problems with memory and in social skills, behavioral abilities) occur [15]. Problems in cognition are associated with two or more of the following domains: acquisition and recall of information, reasoning and judgment in complex tasks, visuospatial ability, language function, and normal personality or behavior [15]. AD can be divided in different clinical stages [16]. The first is a pre-clinical or pre-symptomatic stage, lasting for several years or more, with characteristic mild memory loss and early pathological changes in cortex and hippocampus, but without any functional impairment in the daily activities and there are no clinical symptoms of AD [16]. After that a mild or early stage of AD occurs, with development of particular symptoms (i.e. changes in mood, problems in concentration and memory, disorientation of place and time, and sometimes depression) [16]. A moderate stage of AD is characterized with elevated loss of memory, troubles in recognizing family and friends, deficit in impulse control, and problems in reading, writing, and speaking, while a severe stage of AD or a late-stage of AD is associated with advanced functional and cognitive deterioration, where patients are not able to recognize their family, have problems in swallowing and urination, and this is an end-stage of AD where affected patients die from various complications [16]. Since AD is presented as a continuum, different stages associated with time course of AD exist, depending on the working group that defines them (International Working Group or NIA-AA), and can be divided into: asymptomatic at risk or preclinical AD; prodromal phase or AD with MCI; mild AD dementia or AD with mild dementia; moderate AD dementia or AD with moderate dementia; and the last severe AD dementia or AD with severe

dementia [17]. All these stages are characterized with specific biomarkers, and therefore evaluation of these biomarkers in early stages in asymptomatic patients or those with MCI might offer personalized approach and therapy that targets specific pathological processes and corresponding biomarkers [2].

In the possible prevention, treatment and reduction of the symptoms of AD, early screening and accurate diagnosis are the most important. Biomarkers should be used for the early diagnosis of AD, and this approach offers an improved therapeutic strategy to slow the progression of disease and treat or reduce the symptoms [18]. Following recent recommendations of the International Working Group, biomarkers should detect individuals in early stages who are at risk for progression to AD dementia, or to AD, and will differentiate various types of dementia and different AD phenotypes and assist in identifying those at risk for symptomatic AD [18,19]. However, the prediction or diagnosis of AD should not be based exclusively on AD biomarkers, but should be combined with the clinical assessment [18]. The diagnosis of AD already starts in primary health care with taking patients' medical history, which is afterwards complemented by physical examination, laboratory tests and cognitive screening (Figure 1). The importance of including both neuroimaging and CSF biomarkers is in ruling out AD as the underlying cause of cognitive disfunction (Figure 1), in enabling earlier, more accurate and differential diagnosis of AD, and in enabling personalized management and treatment of AD [20].

2 CSF biomarkers in the diagnosis of AD

The importance of CSF biomarkers in the diagnosis of AD is rapidly increasing, and this interest is also growing in terms of monitoring the response to therapy, especially in the light of newly available approaches to the therapy of neurodegenerative diseases. Determination of CSF amyloid beta (Aβ), total tau (t-tau), and phosphorylated tau (p-tau) levels has been included in both clinical and research diagnostic criteria [18,19,21].

The existing literature is mostly focused on biomarkers that are associated with AD pathogenic process, and the main clinical value of these biomarkers is to facilitate the diagnosis of AD and help the clinician to discriminate between AD associated phenotypes and non-AD pathologies. Diagnostic biomarkers in AD focus on three main pathological features of AD which reflect the process underlying AD: accumulation of Aβ, hyperphosphorylation of tau, and neuronal degeneration (less specific markers of AD) [11]. CSF biomarkers that have been researched the most and have shown the best biomarker properties so far include $A\beta_{42}$, $A\beta_{40}$, p-tau_{181}, p-tau_{217}, and t-tau. These markers accurately identify the pathological changes associated with AD, even in early stages of the disease (asymptomatic and prodromal stages), and they have potential to predict cognitive decline [22]. Markers associated with Aβ accumulation and amyloid processing and the markers of tau pathology are useful for the differentiating AD patients from those with non-AD dementia which will be discussed in more details in the following sections of this review. Some clinical practices have replaced t-tau as a measure of neurodegeneration with neurofilament light chain (NfL) because of its high sensitivity [23]. Other CSF biomarkers which are currently being researched, and will be discussed in the further text, have a better potential to one day be used as biomarkers for staging of disease and prognosis, less for the differential diagnoses [18].

This review concentrates on the most relevant measures of neurodegeneration that are being determined in order to distinguish patients with AD from healthy control subjects and individuals with mild cognitive impairment (MCI) in order to provide an overview of the latest information available in the scientific literature. The review focuses on markers related to

amyloid processing, markers of tau pathology, neuroinflammation, neuroaxonal injury and degeneration, synaptic loss and dysfunction, and markers of α-synuclein pathology (Figure 2).

2.1 Markers of beta-amyloid accumulation and amyloid processing

A significant progress in AD research was the identification of Aβ as the primary protein component of amyloid plaques, formed through the enzymatic breakdown of its precursor, the amyloid precursor protein (APP). In the amyloidogenic pathway, APP is cleaved by β-secretase, resulting in the production of sAPPβ and βCTF 99 fragments [24]. Subsequently, βCTF 99 is cleaved by γ-secretase, leading to the generation of Aβ peptides of various lengths, predominantly $A\beta_{42}$ and $A\beta_{40}$, with the former being much more prone to aggregation [25] and the formation of amyloid plaques associated with AD. In contrast, the nonamyloidogenic pathway involves cleavage by α-secretase and production of sAPPα and αCTF 83 fragments, while further processing by γ-secretase generates peptides like p3, which is less implicated in AD pathology [26].

The CSF levels of $A\beta_{42}$, when combined with total tau (t-tau) and phosphorylated tau (p-tau), form the recognized CSF pattern used to diagnose AD, with $A\beta_{42}$ demonstrating the highest diagnostic accuracy [27]. The sensitivity for predicting progression to AD in patients with MCI was found to be 95%, and the specificity 87% [28]. For distinguishing AD from other conditions, the sensitivity of CSF $A\beta_{42}$ was 85% and the specificity 42% in case of Lewy body disease (LBD), 85% and 77% in case of frontotemporal dementia (FTD) [27], and 77% and 80% for vascular dementia (VaD) [29]. Moreover, evidence suggests that $A\beta_{42}$ levels may serve as a predictor of disease progression in individuals with normal cognition and those with MCI [27]. Levels of CSF $A\beta_{42}$ were significantly reduced in AD patients compared to normal individuals [30]. This reduction is attributed to increased deposition of amyloid plaques in the brain, leading to decreased levels of $A\beta_{42}$ in CSF. In previous studies, reduced CSF levels of $A\beta_{42}$ have also been observed in other non-AD disorders, such as FTD, LBD, and VaD [30]. Nevertheless, these observations could be linked to mixed pathology, suggesting the existence of various pathological conditions or overlapping characteristics among the aforementioned diseases. According to the recent study, it is proposed that the p-tau/$A\beta_{42}$ ratio could serve as a valuable tool in distinguishing AD from FTD with primary language difficulties [31]. However, the same ratio does not effectively differentiate AD from behavioral variant or FTD nor from FTD as a collective entity [31]. In addition, CSF $A\beta_{42}$ could be a differentiating marker for the detection of prodromal AD in clinically diagnosed amnestic MCI patients [32]. Conversely, another study found that, compared to AD, cerebral amyloid angiopathy (CAA) showed similar levels of $A\beta_{42}$, but lower levels of $A\beta_{40}$ [33].

Previous study has reported varied findings regarding CSF $A\beta_{40}$ levels [34]. The reports showed either decreased, unchanged, or elevated CSF $A\beta_{40}$ levels in AD compared to other forms of dementia [34]. Recent research highlighted a significant age-independent rise in CSF $A\beta_{40}$ levels in AD, along with a positive association between CSF $A\beta_{40}$ and p-tau_{181} concentration, even in non-AD individuals, suggesting that initial amyloid peptide levels may serve as a risk factor for sporadic AD [34]. On the other hand, diminished CSF $A\beta_{40}$ levels could indicate alternative conditions such as FTD, CAA [34], normal pressure hydrocephalus [35] and multiple sclerosis [36].

Besides $A\beta_{42}$ and $A\beta_{40}$, $A\beta_{37}$ and $A\beta_{38}$ may help in distinguishing AD from other dementias, such as FTD and LBD [30]. The noticeable decrease in CSF $A\beta_{42}$ levels was reported in AD, while in LBD the CSF levels of $A\beta_{38}$, $A\beta_{40}$ and $A\beta_{42}$ were found to be decreased [37]. This indicates that amyloid metabolism is altered in LBD, even when there is no concurrent

presence of AD pathology. Furthermore, differentiating AD from CAA using CSF biomarkers is challenging due to symptom overlap between these diseases [38]. It was suggested that adding $A\beta_{38}$ and $A\beta_{43}$ to standard AD biomarkers could improve this differentiation [39], while others found no enhancement in distinguishing between AD and CAA with their inclusion [40]. The significant improvement in AD diagnosis was achieved through calculating the CSF $A\beta_{42}/A\beta_{40}$ ratio [41]. The CSF $A\beta_{40}$ initially showed limited potential as a stand-alone biomarker. However, it quickly became apparent that normalizing $A\beta_{42}$ levels to the total Aβ quantity, represented by the most abundant isoform $A\beta_{40}$, yields superior diagnostic performance compared to $A\beta_{42}$ alone [42]. Consistent $A\beta_{40}$ levels were observed across AD patients, non-AD patients and controls, but the reduction in $A\beta_{42}$ levels significantly elevated the $A\beta_{42}/A\beta_{40}$ ratio [27]. Subsequent study revealed notable differences in $A\beta_{42}/A\beta_{38}$ and $A\beta_{42}/A\beta_{40}$ ratios in AD, suggesting superior diagnostic potential compared to individual biomarkers [27]. Further, CSF $A\beta_{42}/A\beta_{38}$ and $A\beta_{42}/A\beta_{40}$ ratios exhibited enhanced performance in distinguishing AD from other forms of dementia, including Parkinson's disease dementia (PD), LBD and subcortical VaD [43]. In addition, it was proposed that the $A\beta_{42}/A\beta_{40}$ ratio is highly valuable for differentiating between AD and LBD patients, especially during the prodromal stage when clinical diagnosis proves to be particularly challenging [44]. Moreover, recent study showed that the $A\beta_{42}/A\beta_{40}$ ratio outperformed $A\beta_{42}$ alone in distinguishing AD from FTD [45].

In addition to these findings, CSF sAPPα and sAPPβ have been proposed as potential novel CSF biomarkers for AD and several other neurodegenerative conditions [46]. Their effectiveness has not met expectations, often yielding conflicting results, with limited studies examining their utility in distinguishing between different neurodegenerative diseases beyond comparisons with healthy elderly controls [47,48]. Significant elevations in both sAPPα and sAPPβ levels in individuals in the MCI-AD group compared to those in the MCI-others group were reported [47]. The same group also verified a strong correlation between levels of sAPPα, sAPPβ, p-tau, as well as t-tau, suggesting potential pathological connections between tau and sAPPs [48]. This correlation is significant as the increase in p-tau and t-tau levels is believed to indicate the neurodegenerative alterations linked to AD [48]. Finally, the study by Alcolea et al. offers pathological confirmation that low levels of sAPPβ and high levels of chitinase-3-like protein 1 (CHI3L1 or YKL-40) in the CSF are linked to FTD [49]. Thus, these biomarkers may be valuable, especially in specific clinical situations where FTD is suspected.

2.2 Markers of tau pathology

P-tau protein levels along with $A\beta_{42}$ levels are known as ''core CSF biomarkers'' and are assumed to have the highest diagnostic accuracy for the early diagnosis of AD [50–52]. The NFTs are considered as second neuropathological hallmarks of AD, after Aß plaques [53]. NFTs are composed of a highly-phosphorylated form of the microtubule-associated protein tau [54]. Tau protein is mainly present in axons and plays an important role in connecting microtubules and controlling axonal length and stability [55]. Abnormal phosphorylation of tau proteins causes detachment of tau from microtubules, degradation of microtubules, which affects axons, and ultimately leads to neuronal death [56]. Injury and degradation of axons and neuronal cell death, lead to the release of tau protein to CSF, which is reflected as t-tau levels [57,58]. Consequently, t-tau levels indicate the degree of neuronal loss and neurodegeneration in AD [59].

A key component of NFTs in AD pathology, and more accurate biomarker for differentiating AD from other dementias in contrast to t-tau, is p-tau. Recent study revealed at least 59 different p-tau phosphorylation sites which could be related to AD using mass spectrometry [60]. CSF p-tau levels have demonstrated strong prognostic accuracy in AD, especially in predicting cognitive decline in patients with AD and MCI [61,62]. Furthermore, the elevated levels of p-tau in AD, compared to other neurodegenerative conditions, such as VaD, FTD, progressive supranuclear palsy (PSP) or corticobasal syndrome (CBS), make it a valuable marker in differentiating AD from other types of dementia [63,64]. Several p-tau species have been reported to be increased in the very early stages of AD such as p-tau$_{181}$, p-tau$_{231}$ and p-tau$_{217}$ [65]. P-tau$_{181}$ is one of the most studied variants of phosphorylated tau protein, and it is considered a gold standard for AD diagnosis [66]. It is reported to be elevated in patients with MCI and AD continuum [67,68]. Even though p-tau$_{181}$ is elevated in AD, in other types of dementia, such as FTD, it is significantly decreased compared to healthy controls [69,70]. However, p-tau$_{181}$ is not significantly different between progressive supranuclear palsy, corticobasal degeneration, or other variants of FTD [70]. Another variant of p-tau is p-tau$_{231}$ that has been reported to be elevated in patients with AD and MCI, compared to controls [71,72]. Previous studies also demonstrated the role of p-tau$_{231}$ in differentiating AD patients from FTD, VaD and LBD with the sensitivity of 90.2% and with 80% of specificity [73]. More recent research has shown that p-tau$_{217}$ exhibited higher accuracy than p-tau$_{181}$ and p-tau$_{231}$ in distinguishing AD from non-AD dementia [74,75]. In a study conducted by Barthélemy and colleagues, p-tau$_{217}$ was a better predictor of Aβ positivity than p-tau$_{181}$ [74]. Compared to CSF p-tau$_{181}$, CSF p-tau$_{217}$ showed a stronger correlation with CSF Aβ$_{42}$ and with Aβ and tau-PET [76]. Similarly, another study also reported better correlation of p-tau$_{217}$ in differentiating AD from other neurodegenerative diseases with a 91% sensitivity and specificity [77]. Additionally, in comparison to p-tau$_{181}$, p-tau$_{217}$ had a 90% accuracy rate in separating AD from tauopathies such as Pick disease, progressive supranuclear palsy, and corticobasal degeneration [78]. These studies suggest that p-tau$_{217}$ is the most accurate tau biomarker for AD in both pre-clinical and advanced stages.

Although CSF t-tau and p-tau are well recognized biomarkers for differentiating AD from other dementias, their diagnostic value is significantly enhanced when measured in combination with Aβ$_{42}$ [22,79–81]. Furthermore, the combination of tau and Aβ markers was demonstrated to be useful in predicting disease progression [82,83]. A recent study revealed that CSF p-tau/Aß$_{42}$ ratio could be accurate predictor of conversion from MCI to AD dementia, with 82.9% sensitivity and 90% specificity [84]. It was previously reported that individuals with FTD exhibited the highest levels of Aß$_{42}$ and the lowest levels of t-tau and p-tau in FTD, whereas AD patients showed the highest levels of t-tau and p-tau, and the lowest levels of Aß$_{42}$ and Aß$_{42}$/p-tau ratios [85]. More recent study aimed to determine the CSF levels of tau and Aβ for distinguishing FTD from AD [31]. This study reported that the p-tau/Aß$_{42}$ ratio might be beneficial in distinguishing between AD and FTD characterized by primary language impairments, but was not effective in discriminating AD from the behavioral variant of FTD, or from FTD as a collective group [31]. Therefore, investigating and gaining a deeper understanding of the role of the tau protein in the mechanisms and underlying pathology of AD could enhance diagnostic and prognostic accuracy, particularly in distinguishing AD from other forms of dementia.

2.3 Markers of neuroaxonal injury and degeneration

Recently, the question has been raised whether AD can be considered as an axonal degeneration disease [86]. Both key factors in the pathogenesis of AD, tau and Aβ, have a predominant expression and an important role in the physiological functions of axons. When microtubules are broken or other axonal injuries occur, Aβ and tau may be abnormally modified and the result is deteriorated neuroaxonal damage. Neuroaxonal injury and consequential synaptic dysfunction are key features of AD, and synaptic loss is closely associated with cognitive decline in the disease [87]. One of the earliest changes that occur in early AD is axonal dystrophy which is associated with extracellular depositions of Aβ and has been observed to contribute to synaptic alterations occurring in AD [88]. As previously mentioned, t-tau levels represent a general signal of neurodegeneration [65]. Different studies reported a higher concentration of t-tau levels in CSF of AD patients in comparison to healthy subjects [89–91]. Since CSF levels of t-tau protein are believed to reflect the extent of neuronal damage, it is hypothesized that very high CSF t-tau levels, compared to moderately elevated levels, may correspond to differences in the degree of cortical atrophy and various clinical subtypes of AD [91]. Previous studies reported a faster rate of clinical progression in AD patients with high CSF t-tau levels [92,93]. Recent studies also reported elevated CSF t-tau levels in rapidly progressive AD (rpAD) [94] and in patients with atypical AD clinical phenotypes [91]. High concentration of CSF t-tau levels may not be very specific for AD, since high levels were observed in other types of dementia such as VaD [95] and FTD [95,96]. Furthermore, fluctuations in CSF t-tau levels also occur in cerebral ischemia [97], hemorrhage and seizures [98], as well as in cases of encephalitis and acute neurodegenerative diseases such as Creutzfeldt-Jakob disease (CJD) [99]. As a consequence of severe neuronal damage in CJD, studies reported t-tau levels are much higher in CJD than in AD.

Neurofilament light chain (Nfl) is one of four subunits of neurofilaments, which are proteins that are located in the neuronal cytoplasm that help maintain the structural stability of neurons and enable the growth of axons [100]. Due to its presence in neurons, when neuroaxonal damage occurs, Nfl levels increase in interstitial fluid and consequently, in CSF and plasma [101]. Therefore, the Nfl level is increased in a whole variety of neurological disorders, including neurodegenerative, inflammatory, traumatic and cerebrovascular diseases and is not specific to the neurodegenerative changes present in AD [102]. Although nonspecific, Nfl provides important information about the progress of neurodegeneration and should be used as a biomarker in AD, but not as a biomarker of AD [66]. The presence of Nfl in CSF and plasma correlates strongly, but concentrations in CSF are much higher and that is why it is the sample of choice for clinical use [103]. The disadvantage of CSF sampling is the invasiveness of the method and, consequently, the difficult implementation of longitudinal studies. With the development of ultra-sensitive techniques for the determination of biomarkers in plasma, such as Single molecule array (SIMOA), the determination of Nfl from plasma has become much more accurate. Measuring Nfl from plasma would solve the problem of invasiveness of sampling and enable longitudinal studies [104]. There is an increasing number of studies that indicate that both CSF and plasma Nfl may serve as diagnostic, prognostic and monitoring biomarkers in differential diagnosis between neurodegenerative diseases, including AD, and nondegenerative disorder, highlighting the Nfl as one of the most promising biomarkers to be used in clinical and research settings in the future [100]. This conclusion is also supported by the results of a recent study that reported a good accuracy for plasma Nfl levels in distinguishing between ATN+ and ATN− subjects in the group of patients with subjective cognitive decline (AUC=0.815) and MCI patients (AUC=0.818) [105].

Visinin-like protein 1 (VILIP-1) is a neuronal calcium-sensor protein and a member of the visinin-like protein subfamily [106]. Its physiological role is to regulate neuronal growth,

survival, and synaptic plasticity [107]. Disturbance of calcium homeostasis in neurons, that occurs in AD, causes degeneration of vulnerable neurons and release of VILIP-1 into the extracellular fluid [108]. Because of this characteristic, VILIP-1 has been rated as a marker of neuronal injury. Other biomarkers based on the same pathophysiological process are stanniocalcin-1 [109] and pre-synaptic vesicle protein synaptotagmin [110]. Many studies have confirmed that CSF VILIP-1 is significantly increased in both AD and AD-MCI patients compared to controls and therefore VILIP-1 represents a good diagnostic and prognostic biomarker [111] but unlike Nfl, the correlation between plasma and CSF levels is poor [108].

2.4 Markers of synaptic dysfunction

Synaptic loss and dysfunction are considered to be one of the earliest signs of neurodegeneration [112]. This has led to a high interest in researching synaptic proteins as potential biomarkers that could be useful in diagnosis, prognosis, and guiding treatment of neurodegenerative diseases. Synaptic proteins were first detected in CSF almost 30 years ago [113] and since then the methods for their detection and quantification have advanced significantly, thus increasing their biomarker potential. Today, markers of synaptic dysfunction are mainly focused on one postsynaptic protein, neurogranin, and three presynaptic markers, synaptosomal-associated protein 25 (SNAP-25), synaptotagmin-1, and growth-associated protein 43 (GAP-43) [114].

Neurogranin (Ng), a small calmodulin-binding protein, is expressed mainly by pyramidal cells in the hippocampus and cortex, where it forms granule-like structures [115]. This postsynaptic protein regulates calcium influx via calmodulin and mediates the plasticity, regeneration of synapses, and long-term potentiation [116]. Despite being a good general marker of neurodegeneration, Ng has actually been shown to be more specific for AD then for other neurodegenerative diseases [117,118]. In CSF samples it is possible to detect both full-length Ng and its fragments, mostly short C-terminal peptide species [119]. The function of these Ng fragments is still unknown. However, the evidence suggests that these different fragments have similar predictive value regarding AD [120]. Overall, the research so far points to elevated CSF Ng levels in patients diagnosed with AD, even in prodromal phase of the disease [119,121–133]. Also, it has been show that CSF Ng levels could be used to predict the progress from MCI to AD [119] and distinct typical from atypical AD forms [134]. A recent meta-analysis [135] confirmed the potential of CSF Ng in predicating memory and executive function decline in subjects diagnosed with MCI. The study also suggests the potential use of CSF Ng/$A\beta_{42}$ as cognitive function biomarker [135]. These results point to CSF Ng as an useful biomarker for detecting neurodegeneration in the early stages of AD and for differentiating AD from several other AD tauopathies. However, it should be kept in mind that there are also data that do not support the specificity of Ng in AD diagnosis [136].

Synaptosomal-Associated Protein, 25kDa (SNAP-25) is a presynaptic protein which is, along with the vesicle-associated membrane proteins (VAMPs) and syntaxins, a component of the SNARE (soluble N-ethylmaleimide-sensitive factor activating protein receptor) complex, thus playing a role in vesicle formation and neurotransmitter release during synaptic transmission [137]. This presynaptic protein is important in neuronal survival, in the process of neurite outgrowth and long-term potentiation (LTP) [138]. Studies focused on the role of SNAP-25 in AD confirmed higher CSF SNAP-25 levels in AD patents compared to healthy controls, and have demonstrated that these changes can even be detected in the early stages of the disease [75,122–124,139–145]. A recent meta-analyses confirmed that increased CSF SNAP-25 levels can be used to differentiate AD and/or MCI patients from healthy control subjects, which

confirms the potential of this biomarker in the early diagnosis of AD [125,139]. However, elevated CSF levels of SNAP-25 have also been detected in patients diagnosed with other neurodegenerative disorders, including PD, CJD, HD, and it has been associated with psychiatric conditions, including attention deficiency hyperactivity disorder, schizophrenia, and bipolar disorder [146]. The other two presynaptic markers, GAP-43 and synaptotagmin-1, have been less investigated as potential CSF biomarkers in AD. Nevertheless, the level of GAP-43 has been found to be increased in preclinical AD [147–149]. The above discussed synaptic biomarkers were found to have a good discriminating power when trying to distinguish patients with AD from the ones with non-AD dementia [124]. The highest discriminating power for distinguish patients with AD from neurologic controls was suggested for the soluble form of SNAP-25 (SNAP-25aa40) [124].

In addition to the previously mentioned reliable biomarkers of synaptic impairment, other markers of synaptic disfunction should also be considered in the future studies. One of these markers is a member of the epithelial growth factor (EGF) family, neuregulin 1 (NRG1). NRG1 is involved in neural development, migration and survival of neurons, axon pathfinding, development of glia cells, myelination, and synaptogenesis [150,151]. Proteolytic processing of NGR1 leads to the formation and secretion of soluble forms which interact with post-synaptic receptor tyrosine-protein kinase erbB4 (ErbB4). The levels of NRG1 and ErbB4 were found to be altered in hippocampus and cortex of subjects diagnosed with AD [152,153]. Recent study has shown increased CSF NGR1 levels in subjects with AD and MCI-AD, in comparison to healthy controls and other non-AD dementias [154]. The CSF levels of NGR1 also positively correlated with CSF $A\beta_{42,}$ and negatively with MMSE scores [154]. However, the results showed that CSF NRG1 levels, which were found increased in AD and MCI-AD subject compared to controls, had lower discriminatory power than $A\beta_{42}$, t-tau, and p-tau [154]. These results lead to conclusion that markers of synaptic loss and dysfunction poses a great promise as biological measures that could be useful in diagnostics and in therapeutic successes monitoring.

2.5 Markers of neuroinflammation

The presence of neuroinflammation has been well recognized as a concurrent pathological condition in AD. The existence of localized low-level inflammation in the initial stages of AD has been firmly confirmed [155]. Neuroinflammation and cerebrovascular dysfunction are the first occurrences that manifest during the presymptomatic phases of AD and have a role in the further development of the disease [67,83,156]. Both microglia and astrocytes are essential for the initiation and regulation of neuroinflammation [157]. Activated microglia are present in the vicinity of amyloid plaques and play a role in the generation of neurotoxic substances that accelerate neuronal harm [158]. Pathogenic stimuli also trigger astrocytes in AD. Astrocytes have a role in neuroinflammation through the release of pro-inflammatory cytokines, reactive oxygen species, and the facilitation of blood-brain barrier dysfunction [159]. Consequently, this process intensifies the damage to neurons.

The glycoprotein YKL-40, often referred to as Chitinase-3-like protein-1 (CHI3L1), is classified as a member of the chitinase family, although it lacks chitinase activity [160]. In the central nervous system, activated astrocytes and microglia are the main source of YKL-40, which they secrete in response to different inflammatory stimuli and neurodegeneration [161,162]. YKL-40 is involved in several biological processes, including inflammation, extracellular matrix remodeling, cell proliferation, and tissue healing [163]. However, its precise biological role remains incompletely elucidated. The role of YKL-40 in the development of AD and in the

disease's progression is still unclear, but it may be useful as a biomarker for long-lasting brain diseases that have an inflammatory background [164]. People with neurodegenerative diseases have elevated YKL-40 CSF levels, prompting research into its potential involvement in neuroinflammation and neuronal injury [156,165]. YKL-40 has been investigated as a potential therapeutic target, considering its role in various physiological processes and pathological disorders. Modifying its activity or expression may offer promise for treating inflammation, tissue remodeling, and related disorders. A meta-analysis demonstrated that individuals with AD exhibit higher levels of YKL-40 in CSF and plasma compared to healthy controls [166]. These data also suggest a noteworthy association between elevated levels of YKL-40 in CSF and AD. An additional cohort study encompassing 288 people, including both healthy controls and patients diagnosed with various kinds of dementia, assessed the amounts of CSF YKL-40 [167]. Compared to controls, CJD and AD showed higher YKL-40 levels. However, these levels were not significantly higher in VaD or DLB [167]. Wang et al. studied how APOE ε4 affected the levels of YKL-40 in CSF in subjects who were cognitively normal, or were diagnosed with MCI or AD [168]. APOE ε4 carriers had higher levels of CSF YKL-40 than noncarriers with MCI [167]. CSF tau and p-tau concentrations significantly correlated with CSF YKL-40 concentrations in the MCI group [167]. These results show that APOE ε4 may be associated with the amount of CSF YKL-40 in MCI subjects [168]. Another study found an increase in CSF YKL-40 levels in individuals with MCI compared to controls [166]. However, the area under the curve (AUC) was smaller, indicating that YKL-40 has only a modest potential as a biomarker in the context of AD. In a recent study, Abu-Rumeileh et al. discovered that YKL-40 had a moderate diagnostic value, with a sensitivity and specificity of 80% or higher, when comparing controls to AD [169]. A novel study showed that reactive astrocyte biomarkers, like YKL-40, contribute to the impairment of neuronal function and cognitive impairment [170].

Microglia in the CNS predominantly express the cell surface receptor Triggering receptor expressed on myeloid cells 2 (TREM2) [171]. It is a member of the immunoglobulin superfamily and has a vital function in controlling innate immune responses and phagocytosis [171]. The role of TREM2 in microglial activation and response to Aβ pathology has attracted major attention in the area of AD research. Microglia activation leads to the cleavage and subsequent generation of soluble TREM2 (sTREM2), a measurable indicator of microglial activity in the CSF [172]. Previous research has shown a link between AD pathology and elevated CSF sTREM2, suggesting a strong diagnostic capability [173,174]. The increase in CSF sTREM2 occurs before symptoms appear, but after amyloidosis and neuronal damage [175]. According to a recent study, CSF sTREM2 levels drop in the presence of Aβ pathology but not tau-related neurodegeneration [175,176]. Just the opposite, the levels of CSF sTREM2 rise in the interaction with tau-related neurodegeneration [175,176]. A notable elevation in CSF sTREM2 levels in non-AD neurodegenerative disorders was found [177]. However, each neurodegenerative disorder has distinct pathological and clinical characteristics. Monitoring TREM2 and YKL-40 may facilitate the assessment of microglial activation and its involvement in the pathogenesis of AD. Moreover, it is important to acknowledge that these indicators possess the capacity to serve as targets for treatment strategies aimed at modulating microglial activation. The therapeutic implications of targeting microglial activation through TREM2 and YKL-40 modulation require further research.

Glial Fibrillary Acidic Protein (GFAP) is a protein that is fundamental in the formation and maintenance of the cytoskeleton of glial cells, specifically astrocytes. It is a reliable marker for reactive astrogliosis and a biomarker for neurodegenerative disorders [178,179]. GFAP can serve as a potent biomarker for predicting dementia risk over a decade before clinical

diagnosis [180]. Elevated CSF GFAP initial levels are indicative of accelerated cognitive decline and associated with significant alterations in other AD biomarkers [181,182]. Given its close proximity to the brain, CSF GFAP is considered to be a precise indicator of brain pathological processes [183]. In the hippocampus of AD patients, especially those with the APOE ε4/ε4 genotype, GFAP levels are significantly elevated [184]. Greater accumulation of tau in the lateral temporal and frontal areas of the brain was found to strongly correlate with higher levels of GFAP in the periphery [185]. Blood GFAP levels were shown to exhibit a robust correlation with amyloid pathology, making it a more reliable indicator then CSF GFAP [186–188], particularly in predicting the transition from MCI to dementia [189–191]. It is more appropriate to use GFAP as an initial screening strategy rather than a final diagnostic indicator, as it is not exclusive to AD and is implicated also in other neurological disorders [192–194]. Higher levels of GFAP generally correlate with faster declines in cognitive function and contribute to the connection between amyloid pathology and tau protein buildup, ultimately leading to cognitive decline.

2.6 Markers of α-synuclein pathology

Alpha-synuclein (α-syn) is a small (140 amino acids) ubiquitously expressed protein, predominantly found in presynaptic sites in the central and peripheral nervous system [195]. It is encoded by *SNCA* gene and normally, it can exist in two forms, as unfolded monomer or as a folded tetramer of about 58 KDa [196]. It participates in the regulation of synaptic vesicle pool and trafficking [197] and has an important role in assembly of exocytosis mediating SNARE complex [198]. Not only it can form a broad range of structures and associate with lipid and protein chaperones, but under certain circumstances α-syn folds and aggregates into pathogenic forms comprising oligomers, protofibrils, fibrils, which further bring to the formation of protein inclusions [199]. Some post-translational modifications, including phosphorylation, can promote α-syn folding and aggregation which are critical steps in synucleinopathies. Namely, under physiological conditions, less than 5% of monomeric α-syn is phosphorylated, while in pathological protein aggregations such as Lewy bodies, approximately 90% of α-syn is phosphorylated. In typical synuleinopathies, PD, DLB, and multiple system atrophy, neuronal loss is accompanied by the presence of α-syn inclusions. In AD there is an overlap of α-syn, Aβ plaques, and tau tangle pathologies [200].

Accumulating evidence imply reduced levels of α-syn in CSF of patients with typical synucleinopathies, PD and DLB [201], but a lack of association between PD severity and CSF levels [202]. In a study by Lilamand et al. [203] α-syn levels were found to be significantly lower in CSF of patients with DLB than in patients with AD and authors suggested that CSF α-syn evaluation could improve the early differentiation between DLB and AD. There are also studies showing increased CSF α-syn levels in patients with MCI and AD [204–206], with positive correlation detected between α-syn levels and disease progression [201] or severity of cognitive decline [204,205]. More precisely, CSF α-syn levels were found to be significantly higher in patients with AD with all positive CSF triple markers (Aβ42, total tau, and phosphorylated tau) [205,207]. However, there are also opposite results indicating decreased levels of CSF total-α-syn not only in patients with PD and DLB, but also in AD patients compared to healthy control subjects [208]. Some studies demonstrated associations of CSF a-syn concentrations with brain Aβ deposition measures as well as with CSF t-tau and p-tau181 concentrations [209,210]. This can be further explained by functional studies indicating that α-syn interacts with AD-related proteins. According to results of *in vitro* studies, the interaction of Aβ with α-syn can accelerate the fibril formation by increasing the aggregation

rate of α-syn [211]. Also, α-syn oligomers can generate and stabilize Aβ oligomers, leading to fibril-like conformations [212]. It was also shown that α-syn induces tau aggregation, while tau accelerates the fibrillization of α-syn [213]. A study dealing with several plasma and CSF biomarkers in different neurodegenerative disorders, reported significantly higher CFS α-syn levels in patients with AD and MCI in comparison to respective control subjects [214]. However, no significant difference in plasma α-syn levels was found among the same groups of subjects [214].

The α-syn levels are not associated only with AD-related proteins, but also with AD-related genes, such as those coding for presenilin 1 (*PSEN1*) and apolipoprotein E (*APOE*). A recent study found significantly increased tau/α-syn ratio in AD when compared with healthy controls [207]. Additionally, the ratio was significantly higher in early than in late onset AD [207]. There are reports about the dose-response relationship between the AD risk increasing allele, *APOEε4*, and CSF α-syn levels, with *APOEε4* homozygotes having the highest CSF α-syn levels [204]. Lewy body pathology in the amygdala was reported in the carriers of *PSEN 1* mutation among AD patients [215]. Moreover, the frequency of Lewy body deposition was higher in the cases with mutations in *PSEN1* than in those with mutations in *PSEN2* [215].

Although not completely straightforward, these findings imply the important role of α-syn in AD pathology which can be reflected in α-syn measurable manifestations at the periphery. Certainly, a complex interplay between numerous biological processes leading to synucleinopathies still has to be investigated, but results of the studies so far support the idea of α-syn as a biomarker that at least could add to the sensitivity and specificity of standard AD biomarker panel.

3 Conclusions

Biomarkers that have entered routine clinical use ($A\beta_{42}$, t-tau, p-tau181, t-tau/$A\beta_{42}$ ratio), along with other markers that show great potential to be included in the practice itself, are of the great importance in AD diagnosis, not only because they can help distinguish AD from healthy controls, but also due to their ability to help differentiate between different neurodegenerative disorders. In conclusion, CSF biomarkers provide significant additional value in the AD assessment by offering more precise, timely and differential clinical diagnosis of AD at different stages and across different ages. In future, the role of CSF biomarkers in AD will be even more prominent in guiding targeted therapeutic interventions and tailoring patient individual management and support in order to improve the quality of life of both AD patients and their caregivers.

4 Expert Opinion

Considering the direct interaction of CSF with the brain, CSF biomarkers closely reflect the pathophysiological alterations occurring in AD brain. Therefore, it is not surprising that, in addition to neuropsychological evaluation, core CSF biomarkers ($A\beta_{42}$, t-tau, and p-tau181) have been recommended for improvement of timely, accurate and differential diagnosis of AD, as well as to assess the risk and rate of disease progression [20]. Specifically, decreased Aβ42 and elevated p-tau levels in CSF suggest Aβ and tau neuropathology of AD, while increased total-tau concentrations represent a non-specific marker of injured neurons.

There are various advantages of CSF biomarkers. In contrast to PET imaging biomarkers, CSF biomarkers are much cheaper, and could be quickly and simply obtained in a clinical setting [216]. In addition, CSF may enable detection of some biomarkers, which could not be

identified by brain imaging; whereas some CSF biomarker alternations may precede PET biomarker changes; such as elevation of p-tau during AD progression and related cognitive decline [20,83]. However, CSF biomarkers are unable to reflect regional differences in the brain neuropathology that may be particularly important during early AD [83].

Moreover, studies demonstrated that CSF biomarker ratios ($A\beta_{42}/A\beta_{40}$, p-tau/$A\beta_{42}$, t-tau/$A\beta_{42}$) could perform even better than individually measured values and may correct for inter-individual differences. In that way, CSF AD biomarker ratios may add relevant information in the differential diagnosis of neurodegenerative diseases, as well as predict the risk of progression from MCI to AD [217].

On the other hand, use of CSF testing may be limited due to perceived invasive nature of lumbar puncture (LB), although several large multicenter studies demonstrated that this procedure is easy, safe and tolerable, with very rare occurrence of serious complications. Further limitations to the routine use of CSF biomarkers include a lack of skills and training in LB procedure, the inability to collect samples from large populations, complex interpretation of the test results, and still present skepticism about their clinical value.

Nevertheless, advanced detection technologies, uniform protocols and standards, as well as fully automated testing procedures that can measure multiple CSF biomarkers in the same sample are now available [20]. Therefore, CSF biomarkers hold promise for a more personalized medicine approach in staging, tracking, and categorization of AD, as well as for the assessing the effects of potential therapeutics [83].

More precise and personalized AD diagnosis may result in lower care costs, delayed institutionalization and reduced mortality, and could be useful for selection of patients suitable to receive novel disease-modifying therapies (DMTs), such as immunotherapies (aducanumab, lecanemab, donanemab) targeting aggregated forms of Aβ [218]. Specifically, via CSF analysis these anti-Aβ mAbs have been confirmed to affect both Aβ plaques, as well as t-tau and p-tau levels [219].

In addition to the core CSF biomarkers, various other CSF markers related to synaptic dysfunction, neuroinflammation, and glial activation (neurogranin, SNAP-25, Nfl, YKL-40, TREM2) are now investigated and have yet to be validated for future potential clinical use in early and differential diagnosis, as well as prognosis of AD [20,83].

Moreover, although intensively investigated blood-based biomarkers, as well as biomarkers measured in other fluids, offer relatively non-invasive approach, which is cost-effective and simple to carry, further studies are needed to establish if their clinical utility in AD is comparable to CSF biomarkers [216].

Disclosure statement: The authors report there are no competing interests to declare.

References

[1] Joe E, Ringman JM. Cognitive symptoms of Alzheimer's disease: clinical management and prevention. BMJ. 2019;367:l6217.

[2] Wang H, Sun M, Li W, et al. Biomarkers associated with the pathogenesis of Alzheimer's disease. Front Cell Neurosci. 2023;17:1279046.

*** The paper discusses the application of biomarkers for AD and the progress of related pathogenesis research.**

[3] Kumar A, Sidhu J, Lui F, et al. Alzheimer Disease [Internet]. StatPearls [Internet]. Treasure Isl. StatPearls Publ. Treasure Island (FL): StatPearls Publishing; 2024. Available from: https://www.ncbi.nlm.nih.gov/books/NBK499922/.

[4] Planche V, Villain N. US Food and Drug Administration Approval of Aducanumab-Is Amyloid Load a Valid Surrogate End Point for Alzheimer Disease Clinical Trials? JAMA Neurol. 2021;78:1307–1308.

[5] Verger A, Yakushev I, Albert NL, et al. FDA approval of lecanemab: the real start of widespread amyloid PET use? - the EANM Neuroimaging Committee perspective. Eur. J. Nucl. Med. Mol. Imaging. Germany; 2023. p. 1553–1555.

[6] Söderberg L, Johannesson M, Nygren P, et al. Lecanemab, Aducanumab, and Gantenerumab - Binding Profiles to Different Forms of Amyloid-Beta Might Explain Efficacy and Side Effects in Clinical Trials for Alzheimer's Disease. Neurother J Am Soc Exp Neurother. 2023;20:195–206.

[7] Swanson CJ, Zhang Y, Dhadda S, et al. A randomized, double-blind, phase 2b proof-of-concept clinical trial in early Alzheimer's disease with lecanemab, an anti-Aβ protofibril antibody. Alzheimers Res Ther. 2021;13:80.

[8] van Dyck CH, Swanson CJ, Aisen P, et al. Lecanemab in Early Alzheimer's Disease. N Engl J Med. 2023;388:9–21.

[9] Dhadda S, Kanekiyo M, Li D, et al. Consistency of efficacy results across various clinical measures and statistical methods in the lecanemab phase 2 trial of early Alzheimer's disease. Alzheimers Res Ther. 2022;14:182.

[10] Cummings J. The National Institute on Aging—Alzheimer's Association Framework on Alzheimer's disease: Application to clinical trials. Alzheimer's Dement. 2019;15:172–178.

[11] Jack CRJ, Bennett DA, Blennow K, et al. A/T/N: An unbiased descriptive classification scheme for Alzheimer disease biomarkers. Neurology. 2016;87:539–547.

**** Proposal of the "A/T/N" system in which 7 major AD biomarkers are divided into 3 binary categories based on the nature of the pathophysiology that each measures.**

[12] Liu C, Li Y, Nwosu A, et al. Sex-specific biomarkers in Alzheimer's disease progression: Framingham Heart Study. Alzheimer's Dement (Amsterdam, Netherlands). 2022;14:e12369.

[13] Morris JC, Schindler SE, McCue LM, et al. Assessment of Racial Disparities in Biomarkers for Alzheimer Disease. JAMA Neurol. 2019;76:264–273.

[14] APA. Diagnostic and statistical manual of mental disorders. 5th ed. Association AP, editor. Arlington, VA; 2013.

[15] Madnani RS. Alzheimer's disease: a mini-review for the clinician. Front Neurol. 2023;14:1178588.

[16] Breijyeh Z, Karaman R. Comprehensive Review on Alzheimer's Disease: Causes and Treatment. Molecules. 2020;25.

[17] Porsteinsson AP, Isaacson RS, Knox S, et al. Diagnosis of Early Alzheimer's Disease: Clinical Practice in 2021. J Prev Alzheimer's Dis. 2021;8:371–386.

[18] Dubois B, von Arnim CAF, Burnie N, et al. Biomarkers in Alzheimer's disease: role in early and differential diagnosis and recognition of atypical variants. Alzheimers Res Ther. 2023;15:175.

*** The review discusses the phenotypic presentation and use of biomarkers for the early diagnosis of AD.**

[19] Dubois B, Villain N, Frisoni GB, et al. Clinical diagnosis of Alzheimer's disease:

recommendations of the International Working Group. Lancet Neurol. 2021;20:484–496.

**** Recommendations of the International Working Group regarding the current limitations of biomarkers in the diagnosis of AD.**

[20] Bouwman FH, Frisoni GB, Johnson SC, et al. Clinical application of CSF biomarkers for Alzheimer's disease: From rationale to ratios. Alzheimer's Dement (Amsterdam, Netherlands). 2022;14:e12314.

[21] McKhann GM, Knopman DS, Chertkow H, et al. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement. 2011;7:263–269.

[22] Hansson O, Seibyl J, Stomrud E, et al. CSF biomarkers of Alzheimer's disease concord with amyloid-β PET and predict clinical progression: A study of fully automated immunoassays in BioFINDER and ADNI cohorts. Alzheimer's Dement. 2018;14:1470–1481.

[23] Hampel H, Cummings J, Blennow K, et al. Developing the ATX(N) classification for use across the Alzheimer disease continuum. Nat Rev Neurol. 2021;17:580–589.

*** State-of-the-art summary of the conceptual framework and clinical importance of the existing AT(N) system and the evolving ATX(N) system.**

[24] Uddin MS, Kabir MT, Jeandet P, et al. Novel anti-Alzheimer's therapeutic molecules targeting amyloid precursor protein processing. Oxid Med Cell Longev. 2020;2020.

[25] Itoh SG, Yagi-Utsumi M, Kato K, et al. Key Residue for Aggregation of Amyloid-β Peptides. ACS Chem Neurosci. 2022;13:3139–3151.

[26] Kuhn AJ, Abrams BS, Knowlton S, et al. Alzheimer's Disease "Non-amyloidogenic" p3 Peptide Revisited: A Case for Amyloid-α. ACS Chem Neurosci. 2020;11:1539–1544.

[27] McGrowder DA, Miller F, Vaz K, et al. Cerebrospinal fluid biomarkers of alzheimer's disease: Current evidence and future perspectives. Brain Sci. 2021;11:1–56.

[28] Blennow K, Zetterberg H. Biomarkers for Alzheimer's disease: current status and prospects for the future. J Intern Med. 2018;284:643–663.

[29] Llorens F, Schmitz M, Knipper T, et al. Cerebrospinal Fluid Biomarkers of Alzheimer's Disease Show Different but Partially Overlapping Profile Compared to Vascular Dementia. Front Aging Neurosci. 2017;9.

[30] Papaliagkas V, Kalinderi K, Vareltzis P, et al. CSF Biomarkers in the Early Diagnosis of Mild Cognitive Impairment and Alzheimer's Disease. Int J Mol Sci. 2023;24.

[31] Casoli T, Paolini S, Fabbietti P, et al. Cerebrospinal fluid biomarkers and cognitive status in differential diagnosis of frontotemporal dementia and Alzheimer's disease. J Int Med Res. 2019;47:4968–4980.

**** The ratio of CSF p-tau/Aβ42 is a sensitive and specific biomarker for distinguishing patients with primary progressive aphasia from those with AD.**

[32] Park JE, Choi KY, Kim BC, et al. Cerebrospinal Fluid Biomarkers for the Diagnosis of Prodromal Alzheimer's Disease in Amnestic Mild Cognitive Impairment. Dement Geriatr Cogn Dis Extra. 2019;9:100–113.

[33] Grangeon L, Paquet C, Guey S, et al. Cerebrospinal Fluid Profile of Tau, Phosphorylated Tau, Aβ42, and Aβ40 in Probable Cerebral Amyloid Angiopathy. J Alzheimer's Dis. 2022;87:791–802.

[34] Lehmann S, Dumurgier J, Ayrignac X, et al. Cerebrospinal fluid A beta 1-40 peptides increase in Alzheimer's disease and are highly correlated with phospho-Tau in control individuals. Alzheimer's Res Ther. 2020;12:1–12.

* **A significant age-independent increase in CSF Aβ40 observed in AD, along with a strong positive correlation between Aβ40 and p-tau181, especially in control patients.**

[35] Chen Z, Liu C, Zhang J, et al. Cerebrospinal fluid Aβ42, t-tau, and p-tau levels in the differential diagnosis of idiopathic normal-pressure hydrocephalus: a systematic review and meta-analysis. Fluids Barriers CNS. 2017;14:13.

[36] Pietroboni AM, Caprioli M, Carandini T, et al. CSF β-amyloid predicts prognosis in

patients with multiple sclerosis. Mult Scler J. 2019;25:1223–1231.
[37] Van Steenoven I, Van Der Flier WM, Scheltens P, et al. Amyloid-β peptides in cerebrospinal fluid of patients with dementia with Lewy bodies. Alzheimer's Res Ther. 2019;11:8–10.
*** Distinct CSF Aβ profiles detected in AD and LBD, with AD showing a decrease in Aβ42 and LBD in Aβ38, Aβ40, and Aβ42.**
[38] Jäkel L, De Kort AM, Klijn CJM, et al. Prevalence of cerebral amyloid angiopathy: A systematic review and meta-analysis. Alzheimer's Dement. 2022;18:10–28.
[39] De Kort AM, Kuiperij HB, Marques TM, et al. Decreased Cerebrospinal Fluid Amyloid β 38, 40, 42, and 43 Levels in Sporadic and Hereditary Cerebral Amyloid Angiopathy. Ann Neurol. 2023;93:1173–1186.
[40] Dargvainiene J, Jensen-Kondering U, Bender B, et al. Aβ38 and Aβ43 do not differentiate between Alzheimer's disease and cerebral amyloid angiopathy. Ann Clin Transl Neurol. 2024;1–6.
[41] Hansson O, Lehmann S, Otto M, et al. Advantages and disadvantages of the use of the CSF Amyloid β (Aβ) 42/40 ratio in the diagnosis of Alzheimer's Disease. Alzheimer's Res Ther. 2019;11:1–15.
[42] Lewczuk P, Wiltfang J, Kornhuber J, et al. Distributions of Aβ42 and Aβ42/40 in the Cerebrospinal Fluid in View of the Probability Theory. Diagnostics. 2021;11:2372.
[43] Janelidze S, Zetterberg H, Mattsson N, et al. CSF Aβ42/Aβ40 and Aβ42/Aβ38 ratios: Better diagnostic markers of Alzheimer disease. Ann Clin Transl Neurol. 2016;3:154–165.
[44] Bousiges O, Cretin B, Lavaux T, et al. Diagnostic value of cerebrospinal fluid biomarkers (phospho-Tau181, total-Tau, Aβ42, and Aβ40) in prodromal stage of Alzheimer's disease and dementia with lewy bodies. J Alzheimer's Dis. 2016;51:1069–1083.
[45] Constantinides VC, Paraskevas GP, Boufidou F, et al. CSF Aβ42 and Aβ42/Aβ40 Ratio in Alzheimer's Disease and Frontotemporal Dementias. Diagnostics. 2023;13:1–13.
[46] Tang W, Wang Y, Cheng J, et al. CSF sAPPα and sAPPβ levels in Alzheimer's Disease and Multiple Other Neurodegenerative Diseases: A Network Meta-Analysis. NeuroMolecular Med. 2020;22:45–55.
[47] Araki W, Kanemaru K, Hattori K, et al. Soluble APP-α and APP-β in cerebrospinal fluid as potential biomarkers for differential diagnosis of mild cognitive impairment. Aging Clin Exp Res. 2022;34:341–347.
[48] Araki W, Hattori K, Kanemaru K, et al. Re-evaluation of soluble APP-α and APP-β in cerebrospinal fluid as potential biomarkers for early diagnosis of dementia disorders. Biomark Res. 2017;5:1–9.
*** sAPPα and sAPPβ highly correlate with p-tau and t-tau, with sAPPα levels significantly elevated in MCI-AD and sAPPβ levels similarly elevated in both MCI-AD and AD cases relative to other groups.**
[49] Alcolea D, Irwin DJ, Illán-Gala I, et al. Elevated YKL-40 and low sAPPβ:YKL-40 ratio in antemortem cerebrospinal fluid of patients with pathologically confirmed FTLD. J Neurol Neurosurg Psychiatry. 2019;90:180–186.
[50] Hampel H, Mitchell A, Blennow K, et al. Core biological marker candidates of Alzheimer's disease - perspectives for diagnosis, prediction of outcome and reflection of biological activity. J Neural Transm. 2004;111:247–272.
[51] Blennow K, Zetterberg H. Cerebrospinal fluid biomarkers for Alzheimer's disease. J Alzheimers Dis. 2009;18:413–417.
[52] Frisoni GB, Prestia A, Zanetti O, et al. Markers of Alzheimer's disease in a population attending a memory clinic. Alzheimers Dement. 2009;5:307–317.
[53] Hardy JA, Higgins GA. Alzheimer's disease: the amyloid cascade hypothesis. Science. 1992;256:184–185.
[54] Wattmo C, Blennow K, Hansson O. Cerebro-spinal fluid biomarker levels: Phosphorylated tau (T) and total tau (N) as markers for rate of progression in Alzheimer's disease. BMC Neurol. 2020;20:1–12.

[55] Johnson G V, Jenkins SM. Tau protein in normal and Alzheimer's disease brain. J Alzheimers Dis. 1999;1:307–328.
[56] Mandelkow EM, Mandelkow E. Tau in Alzheimer's disease. Trends Cell Biol. 1998;8:425–427.
[57] Grundke-Iqbal I, Iqbal K, Tung YC, et al. Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer cytoskeletal pathology. Proc Natl Acad Sci. 1986;83:4913–4917.
[58] Johnson GVW, Stoothoff WH. Tau phosphorylation in neuronal cell function and dysfunction. J Cell Sci. 2004;117:5721–5729.
[59] Jack CRJ, Knopman DS, Jagust WJ, et al. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. Lancet Neurol. 2010;9:119–128.
[60] Wesseling H, Mair W, Kumar M, et al. Tau PTM Profiles Identify Patient Heterogeneity and Stages of Alzheimer's Disease. Cell. 2020;183:1699-1713.e13.
**** High-resolution quantitative proteomics analysis revealed at least 59 different p-tau phosphorylation sites which could be related to AD.**
[61] Hansson O, Zetterberg H, Buchhave P, et al. Association between CSF biomarkers and incipient Alzheimer's disease in patients with mild cognitive impairment: a follow-up study. Lancet Neurol. 2006;5:228–234.
[62] Petersen RC, Aisen P, Boeve BF, et al. Mild cognitive impairment due to Alzheimer disease in the community. Ann Neurol. 2013;74:199–208.
[63] Lleó A, Irwin DJ, Illán-Gala I, et al. A 2-Step Cerebrospinal Algorithm for the Selection of Frontotemporal Lobar Degeneration Subtypes. JAMA Neurol. 2018;75:738–745.
[64] Schoonenboom NSM, Reesink FE, Verwey NA, et al. Cerebrospinal fluid markers for differential dementia diagnosis in a large memory clinic cohort. Neurology. 2012;78:47–54.
[65] Avila J, Lucas JJ, Perez M, et al. Role of tau protein in both physiological and pathological conditions. Physiol Rev. 2004;84:361–384.
[66] Jack CRJ, Bennett DA, Blennow K, et al. NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. Alzheimers Dement. 2018;14:535–562.
[67] 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. Lancet Neurol. 2016;15:673–684.
[68] Tondelli M, Bedin R, Chiari A, et al. Role of cerebrospinal fluid biomarkers to predict conversion to dementia in patients with mild cognitive impairment: a clinical cohort study. Clin Chem Lab Med. 2015;53:453–460.
[69] Rojas JC, Bang J, Lobach I V, et al. CSF neurofilament light chain and phosphorylated tau 181 predict disease progression in PSP. Neurology. 2018;90:e273–e281.
[70] Meeter LHH, Vijverberg EG, Del Campo M, et al. Clinical value of neurofilament and phospho-tau/tau ratio in the frontotemporal dementia spectrum. Neurology. 2018;90:e1231–e1239.
[71] Santos JRF, Bauer C, Schuchhardt J, et al. Validation of a prototype tau Thr231 phosphorylation CSF ELISA as a potential biomarker for Alzheimer's disease. J Neural Transm. 2019;126:339–348.
[72] Nabizadeh F, Salehi N, Ramezannezhad E, et al. P-tau231 as a Diagnostic Biomarker for Alzheimer's Disease and Mild Cognitive Impairment: A Systematic Review and Meta-Analysis. Ann Indian Acad Neurol. 2022;25:845–851.
**** A meta-analysis showing that the CSF p-tau231 can reliably differentiate AD patients from MCI and control subjects.**
[73] Buerger K, Zinkowski R, Teipel SJ, et al. Differential diagnosis of Alzheimer disease with cerebrospinal fluid levels of tau protein phosphorylated at threonine 231. Arch Neurol. 2002;59:1267–1272.
[74] Barthélemy NR, Bateman RJ, Hirtz C, et al. Cerebrospinal fluid phospho-tau T217 outperforms T181 as a biomarker for the differential diagnosis of Alzheimer's disease and PET amyloid-positive patient identification. Alzheimer's Res Ther. 2020;12:1–11.
[75] Nilsson J, Ashton NJ, Benedet AL, et al. Quantification of SNAP-25 with mass

spectrometry and Simoa: a method comparison in Alzheimer's disease. Alzheimers Res Ther. 2022;14:78.
[76] Leuzy A, Janelidze S, Mattsson-Carlgren N, et al. Comparing the Clinical Utility and Diagnostic Performance of CSF P-Tau181, P-Tau217, and P-Tau231 Assays. Neurology. 2021;97:e1681–e1694.
[77] Janelidze S, Stomrud E, Smith R, et al. Cerebrospinal fluid p-tau217 performs better than p-tau181 as a biomarker of Alzheimer's disease. Nat Commun. 2020;11:1–12.
[78] 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–e3035.
[79] Abildgaard A, Parkner T, Knudsen CS, et al. Diagnostic Cut-offs for CSF β-amyloid and tau proteins in a Danish dementia clinic. Clin Chim Acta. 2023;539:244–249.
[80] van Harten AC, Wiste HJ, Weigand SD, et al. Detection of Alzheimer's disease amyloid beta 1-42, p-tau, and t-tau assays. Alzheimer's \& Dement. 2022;18:635–644.
[81] Dakterzada F, López-Ortega R, Arias A, et al. Assessment of the Concordance and Diagnostic Accuracy Between Elecsys and Lumipulse Fully Automated Platforms and Innotest. Front Aging Neurosci. 2021;13.
[82] Khoonsari PE, Shevchenko G, Herman S, et al. Improved Differential Diagnosis of Alzheimer's Disease by Integrating ELISA and Mass Spectrometry-Based Cerebrospinal Fluid Biomarkers. J Alzheimers Dis. 2019;67:639–651.
[83] Molinuevo JL, Ayton S, Batrla R, et al. Current state of Alzheimer's fluid biomarkers. Acta Neuropathol. Springer Berlin Heidelberg; 2018.
[84] Santangelo R, Masserini F, Agosta F, et al. CSF p-tau/Aβ42 ratio and brain FDG-PET may reliably detect MCI "imminent" converters to AD. Eur J Nucl Med Mol Imaging. 2020;47:3152–3164.
**** CSF p-tau/Aß42 proposed as an accurate predictor of conversion from MCI to AD dementia.**
[85] Skillbäck T, Rosén C, Asztely F, et al. Diagnostic performance of cerebrospinal fluid total tau and phosphorylated tau in Creutzfeldt-Jakob disease: results from the Swedish Mortality Registry. JAMA Neurol. 2014;71:476–483.
[86] Dan L, Zhang Z. Alzheimer's disease: an axonal injury disease? Front Aging Neurosci. 2023;15.
[87] Mecca AP, O'Dell RS, Sharp ES, et al. Synaptic density and cognitive performance in Alzheimer's disease: A PET imaging study with [11 C]UCB-J. Alzheimer's Dement. 2022;18:2527–2536.
[88] Koffie RM, Hyman BT, Spires-Jones TL. Alzheimer's disease: synapses gone cold. Mol Neurodegener. 2011;6:63.
[89] Visser PJ, Reus LM, Gobom J, et al. Cerebrospinal fluid tau levels are associated with abnormal neuronal plasticity markers in Alzheimer's disease. Mol Neurodegener. 2022;17:1–16.
[90] Duits FH, Wesenhagen KEJ, Ekblad L, et al. Four subgroups based on tau levels in Alzheimer's disease observed in two independent cohorts. Alzheimer's Res Ther. 2021;13.
[91] Pillai JA, Bonner-Jackson A, Bekris LM, et al. Highly Elevated Cerebrospinal Fluid Total Tau Level Reflects Higher Likelihood of Non-Amnestic Subtype of Alzheimer's Disease. J Alzheimers Dis. 2019;70:1051–1058.
[92] Sämgård K, Zetterberg H, Blennow K, et al. Cerebrospinal fluid total tau as a marker of Alzheimer's disease intensity. Int J Geriatr Psychiatry. 2010;25:403–410.
[93] Wallin AK, Blennow K, Zetterberg H, et al. CSF biomarkers predict a more malignant outcome in Alzheimer disease. Neurology. 2010;74:1531–1537.
[94] Pillai JA, Appleby BS, Safar J, et al. Rapidly Progressive Alzheimer's Disease in Two Distinct Autopsy Cohorts. J Alzheimers Dis. 2018;64:973–980.
[95] Kiđemet-Piskač S, Babić Leko M, Blažeković A, et al. Evaluation of cerebrospinal fluid phosphorylated tau(231) as a biomarker in the differential diagnosis of Alzheimer's disease and vascular dementia. CNS Neurosci Ther. 2018;24:734–740.

[96] Babić M, Svob Štrac D, Mück-Šeler D, et al. Update on the core and developing cerebrospinal fluid biomarkers for Alzheimer disease. Croat Med J. 2014;55:347–365.
[97] Hesse C, Rosengren L, Andreasen N, et al. Transient increase in total tau but not phospho-tau in human cerebrospinal fluid after acute stroke. Neurosci Lett. 2001;297:187–190.
[98] Halawa I, Vlachogiannis P, Amandusson Å, et al. Seizures, CSF neurofilament light and tau in patients with subarachnoid haemorrhage. Acta Neurol Scand. 2018;137:199–203.
[99] Stoeck K, Sanchez-Juan P, Gawinecka J, et al. Cerebrospinal fluid biomarker supported diagnosis of Creutzfeldt-Jakob disease and rapid dementias: a longitudinal multicentre study over 10 years. Brain. 2012;135:3051–3061.
[100] Gaetani L, Blennow K, Calabresi P, et al. Neurofilament light chain as a biomarker in neurological disorders. J Neurol Neurosurg Psychiatry. 2019;90:870–881.
[101] Petzold A. Neurofilament phosphoforms: Surrogate markers for axonal injury, degeneration and loss. J Neurol Sci. 2005;233:183–198.
[102] Xiong Y, Meng T, Luo J, et al. The Potential of Neurofilament Light as a Biomarker in Alzheimer's Disease. Eur Neurol. 2021;84:6–15.
[103] Bacioglu M, Maia LF, Preische O, et al. Neurofilament Light Chain in Blood and CSF as Marker of Disease Progression in Mouse Models and in Neurodegenerative Diseases. Neuron. 2016;91:56–66.
[104] Kuhle J, Barro C, Andreasson U, et al. Comparison of three analytical platforms for quantification of the neurofilament light chain in blood samples: ELISA, electrochemiluminescence immunoassay and Simoa. Clin Chem Lab Med. 2016;54:1655–1661.
[105] Mazzeo S, Lassi M, Padiglioni S, et al. PRedicting the EVolution of SubjectIvE Cognitive Decline to Alzheimer's Disease With machine learning: the PREVIEW study protocol. BMC Neurol. 2023;23:300.
[106] Braunewell K-H, Szanto AJK. Visinin-like proteins (VSNLs): interaction partners and emerging functions in signal transduction of a subfamily of neuronal Ca2+-sensor proteins. Cell Tissue Res. 2009;335:301–316.
[107] Braunewell KH. The visinin-like proteins VILIP-1 and VILIP-3 in Alzheimer's disease-old wine in new bottles. Front Mol Neurosci. 2012;5:20.
[108] Halbgebauer S, Steinacker P, Riedel D, et al. Visinin-like protein 1 levels in blood and CSF as emerging markers for Alzheimer's and other neurodegenerative diseases. Alzheimers Res Ther. 2022;14:175.
*** Report on successful establishment of a novel Simoa assay for VILIP-1 detection and presentation of CSF and serum VILIP-1 as a marker for differential diagnosis of AD.**
[109] Shahim P, Blennow K, Johansson P, et al. Cerebrospinal Fluid Stanniocalcin-1 as a Biomarker for Alzheimer's Disease and Other Neurodegenerative Disorders. NeuroMolecular Med. 2017;19:154–160.
[110] 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.
[111] Dulewicz M, Kulczyńska-Przybik A, Mroczko B. Neurogranin and VILIP-1 as Molecular Indicators of Neurodegeneration in Alzheimer's Disease: A Systematic Review and Meta-Analysis. Int J Mol Sci. 2020;21.
**** A meta-analysis confirming that Ng and VILIP-1 can be useful CSF biomarkers in differential diagnosis and monitoring progression of cognitive decline.**
[112] Masliah E, Mallory M, Alford M, et al. Altered expression of synaptic proteins occurs early during progression of Alzheimer's disease. Neurology. 2001;56:127–129.
[113] Davidsson P, Jahn R, Bergquist J, et al. Synaptotagmin, a synaptic vesicle protein, is present in human cerebrospinal fluid: a new biochemical marker for synaptic pathology in Alzheimer disease? Mol Chem Neuropathol. 1996;27:195–210.
[114] Camporesi E, Nilsson J, Brinkmalm A, et al. Fluid Biomarkers for Synaptic Dysfunction

and Loss. Biomark Insights. 2020;15:1177271920950319.
[115] Represa A, Deloulme JC, Sensenbrenner M, et al. Neurogranin: immunocytochemical localization of a brain-specific protein kinase C substrate. J Neurosci Off J Soc Neurosci. 1990;10:3782–3792.
[116] Zhong L, Cherry T, Bies CE, et al. Neurogranin enhances synaptic strength through its interaction with calmodulin. EMBO J. 2009;28:3027–3039.
[117] Kvartsberg H, Lashley T, Murray CE, et al. The intact postsynaptic protein neurogranin is reduced in brain tissue from patients with familial and sporadic Alzheimer's disease. Acta Neuropathol. 2019;137:89–102.
[118] Wellington H, Paterson RW, Portelius E, et al. Increased CSF neurogranin concentration is specific to Alzheimer disease. Neurology. 2016;86:829–835.
[119] Kvartsberg H, Duits FH, Ingelsson M, et al. Cerebrospinal fluid levels of the synaptic protein neurogranin correlates with cognitive decline in prodromal Alzheimer's disease. Alzheimers Dement. 2015;11:1180–1190.
[120] Willemse EAJ, De Vos A, Herries EM, et al. Neurogranin as Cerebrospinal Fluid Biomarker for Alzheimer Disease: An Assay Comparison Study. Clin Chem. 2018;64:927–937.
[121] Thorsell A, Bjerke M, Gobom J, et al. Neurogranin in cerebrospinal fluid as a marker of synaptic degeneration in Alzheimer's disease. Brain Res. 2010;1362:13–22.
[122] Dage JL, Eloyan A, Thangarajah M, et al. Cerebrospinal fluid biomarkers in the Longitudinal Early-onset Alzheimer's Disease Study. Alzheimers Dement. 2023;19 Suppl 9:S115–S125.
**** A comprehensive analysis of CSF biomarkers in sporadic EOAD that can inform EOAD clinical trial design.**
[123] Kivisäkk P, Carlyle BC, Sweeney T, et al. Increased levels of the synaptic proteins PSD-95, SNAP-25, and neurogranin in the cerebrospinal fluid of patients with Alzheimer's disease. Alzheimers Res Ther. 2022;14:58.
*** Study presenting SNAP-25 and Ng as promising CSF markers for neurodegenerative diseases with highest specificity for AD.**
[124] Tible M, Sandelius Å, Höglund K, et al. Dissection of synaptic pathways through the CSF biomarkers for predicting Alzheimer disease. Neurology. 2020;95:e953–e961.
[125] Roveta F, Cermelli A, Boschi S, et al. Synaptic Proteins as Fluid Biomarkers in Alzheimer's Disease: A Systematic Review and Meta-Analysis. J. Alzheimers. Dis. Netherlands; 2022. p. 1381–1393.
**** A meta-analysis showing that neurogranin, SNAP-25, and GAP-43 are possible biomarkers of synaptic damage in AD.**
[126] Kester MI, Teunissen CE, Crimmins DL, et al. Neurogranin as a Cerebrospinal Fluid Biomarker for Synaptic Loss in Symptomatic Alzheimer Disease. JAMA Neurol. 2015;72:1275–1280.
[127] Portelius E, Zetterberg H, Skillbäck T, et al. Cerebrospinal fluid neurogranin: relation to cognition and neurodegeneration in Alzheimer's disease. Brain. 2015;138:3373–3385.
[128] De Vos A, Jacobs D, Struyfs H, et al. C-terminal neurogranin is increased in cerebrospinal fluid but unchanged in plasma in Alzheimer's disease. Alzheimers Dement. 2015;11:1461–1469.
[129] Tarawneh R, D'Angelo G, Crimmins D, et al. Diagnostic and Prognostic Utility of the Synaptic Marker Neurogranin in Alzheimer Disease. JAMA Neurol. 2016;73:561–571.
[130] Sanfilippo C, Forlenza O, Zetterberg H, et al. Increased neurogranin concentrations in cerebrospinal fluid of Alzheimer's disease and in mild cognitive impairment due to AD. J Neural Transm. 2016;123:1443–1447.
[131] Sutphen CL, McCue L, Herries EM, et al. Longitudinal decreases in multiple cerebrospinal fluid biomarkers of neuronal injury in symptomatic late onset Alzheimer's disease. Alzheimers Dement. 2018;14:869–879.
[132] Lista S, Toschi N, Baldacci F, et al. Cerebrospinal Fluid Neurogranin as a Biomarker of Neurodegenerative Diseases: A Cross-Sectional Study. J Alzheimers Dis. 2017;59:1327–1334.

[133] Mavroudis IA, Petridis F, Chatzikonstantinou S, et al. A meta-analysis on CSF neurogranin levels for the diagnosis of Alzheimer's disease and mild cognitive impairment. Aging Clin Exp Res. 2020;32:1639–1646.

*** A meta-analysis showing that neurogranin CSF levels are significantly higher in AD patients compared to controls.**

[134] Wellington H, Paterson RW, Suárez-González A, et al. CSF neurogranin or tau distinguish typical and atypical Alzheimer disease. Ann Clin Transl Neurol. 2018;5:162–171.

[135] Yoong SQ, Lu J, Xing H, et al. The prognostic utility of CSF neurogranin in predicting future cognitive decline in the Alzheimer's disease continuum: A systematic review and meta-analysis with narrative synthesis. Ageing Res Rev. 2021;72:101491.

[136] Willemse EAJ, Sieben A, Somers C, et al. Neurogranin as biomarker in CSF is non-specific to Alzheimer's disease dementia. Neurobiol Aging. 2021;108:99–109.

[137] Chen F, Chen H, Chen Y, et al. Dysfunction of the SNARE complex in neurological and psychiatric disorders. Pharmacol Res. 2021;165:105469.

[138] Antonucci F, Corradini I, Fossati G, et al. SNAP-25, a Known Presynaptic Protein with Emerging Postsynaptic Functions. Front Synaptic Neurosci. 2016;8:7.

[139] Liu Q, Liu H, Zhang S, et al. Cerebrospinal Fluid Synaptosomal-Associated Protein 25 Levels in Patients with Alzheimer's Disease: A Meta-Analysis. J Alzheimers Dis. 2022;89:121–132.

**** A meta-analysis showing that increased CSF SNAP-25 levels differentiated patients with AD or MCI from controls.**

[140] Brinkmalm A, Brinkmalm G, Honer WG, et al. SNAP-25 is a promising novel cerebrospinal fluid biomarker for synapse degeneration in Alzheimer's disease. Mol Neurodegener. 2014;9:53.

[141] Galasko D, Xiao M, Xu D, et al. Synaptic biomarkers in CSF aid in diagnosis, correlate with cognition and predict progression in MCI and Alzheimer's disease. Alzheimer's Dement (New York, N Y). 2019;5:871–882.

[142] Wang S, Zhang J, Pan T. APOE ε4 is associated with higher levels of CSF SNAP-25 in prodromal Alzheimer's disease. Neurosci Lett. 2018;685:109–113.

[143] 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.

[144] Halbgebauer S, Steinacker P, Hengge S, et al. CSF levels of SNAP-25 are increased early in Creutzfeldt-Jakob and Alzheimer's disease. J Neurol Neurosurg Psychiatry. 2022;

[145] Wang Q, Zhou W, Zhang J. Levels of Cortisol in CSF Are Associated With SNAP-25 and Tau Pathology but Not Amyloid-β. Front Aging Neurosci. 2018;10:383.

[146] Noor A, Zahid S. A review of the role of synaptosomal-associated protein 25 (SNAP-25) in neurological disorders. Int J Neurosci. 2017;127:805–811.

[147] Andersson A, Remnestål J, Nellgård B, et al. Development of parallel reaction monitoring assays for cerebrospinal fluid proteins associated with Alzheimer's disease. Clin Chim Acta. 2019;494:79–93.

[148] Remnestål J, Just D, Mitsios N, et al. CSF profiling of the human brain enriched proteome reveals associations of neuromodulin and neurogranin to Alzheimer's disease. Proteomics Clin Appl. 2016;10:1242–1253.

[149] 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.

*** Study showing significantly increased GAP-43 levels in AD compared to controls and most neurodegenerative diseases.**

[150] Wolpowitz D, Mason TB, Dietrich P, et al. Cysteine-rich domain isoforms of the neuregulin-1 gene are required for maintenance of peripheral synapses. Neuron. 2000;25:79–91.

[151] Meyer D, Birchmeier C. Multiple essential functions of neuregulin in development.

Nature. 1995;378:386–390.
[152] Chaudhury AR, Gerecke KM, Wyss JM, et al. Neuregulin-1 and erbB4 immunoreactivity is associated with neuritic plaques in Alzheimer disease brain and in a transgenic model of Alzheimer disease. J Neuropathol Exp Neurol. 2003;62:42–54.
[153] Woo R-S, Lee J-H, Yu H-N, et al. Expression of ErbB4 in the neurons of Alzheimer's disease brain and APP/PS1 mice, a model of Alzheimer's disease. Anat Cell Biol. 2011;44:116–127.
[154] Mouton-Liger F, Dumurgier J, Cognat E, et al. CSF levels of the BACE1 substrate NRG1 correlate with cognition in Alzheimer's disease. Alzheimers Res Ther. 2020;12:88.
[155] Colonna M, Butovsky O. Microglia Function in the Central Nervous System During Health and Neurodegeneration. Annu Rev Immunol. 2017;35:441–468.
[156] Janelidze S, Mattsson N, Stomrud E, et al. CSF biomarkers of neuroinflammation and cerebrovascular dysfunction in early Alzheimer disease. Neurology. 2018;91:e867–e877.
[157] Hampel H, Caraci F, Cuello AC, et al. A Path Toward Precision Medicine for Neuroinflammatory Mechanisms in Alzheimer's Disease. Front Immunol. 2020;11:456.
[158] Block ML, Zecca L, Hong J-S. Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. Nat Rev Neurosci. 2007;8:57–69.
[159] Sofroniew M V, Vinters H V. Astrocytes: biology and pathology. Acta Neuropathol. 2010;119:7–35.
[160] Zhao T, Su Z, Li Y, et al. Chitinase-3 like-protein-1 function and its role in diseases. Signal Transduct Target Ther. 2020;5:201.
[161] Talaat F, Abdelatty S, Ragaie C, et al. Chitinase-3-like 1-protein in CSF: a novel biomarker for progression in patients with multiple sclerosis. Neurol Sci Off J Ital Neurol Soc Ital Soc Clin Neurophysiol. 2023;44:3243–3252.
[162] Park SA, Han SM, Kim CE. New fluid biomarkers tracking non-amyloid-β and non-tau pathology in Alzheimer's disease. Exp. Mol. Med. Springer Nature; 2020. p. 556–568.
[163] Lee CG, Da Silva CA, Dela Cruz CS, et al. Role of chitin and chitinase/chitinase-like proteins in inflammation, tissue remodeling, and injury. Annu Rev Physiol. 2011;73:479–501.
[164] Muszyński P, Groblewska M, Kulczyńska-Przybik A, et al. YKL-40 as a Potential Biomarker and a Possible Target in Therapeutic Strategies of Alzheimer's Disease. Curr Neuropharmacol. 2017;15:906–917.
[165] Alcolea D, Vilaplana E, Pegueroles J, et al. Relationship between cortical thickness and cerebrospinal fluid YKL-40 in predementia stages of Alzheimer's disease. Neurobiol Aging. 2015;36:2018–2023.
[166] Zhang Y, Tian J, Ni J, et al. Peripheral Blood and Cerebrospinal Fluid Levels of YKL-40 in Alzheimer's Disease: A Systematic Review and Meta-Analysis. Brain Sci. 2023;13.
**** A meta-analysis supporting the significance of YKL-40 CSF and plasma levels in early screening of AD.**
[167] Llorens F, Thüne K, Tahir W, et al. YKL-40 in the brain and cerebrospinal fluid of neurodegenerative dementias. Mol Neurodegener. 2017;12:83.
[168] Wang L, Gao T, Cai T, et al. Cerebrospinal fluid levels of YKL-40 in prodromal Alzheimer's disease. Neurosci Lett. 2020;715:134658.
[169] Abu-Rumeileh S, Steinacker P, Polischi B, et al. CSF biomarkers of neuroinflammation in distinct forms and subtypes of neurodegenerative dementia. Alzheimers Res Ther. 2019;12:2.
[170] Ferrari-Souza JP, Ferreira PCL, Bellaver B, et al. Astrocyte biomarker signatures of amyloid-β and tau pathologies in Alzheimer's disease. Mol Psychiatry. 2022;27:4781–4789.
[171] Ennerfelt HE, Lukens JR. The role of innate immunity in Alzheimer's disease. Immunol Rev. 2020;297:225–246.
[172] Ulland TK, Colonna M. TREM2 — a key player in microglial biology and Alzheimer

disease. Nat Rev Neurol. 2018;14:667–675.
[173] Yang J, Fu Z, Zhang X, et al. TREM2 ectodomain and its soluble form in Alzheimer's disease. J Neuroinflammation. 2020;17:204.
[174] Španić Popovački E, Babić Leko M, Langer Horvat L, et al. Soluble TREM2 Concentrations in the Cerebrospinal Fluid Correlate with the Severity of Neurofibrillary Degeneration, Cognitive Impairment, and Inflammasome Activation in Alzheimer's Disease. Neurol Int. 2023;15:842–856.
**** Study showing that CSF sTREM2 levels significantly correlate with neurofibrillary degeneration, cognitive decline, and inflammasome activity in AD patients.**
[175] Suárez-Calvet M, Morenas-Rodríguez E, Kleinberger G, et al. Early increase of CSF sTREM2 in Alzheimer's disease is associated with tau related-neurodegeneration but not with amyloid-β pathology. Mol Neurodegener. 2019;14:1.
[176] Piccio L, Deming Y, Del-Águila JL, et al. Cerebrospinal fluid soluble TREM2 is higher in Alzheimer disease and associated with mutation status. Acta Neuropathol. 2016;131:925–933.
[177] Zhou W, Zhou Y, Li J. Association between Cerebrospinal Fluid Soluble TREM2, Alzheimer's Disease and Other Neurodegenerative Diseases. J Clin Med. 2023;12.
[178] Petzold A. Glial fibrillary acidic protein is a body fluid biomarker for glial pathology in human disease. Brain Res. 2015;1600:17–31.
[179] Abdelhak A, Foschi M, Abu-Rumeileh S, et al. Blood GFAP as an emerging biomarker in brain and spinal cord disorders. Nat Rev Neurol. 2022;18:158–172.
[180] Guo Y, You J, Zhang Y, et al. Plasma proteomic profiles predict future dementia in healthy adults. Nat Aging. 2024;4:247–260.
[181] Liu T, Zuo H, Ma D, et al. Cerebrospinal fluid GFAP is a predictive biomarker for conversion to dementia and Alzheimer's disease-associated biomarkers alterations among de novo Parkinson's disease patients: a prospective cohort study. J Neuroinflammation. 2023;20:1–12.
[182] Ferrari-Souza JP, Ferreira PCL, Bellaver B, et al. Astrocyte biomarker signatures of amyloid-β and tau pathologies in Alzheimer's disease. Mol Psychiatry. 2022;27:4781–4789.
[183] Kim KY, Shin KY, Chang KA. GFAP as a Potential Biomarker for Alzheimer's Disease: A Systematic Review and Meta-Analysis. Cells. 2023;12.
[184] Ganne A, Balasubramaniam M, Griffin WST, et al. Glial Fibrillary Acidic Protein: A Biomarker and Drug Target for Alzheimer's Disease. Pharmaceutics. 2022;14.
[185] Boccalini C, Ribaldi F, Scheffler M, et al. disease pathology and cognitive decline SC.
[186] Pereira JB, Janelidze S, Smith R, et al. Plasma GFAP is an early marker of amyloid-β but not tau pathology in Alzheimer's disease. Brain. 2021;144:3505–3516.
[187] Verberk IMW, Thijssen E, Koelewijn J, et al. Combination of plasma amyloid beta(1-42/1-40)and glial fibrillary acidic protein strongly associates with cerebral amyloid pathology. Alzheimer's Res Ther. 2020;12:1–14.
[188] Chiotis K, Johansson C, Rodriguez-Vieitez E, et al. Tracking reactive astrogliosis in autosomal dominant and sporadic Alzheimer's disease with multi-modal PET and plasma GFAP. Mol Neurodegener. 2023;18:1–14.
[189] Shen X-N, Huang S-Y, Cui M, et al. Plasma Glial Fibrillary Acidic Protein in the Alzheimer Disease Continuum: Relationship to Other Biomarkers, Differential Diagnosis, and Prediction of Clinical Progression. Clin Chem. 2023;69.
[190] Tang Y, Han L, Li S, et al. Plasma GFAP in Parkinson's disease with cognitive impairment and its potential to predict conversion to dementia. npj Park Dis. 2023;9:1–5.
[191] Sanchez-Rodriguez LM, Bezgin G, Carbonell F, et al. Revealing the combined roles of Aβ and tau in Alzheimer's disease via a pathophysiological activity decoder. bioRxiv (under-revision Nat Comms). 2023;2023.02.21.529377.
[192] Bernick C, Shan G, Ritter A, et al. Blood biomarkers and neurodegeneration in individuals exposed to repetitive head impacts. Alzheimer's Res Ther. 2023;15:1–11.
[193] Wang X, Shi Z, Qiu Y, et al. Peripheral GFAP and NfL as early biomarkers for dementia:

longitudinal insights from the UK Biobank. BMC Med. 2024;22:1–13.
[194] Cicognola C, Janelidze S, Hertze J, et al. Plasma glial fibrillary acidic protein detects Alzheimer pathology and predicts future conversion to Alzheimer dementia in patients with mild cognitive impairment. Alzheimer's Res Ther. 2021;13:1–9.
[195] Burré J, Sharma M, Südhof TC. Cell Biology and Pathophysiology of α-Synuclein. Cold Spring Harb Perspect Med. 2018;8.
[196] Bartels T, Choi JG, Selkoe DJ. α-Synuclein occurs physiologically as a helically folded tetramer that resists aggregation. Nature. 2011;477:107–110.
[197] Bridi JC, Hirth F. Mechanisms of α-Synuclein Induced Synaptopathy in Parkinson's Disease. Front Neurosci. 2018;12:80.
[198] Burré J, Sharma M, Tsetsenis T, et al. Alpha-synuclein promotes SNARE-complex assembly in vivo and in vitro. Science. 2010;329:1663–1667.
[199] Kumari P, Ghosh D, Vanas A, et al. Structural insights into α-synuclein monomer-fibril interactions. Proc Natl Acad Sci U S A. 2021;118.
[200] Larson ME, Sherman MA, Greimel S, et al. Soluble α-synuclein is a novel modulator of Alzheimer's disease pathophysiology. J Neurosci Off J Soc Neurosci. 2012;32:10253–10266.
**** One of the first studies showing evidence of the potential contribution of soluble α-syn to AD pathogenesis.**
[201] Wennström M, Surova Y, Hall S, et al. Low CSF levels of both α-synuclein and the α-synuclein cleaving enzyme neurosin in patients with synucleinopathy. PLoS One. 2013;8:e53250.
[202] van Dijk KD, Bidinosti M, Weiss A, et al. Reduced α-synuclein levels in cerebrospinal fluid in Parkinson's disease are unrelated to clinical and imaging measures of disease severity. Eur J Neurol. 2014;21:388–394.
[203] Lilamand M, Clery J, Vrillon A, et al. Cerebrospinal Fluid Alpha-Synuclein Improves the Differentiation between Dementia with Lewy Bodies and Alzheimer's Disease in Clinical Practice. Int J Mol Sci. 2022;23.
[204] Twohig D, Rodriguez-Vieitez E, Sando SB, et al. The relevance of cerebrospinal fluid α-synuclein levels to sporadic and familial Alzheimer's disease. Acta Neuropathol Commun. 2018;6:130.
[205] Majbour NK, Chiasserini D, Vaikath NN, et al. Increased levels of CSF total but not oligomeric or phosphorylated forms of alpha-synuclein in patients diagnosed with probable Alzheimer's disease. Sci Rep. 2017;7:40263.
[206] Shi M, Tang L, Toledo JB, et al. Cerebrospinal fluid α-synuclein contributes to the differential diagnosis of Alzheimer's disease. Alzheimers Dement. 2018;14:1052–1062.
[207] 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. Alzheimers Res Ther. 2020;12:83.
[208] Førland MG, Tysnes O-B, 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. Eur J Neurol. 2020;27:43–50.
[209] Vergallo A, Bun R-S, Toschi N, et al. Association of cerebrospinal fluid α-synuclein with total and phospho-tau(181) protein concentrations and brain amyloid load in cognitively normal subjective memory complainers stratified by Alzheimer's disease biomarkers. Alzheimers Dement. 2018;14:1623–1631.
[210] Wang H, Stewart T, Toledo JB, et al. A Longitudinal Study of Total and Phosphorylated α-Synuclein with Other Biomarkers in Cerebrospinal Fluid of Alzheimer's Disease and Mild Cognitive Impairment. J Alzheimers Dis. 2018;61:1541–1553.
[211] Köppen J, Schulze A, Machner L, et al. Amyloid-Beta Peptides Trigger Aggregation of Alpha-Synuclein In Vitro. Molecules. 2020;25.
[212] Atsmon-Raz Y, Miller Y. Non-Amyloid-β Component of Human α-Synuclein Oligomers Induces Formation of New Aβ Oligomers: Insight into the Mechanisms That Link Parkinson's and Alzheimer's Diseases. ACS Chem Neurosci. 2016;7:46–55.

*** A study indicating the molecular mechanisms explaining the link between AD and α-syn pathology.**

[213] Dasari AKR, Kayed R, Wi S, et al. Tau Interacts with the C-Terminal Region of α-Synuclein, Promoting Formation of Toxic Aggregates with Distinct Molecular Conformations. Biochemistry. 2019;58:2814–2821.

[214] Seino Y, Nakamura T, Kawarabayashi T, et al. Cerebrospinal Fluid and Plasma Biomarkers in Neurodegenerative Diseases. J Alzheimers Dis. 2019;68:395–404.

[215] Leverenz JB, Fishel MA, Peskind ER, et al. Lewy Body Pathology in Familial Alzheimer Disease: Evidence for Disease- and Mutation-Specific Pathologic Phenotype. Arch Neurol. 2006;63:370–376.

[216] Hazan J, Wing M, Liu KY, et al. Clinical utility of cerebrospinal fluid biomarkers in the evaluation of cognitive impairment: a systematic review and meta-analysis. J Neurol Neurosurg Psychiatry. 2023;94:113–120.

[217] Paterson RW, Slattery CF, Poole T, et al. Cerebrospinal fluid in the differential diagnosis of Alzheimer's disease: clinical utility of an extended panel of biomarkers in a specialist cognitive clinic. Alzheimers Res Ther. 2018;10:32.

[218] Dolphin H, Dyer AH, Morrison L, et al. New horizons in the diagnosis and management of Alzheimer's Disease in older adults. Age Ageing. 2024;53.

[219] Wu W, Ji Y, Wang Z, et al. The FDA-approved anti-amyloid-β monoclonal antibodies for the treatment of Alzheimer's disease: a systematic review and meta-analysis of randomized controlled trials. Eur J Med Res. 2023;28:544.

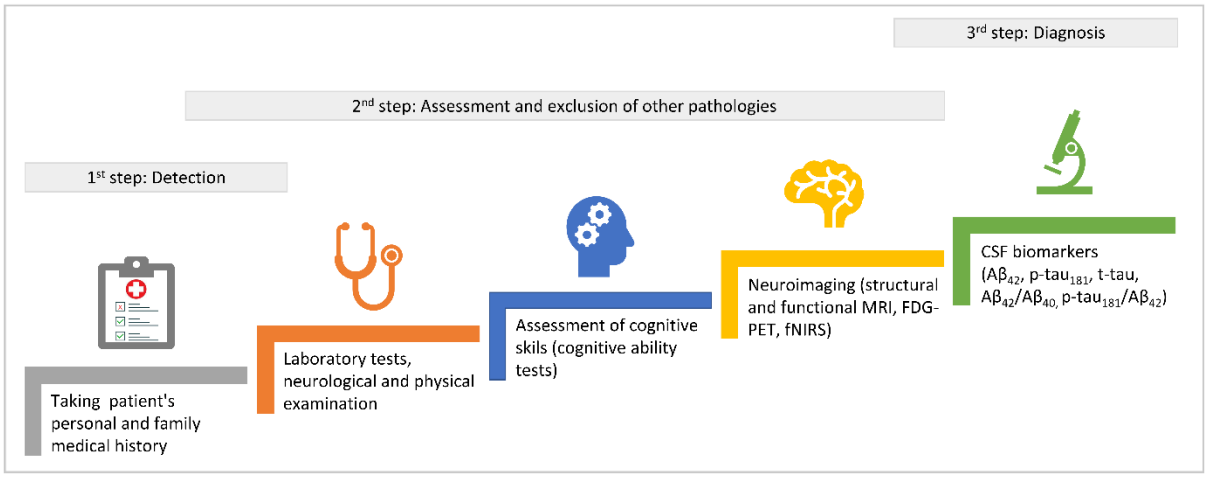

Figure 1. Diagnostic Process in Alzheimer's Disease.

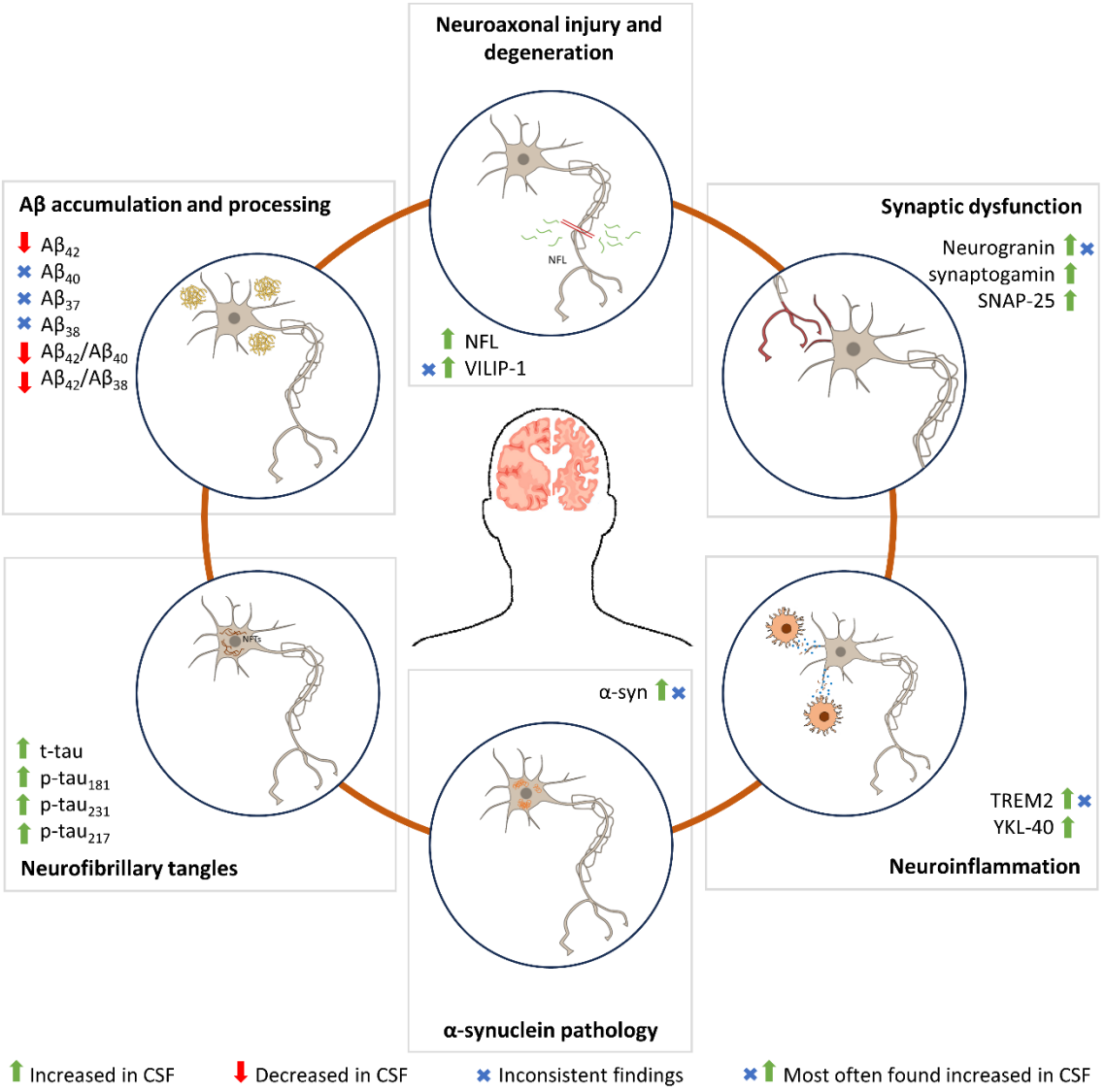

Figure 2. Overview of the most promising CSF biomarkers for Alzheimer's disease diagnosis. Aβ, Amyloid-beta; α-syn, Alpha-synuclein; NFL, Neurofilament light protein; SNAP-25; Synaptosomal-Associated Protein, 25kDa; TREM2, Triggering receptor expressed on myeloid cells 2; VILIP-1, Visinin-like protein 1; YKL-40, Chitinase-3-like protein-1.