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Cerebrospinal fluid and plasma biomarkers in Alzheimer disease

Kaj Blennow, Harald Hampel, Michael Weiner and Henrik Zetterberg

Abstract | Intense multidisciplinary research has provided detailed knowledge of the molecular pathogenesis of Alzheimer disease (AD). This knowledge has been translated into new therapeutic strategies with putative disease-modifying effects. Several of the most promising approaches, such as amyloid-β immunotherapy and secretase inhibition, are now being tested in clinical trials. Disease-modifying treatments might be at their most effective when initiated very early in the course of AD, before amyloid plaques and neurodegeneration become too widespread. Thus, biomarkers are needed that can detect AD in the predementia phase or, ideally, in presymptomatic individuals. In this Review, we present the rationales behind and the diagnostic performances of the core cerebrospinal fluid (CSF) biomarkers for AD, namely total tau, phosphorylated tau and the 42 amino acid form of amyloid-β. These biomarkers reflect AD pathology, and are candidate markers for predicting future cognitive decline in healthy individuals and the progression to dementia in patients who are cognitively impaired. We also discuss emerging plasma and CSF biomarkers, and explore new proteomics-based strategies for identifying additional CSF markers. Furthermore, we outline the roles of CSF biomarkers in drug discovery and clinical trials, and provide perspectives on AD biomarker discovery and the validation of such markers for use in the clinic.

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Introduction

Alois Alzheimer presented the first case of the disease that was to bear his name at a congress in Tübingen, Germany, in 1906.[1] In this presentation, he described the "miliary bodies" (amyloid plaques) and "dense bundles of fibrils" (neurofibrillary tangles) that we now recognize as neuropathological hallmarks of Alzheimer disease (AD). In 1985, researchers succeeded in purifying amyloid plaque cores and, in so doing, identified the 4 kDa amyloid-β (Aβ) peptide as the main component of these extracellular deposits.[2] This breakthrough led to the cloning of the gene encoding the amyloid precursor protein (APP),[3] the molecule from which Aβ is derived. In 1986, neurofibrillary tangles were shown to be composed of abnormally hyperphosphorylated forms of the protein tau.[4] These important achievements in the 1980s marked the start of modern AD research, and have led to a detailed knowledge of APP metabolism and Aβ generation (Figure 1), and of tau homeostasis (Figure 2).

Mutations in *APP* or in one of the presenilin genes (*PSEN1* or *PSEN2*), which encode proteins involved in APP metabolism, have been found to cause rare familial forms of AD.[5] Largely on the basis of these mutations, Aβ—in particular the 42 amino acid form of this peptide ($Aβ_{1–42}$)—has been proposed as the driving force in the disease process. Indeed, the 'amyloid cascade hypothesis'[6] posits that an imbalance between the production and clearance of Aβ is the initiating event in AD, with the increase in Aβ load ultimately leading to tau pathology, neuronal degeneration and dementia (Figure 3). Progress in AD research has been translated into novel treatment strategies with disease-modifying potential, and a large number of candidate anti-Aβ drugs, such as Aβ immunotherapies, secretase inhibitors and Aβ aggregation inhibitors, are in various phases of clinical trials.[5] Despite this progress, one should note that the amyloid cascade hypothesis has not been proven with certainty for late-onset AD.

Disease-modifying drugs will probably be at their most effective in patients in the earliest stages of AD, before amyloid plaques and neurofibrillary tangles become prevalent and neurodegeneration becomes too severe.[7–9] Thus, patients will need to be identified in the predementia stage (prodromal AD), or even the asymptomatic phase of the disease (preclinical AD). Prodromal AD is defined as mild cognitive impairment (MCI) resulting from underlying AD pathology, whereas preclinical AD is characterized by progressive AD pathology in the brain that is insufficiently severe to affect cognition. For an AD drug to be labeled as disease-modifying, evidence must be available that the agent affects the central disease processes and hallmark neuropathology, in addition to a beneficial effect on cognition.[10]

The challenges of early diagnosis and identification of disease-modifying drugs have created a need for biomarkers that reflect core elements of the disease

Competing interests
K. Blennow and M. Weiner declare an association with the following company: Innogenetics. H. Hampel declares an association with the following company: BRAHMS AG. See the article online for full details of the relationships. H. Zetterberg declares no competing interests.

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Key points

- Current clinical diagnostic criteria for Alzheimer disease (AD) require a patient to have dementia before a diagnosis can be made, and are largely based on the exclusion of other disorders
- Disease-modifying drugs for AD, when they become available, will need to be administered very early in the course of the disease, before neurodegeneration is too severe and widespread
- No clinical method is available for identifying prodromal AD in patients with mild cognitive impairment (MCI), as such individuals have only mild disturbances in episodic memory
- The cerebrospinal fluid (CSF) biomarkers total tau, phosphorylated tau (p-tau$_{181}$ and p-tau$_{231}$) and β-amyloid$_{1–42}$ have a high diagnostic accuracy for AD, and for prodromal AD in patients with MCI
- CSF biomarkers are increasingly being used in the clinic for diagnosing AD, and will also be valuable in clinical trials, allowing enrichment of patient populations with pure AD cases
- Biomarker evidence that a candidate drug affects the central disease processes in AD will, together with a beneficial effect on cognition, be essential for labeling the drug as disease modifying

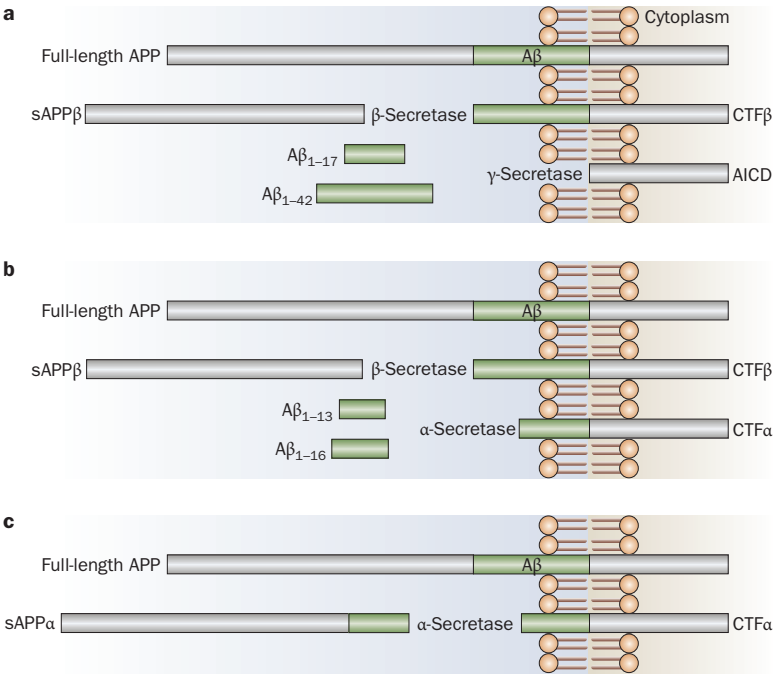

Figure 1 | Metabolic pathways for the generation of APP fragments detected in the CSF. **a** | In the amyloidogenic pathway, APP is cleaved by β-secretase, releasing sAPPβ into the extracellular fluid and CSF. The remaining fragment in the plasma membrane (CTFβ) is cleaved by γ-secretase, generating Aβ$_{1–42}$ and several carboxy-terminal truncated Aβ isoforms (Aβ$_{1–40}$ down to Aβ$_{1–17}$).[81,145] β-Secretase has been identified as β-site APP-cleaving enzyme 1, also known as BACE1,[146] whereas γ-secretase is an enzyme complex consisting of four components: presenilin, nicastrin, PEN2 and APH1.[147] **b** | In a second pathway, APP is cleaved by β-secretase followed by α-secretase, resulting in the release of several short Aβ isoforms (Aβ$_{1–16}$ down to Aβ$_{1–13}$).[81,145] **c** | In a third pathway, APP is cleaved in the middle of the Aβ domain by α-secretase, releasing the large amino-terminal derivative sAPPα into the extracellular fluid and CSF and leaving CTFα in the plasma membrane. α-Secretase activity has been attributed to the ADAM family of proteases.[148] The p3 peptide, which has been found in cell culture experiments,[149] is not present in CSF.[75,150] Abbreviations: Aβ, amyloid-β; AICD, amyloid precursor protein intracellular domain; APP, amyloid precursor protein; CSF, cerebrospinal fluid; CTF, carboxy-terminal fragment; sAPP, soluble APP extracellular domain.

process. Here, we review the development of candidate cerebrospinal fluid (CSF) and plasma biomarkers for AD. We focus on established biomarkers (biomarkers evaluated in several studies by various research groups), providing a practical guide to their implementation in the clinic and discussing their potential roles in clinical trials.

Cerebrospinal fluid biomarkers

A biomarker is an objective measure of a biological or pathogenic process that can be used to evaluate disease risk or prognosis, to guide clinical diagnosis or to monitor therapeutic interventions. The CSF is in direct contact with the extracellular space of the brain and can reflect biochemical changes that occur in the latter. For these reasons, the CSF is the optimal source of AD biomarkers.

CSF biomarkers for AD can be divided into basic and core biomarkers (Table 1). Basic biomarkers are used to identify conditions that might mimic or coexist with AD, while core biomarkers have been developed to identify the central pathogenic processes in AD. The procedure for obtaining CSF by lumbar puncture is outlined in Supplementary Figure 1 and Supplementary Table 1 online, with the latter also providing a standardized protocol for CSF sample handling.

Basic biomarkers

Basic biomarkers include assays for blood–brain barrier (BBB) status and inflammatory processes in the brain (Supplementary Figure 2 online). The BBB is formed from the restricted permeability of the capillaries in the brain, and serves to maintain a controlled milieu for neurons. The CSF:serum albumin ratio is the standard biomarker for BBB function.[11] An increase in this ratio indicates BBB damage and is found in a variety of disorders, such as infections (for example, neuroborreliosis) and inflammatory diseases (for example, Guillain–Barré syndrome), brain tumors, and cerebrovascular disease, including many cases of vascular dementia (Table 1). The CSF:serum albumin ratio is normal in patients with pure AD, but often increases in cases of the disease that show concomitant cerebrovascular pathology.[12] Thus, this ratio might be of value in excluding various causes of brain damage and for identifying patients with pure AD.

The immune system responds to chronic inflammatory or infectious disorders in the CNS, such as multiple sclerosis and neuroborreliosis, by producing antibodies—a process called intrathecal immunoglobulin production. This response can be measured either quantitatively, by the IgG and IgM indices, or qualitatively, by identification of oligoclonal bands in the CSF (Supplementary Figure 3 online).[13] The vast majority of patients with AD have no, or only very mild signs of, intrathecal immunoglobulin production (Table 1), making measurement of this process a valuable tool for excluding chronic inflammatory and infectious disorders in the clinical work-up of AD.

Core biomarkers

Ideally, a core biomarker should be coupled to the underlying molecular pathology of a disease.[14] In AD, the core biomarkers that have been developed reflect amyloid and

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neurofibrillary tangle pathology, and axonal degeneration (Supplementary Figure 2 online). Few methods have been available in living patients for measuring amyloid plaque and neurofibrillary tangle loads, and the severity of neuronal and synaptic degeneration. Thus, most studies of core biomarkers for AD have looked for correlations between CSF biomarkers measured during life and neuropathological findings at autopsy. The time lag between CSF tapping and autopsy, and other methodological issues of such studies, have made such correlations difficult to find.

One autopsy study found a correlation between postmortem ventricular CSF $A\beta_{1-42}$ and amyloid plaque load,[15] and another study demonstrated that $A\beta_{1-42}$ in lumbar CSF antemortem also correlated with amyloid plaque load at autopsy.[16] The development of Aβ PET ligands, notably Pittsburgh compound B (PIB), has enabled direct visualization of the fibrillar Aβ load in the brain when a patient is still alive. Several studies have reported a relationship between ^{11}C-PIB retention and CSF $A\beta_{1-42}$, with high ^{11}C-PIB binding correlating with low CSF $A\beta_{1-42}$ levels.[17,18] These data support the notion that CSF $A\beta_{1-42}$ levels reflect fibrillar $A\beta_{1-42}$ levels and amyloid plaque load in the brain. The most widely accepted explanation for the reduction in CSF $A\beta_{1-42}$ in AD is that aggregation of Aβ into plaques (and, hence, retention of the peptide in the brain parenchyma) results in reduced availability of Aβ to diffuse into the CSF.

Data from various studies suggest that CSF total tau (t-tau) levels reflect the intensity of neuronal and axonal degeneration and damage in the brain. Patients who experienced an acute disorder, such as stroke or brain trauma, were reported to have a transient increase in CSF t-tau, the magnitude of which correlated both with the extent of tissue damage and the probability of poor clinical outcome.[19–21] High CSF t-tau has also been associated with fast progression from MCI to AD,[22] and with rapid cognitive decline and a high mortality rate in patients with AD.[23,24] The highest increases in CSF levels of t-tau, however, have been reported in disorders with the most rapid neuronal degeneration, such as Creutzfeldt–Jakob disease.[25] One study found that CSF t-tau correlates with postmortem neurofibrillary tangle load,[16] suggesting that neurofibrillary tangle-bearing neurons might contribute to the CSF level of t-tau.

CSF levels of phosphorylated tau (p-tau) seem to reflect both the phosphorylation state of tau and the formation of neurofibrillary tangles in the brain. In some studies that involved measuring p-tau in CSF samples taken during life and at autopsy, correlations were reported for CSF p-tau—phosphorylated at Thr181 ($p\text{-}tau_{181}$) or Thr231 ($p\text{-}tau_{231}$)—with neocortical neurofibrillary tangle pathology, as well as with the rate of hippocampal atrophy in the brain.[16,26,27] High CSF $p\text{-}tau_{181}$ has also been associated with a fast progression from MCI to AD,[22] and with rapid cognitive decline in AD.[23]

Several studies have reported strong correlations between the levels of CSF t-tau and p-tau in patients with AD and in healthy elderly individuals.[28,29] Such correlations have not been found in Creutzfeldt–Jakob disease or acute stroke. In both of these conditions, t-tau is found at very high levels, reflecting intense neuronal damage, while p-tau levels are normal.[19,30] Data are accumulating that the main use of p-tau could be in differentiating AD from other forms of dementia.[31–33]

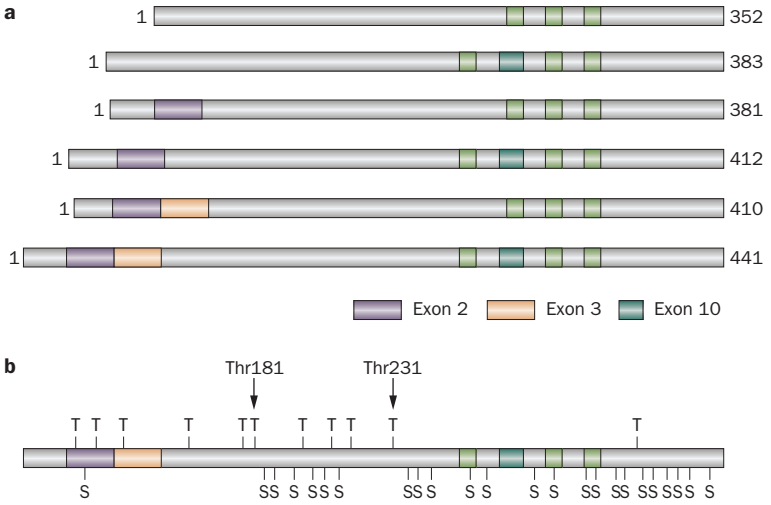

Figure 2 | Tau isoforms and phosphorylation sites. Tau is an axonal protein that binds to microtubules, promoting microtubule assembly and stability. Tau expression is high in nonmyelinated cortical axons, especially in the regions of the brain that are involved in memory consolidation, such as the limbic cortex.[151] **a** | Six isoforms of tau exist as a result of alternative splicing of exons 2, 3 and 10.[152] These isoforms contain three or four microtubule-binding domains (green boxes; the fourth domain is in exon 10). **b** | Numerous threonine and serine phosphorylation sites have been identified in tau, but the level of phosphorylated tau in cerebrospinal fluid is usually quantified by measuring phosphorylation at Thr181 or Thr231.[37,38] Tau phosphorylation is regulated by the balance between multiple kinases and phosphatases.[153] Hyperphosphorylated tau sequesters normal tau and other microtubule-associated proteins (MAP1 and MAP2), and causes disassembly of microtubules, which disrupts axonal transport. Furthermore, hyperphosphorylated tau becomes prone to aggregation into insoluble fibrils called paired helical filaments, which can form larger aggregates, namely neurofibrillary tangles.[153] Both the loss of microtubule stabilization and neurofibrillary tangle formation compromise neuronal and synaptic function, although whether tau hyperphosphorylation and aggregation is a cause or a consequence of Alzheimer disease is unknown. Abbreviations: S, serine; T, threonine.

Assay development for core biomarkers

The discovery that Aβ is produced during normal cell metabolism and secreted into the CSF was the basis for developing an Aβ biomarker for AD.[34] The subsequent finding that $A\beta_{1-42}$ is the most abundant species of Aβ in amyloid plaques led to the development of assays for this Aβ isoform.[35] Studies using various enzyme-linked immunosorbent assays (ELISAs) have shown that patients with AD consistently exhibit a decrease in CSF $A\beta_{1-42}$, to approximately 50% of the levels found in age-matched healthy elderly individuals.[36]

Tau has several isoforms and numerous phosphorylation sites (Figure 2). The most common ELISA for t-tau uses monoclonal antibodies that detect all isoforms of tau independently of phosphorylation state.[28] By use of this assay, numerous studies have reported that patients with AD have an increase in CSF t-tau of around 300% of the levels found in healthy elderly individuals.[36]

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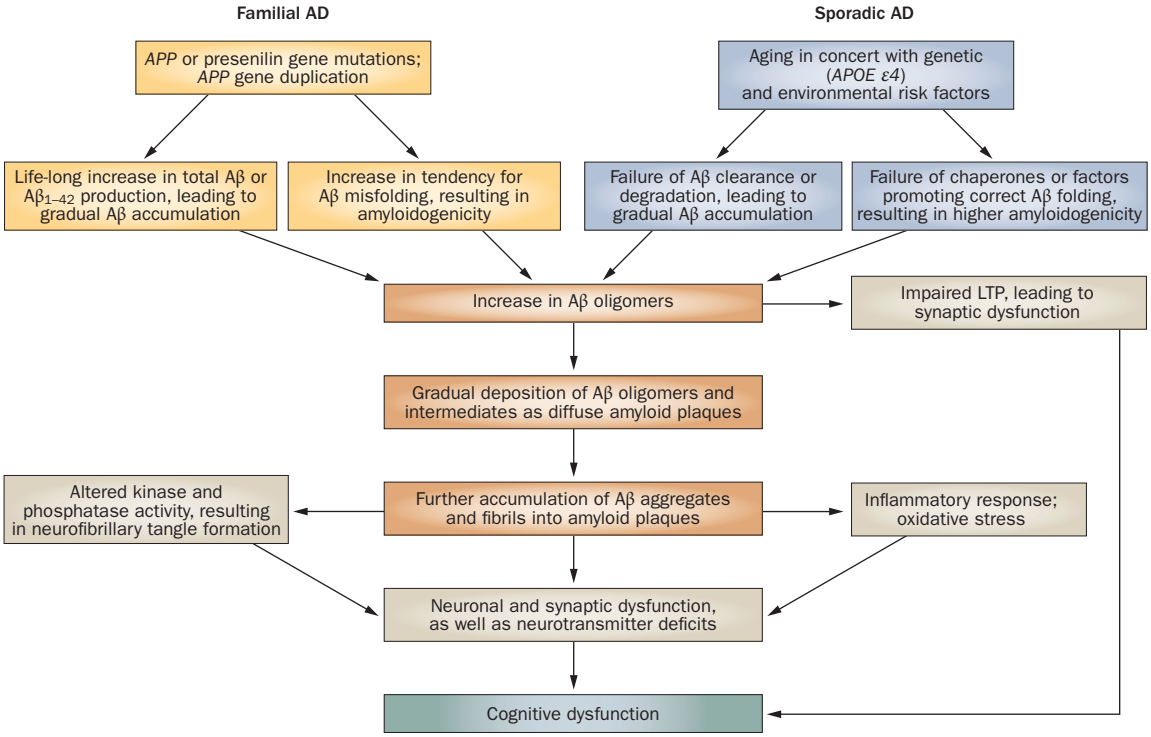

Figure 3 | The amyloid cascade hypothesis of AD. The amyloid cascade hypothesis states that an imbalance between the production and clearance of Aβ in the brain, causing an increase in the level of the peptide, is the initiating event in AD, and ultimately leads to neuronal degeneration and dementia.[6] An increase in production of either total Aβ or the amyloidogenic $Aβ_{1–42}$ isoform is well established in familial AD, but only limited evidence exists for a specific disturbance in Aβ clearance in sporadic AD. In both familial and sporadic AD, soluble Aβ is believed to undergo a conformational change that renders it prone to aggregation into soluble oligomers and the larger insoluble fibrils found in plaques. The specific molecular mechanisms underlying this conformational change are largely unknown. Fibrillar Aβ deposited in plaques might be neurotoxic; however, synaptic loss and clinical progression of the disease mainly correlate with soluble Aβ levels.[154] Data suggest that soluble Aβ oligomers might inhibit LTP in the hippocampus and, hence, disrupt synaptic plasticity.[82] Tau phosphorylation and subsequent neurofibrillary tangle formation, as well as inflammation and oxidative stress, are regarded as downstream events. Abbreviations: Aβ, amyloid-β; AD, Alzheimer disease; *APOE*, apolipoprotein E; *APP*, amyloid precursor protein; LTP, long-term potentiation.

The most common ELISAs for p-tau in CSF use antibodies specific for either $p\text{-}tau_{181}$ or $p\text{-}tau_{231}$ (Figure 2).[37,38] Studies using these assays have consistently reported a marked increase in CSF p-tau in patients with AD.[36] A study directly comparing the two p-tau assays found that they had similar diagnostic performances.[31]

The diagnostic accuracy of CSF t-tau, p-tau and $Aβ_{1–42}$ when considered together—in terms of identifying cases of AD or prodromal AD, and for differentiating AD from other disorders—is higher than for any of these biomarkers alone.[39–42] Thus, a multiparameter assay was developed to simultaneously quantify these CSF biomarkers. This assay, which was based on xMAP® technology (Luminex, Austin, TX, USA),[43] has been used in several large multicenter studies of CSF biomarkers in AD, and showed a high diagnostic performance.[39,44,45] Interestingly, the absolute values for the biomarker levels detected in CSF vary between the xMAP® system and ELISA methods.[43] Several factors probably account for this finding, including differences in the pairs of antibodies used in the assays, the methods for coupling the antibodies to the beads and coating the plates, and the calibrators and incubation conditions. Correction factors can be used to allow direct comparisons of the results from the xMAP® system and the various ELISA methods.[43,44]

Performance of core biomarkers

Alzheimer disease

Numerous studies have found that patients with AD have a marked increase in CSF levels of t-tau and p-tau and a substantial reduction in $Aβ_{1–42}$ levels. Each of these biomarkers has been reported to differentiate patients with AD from healthy elderly individuals with 80–90% sensitivity and specificity (Box 1).[36,46] Moreover, t-tau, p-tau and $Aβ_{1–42}$ CSF levels have been found to be normal in several important differential diagnoses of AD, including depression and Parkinson disease.[47] A combined analysis of two or more of these biomarkers more accurately diagnoses AD than any of these biomarkers alone.[39,40,44] For example, one study showed that a combined analysis of $Aβ_{1–42}$ and t-tau improved the sensitivity of a diagnosis of AD from 78–84% (using one of these biomarkers alone) to 86% and the specificity from 84–90% to 97%.[40]

CSF p-tau aids the differentiation of AD from other dementias, including frontotemporal dementia and dementia with Lewy bodies.[31] The diagnostic performance

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Table 1 | CSF biomarkers for AD

Biomarker	Pathogenic process	Change in biomarker level in AD	Comment
Basic biomarkers			
CSF cell count	Inflammation	Unchanged[155]	CSF cell count is used to exclude infectious disorders[13]
CSF:serum albumin ratio	BBB function	Unchanged in cases of pure AD;[12] mild to moderate increase in AD with concomitant cerebrovascular pathology[12]	Increase in CSF:serum albumin ratio is an indicator of BBB damage;[11] BBB damage is found in CNS infections (for example, neuroborreliosis), inflammatory disorders (for example, Guillain–Barré syndrome), brain tumors and cerebrovascular disease (including vascular dementia)[13]
IgG or IgM index; IgG or IgM oligoclonal bands	Intrathecal immunoglobulin production	Unchanged[156]	These analyses are used to exclude cases with inflammatory (for example, multiple sclerosis or cerebral systemic lupus erythematosus) and chronic infectious (for example, *Borrelia* encephalitis or syphilis) disorders[13]
Core biomarkers			
$A\beta_{1–42}$	Amyloidogenic pathway of APP metabolism	Marked reduction in AD and prodromal AD[36,46]	CSF $A\beta_{1–42}$ is the central CSF biomarker for brain Aβ metabolism and plaque formation;[15,17,18] low CSF $A\beta_{1–42}$ is found in patients with dementia with Lewy bodies[157]
p-tau_{181} and p-tau_{231}	Tau phosphorylation	Marked increase in AD and prodromal AD[36,46]	High CSF p-tau has only been found in AD;[36,46] CSF p-tau_{181} and p-tau_{231} levels correlate tightly and give similar diagnostic accuracy[31]
t-tau	Axonal (neuronal) degeneration	Marked increase in AD and prodromal AD[36,46]	High CSF t-tau is found in disorders with acute brain damage, such as stroke, trauma and encephalitis;[19–21] very high CSF t-tau, together with normal p-tau, is found in cases of Creutzfeldt–Jakob disease[30]

Abbreviations: Aβ, amyloid-β; AD, Alzheimer disease; BBB, blood–brain barrier; CSF, cerebrospinal fluid; p-tau, phosphorylated tau; t-tau, total tau.

of CSF biomarkers in differentiating AD from other dementias, however, is far from optimal. Several factors could explain this finding. First, most studies of CSF biomarkers are based on clinically diagnosed cases, which introduces a relatively large percentage of misdiagnoses.[48,49] Second, a sizeable percentage of elderly individuals without dementia have enough amyloid plaques and neurofibrillary tangles to warrant a neuropathological diagnosis of AD.[50,51] Last, AD exhibits a large overlap in pathology with some other forms of dementia, notably dementia with Lewy bodies and vascular dementia.[52–54] This overlap in pathology essentially precludes the possibility of finding CSF biomarkers that have close to 100% sensitivity and specificity for AD.

Prodromal Alzheimer disease

Studies have consistently shown that the combination of t-tau, p-tau and $A\beta_{1–42}$ has a high predictive value for identifying cases of prodromal AD in patients with MCI,[46] with one study reporting a sensitivity of 95% (Box 1).[39] This high predictive value has been verified in large multicenter studies, including the Alzheimer's Disease Neuroimaging Initiative study,[45] the DESCRIPA study,[55] and the Swedish Brain Power project.[44] The results from these studies show that CSF biomarkers might be valuable clinical diagnostic tools for identifying prodromal AD in individuals with cognitive impairment.

Presymptomatic Alzheimer disease

Some studies have examined whether CSF biomarkers might be useful in predicting AD in the preclinical stage of the disease. Two population-based studies found a marked reduction in CSF $A\beta_{1–42}$ levels in cognitively normal elderly people who later developed AD, although no changes were observed in CSF t-tau or p-tau.[56,57] In addition, a clinical study reported that CSF $A\beta_{1–42}$, but not t-tau and p-tau, predicted cognitive decline in healthy elderly individuals.[58] Moreover, asymptomatic individuals with familial AD mutations had low CSF $A\beta_{1–42}$,[59] yet high CSF t-tau and p-tau.[60] Together, these results support earlier animal data suggesting that the amyloidogenic process (the process of generating Aβ) is upstream of tau pathology.[61,62]

A large study showed that cognitively normal elderly individuals who exhibited cortical ^{11}C-PIB binding on PET had low CSF $A\beta_{1–42}$ levels, although the same study revealed that low CSF $A\beta_{1–42}$ was also found in some individuals who did not exhibit ^{11}C-PIB binding.[63] These findings might be explained by the fact that ^{11}C-PIB binds fibrillary Aβ, but not the Aβ oligomers or diffuse plaques that are found in the earliest stages of the disease process.[64] Furthermore, these data suggest that CSF $A\beta_{1–42}$ might predict AD in its very early stages in cognitively normal elderly individuals. The overlap in variation between CSF $A\beta_{1–42}$ levels in individuals with presymptomatic AD and healthy elderly people, however, might be too large for presymptomatic AD to be predicted in individual cases. Moreover, the use of biomarkers to predict AD in asymptomatic people is not warranted until registered drugs with distinct disease-modifying effects, and few adverse effects, become available.

Validated Alzheimer disease cases

The diagnostic performance of CSF biomarkers has been examined in several patient series in which the diagnosis of dementia was confirmed at autopsy. In these studies, the combination of CSF t-tau, p-tau and $A\beta_{1–42}$ differentiated people with AD from both cognitively normal elderly individuals and cases of other dementias—including dementia with Lewy bodies, frontotemporal dementia and vascular dementia—with high specificity

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Box 1 | Criteria for evaluation of Alzheimer disease biomarkers

Studies evaluating the diagnostic performance of a biomarker for Alzheimer disease should include determination of the molecule's sensitivity, specificity, positive predictive value and negative predictive value for the disorder.[158] Sensitivity refers to the capacity of a biomarker to identify patients who have disease (the number of true positive cases divided by all cases with disease), while specificity refers to the capacity of a biomarker to identify patients who do not have disease (the number of true negative cases divided by all cases without disease). The positive predictive value refers to the percentage of cases with a positive test who prove to have the disease (the number of true positive cases divided by all cases with a positive test), while the negative predictive value refers to the percentage of cases with a negative test who prove not to have the disease (the number of true negative cases divided by all cases with a negative test). According to the criteria for an ideal Alzheimer disease diagnostic biomarker, outlined by the Ronald and Nancy Reagan Research Institute–National Institute on Aging Working Group, sensitivity and specificity should exceed 80%, whereas the predictive values should be ≥80%.[14]

and sensitivity.[33,45,65–67] Thus, CSF biomarkers have been validated in patient series with a neuropathological follow-up, showing similar or better discriminatory power than in patient series with only clinical diagnoses.

Novel candidate biomarkers

Many publications can be found for candidate CSF AD biomarkers other than Aβ and tau, although the initially promising results from such studies have often not been reproduced. Here, we review novel biomarkers that have shown high sensitivity and specificity for AD in at least two independent studies. We also discuss selected candidate biomarkers related to Aβ and APP metabolism.

BACE1

Aβ is generated following the sequential actions of β-secretase and γ-secretase on APP (Figure 1). The main enzyme responsible for β-secretase activity is β-site APP-cleaving enzyme 1 (BACE1). Increases in BACE1 expression and enzymatic activity have been reported in AD brain tissue at postmortem.[68,69] BACE1 can also be measured in the CSF, and increases in BACE1 concentration and activity have been found in patients with AD and in cases of prodromal AD.[70–72] Together, these data suggest that upregulation of BACE1 might be an early event in AD.

Amyloid precursor protein isoforms

During APP processing, the large soluble amino-terminal domain of APP, sAPPα or sAPPβ (depending on whether APP is first cleaved by α-secretase or β-secretase, respectively), is secreted into the extracellular space and also into the CSF (Figure 1). In sporadic AD and MCI, CSF levels of both sAPPα and sAPPβ have been reported to remain unaltered or to increase slightly.[71,73,74] Despite the absence of a consistent change in sAPP levels in AD, these CSF biomarkers might be valuable tools in treatment trials for monitoring the effect of a drug on APP processing (Table 2).

Truncated amyloid-β isoforms

$A\beta_{1–40}$ is the most abundant Aβ isoform in CSF.[75] In AD and MCI, no major change has been detected in CSF $A\beta_{1–40}$; however, a marked decrease has been observed in the $A\beta_{1–42}$:$A\beta_{1–40}$ ratio, and this change was more pronounced than the reduction in CSF $A\beta_{1–42}$.[76,77] Other carboxy-terminal truncated Aβ peptides, including $A\beta_{1–37}$, $A\beta_{1–38}$ and $A\beta_{1–39}$, have also been identified in the CSF of patients with AD.[78] An increase in the CSF level of $A\beta_{1–38}$ was found together with a decrease in $A\beta_{1–42}$ in such individuals,[78,79] suggesting that the $A\beta_{1–42}$:$A\beta_{1–38}$ ratio might improve diagnostic accuracy in cases of AD.

Several short carboxy-terminal truncated Aβ isoforms have been identified and quantified by a combination of immunoprecipitation with an anti-Aβ monoclonal antibody and matrix-assisted laser desorption–ionization time-of-flight mass spectrometry.[75] A marked increase in $A\beta_{1–16}$ together with the expected decrease in $A\beta_{1–42}$ was reported in CSF from patients with AD.[80] Data from experimental studies showed that the short Aβ isoforms $A\beta_{1–14}$, $A\beta_{1–15}$ and $A\beta_{1–16}$ were produced by a novel pathway of APP processing involving the concerted actions of β-secretase and α-secretase, whereas the longer isoforms ($A\beta_{1–17}$ up to $A\beta_{1–42}$) were produced by the γ-secretase pathway (Figure 1).[81]

Amyloid-β oligomers

The aggregation of soluble Aβ peptides to form insoluble fibrillar Aβ in plaques has long been regarded to be the central pathogenic event in AD (Figure 3). Experimental data, however, have suggested that soluble Aβ oligomers might inhibit long-term potentiation and, thereby, have a role in AD pathogenesis.[82] Thus, CSF Aβ oligomers might be important core biomarkers for AD.

Some preliminary studies on Aβ oligomers in CSF have been published. In one study, antibodies coupled to DNA-tagged nanoparticles were used to capture Aβ oligomers from the CSF of patients with AD and healthy aged-matched controls at postmortem. PCR-based amplification revealed a higher assay signal in the CSF from patients with AD than from the control samples.[83] A study using flow cytometry technology also suggested the presence of Aβ oligomers in lumbar CSF from neurological patients, although no data on the diagnostic utility of this technique in the context of AD were presented.[84] Following immunoprecipitation of CSF samples using an anti-Aβ antibody, immunoblotting revealed a weak band migrating at the size expected for Aβ dimers in some samples from patients with AD and cognitively normal elderly individuals. No consistent change in this band, however, could be found in the AD group.[85] Thus, although Aβ oligomers are attractive AD biomarker candidates, several issues relating to these molecules persist. The level of these Aβ species in CSF seems to be very low in comparison with Aβ monomers. Importantly, mass spectrometry analyses are needed to verify that the signals measured using the various techniques described above actually represent changes in Aβ oligomers. Furthermore, assays suitable for large clinical studies have yet to be developed for these molecules.

Endogenous amyloid-β autoantibodies

Several studies have reported the existence of naturally occurring Aβ antibodies (either in free form or in complexes with Aβ) in CSF and/or blood. The results

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Table 2 | Applications of CSF biomarkers in AD clinical trials

Application	Details	Time point for use	Possible biomarker and their role
Improving the accuracy of diagnosis	CSF biomarkers could be used in clinical trials to improve diagnostic accuracy in trial participants, enabling patient cohorts to be enriched with cases of AD	Before trial initiation	High T-tau and P-tau and low $A\beta_{1–42}$ are indicative of AD
Stratification of AD cases	AD cases with CSF biomarker evidence of a disturbance in Aβ metabolism might be more responsive to anti-Aβ drugs than patients who do not exhibit such a disturbance	Post hoc analysis	$A\beta_{1–42}$ might be used to stratify cases in trials of anti-Aβ disease-modifying drug candidates; p-tau might be used to stratify cases in trials of drugs that aim to reduce tau phosphorylation and neurofibrillary tangle pathology
Safety monitoring	Anti-Aβ drug candidates, such as Aβ immunotherapy, might elicit adverse effects, such as meningoencephalitis or vasogenic oedema	Baseline evaluation and assessment during trial	CSF cell count, IgG or IgM index and IgG or IgM oligoclonal bands are standard measures for identifying and monitoring inflammatory processes, such as meningoencephalitis, in the CNS; the CSF:serum albumin ratio is the standard measure to identify and monitor a disturbance in the blood–brain barrier, which can lead to cerebral edema
Theragnostics	CSF biomarkers might indicate whether a drug has an effect on the molecular pathology of AD in living patients	Baseline evaluation and at time points throughout the trial, including the last week of the study	$A\beta_{1–42}$ is the main biomarker for Aβ metabolism and deposition; APP isoforms (sAPPα and sAPPβ) and BACE1 activity might be valuable in clinical trials of BACE1 inhibitors; p-tau is the main biomarker for monitoring the phosphorylation state of tau; t-tau might be a valuable biomarker for identifying and monitoring a downstream effect on the intensity of neuronal or axonal degeneration

Abbreviations: Aβ, amyloid-β; AD, Alzheimer disease; APP, amyloid precursor protein; BACE1, β-site APP cleaving enzyme 1; CSF, cerebrospinal fluid; p-tau, phosphorylated tau; sAPP, soluble APP extracellular domains; t-tau, total tau.

from these studies, however, have been inconsistent, with increases,[86,87] decreases[88,89] or unchanged[90] titers of such antibodies all having been reported in cases of AD. These studies were all based on assays that could not differentiate between antibodies against various Aβ isoforms or different assembly states of the peptide (monomeric versus oligomeric Aβ). Human plasma has been shown to contain autoantibodies against a broad range of Aβ peptides, including oxidized, pyroglutamate and mutated variants.[91] Antibody reactivity was highest for oligomeric Aβ, but the level of this reactivity did not differ between cases of AD and controls.[91]

Neuronal and synaptic markers

Neuronal and synaptic proteins could prove to be valuable CSF biomarkers for AD, as these molecules might correlate with cognitive function and disease progression. Visinin-like protein 1 (VLP-1) is a highly expressed neuronal calcium sensor protein that was identified by gene array analyses during a search for brain-specific protein biomarkers.[92] In the first clinical study of this protein, a marked increase in CSF VLP-1 was found in patients with AD. Moreover, the diagnostic performance of VLP-1 in distinguishing patients with AD from healthy elderly individuals was similar to that of CSF t-tau, p-tau and $A\beta_{1–42}$, with a sensitivity and a specificity both close to 80%.[93] CSF levels of VLP-1 were high in patients with AD who carried the apolipoprotein E (*APOE*) *ε4* allele (a genetic risk factor for AD), and also negatively correlated with Mini-Mental State Examination scores. Thus, VLP-1 is a promising candidate CSF biomarker for AD.

Neurofilaments are structural components of axons, and show particularly high expression in large myelinated axons.[94] Accordingly, high CSF levels of neurofilaments are found in disorders with subcortical pathology, such as vascular dementia and normal pressure hydrocephalus.[95,96] High CSF levels are also found in frontotemporal dementia, while normal levels are found in most patients with AD.[97] CSF neurofilament proteins might, therefore, be useful in differentiating between AD, frontotemporal dementia, and subcortical dementia disorders.

Synaptic protein biomarkers should ideally reflect synaptic functioning and, hence, cognition. Several presynaptic and postsynaptic proteins have been identified in CSF using a procedure that involved protein precipitation followed by liquid-phase isoelectric focusing and Western blotting. These proteins included RAB3A, synaptotagmin, growth-associated protein (GAP-43), synaptosomal-associated protein 25 and neurogranin.[98] An immunoassay for GAP-43 revealed increased levels of the protein in CSF from patients with AD compared with controls and individuals with frontotemporal dementia.[99] Furthermore, the same study showed that GAP-43 and t-tau levels in CSF were positively correlated, suggesting that both biomarkers reflect axonal and synaptic degeneration. When validated assays are available for measuring synaptic proteins in CSF, these proteins might serve as tools for monitoring the effect of novel drug candidates on synaptic function in clinical trials.

F2-isoprostanes

AD pathogenesis includes free radical-mediated injury to neurons (Supplementary Figure 2 online). Lipid peroxidation is an important consequence of free radical-mediated damage and leads to the generation of F2-isoprostanes, which might serve as biomarkers for this pathogenic mechanism. Several studies have shown that CSF F2-isoprostane levels are increased in patients with AD compared with healthy elderly individuals or patients with non-AD forms of dementia.[100] Levels of CSF F2-isoprostanes have also

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been demonstrated to increase in cognitively impaired individuals with prodromal AD,[101] and in asymptomatic carriers of familial AD mutations.[60] By contrast, the results from studies of F2-isoprostanes in plasma have been conflicting, probably because the contribution of brain-derived F2-isoprostanes to the total level of these molecules in plasma is much smaller than the contribution from peripherally derived F2-isoprostanes.[100]

Roles in clinical trials

Aside from their potential as tools for clinical diagnosis, CSF biomarkers might be valuable in drug development. Such biomarkers could be used as diagnostic markers for enriching the number of AD cases, for patient stratification, as safety markers, and to detect and monitor the biochemical effects of drugs (Table 2).

Enrichment of AD cases

Making a diagnosis of AD during the early stages of the disease is a great challenge for clinicians, as patients with MCI only have a mild disturbance in episodic memory. Moreover, in such patients, other symptoms of AD can be absent or seem vague. The only clinical method available for determining which patients with MCI have prodromal AD is to follow their cognitive function over several years. Even at specialized academic centers, however, the accuracy of the clinical diagnosis of AD in cases that have been followed up for several years is relatively low, with sensitivity and specificity values of 70–80%.[102] These figures are considerably lower for patients with early AD,[103] and in primary care settings.[104]

Clinical trials of cholinesterase inhibitors in patients with MCI have failed to find any marked benefit of these drugs. The clinical end point in these trials was a reduction in the conversion rate to AD.[105] These studies involved patients with unspecified MCI, meaning that around half the participants did not have prodromal AD, and would not have converted. Thus, the inclusion of such patients might have seriously affected any possibility of identifying clinical effects of the drugs.[106] The addition of a positive CSF biomarker as an inclusion criterion in MCI trials will increase the proportion of individuals with underlying AD pathology and, thereby, increase the possibility of identifying a positive effect of a drug (Table 2).

Patient stratification

AD is a heterogeneous disorder, both at clinical and neuropathological levels.[5] Thus, the effectiveness of any potential disease-modifying drug could plausibly vary between subgroups of patients with this disease. Indeed, the effectiveness and adverse effects of one passive Aβ immunotherapy for AD were reported to differ between *APOE ε4* carriers and noncarriers.[107]

As CSF biomarkers reflect the central pathogenic processes in AD, these molecules might be used in post hoc data analyses of clinical trials to stratify patient cohorts on the basis of underlying pathology. Indeed, a patient subgroup with a certain biomarker trait that indicates amyloid plaque pathology, such as low CSF $A\beta_{1-42}$, might be more responsive to anti-Aβ disease-modifying drugs than a subgroup of patients with normal CSF $A\beta_{1-42}$ levels (Table 2).

Safety measures

Clinical trials of disease-modifying treatments for AD have been hampered by adverse effects. In the clinical trial of the Aβ vaccine AN1792, a small but notable number of patients developed meningoencephalitis, and treatment with the passive Aβ immunotherapy AAB-001 led to vasogenic edema in some individuals.[107,108] CSF analysis is a standard method for diagnosing encephalitis and BBB damage associated with disorders causing edema,[11,13] and could be employed usefully in clinical trials of AD-modifying drugs.

Analysis of CSF taken from patients at baseline, before treatment, could be useful in clinical trials for identifying and excluding individuals with chronic infectious or inflammatory CNS disorders that can mimic AD, such as neuroborreliosis. The inclusion of such cases in trials could result in the erroneous conclusion that an adverse effect, such as encephalitis, was related to the drug being tested. Baseline CSF samples can also be used in comparisons with CSF removed after treatment initiation. The benefit of such comparisons is that even minor inflammatory activation within the CNS, as a result of adverse effects of the drug, can be identified. Thus, CSF biomarkers could allow safety monitoring during clinical trials (Table 2). Longitudinal CSF sampling during the treatment period might indicate whether a certain drug induces harmful immune activation over the long term.

Theragnostic markers

The effect of disease-modifying anti-Aβ drugs on amyloid plaque pathology is commonly evaluated in AD transgenic mice; however, these animal models have a low predictive power for treatment success in patients with sporadic AD.[5] Biomarkers might help bridge the gap between animal studies and large clinical trials by providing a means of evaluating whether a drug has a true disease-modifying effect in humans in small-scale clinical studies. Only the most promising drug candidates would then be selected for further study, thereby improving the success rate of large phase II and III trials.

In slowly progressive disorders such as AD, the clinical evaluation of a drug by use of rating scales requires large patient numbers and extended treatment periods. For drugs with symptomatic effects, such as cholinesterase inhibitors, an improvement in cognitive function can be expected in the short term (Figure 4). By contrast, disease-modifying drugs cannot be expected to have an early effect on symptoms. Instead, such therapies might lead to a reduction in the rate of cognitive decline over several years (Figure 4). Thus, the number of patients needed to detect an effect on cognition is probably larger, and the treatment period longer, for a disease-modifying drug than for symptomatic therapies.

Biomarkers that are used to identify and monitor the biochemical effect of drugs are called theragnostic markers. Such biomarkers can be used to identify and monitor both the specific effect of the drug and downstream effects on

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pathogenic mechanisms (Table 2). Trials employing theragnostic biomarkers can be based on relatively small patient numbers and short treatment periods and, thus, might be valuable for deciding whether to proceed with large and expensive phase II or III clinical trials. Theragnostic biomarker trials are feasible in AD, as CSF t-tau, p-tau and $A\beta_{1-42}$ levels have shown low intra-individual variability over time in longitudinal samples.[109,110] Some of these biomarkers might also serve as substitutes for clinical end points (surrogate biomarkers), although this possibility needs to be evaluated in full-scale clinical trials. Finally, theragnostic biomarkers are important for regulatory purposes, as a drug can only really be labeled as disease modifying if it has an effect on cognition, and if biomarker evidence can be presented that the drug affects the central pathogenic processes.[10,111]

To date, only preliminary evidence exists to suggest that CSF biomarkers might be useful as theragnostic markers. Importantly, cholinesterase inhibitors and lithium—drug candidates with no proven effect on the molecular pathogenesis of AD—had no effect on AD CSF core biomarkers.[109,112] Data from animal studies, however, demonstrated that γ-secretase inhibitor treatment resulted in a reduction in cortical, CSF and plasma levels of Aβ.[113,114] Similarly, in nonhuman primates, BACE1 inhibitor treatment resulted in a reduction in CSF $A\beta_{1-42}$, $A\beta_{1-40}$ and sAPPβ levels.[115] Whether CSF Aβ levels in patients with AD will be altered in response to treatment with efficacious anti-Aβ drugs remains unclear. A phase IIa study of the Aβ clearance-enhancing compound PBT2 demonstrated that CSF $A\beta_{1-42}$ underwent a dose-dependent reduction during the treatment period.[116] Furthermore, data from a clinical study of the amyloid-targeting drug phenserine also suggested that CSF Aβ might be of value in the evaluation of treatment effects.[117] In the interrupted phase IIa AN1792 trial, however, no change in CSF $A\beta_{1-42}$ was detected in treated patients, despite a decrease towards normal levels of the downstream biomarker t-tau.[118] A clinical trial of the γ-secretase inhibitor LY450139 also failed to find any effect on CSF $A\beta_{1-42}$ levels, as measured by ELISA, in patients with AD.[119] However, acute treatment with the same compound in young healthy volunteers resulted in a clear inhibitory effect on the Aβ production rate, as determined by measuring the ratio of newly synthesized (isotope-labeled) Aβ to pre-existing (unlabeled) Aβ in CSF.[120] Several other clinical trials of disease-modifying drug candidates that include biomarkers as end points are currently ongoing. These trials will provide further evidence to indicate whether biomarkers can be used to assess disease modification, and as surrogate markers for predicting clinical outcomes.

Plasma biomarkers

Efforts to find reliable biomarkers for AD in peripheral blood have met with little success. Several candidate blood biomarkers have been proposed, yet changes in the levels of these molecules have proved difficult to verify in independent studies. In the section below, we focus on plasma Aβ, which has been the most extensively examined peripheral biomarker for AD. We also review some explorative pilot studies of novel plasma protein biomarkers that have shown promising results.

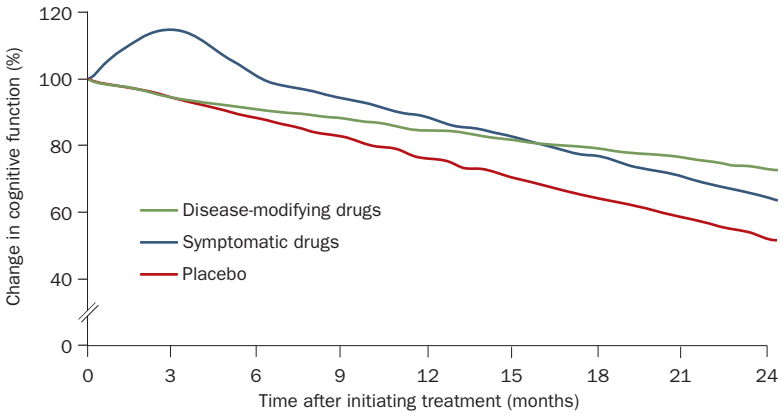

Figure 4 | Evaluation of Alzheimer disease therapies by cognitive scales. Theoretical differences in cognitive effect of treatments for Alzheimer disease are shown over a 24 month period. During treatment with symptomatic drugs, such as cholinesterase inhibitors, an improvement can be observed in cognitive function during the initial phase of therapy (~6 months). Beyond this initial phase a decline in cognition occurs, although a marked difference in cognitive function exists between patient treated with symptomatic drugs and placebo-treated individuals.[159] A less pronounced decline in cognitive function can be seen in patients treated with disease-modifying drugs than in individuals receiving placebo; however, no initial improvement in cognition is observed in the former group. Thus, to find an effect of a disease-modifying drug on cognition in a clinical trial, the number of patients needed is larger and the treatment period markedly longer than in a trial of a symptomatic drug. CSF biomarkers might be valuable in clinical trials of disease-modifying drugs, by providing objective evidence that a drug affects the underlying pathogenic processes. Indeed, such evidence, alongside an effect on cognitive decline, is essential to claim that a drug is disease modifying.[111]

Plasma amyloid-β

Many studies have examined plasma Aβ as a biomarker for AD; however, the findings from these studies have been contradictory. Some investigators have reported slightly higher $A\beta_{1-42}$ or $A\beta_{1-40}$ plasma levels in patients with AD than in healthy age-matched controls, although most studies have found no change in plasma Aβ between these groups.[121] In addition, studies examining the value of plasma Aβ tests for predicting AD in cognitively normal elderly individuals have shown a broad overlap in plasma $A\beta_{1-42}$ and $A\beta_{1-40}$ levels between individuals with preclinical AD and those people who do not develop AD. Some studies have reported that a high level of plasma $A\beta_{1-42}$ or a large $A\beta_{1-42}$:$A\beta_{1-40}$ ratio are risk factors for future AD, while others studies have reported opposing data.[122–125] These discouraging results are probably explained by the fact that plasma Aβ is derived from peripheral tissues, and does not reflect brain Aβ turnover or metabolism.[76] Furthermore, the hydrophobic nature of Aβ makes the peptide bind to plasma proteins, which could result in epitope masking and other analytical interferences.[126]

Novel blood biomarkers

Several promising novel blood biomarkers for AD have been documented. The combined multivariate analysis of 18 plasma signaling and inflammatory proteins accurately identified patients with AD and predicted the onset of

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Box 2 | Research criteria for a diagnosis of Alzheimer disease

The diagnostic criteria for probable Alzheimer disease (AD) described below are based on the core criterion of early memory disturbances together with supportive criteria that include positive findings for one or more biomarkers.[140]

Core diagnostic criterion

Evidence of progressive episodic memory impairment lasting >6 months (reported by patients or informants) that can be verified by objective testing; memory impairment can be isolated or associated with other cognitive changes

Selected supportive criteria

Presence of medial temporal lobe atrophy (in the hippocampus, entorhinal cortex or amygdala) on MRI, measured by either qualitative rating or quantitative volumetry, and referenced to a well-characterized age-matched population

Positive cerebrospinal fluid biomarker result (low amyloid-β_{1-42}, high total tau and/or high phosphorylated tau)

Reduction in glucose metabolism in bilateral temporal parietal regions or increase in binding of amyloid-β ligands (^{18}F-FDDNP or ^{11}C-labeled Pittsburgh compound B), as measured by PET

Presence of a familial AD-causing mutation

Selected exclusion criteria

History of sudden onset of symptoms or early symptoms, including gait disturbances, seizures or behavioral changes

Clinical features of focal neurological signs, such as hemiparesis, sensory loss, visual field deficits or early extrapyramidal signs

Other medical disorders severe enough to account for memory and related symptoms—including non-AD dementia, major depression, cerebrovascular disease, toxic and metabolic abnormalities—or MRI fluid-attenuated inversion recovery or T2-weighted signal abnormalities in the medial temporal lobe that are consistent with infectious or vascular insults

Box 3 | The Alzheimer's Association quality control program

The aim of the quality control program is to standardize cerebrospinal fluid (CSF) biomarker measurements between both research and clinical laboratories. Achievement of this aim will increase the analytical precision and improve the longitudinal stability of biomarker measurements. The program will allow direct comparisons of biomarker levels between laboratories and, thus, between publications.

The program is run by the Clinical Neurochemistry Laboratory in Gothenburg, Sweden in conjunction with the Alzheimer's Association. Biotech companies and a number of reference laboratories, including the Alzheimer's Disease Neuroimaging Initiative Biomarker Core, are also represented. Both research and clinical CSF laboratories, as well as pharmaceutical companies, are enrolled in the program.

The program is open for generally (commercially) available assay formats, but not for in-house assays, and consists of two parts. The first part involves a standardized flow chart for lumbar puncture and CSF processing (Supplementary Table 1 online). The second part is an external quality control program, in which samples (aliquots of pooled CSF) are sent out to the participating laboratories for CSF biomarker analysis, after which biomarkers levels are entered into a report form and returned.

The final report for each quality control round includes information on the measured biomarker levels for the individual laboratory and, for comparison, the mean and variation in biomarker levels across all laboratories involved in the program. In addition, the longitudinal stability in CSF biomarker levels for the individual laboratory, expressed as percent deviation over time, will be reported. These reports will serve as feedback for the participating laboratories, to identify whether the level of a biomarker is outside an acceptable range and to note sudden changes or longitudinal drifts in CSF biomarker levels.

AD in individuals with MCI.[127] This panel of proteins was identified after screening 120 known signaling proteins using a filter-based, arrayed sandwich ELISA. Further independent studies are needed to examine whether this set of proteins is the optimal combination of plasma biomarkers for diagnosing prodromal AD, and to further assess the diagnostic value of this approach. Another study that used explorative proteomics technology identified AD-associated increases in the plasma levels of complement factor H and α_2-macroglobulin—findings that were replicated using semiquantitative immunoblotting techniques.[128] The midregional pro-atrial natriuretic peptide:carboxy-terminal endothelin-1 precursor fragment ratio has also been reported to be elevated in plasma from patients with AD.[129] If this finding and other results for candidate plasma biomarkers could be replicated in independent studies using immunoassay techniques suitable for routine diagnostic laboratories, plasma protein panels might serve as screening tests for AD.

Future perspectives

CSF biomarkers have a high diagnostic value in the context of AD. The combination of these biomarkers and structural (CT or MRI) and/or functional (single photon emission CT [SPECT] or PET) brain imaging should provide increased diagnostic accuracy compared with CSF biomarkers or one type of imaging used in isolation. To date, only a few studies have directly examined this possibility. CSF biomarkers combined with either CT or MRI measurements of medial temporal lobe atrophy have been reported to increase the accuracy of AD diagnosis.[130–132] In addition, assessment of both CSF biomarker levels and the degree of structural AD-like abnormalities on MRI more accurately predicted which patients with amnestic MCI would convert to AD than either biomarker alone.[133] Similarly, the measurement of CSF biomarkers alongside the assessment of regional cerebral blood flow, using the ^{133}Xe inhalation technique or SPECT, has been shown to improve diagnostic accuracy of either biomarker alone in cases of prodromal AD.[134,135] Furthermore, although no study has examined the added diagnostic value of ^{11}C-PIB PET when combined with CSF biomarkers, a strong negative correlation exists between the degree of ^{11}C-PIB binding and the CSF level of $A\beta_{1-42}$.[17,18]

Large multicenter studies are needed to further define the added diagnostic value when multiple biomarker modalities are combined. Such studies will also provide information on the optimal brain regions to evaluate by MRI (for atrophy) and by PET (for Aβ load) in the context of AD. Complementary data are needed on whether high-resolution MRI scanners and newly developed amyloid ligands, such as AZD2184, will improve diagnostic sensitivity and specificity.[136] When implementing these biomarkers in clinical practice, financial considerations will be of importance. The cost of combined analysis of CSF t-tau, p-tau and $A\beta_{1-42}$ is ~US$200, whereas a structural MRI investigation and a ^{11}C-PIB-PET scan cost ~$500 and ~$5,000, respectively.

The current clinical diagnostic criteria for AD were outlined more than 25 years ago by the National Institute of

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Neurological and Communicative Disorders and Stroke–Alzheimer Disease and Related Disorders Work Group. Diagnosing AD using these criteria largely involves exclusion of other causes of dementia.[137] Moreover, according to these criteria, a diagnosis of AD cannot be made until the patient has dementia, which is defined as cognitive symptoms severe enough to interfere with social or occupational activities. The Diagnostic and Statistical Manual of Mental Disorders 4th Edition and International Classification of Diseases 10 criteria for AD, which are both used in the routine diagnosis of this disorder in the clinic, also require that a patient has dementia before a diagnosis of AD can be made.[138,139] Should disease-modifying drugs become available for AD, these criteria will all hinder patients in the early stages of disease from receiving effective therapy.

New research criteria for AD have been constructed to allow a diagnosis of AD to be made in the early stages of the disease. These criteria are centered on the clinical identification of episodic memory impairment alongside the detection of one or more abnormal biomarkers, including MRI, PET, and CSF biomarkers (Box 2).[140] More detailed guidelines are needed on how biomarkers can be implemented in the diagnostic procedure for early AD in the clinic. Such guidelines should provide details of the scales to be used in measuring memory impairment, the assays and cut-offs to be employed for CSF biomarkers, the brain regions (whole brain, hippocampus or entorhinal cortex) to be evaluated by MRI for atrophy, and the amyloid ligands to be used and brain regions to be evaluated by PET. Studies relating to these issues are only just emerging.[141]

Assays for measuring tau and Aβ in CSF have been well validated[43,142,143] and single-center studies in which samples have been assayed simultaneously using the same batches show that the biological variability for these biomarkers is low.[118,119] Nevertheless, the levels of these biomarkers measured in patients have varied in reports from different research centers, and even between studies that have used the same assay.[44,144] This variation in CSF biomarker levels between laboratories complicates multicenter research studies and trials, and also precludes the introduction of generally applicable cut-off levels.

The variation in CSF biomarker levels between centers is probably the result of variations in clinical procedures—such as the protocols for lumbar puncture, CSF sample processing and other laboratory practices—as well as batch-to-batch variation in the biomarker assays. These types of variation are well known in clinical chemistry, and are routinely controlled by external control programs. Thus, the Clinical Neurochemistry Laboratory in Gothenburg (Sweden), in conjunction with the Alzheimer's Association, has initiated a quality control program for CSF biomarkers (Box 3). This program includes standardization of the procedures for lumbar puncture, CSF processing and CSF analysis. Standardized protocols should minimize variation caused by differences in pre-analytical and laboratory procedures and, thus, allow direct comparisons of biomarker levels between laboratories and between publications. To overcome batch-to-batch variation in CSF biomarker assays, biomarker kit vendors will need to implement new standards for quality control. Assays should exhibit low overall variability in calibration curves and strict limits of variability across batches. To achieve these goals, stringent quality control of critical reagents, including antibodies and calibrators, is needed. In the long term, the aim is that the quality control program will serve as the basis for a more general introduction of CSF biomarkers into routine clinical practice and multicenter clinical trials.

Conclusions

Numerous studies have shown that combined analysis of the core CSF biomarkers $A\beta_{1-42}$, t-tau and p-tau can be used to reliably diagnose patients with AD and identify prodromal AD in cases of MCI. In addition, these biomarkers fulfill the criteria for an ideal AD diagnostic biomarker outlined by the Ronald and Nancy Reagan Research Institute–National Institute on Aging Working Group (Supplementary Table 2 online).[14] Basic CSF biomarkers might also serve as tools for identifying patients with pure AD and to exclude other disorders (Table 1). Thus, CSF biomarkers might be useful in a routine clinical diagnostic setting; however, the low positive predictive value of the combined three-marker test in asymptomatic populations suggests that such markers might be of limited use in screening individuals for AD before cognitive deficits appear.

CSF biomarkers might serve as valuable tools in drug development. CSF $A\beta_{1-42}$, t-tau and p-tau are being increasingly implemented as diagnostic markers in clinical trials to enrich the number of AD cases, while basic CSF biomarkers are used as safety markers. Finally, small-scale trials using CSF biomarkers will be valuable for providing biochemical data that a candidate drug affects AD pathogenesis. Such data will be vital for deciding whether large and expensive phase 2 and 3 trials should go ahead.

Review criteria

We searched PubMed for English language articles on biomarkers for Alzheimer disease using the following keywords: "Alzheimer", "biomarker", "cerebrospinal fluid", "CSF", "diagnosis", "plasma", "serum", "amyloid", "tau", "treatment" and "therapy". We also conducted searches using several keywords relevant to each section. In addition, we identified papers from references in the articles retrieved by the initial searches, and re-read selected articles from our own archives.

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