

Alzheimer's Disease

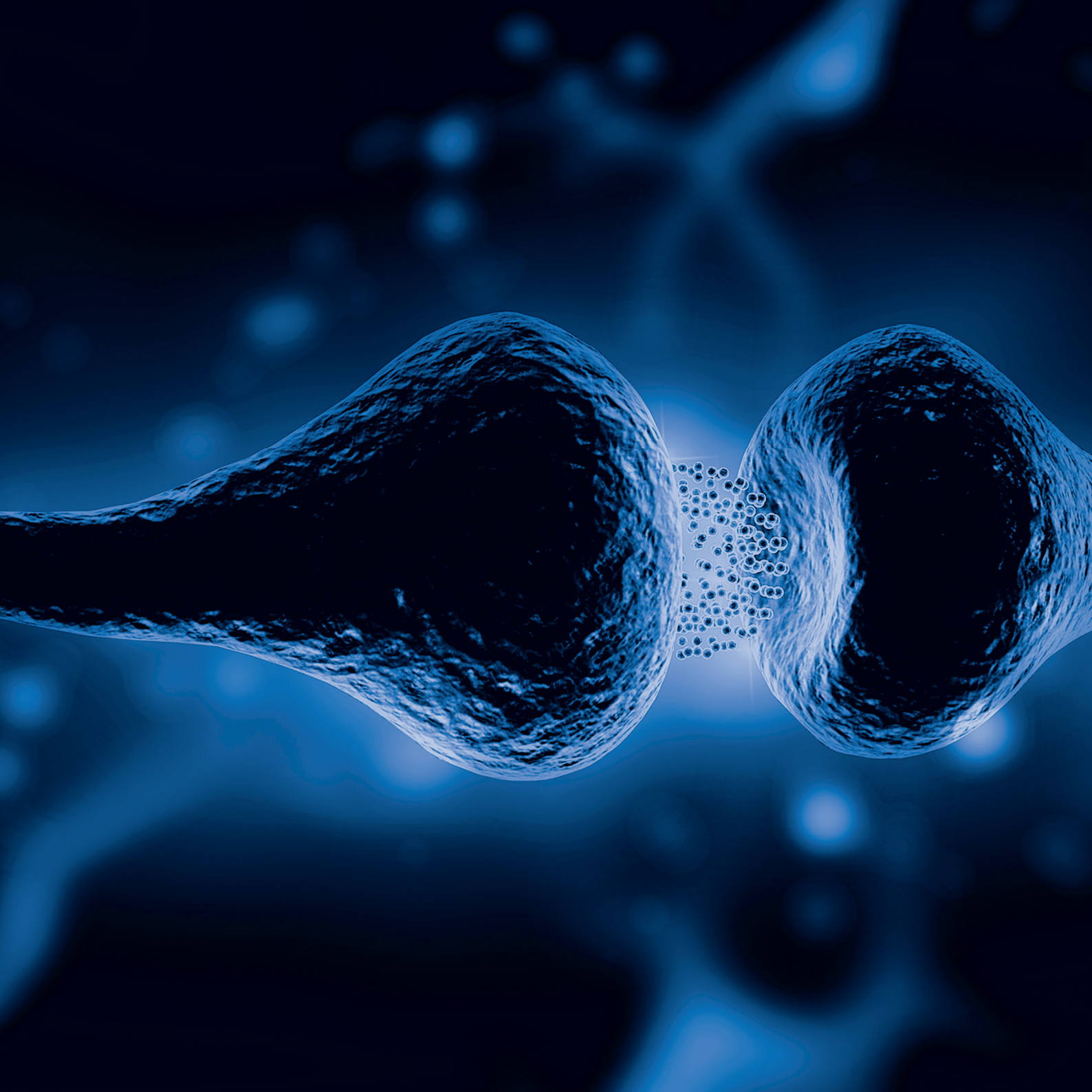
Fujirebio - The Pioneer in Alzheimer Biomarkers

A few drops of insight can lead to
an ocean of understanding



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Why is early detection important?

Early diagnosis is crucial

Alzheimer's disease (AD) is a continuous process leading to an alteration of cognitive and behavioral functions. It is essential to consult a doctor as soon as the first signs appear. By then, the disease might already have progressed insidiously for many years.

A clear diagnosis will allow patients' relatives and closest social network to better understand the symptoms that occur and to anticipate the future in consultation with the patient.

An early detection of the disease allows for quicker action and for saving precious time; a precise diagnosis to prevent complications and rapid worsening!

- **A precise diagnosis** helps choosing the most optimal management of the patient: all dementia disorders do not evolve in the same way. The coexistence of different causes that can affect cognitive abilities is frequent, a good differential diagnosis is essential.
- **General prevention:** stimulate the cognitive reserve by playing on cerebral plasticity, namely the capacity of the brain to establish new connections when some are destroyed by the disease.
 - » Stimulating this reserve involves regular physical activity, the fight against hypertension and the adoption of a diet to avoid the risk of diabetes.
 - » Keeping up intellectual activities and social interactions is essential.

- **Secondary prevention:** protecting the patient from complications.
 - » Falls and surgical operations: the decrease in cerebral functional reserves due to the effects of aging and the impact of diseases, particularly dementias, is exposed when the elderly suffers from surgical stress. Considering local anaesthesia where feasible reduces the risk of acute post-operative confusion syndrome or cognitive dysfunction by 50%. Many demented states attributed to surgery are in fact cerebral neurodegenerative diseases revealed at the time of hospital stay while they evolved until then gradually without attracting the attention of family or physicians.
 - » Medication control: avoid mistakes in taking medication, but also vigilance on what is actually prescribed is warranted! Many medical treatments commonly used by elderly are associated with a risk of cognitive decline.

As a conclusion, early diagnosis is associated with numerous benefits regardless of the availability of drug treatments for Alzheimer's disease.

Alzheimer-related biomarkers

- › Amyloid peptides
- › Tau protein
- › Hyperphosphorylated Tau protein

Lewy-related biomarkers

- › Alpha-synuclein

Frontotemporal dementia associated proteins

- › TAR DNA-binding protein of 43kDa (TDP-43)
- › FET/FUS proteinopathies

Prion protein associated deposits

- › Pathological prion protein (PrPSc)

Additional biomarkers of any cause of neuronal damage or injury

- › Neurofilaments
- › S100B protein
- › NSE enzyme
- › sTREM2 fragment

Bibliography

Section One

Altered proteins in brain neurodegenerative diseases

A biomarker is defined here as a characteristic that is **objectively measured in body fluids** with a level of evidence sufficient to be used in in-vitro diagnostic procedures, i.e. evaluate disease risk, guide clinical diagnosis, monitor therapeutic interventions.

In neurodegenerative diseases, the biomarkers derived from the cerebrospinal fluid (CSF) has been intensely studied. The **CSF biomarkers** reflect molecular events in the brain due to it being in direct contact with the extracellular space of the brain.

Nevertheless, CSF movement is not a unidirectional flow and there is no evidence that the pathological proteins can be found in the extracellular environment. The search for biomarkers for neurodegenerative diseases (NDD) is a challenge because most brain proteins are extensively modified post translationally.

Alzheimer-related biomarkers

Protein markers have been developed that reflect the central pathogenic processes in Alzheimer's pathology, i.e., the disturbance in the metabolism of β -amyloid ($A\beta$) and its subsequent deposition in senile plaques, the hyperphosphorylation of tau protein with subsequent formation of tangles (phosphorylated tau, P-tau) and the neuronal degeneration (total tau, T-tau).

AMYLOID PEPTIDES

The main component of plaques is a peptide called β -amyloid ($A\beta$), which is a cleavage-metabolite of the amyloid precursor protein (APP). APP is a single-transmembrane protein with the $A\beta$ domain partly embedded in the membrane. $A\beta$ is generated by cleavage of APP by two proteases, the β - and γ -secretases. Free $A\beta$ is secreted into the CSF.

There are two major C-terminal variants of $A\beta$, a shorter form ending at amino acid 40 ($A\beta_{40}$) and a longer form ending at amino acid 42 ($A\beta_{42}$). The $A\beta_{42}$ isoform has a high tendency for aggregation and is also the earliest $A\beta$ species deposited into plaques.

TAU PROTEIN

Tau is a normal protein located in the neuronal axons in the brain. Its function is to stabilize the microtubular network in the axons, by binding to the microtubules. There are six different isoforms of tau, depending on which exons of the tau gene are translated to the mature tau protein. There are also numerous phosphorylation sites, i.e. amino acids that can be phosphorylated on the Tau protein.

The CSF level of T-tau reflects the intensity of the neuronal and axonal degeneration and damage in the brain.

HYPERPHOSPHORYLATED TAU PROTEIN

In AD, a phosphate group is attached to several amino acids in tau protein, and tau is thus found in variants with different degrees of phosphorylation. Phosphorylated tau has a reduced ability to bind to the microtubules in the axons, which affect the axonal stability and thus the neuronal function, and also render tau an increased tendency for aggregation into paired helical filaments which then form the larger protein aggregates that make up the tangles.

Lewy-related biomarkers

ALPHA-SYNUCLEIN

Synucleinopathies are characterized by intra-neuronal aggregates consisting mainly of α -synuclein (α -syn) are found in Lewy bodies and Lewy neurites in Parkinson's disease (PD), Parkinson's disease dementia (PDD) and dementia with Lewy bodies (DLB) and in glial cytoplasmic inclusions in multiple system atrophy (MSA). This extracellular form of α -syn seems to be secreted from neuronal cells by exocytosis and detected in CSF as phosphorylated oligomeric α -syn.

Frontotemporal dementia associated biomarkers

The various FTD spectrum disorders are associated with brain accumulation of different proteins: tau, the transactive response DNA binding protein of 43 kDa (TDP43), or fused in sarcoma (FUS) protein, Ewing sarcoma protein and TATA-binding protein-associated factor 15 (TAF15) (the latter three are collectively known as FET proteins).

TAR DNA-BINDING PROTEIN OF 43KDA (TDP-43)

TDP-43 is a major component of ubiquitin-positive inclusions that are one of the neuropathological hallmarks in amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). Only 50% of FTLD patients have aggregates positive for TDP-43. Unfortunately, TDP-43 in CSF originates mainly from blood. Measurements of TDP-43 in CSF and blood are of minor importance as a diagnostic tool but may be important for monitoring therapy effects of TDP-43 modifying drugs in the future.

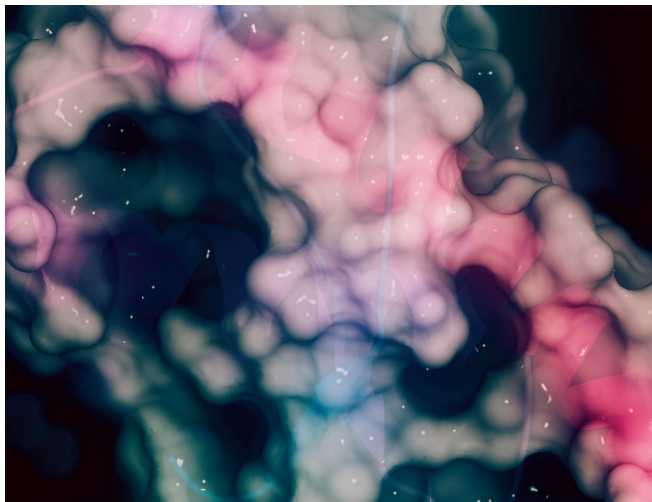
FET/FUS PROTEINOPATHIES

Recent reports identified mutations causative of neurological disorders in the genes encoding a family of RNA-binding proteins named FET. RNA-binding proteins are involved at all stages of RNA metabolism in neurodegenerative diseases. FET proteins are highly conserved and ubiquitously expressed. Recently, the involvement of FET proteins in neurological diseases, such as frontotemporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis (ALS), has been suggested, where they have been found in cytoplasmic aggregates. Abnormal co-accumulation of FET proteins into pathological inclusions has been described in all subtypes of FTLD-FUS. FUS is the most examined protein and is localized in dendritic granules and neuronal spines where it plays a role in mRNA transport to dendrites, which represents an essential process for local protein synthesis and synaptic plasticity. No CSF FET-related markers are available.

Prion protein associated deposits

PATHOLOGICAL PRION PROTEIN (PrP^{Sc})

Human prion diseases are rapidly progressive neurodegenerative disorders caused by prion protein misfolding. The sporadic Creutzfeldt-Jakob disease (sCJD) is the most common form (85–90% of cases), followed by genetic CJD (gCJD) and fatal familial insomnia (FFI) (10–15% of cases), which are linked to point or insertion mutations in the prion protein gene *PRNP*. Several molecular subtypes of sCJD have been identified. Electroencephalogram and CSF biomarkers have been reported to support clinical diagnosis but with variable utility according to subtype (updated WHO criteria for the diagnosis of CJD and related disorders, 2009). The principle for detecting PrP^{Sc} is to exploit the ability of small amounts of CSF PrP^{Sc} to convert native PrP into PrP^{Sc} in a newly described protein aggregation assay known as real-time quaking-induced conversion (RT-QuIC). This technique using a recombinant PrP showed good diagnostic sensitivity (82–96%) and virtually 100% specificity. Prion diseases may trigger biochemical changes similar to AD involving PrP^{Sc}, A β ₄₂, *APOE4* and abnormal tau. Autopsied brains of sCJD patients also showed AD-like changes (17% of cases). Testing of 14-3-3 protein in CSF is a standard biomarker test in suspected sCJD diagnosis by established Western blot method in CJD reference laboratories. Blood-contaminated samples which may result in artificially elevated CSF levels of 14-3-3.



Additional biomarkers of any cause of neuronal damage or injury

NEUROFILAMENTS AS MARKER OF AXONAL INJURY

Neurofilaments are intracellular intermediate filaments found in the central and peripheral nervous systems. In neurons, they control axonal diameter, which is correlated with nerve conduction velocity. Neurofilament protein include three subunits: neurofilament light (NfL) chain of ~68 kDa, Nf medium chain of ~150 kDa, and Nf heavy chain of ~190–210 kDa. After axonal injury, intracellular neurofilaments can leak into the extracellular space, leading to an increased concentration in the CSF. Comparable performance of NfL in blood and CSF demonstrates its promise as a noninvasive biomarker of neurodegeneration.

ASTROGLIAL BIOMARKER

S100B protein is an astroglial 11 kDa calcium-binding protein. In the classic neurodegenerative disorders (AD, PD, ALS) S100B concentration in CSF usually reflects the severity of the pathological condition, whereas, in many cases, S100B levels in blood remains unchanged during the course of the disease. However, serum S100B is valuable in the assessment of mild head injuries.

NEURONAL DAMAGE BIOMARKER

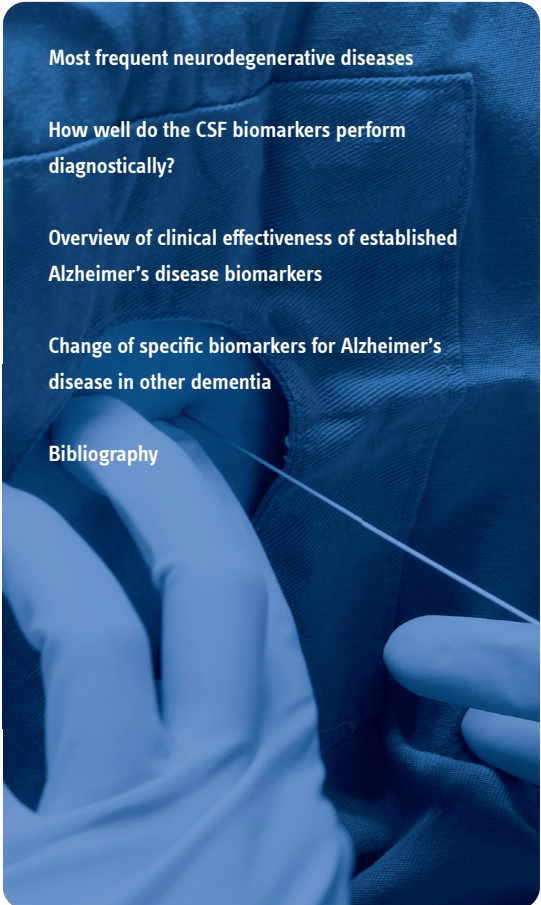
Neuron specific enolase (NSE) is a glycolytic isoenzyme located in central and peripheral neurons and neuroendocrine cells. The measurement of NSE in CSF could be a sensitive index of neuronal damage. NSE is considered a biomarker of neuronal stress and has prognostic potential for a variety of neurological disorders. Serum NSE levels are significantly elevated in patients with unfavorable neurological outcome in a variety of conditions.

INFLAMMATION IN NEURODEGENERATION

Triggering receptor expressed on myeloid cells 2 (TREM2) is a transmembrane protein that is specifically expressed on microglia in the brain. TREM2 is one of the most crucial factors in regulating the innate immune system during AD progression. Soluble TREM2 (sTREM2) is the ectodomain released in a soluble form. sTREM2 is described to be a central regulator of microglial function and CSF sTREM2 is known to increase 5 years before the expected symptom onset in AD.

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Most frequent neurodegenerative diseases

How well do the CSF biomarkers perform
diagnostically?

Overview of clinical effectiveness of established
Alzheimer's disease biomarkers

Change of specific biomarkers for Alzheimer's
disease in other dementia

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Section Two

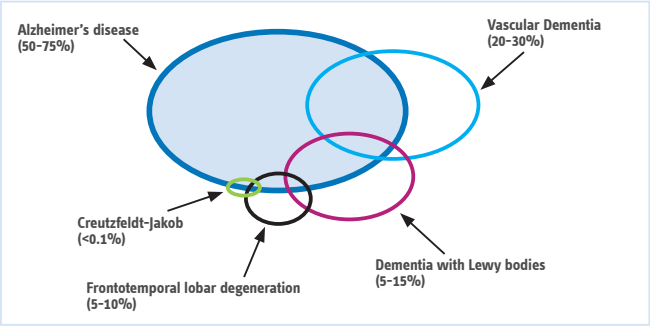
Clinical features and neurodegenerative biomarker changes in CSF

The **cerebrospinal fluid (CSF)** is the optimal source for biomarkers to establish ante-mortem a link between clinical features and underlying pathologic features.

Brain neurodegenerative diseases (NDD) are commonly classified by distinct clinical presentations, e.g., impairment in cognitive functioning involving anatomical regions showing neuronal dysfunction and loss. Most of these diseases follow a deteriorating course to dementia. Fifty to seventy-five percent of dementia are due to Alzheimer's pathology.

Most cases of disease are sporadic, but some are inherited in a dominant manner. In these cases, the overexpression of mutant proteins rises to disease-associated phenotypes with early occurrence of disease often.

Most frequent neurodegenerative dementias



For some diseases, only the criteria referring to the clinical phenotype are used for subtyping, whereas for others biochemical modifications or gene polymorphisms could also to be considered. The classification gains in confidence based on positive evidence of the presence of pathological protein deposits in brain.

The accumulation of brain pathologies seems to be a nearly inevitable consequence of aging; there is frequently an overlap of concomitant pathologies.

How well do the CSF biomarkers perform diagnostically?

Although a multitude of CSF biomarkers for specific pathologic changes and nonspecific markers of oxidative damage or inflammation involved in neurodegenerative diseases were studied, only three core biomarkers are validated for a differential diagnosis of AD, i.e. $A\beta_{1-42}$ peptide ($A\beta_{42}$), total tau (T-tau), and its phosphorylated form (P-tau) measured *in vitro* using CSF specimens.

CSF α -syn is currently studied for its possible value as a PD biomarker and in the differential diagnosis of NDD, but not validated. Current commercial assays detecting total α -syn levels seem not able to distinguish Lewy body disorders from other neurodegenerative disorders.

The detection of CSF PrPSc is performed only in reference laboratories for prion diseases with the nonspecific 14-3-3 protein. For now, only results of CSF $A\beta_{42}$ (or CSF $A\beta_{42/40}$ ratio), T-tau and P-tau can be used in routine clinical setting.

Overview of clinical effectiveness of established Alzheimer's disease biomarkers

Biomarkers	Pathological specificity	Early diagnostic sensitivity	Correlation with disease progression
Neuropsychological testing	+	++	++
$A\beta_{1-42}$	++	+++	+
T-tau	+	++	++
P-tau	+++	++	++
Magnetic resonance imaging	+	++	+++
Positron emission tomography FDG uptake	+	++	+++
Minimum (+), moderate (++), maximum (+++)			

The concomitant pathologies in elderly individuals lead to an artificial clinical subgrouping driven by the dominant clinical phenotype. The common age-associated brain pathologies are amyloid plaques, tangles, ischemic cerebrovascular disease but also microinfarctions, hippocampal sclerosis, α -syn deposits (Lewy bodies), TDP43 inclusions, and argyrophilic grains.

A clinical phenotype (typical, atypical or unclear) and the CSF core biomarkers reflecting the dynamic changes of protein metabolism in the brain help in differentiating pathological neurodegenerative decline from normal aging.

Change of specific biomarkers for Alzheimer's disease in other dementias

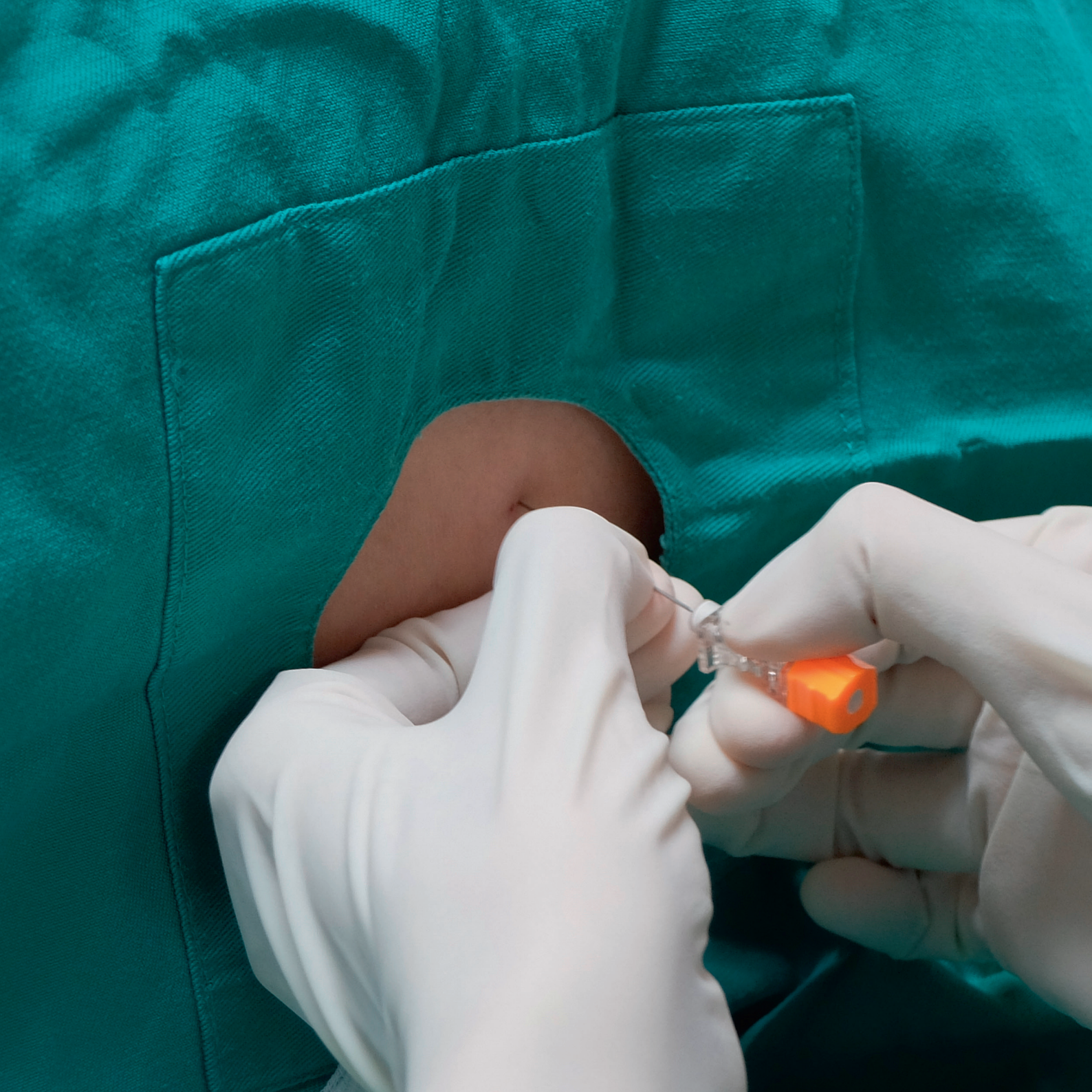
Disorder	Total tau	Phosphorylated tau	β -amyloid ($A\beta_{42}$)
Alzheimer's disease with dementia	Mild to moderate increase	Mild to moderate increase	Mild to moderate decrease
Mild cognitive impairment with incipient AD	Mild to moderate increase	Mild to moderate increase	Mild to moderate decrease
Stable MCI without progression	Normal	Normal	Normal
Normal aging	Normal	Normal	Normal
Depression	Normal	Normal	Normal
Parkinson's disease	Normal	Normal	Normal
Alcohol dementia	Normal	Normal	Normal
Other neurological disorder such as PD, PSP and ALS	Normal	Normal	Normal
Non-acute cerebrovascular disease	Normal	Normal	Normal
Creutzfeldt-Jakob disease	Very marked increase	Normal or mild increase	Normal or mild decrease
Frontotemporal dementia	Normal or mild increase	Normal	Normal or mild decrease
Dementia with Lewy bodies	Normal or mild increase	Normal	Mild to moderate decrease
Vascular dementia	Normal or mild increase	Normal	Normal or mild decrease

AD = Alzheimer's disease; MCI = mild cognitive impairment; PD = Parkinson's disease; PSP = progressive supranuclear palsy; ALS = amyotrophic lateral sclerosis

Source: K. Blennow, Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Sweden

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Recommended procedure

› Illustration

Bibliography

Section Three

Handling and transportation of CSF samples

Cerebrospinal fluid (CSF) can be collected in the lumbar region by an experienced physician. Taking into account the necessary precautions (e.g, sterile environment) and other medical investigations (e.g, brain imaging), the lumbar puncture is generally accepted by the medical society as a safe procedure.

Adsorption to plastic polymers can alter the biomarker concentrations and the extent of this adsorption differs between tubes. Therefore, always use the same polypropylene reference tube (within one lab).

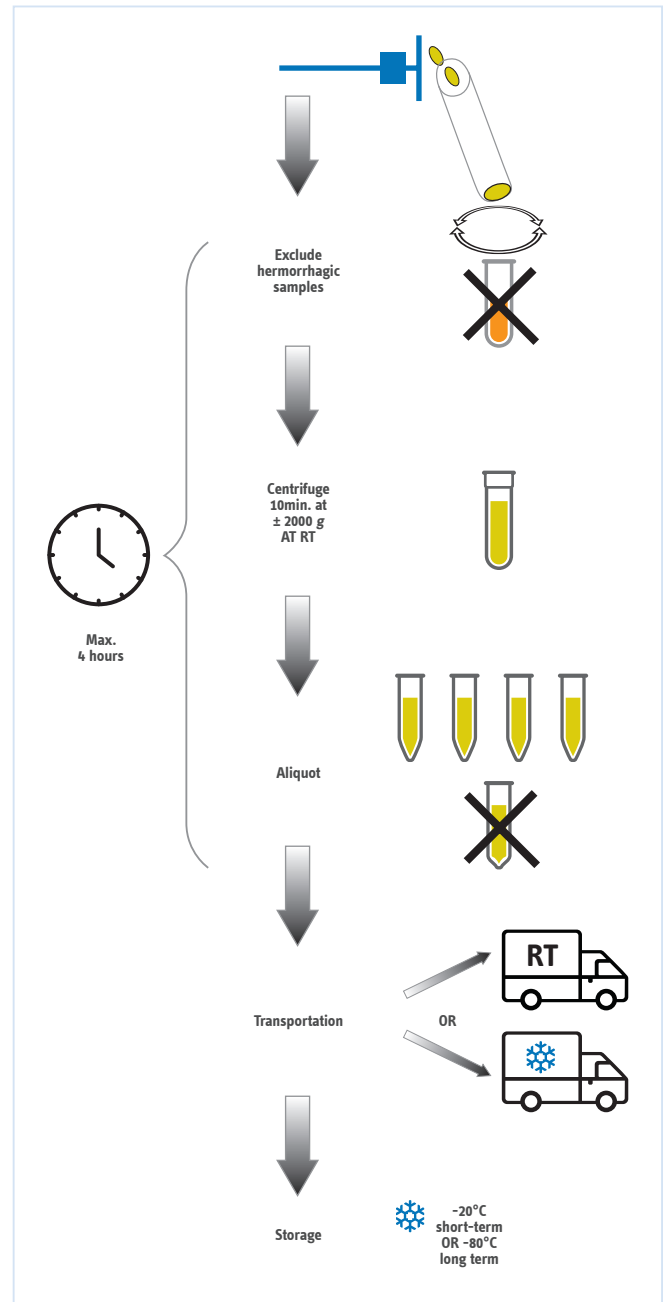
Recommended procedure

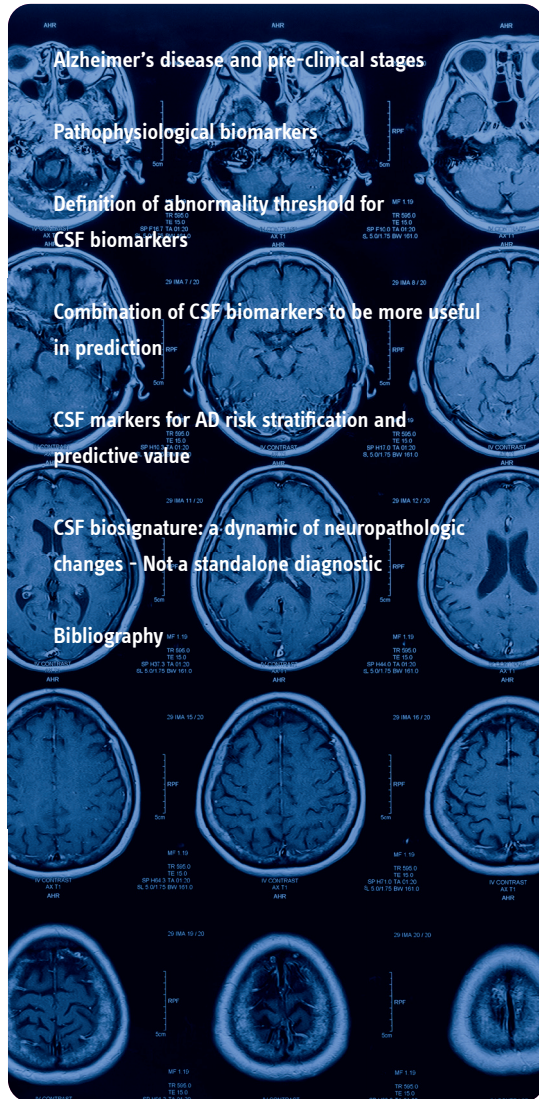
1. The CSF sample must be sent to the local laboratory without delay.
 - » A CSF cell count is usually performed
 - » The CSF sample is centrifuged in the original tube
2. The CSF sample is aliquoted for basic and specific neurodegenerative disease biomarkers
 - » CSF should be aliquoted in tubes made of polypropylene
 - » The volume needed for CSF analyses may vary between laboratories
 - » The volume needed for Alzheimer specific biomarkers is between 0.5 mL and 1.0 mL
3. Transportation: CSF samples can be sent by ordinary mail, at room temperature if the shipping time is less than two days. If the CSF sample is taken on a Friday, it can be frozen and sent to the central laboratory on dry ice the next week.
4. Handling of CSF samples before analyses of AD biomarkers
 - » It is recommended to freeze CSF samples if the analysis cannot be performed within 48 hours. Samples sent frozen should be kept frozen until analysis.
 - » CSF samples sent at room temperature should be frozen, and CSF samples sent frozen should be kept frozen.

Sample analysis on fresh samples upon arrival may result in slightly different CSF biomarker values compared to frozen samples but is unlikely to result in a different CSF biomarker profile.

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Section Four

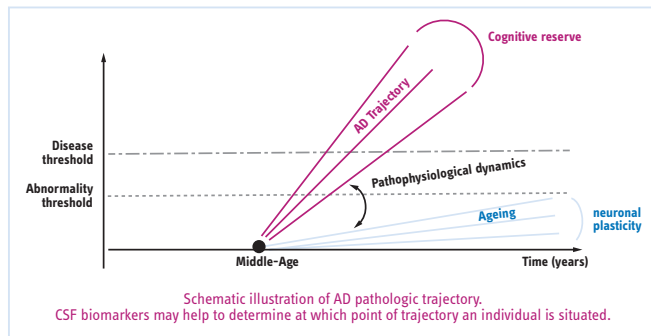
CSF biochemical pattern interpretation

Alzheimer's disease and pre-clinical stages

Alzheimer's disease is the most common neurodegenerative disease and demonstrates exponential increase in prevalence with advancing age beyond 60 years. There are three stages usually described: latent stage when the disease has started but is asymptomatic, prodromal stage when the disease has progressed and very mild clinical signs and symptoms are present, and the clinical stage when the disease has advanced and the full clinical spectrum is expressed.

It is critical to realize that latent disease cannot be distinguished from absence of disease by clinical examination or neuropsychological testing, but rather requires some ensemble of laboratory-based methods to detect disease initiation in the absence of symptoms.

How fast the patient will progress in the disease depends on risk-enhancing factors, such as age, modifying genes, cognitive reserve, comorbidities, and so forth.



Pathophysiological biomarkers

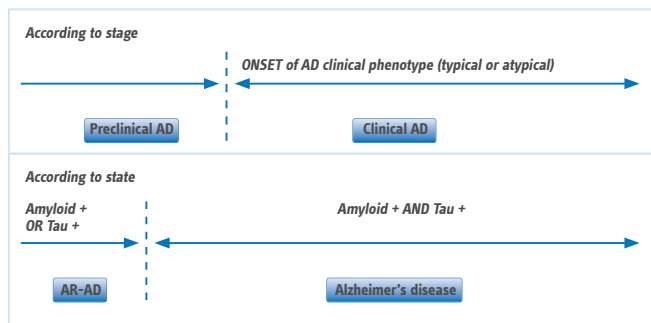
Individuals can now be identified as being in the preclinical state by the *in vivo* evidence of Alzheimer pathology, by a biological or molecular “signature” of AD.

CSF $A\beta_{42}$ and amyloid PET are highly concordant when used to dichotomize individuals as amyloid positive or amyloid-negative, showing 80%–90% agreement across studies. The CSF $A\beta_{42}/A\beta_{40}$ ratio typically shows agreement with PET above 90%.

A Tau PET ligand is not routinely available, CSF T-tau and P-tau are the easiest tools for evidence of tauopathy. Alternatively, topographical markers include volume changes in the brain (hippocampal atrophy, cortical thickness) assessed by MRI and hypometabolism of neocortical regions measured by fluorodeoxyglucose (FDG)-PET.

Optimal and reliable blood-based biomarkers are not yet ready for clinical application.

Only the association of both pathologic hallmarks defines AD even in the absence of cognitive symptoms.



Definition of abnormality threshold for CSF biomarkers

The threshold for “abnormality” for CSF biomarkers is difficult to assess, especially in clinically healthy elderly subjects. There are several approaches to define what is abnormal.

- abnormality may be defined based on comparison between cognitively normal (having a CSF collection for any other causes than NDD) and AD groups.
- abnormality can be defined based on the distribution of values within a cognitively normal population, where subjects with values exceeding, for example, 2 standard deviations below or above the mean can be considered “abnormal”.
- abnormality can be defined based on longitudinal observation of clinical progression in a group starting as healthy and declining to AD at follow-up evaluations.

In healthy control subjects, the cortical uptake of $A\beta$ agents is low in comparison with patients suffering from prodromal AD of the hippocampal type/MCI-due-to-AD(*) or fully developed AD dementia. However, a significant proportion of cognitively healthy elderly show increased cortical $A\beta$ binding and decreased CSF $A\beta_{42}$. This finding is supported by postmortem histopathological data showing $A\beta$ plaques upwards of 30% of the non-demented elderly population above 75 years of age, likely representing preclinical AD. (*) MCI-due-to-AD, *Mild cognitive impairment due to Alzheimer etiology*.

CSF T-tau levels increase with age and are higher in apolipoprotein E (*APOE*) carriers. The *APOE* polymorphism is the most widely accepted genetic factor increasing the risk for sporadic AD. *APOE* $\epsilon 4$ carriers might be predisposed to vascular diseases which in turn could contribute to age-related brain damage and therefore to elevated T-tau levels.

In conclusion, a substantial number of healthy subjects over age 60 (25-40%) has at least one CSF biomarker concentration in range that can be considered abnormal. To minimize the age-related risk factor, “normality” may be defined using results from clinically and cognitively normal individuals below the age of 50.

Remark: Commercial assays for the measurement of CSF biomarkers bearing the CE mark for in vitro diagnostics propose an estimated range of normal values for specific populations.

¶ From Dubois B, et al. *Alzheimers Dement* (2016)
Abbreviations: AD, Alzheimer's disease; AR-AD, at risk for AD

Combination of CSF biomarkers to be more useful in prediction

CSF T-tau, P-tau, and Aβ₄₂ are valuable as biomarkers of AD. At present, their strength lies mostly in their ability to support neurodegenerative etiology criteria for MCI and AD, and their reasonable capacity to predict the conversion from MCI to AD. A combination of biomarkers seems to be more useful in prediction than a single analyte.

The below table published by Mattsson N, *et al.* (2012) summarizes the specificities and likelihood ratios at cutoffs for 85% sensitivity for AD dementia according to age categories. The specificity of CSF biomarkers decreases with age, as an effect of the high AD prevalence in older ages, but the likelihood ratios are improved when CSF biomarkers are combined.

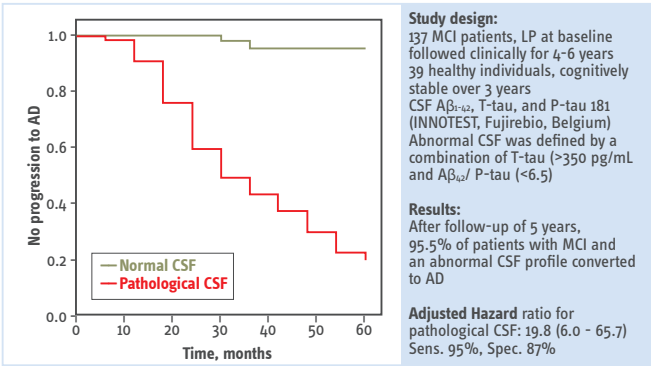
Biomarker and age group (years)	Cross-sectional cohort (AD dementia and controls)			Longitudinal cohort (MCI)		
	Specificity (controls)	LR+	LR-	Specificity (sMCI/MCI-other)	LR+	LR-
Aβ ₄₂						
≤64	82	4.9	0.13	77	3.2	0.33
65-74	82	4.6	0.18	56	1.9	0.28
≥75	73	3.2	0.19	60	2.1	0.29
T-tau						
≤64	74	3.3	0.20	65	2.3	0.29
65-74	53	1.8	0.28	49	1.8	0.19
≥75	61	2.2	0.24	46	1.5	0.38
P-tau						
≤64	67	2.6	0.22	55	1.6	0.48
65-74	46	1.6	0.31	44	1.6	0.23
≥75	37	1.4	0.40	30	1.2	0.54
Combination						
≤64	95	18	0.14	86	5.5	0.27
65-74	83	5.1	0.16	65	2.4	0.21
≥75	80	4.3	0.16	61	2.1	0.29

Abbreviations: AD = Alzheimer’s disease; LR = Likelihood ratio; MCI = Mild cognitive impairment; sMCI = Stable mild cognitive impairment. All measurements are calculated at the 85% sensitivity cutoff value for AD dementia.

The CSF biomarkers in combination, e.g., low CSF Aβ₄₂ peptide with high T-tau and P-tau, are sensitive and specific biomarkers highly predictive of progression to AD dementia in patients with MCI and of presence of AD etiology even in older populations.

CSF markers for AD risk stratification and predictive value

Model 1: AD risk adapted from Hansson O, *et al.*, Lancet Neurol 2006



Association between CSF biomarkers and incipient AD – Monocentric longitudinal study by Hansson O, *et al.* (2006). The follow-up period was extended with 5-10 years and the results were published by Buchhave P, *et al.* (2012).

95.5 % of patients with MCI and abnormal CSF converted to AD.

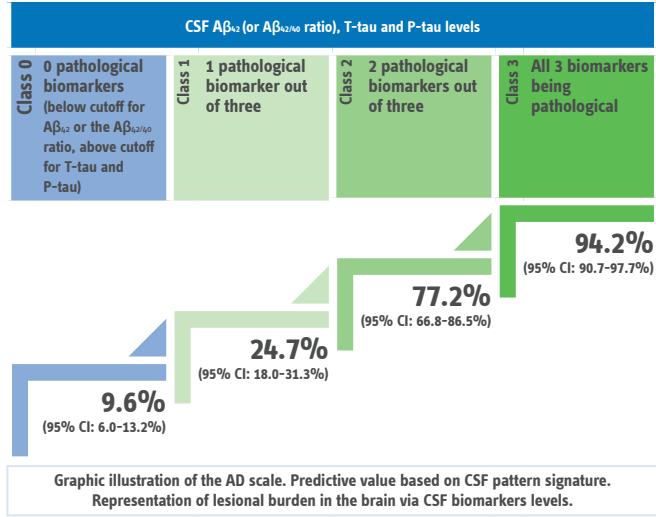
Model 2: AD risk adapted from Lewczuk P, *et al.* J Neural Transm. 2009; J Alzheimers Dis. 2015 ; Somers C, *et al.*, J Alzheimer Dis. 2019

Erlangen Score			
	$A\beta_{1-42}$ normal (+0)	$A\beta_{1-42}$ in border zone (+1)	$A\beta_{1-42}$ pathologic (+2)
T-tau/P-tau ₁₈₁ normal (+0)	0	1	2
T-tau/P-tau ₁₈₁ in border zone (+1)	1	2	3
T-tau/P-tau ₁₈₁ pathologic (+2)	2	3	4
Comprehensive interpretation and semi-quantitative presentation of all possible patterns of CSF AD biomarkers			

The Erlangen score was validated using two cohorts of pre-dementia subjects, the German Dementia Competence Network (n = 190 subjects with MCI) and the US Alzheimer’s Disease Neuroimaging Initiative 1 (n = 292 MCI or cognitively normal subjects). The erlangen score uses a risk-based approach.

The CSF results of a given patient are scored between 0 and 4 points. A CSF result with all biomarkers entirely normal is scored 0 points; a pattern with only marginal alterations in one biomarkers group (either A β or Tau, but not both) results in the score of 1; a CSF result with the alterations in either A β metabolism (decreased A β_{42} concentration and/ or decreased A $\beta_{42/40}$ ratio) or Tau metabolism (increased concentrations of T-tau and/or P-Tau) but not both is scored 2 points; a result with clear alterations in one biomarkers’ group (either A β or Tau) accompanied by marginal alterations in the other group is scored 3 points; clear alterations in both A β and T-tau/P-Tau result in 4 points.

Model 3: AD risk adapted from Lehmann S, *et al.* Alzheimers Res Ther. 2014; Front Aging Neurosci. 2018



The scale’s overall predictive value for AD for the different categories (n = 1,273 patients including 646 AD and 627 non-AD) from six independent memory-clinic cohorts.

AD risk assessment may integrate the A $\beta_{42/40}$ ratio (instead of A β_{42}) which accounts for interindividual difference in amyloidogenic APP-processing.

These simple scales using the presence of two or three pathologic biomarkers as a criterion of AD can be used to facilitate the interpretation of CSF pattern in routine.

Remark: The two last illustrations of risk scoring are cutoff values independent, meaning each laboratory can easily supplement it with the cutoff values and normal/abnormal ranges according to the analytical method used for biomarker measurement.

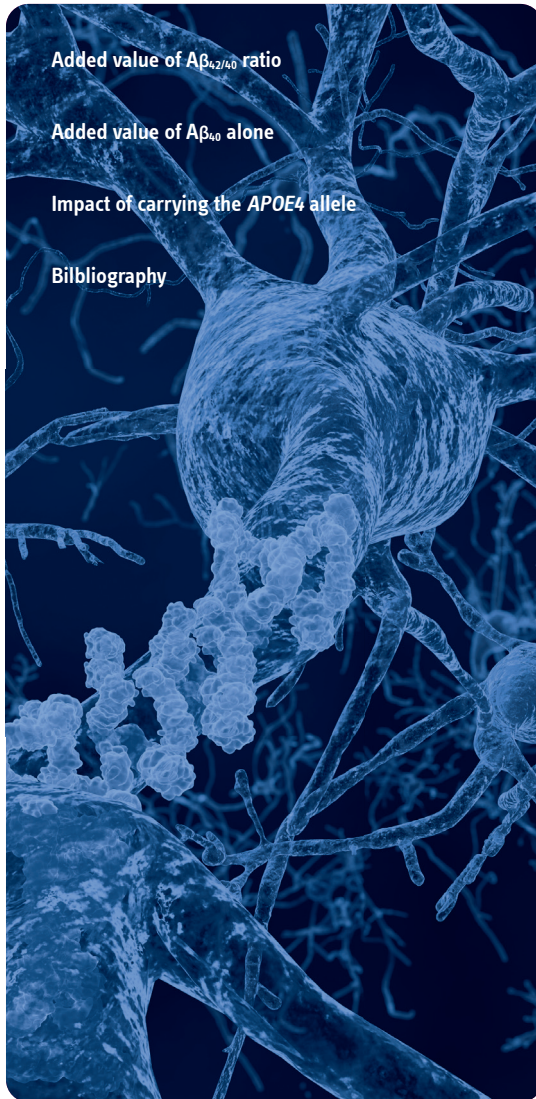
CSF biosignature: a dynamic of neuropathologic changes - Not a standalone diagnostic

The combination of CSF biomarkers permits a diagnosis of AD in earlier stages of the disease. Nevertheless, the clinical identification of cognitive impairment and the use of both structural (CT/MRI) and functional (SPECT/PET) brain imaging are necessary for an accurate differential diagnosis with other neurodegenerative diseases. Mixed pathology, especially in elderly subjects, is frequent.

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Section Five

$A\beta$ deposition and clearance: a key feature of ageing brain

$A\beta_{40}$ is the most abundant amyloid protein and less neurotoxic than $A\beta_{42}$, which is less abundant, highly insoluble, severely neurotoxic, and more aggregation-prone.

Amyloid peptides can be degraded by metalloproteinases, astrocytes, or macrophages, or are transported across the blood-brain barrier via low-density lipoprotein (LDL) receptor-related protein-1 and drained via the lymphatic system.

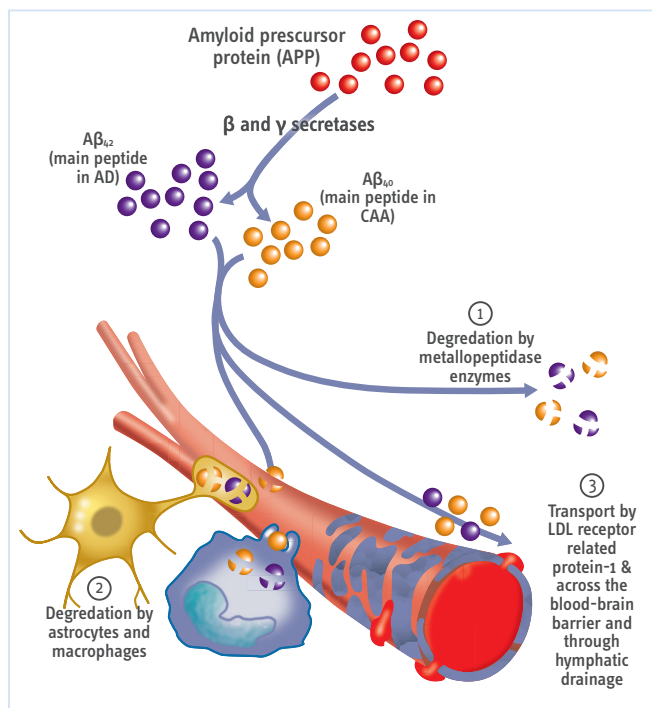
The pathogenic pathways of cerebral amyloid angiopathy (CAA) and AD intersect at the levels of $A\beta$ generation, its circulation within the interstitial fluid and perivascular drainage pathways and its brain clearance:

- $A\beta_{40}$: Primarily associated with vascular $A\beta$ deposition
- $A\beta_{42}$: Primarily associated with plaque $A\beta$ deposition

Vascular deposits also contain $A\beta_{42}$, but the proportion of $A\beta_{40}$ is higher than that found in plaques. Impairment of perivascular drainage induces a self-reinforcing cycle of $A\beta$ deposition, loss of vascular smooth muscle cells and vasoactivity, and further reduction in clearance altogether causing CAA.

The AD and CAA pathways seem to diverge with respect to how they cause tissue injury: AD pathology promotes neuronal and synaptic loss whereas CAA generates focal tissue lesions via haemorrhagic and ischaemic vascular brain injury.

CAA occurs in 85% to 95% of patients with AD, has a significant impact on vessel health, and is an important contributor to cerebrovascular pathology in AD.



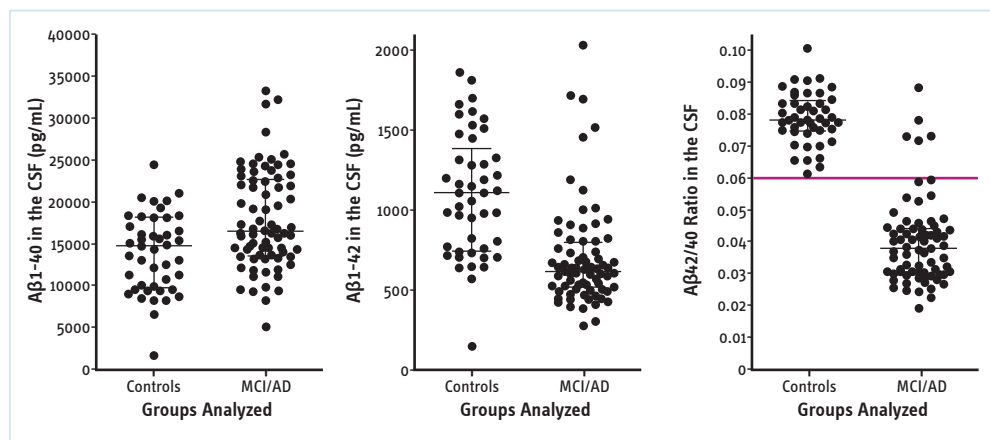
Source: DeSimone CV, *et al.*, J Am Coll Cardiol. 2017; 70(9): 1173–1182. © 2017 by the American College of Cardiology Foundation. Cerebral Amyloid Angiopathy - Mechanism of Production, Degradation, and Deposition of Aβ in Cortical Arteries.

Added value of Aβ_{42/40} ratio

Aβ peptide is produced from a transmembrane Aβ precursor protein, sequentially cleaved by β- and γ-secretase. Cleavage of APP by γ-secretase generates a number of Aβ isoforms. Aβ₄₂, a 42 amino acid-long peptide, has the highest propensity for aggregation and appears to be the predominant species in neuritic plaques. Although the concentration of Aβ₄₀, has been reported to be unaltered in AD, the Aβ_{42/40} ratio has been suggested to be superior to the concentration of Aβ₄₂ alone in discriminating patients with AD.

In a population of normal subjects and AD patients, the distribution of total Aβ (40 and 42) follows a Gaussian distribution for both normal subjects and AD patients, with Aβ₄₀ making up about 70% of total Aβ. Further although many cases fall into the middle of the distribution with the majority having normally total Aβ, outliers are still present. Some AD patients will have high total Aβ (enhanced amyloidogenic processing of APP, also called “high producers” and some cognitively normal subjects will have low total Aβ (reduced amyloidogenic) processing of APP, also called “low producers”.

This means that AD patients with a high total Aβ will show an incomplete CSF pattern and vice versa, normal subjects with low total Aβ will be classify as individual with sign of cerebral amyloidosis. In all three cases (normal, low and high total Aβ) the ratio can correctly classify some doubtful CSF pattern. The ratio led to a reduction by half of the number of indeterminate profiles without changing the conclusion when usual biomarkers (Aβ₄₂ and P-tau) were concordant.



There is a consensus to recognize that the Aβ_{42/40} ratio is helpful to:

- reflect interindividual difference in amyloidogenic-APP processing
- solve undetermined core biomarker profiles of AD
- decrease the impact of preanalytical and analytical sources of variability within and among centers

◄Lewczuk *et al.*, J Alzheimers Dis, 2015

Added value of A β_{40} alone

CAA diagnosis based on MRI findings alone is not clear. Amyloid imaging with amyloid-binding PET ligands can detect CAA, although they cannot discriminate vascular from parenchymal amyloid deposits. In addition, CSF markers may be useful, including levels of A β_{40} for CAA and anti-A β antibody for CAA-related inflammation (CAA-ri).

Recent findings indicate that the presence of one or more biomarkers plus one or more risk factors may be suggestive of CAA:

- Amyloid imaging with greater occipital uptake
- A decrease in CSF A β_{40} levels

Risk factors:

- General factors:
 - » Old age
 - » AD
- Genetic factors:
 - » CAA-related gene mutations in familial cases
 - » *APOE* in sporadic cases: $\epsilon 4$ as a risk factor for CAA

Impact of carrying the *APOE4* allele

The brain A β pathology is inarguably associated with *APOE* $\epsilon 4$ status. Carrying the $\epsilon 4$ allele of the *APOE* gene encoding apolipoprotein E (*APOE* $\epsilon 4$) markedly increases the risk for AD and CAA. *APOE* $\epsilon 4$ -mediated amyloid pathology depends on its neuronal LDL receptor-related protein 1 (LRP1). *APOE* $\epsilon 4$ decreases A β clearance without affecting A β production. According to the current concept, A β that accumulate in the brain in AD is likely due to its faulty clearance from the brain. LRP1 is a major efflux transporter for A β at the blood-brain barrier (BBB). Binding of A β to LRP1 at the abluminal side of the BBB initiates a rapid A β clearance from brain to blood via transcytosis across the BBB.

In summary, cognitive impairment in the ageing brain is typically driven by overlapping neurodegenerative and cerebrovascular pathologies. The impaired perivascular clearance of A β and the deficient neuronal LRP1 exacerbate the brain accumulation of A β peptides and subsequent deposition – the most likely cause of CAA and AD.

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AT(N)(C) system in Alzheimer's disease

- › AT(N) biomarker grouping
- › CSF (cerebrospinal fluid) versus imaging biomarkers
- › Definition of Alzheimer's continuum
- › Staging severity
- › Flexibility of the AT(N) system

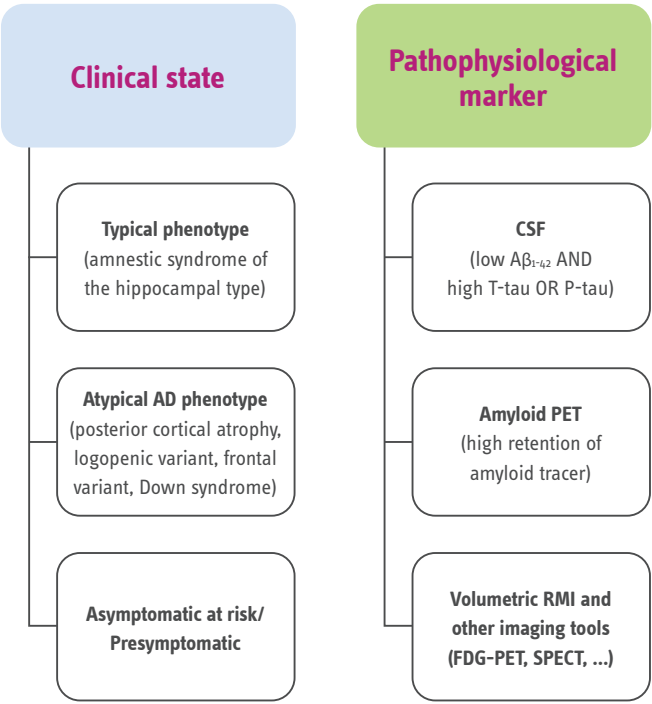
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Section Six

New criteria for Alzheimer's disease

New criteria for different stages of AD have been suggested by the International Working Group (IWG) and the National Institute on Aging-Alzheimer's Association (NIA-AA) (Dubois B, *et al.* 2007; Albert MS, *et al.* 2011; McKhann GM, *et al.* 2011; Sperling M, *et al.* 2011; Dubois B, *et al.* 2014; Dubois B, *et al.* 2021).

These criteria have been constructed to permit a diagnosis of AD in earlier stages of the disease and are centered on the clinical identification of cognitive impairment subtypes together with one or more abnormal biomarkers, including MRI, PET, and CSF markers.

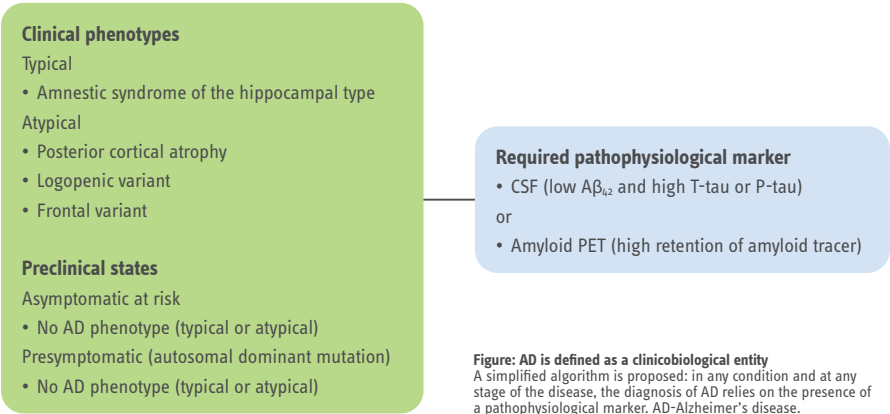


To increase diagnostic certainty, the criteria for AD incorporate biomarker evidence for pathology, which can be obtained by neuroimaging (MRI measures of atrophy, ¹⁸F-FDG PET measures of cerebral hypometabolism, and amyloid PET measures of β-amyloid deposition) and CSF testing (decreased β-amyloid concentrations and increased T-tau or P-tau concentrations).

Abnormality		Pathology
MRI		
Regional anatomy	Decreased volume of hippocampus and other temporal lobe structures	Tissue loss and neurodegeneration
PET		
¹⁸ F-FDG PET	Decreased uptake in posterior cingulate-precuneus and temporoparietal cortex	Glucose hypometabolism and neurodegeneration
¹¹ C-PiB and fluorinated tracers for amyloid PET*	Increased cortical retention	Deposition of β-amyloid in the cortex
CSF measures		
Aβ ₄₂ or Aβ _{42/40}	Decreased volume of hippocampus and other temporal lobe structures	Abnormal metabolism of β-amyloid
T-tau and P-tau	Increased concentration	Neuronal damage and accumulation of tau pathology; hyperphosphorylated tau is more specific for Alzheimer's disease neurodegeneration

Tau-PET is still under development and, therefore, is not included.
PiB=Pittsburgh compound. Aβ=fibrillar β-amyloid. FDG=fluorodeoxyglucose
*Using tracers such as florbetapir, flutemetamol, and florbetaben.

These criteria broaden the spectrum of the disease to include its preclinical states, in which Alzheimer's pathology exists without clinical symptoms.



AT(N)(C) system in Alzheimer's disease

More recently, the National Institute on Aging–Alzheimer's Association (NIA–AA) (Jack CR *et al.* 2018) has proposed to define exclusively AD by its underlying neuropathologic changes that can be documented by the biomarkers in living people. The biomarker profile (pathologic process) and cognitive staging represent independent sources of information. The definition and the grading of disease severity is assessed by the biological construct of AD across its entire spectrum as a continuum.

AT(N) BIOMARKER GROUPING

- A: Aggregated A β amyloid peptides or associated pathologic state (CSF A β_{42} , or A $\beta_{42/40}$ ratio; Amyloid PET)
- T: Aggregated tau (neurofibrillary tangles) or associated pathologic state (CSF phosphorylated tau; Tau PET)
- (N): Neurodegeneration or neuronal injury (Anatomic MRI; FDG PET; CSF total tau)

CSF VERSUS IMAGING BIOMARKERS

- the ongoing active pathologic state is denoted by CSF
- the accumulation of neuropathologic load and location in the brain is denoted by imaging

DEFINITION OF ALZHEIMER'S CONTINUUM

- A: Amyloid biomarkers (A β_{42} or A $\beta_{42/40}$ ratio for CSF) determine whether or not an individual is potentially in the Alzheimer's continuum.
- T: Pathologic tau biomarkers (P-tau for CSF) determine if someone who is in Alzheimer's continuum has Alzheimer's disease.

STAGING SEVERITY

- (N): Neurodegenerative/neuronal injury biomarkers (T-tau for CSF) provide powerful prediction of future cognitive decline.
- (C): Cognitive symptoms determine the syndromal categorical cognitive staging regardless the etiology.

Note: A and T are specific neuropathologic changes of AD, whereas (N) and (C) are not specific to AD and therefore placed in parentheses.

FLEXIBILITY OF THE AT(N) SYSTEM

- each biomarker group is labeled (-) normal, (+) abnormal or (*) not determined

E.g. **A+T+(N)+** to categorize AD-pathologic changes

- alternatively, to the binary approach, each biomarker group could be also labelled semi-quantitatively (0) clearly normal, (1) intermediate / marginally altered or (2) clearly abnormal.

E.g. **A⁺T⁺(N)⁺** categorize AD profile or **A⁺T⁺(N)⁰** if only Amyloid biomarker is clearly abnormal

- new biomarker groups beyond AT(N) can be added when they will become available.

These guidelines would permit an important step toward harmonization of interpretation of biomarkers in a personalized medicine.

- A combination of an abnormal A β and a pathologic tau biomarker constitutes AD regardless of cognitive symptoms, and thus AD is a biologically defined entity.
- The staging of the severity of cognitive symptoms is independent of the underlined pathology. The syndromal categorical scheme preserves the tree clinical categories: cognitively unimpaired, mild cognitive impairment and dementia.

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Section Seven

Future vision on blood markers

Whilst the **cerebrospinal fluid (CSF)** continuously exchanges with the brain extracellular fluid, only a fraction enters the **bloodstream**. The blood-brain barrier (BBB), the perivascular space and the glymphatic system are responsible for the drainage of solutes to blood and for maintaining brain homeostasis across the lifespan.

Brain analytes released into blood may be degraded by proteases, metabolized in the liver and cleared by the kidneys.

As a consequence, the following is often observed:

- A lack of correlation between CSF and plasma concentrations,
- A large overlap between patients and controls,
- An individual variability enhanced by cumulative age-related disorders (comorbidities) introducing a greater variance in blood than in CSF.

Nevertheless, combined with patient-related risk factors (e.g., cardiovascular, metabolic), blood markers may find utility as a screening tool for patients at risk of developing dementia – especially once disease-modifying drugs will be available.

- Significantly lower **plasma $A\beta_{42/40}$** ratio found in both MCI and AD cases as compared with controls.
- In contrast to tau protein, **plasma NfL** might serve to rule out neurodegeneration in the future.
- Longitudinal analysis of **plasma P-tau** as a noninvasive biomarker might help for tracking disease progression in AD and monitoring effects of disease-modifying therapeutics in clinical trials.

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GLOSSARY

AD	Alzheimer's disease
ALS	Amyotrophic lateral sclerosis
APOE	Apolipoprotein E
APP	Amyloid precursor protein
AT(N)(C)	NIA-AA staging and classification system (A = amyloid pathology; T = tau pathology; N = neurodegeneration; C = cognition)
A β	Amyloid-beta, β -amyloid
A β_{40}	Amyloid-beta 40, β -amyloid 40
A β_{42}	Amyloid-beta 42, β -amyloid 42
α -syn	α -synuclein
BBB	Blood-brain barrier
CAA	Cerebral amyloid angiopathy
CAA-ri	CAA-related inflammation
CJD	Creutzfeldt-Jakob disease
CSF	Cerebrospinal fluid
CT	Computed tomography
DLB	Dementia with Lewy bodies
FDG-PET	Fluorodeoxyglucose PET
FET	Fused in sarcoma protein, Ewing sarcoma protein and TATA-binding protein-associated factor 15, collectively known as FET proteins

FFI	Fatal familial insomnia
FTD	Frontotemporal dementia
FTLD	Frontotemporal lobar degeneration
FUS	Fused in sarcoma protein
gCJD	Genetic Creutzfeldt-Jakob disease
LDL	Low-density lipoprotein
LRP1	Low Density Lipoprotein Receptor-related Protein 1
MCI	Mild cognitive impairment
MRI	Magnetic resonance imaging
MSA	Multiple system atrophy
NDD	Neurodegenerative diseases
NfL	Neurofilament light
NIA-AA	National Institute on Aging-Alzheimer's Association
NSE	Neuron specific enolase
PD	Parkinson's disease
PDD	Parkinson's disease dementia
PET	Positron emission tomography
PRNP	Prion protein gene
PrPSc	Pathological prion protein
P-tau	Phosphorylated tau
RBP	RNA-binding protein
RT-QuIC	Real-time quaking-induced conversion
sCJD	Sporadic Creutzfeldt-Jakob disease
SD	Standard deviation
SPECT	Single photon emission computed tomography
stREM2	Soluble triggering receptor expressed on myeloid cells 2
TAF15	TATA-binding protein-associated factor 15
TDP-43	TAR DNA-binding protein of 43kDa
TREM2	Triggering receptor expressed on myeloid cells 2
T-tau	Total tau
WHO	World Health Organisation

We work continuously on the content that you find in this booklet, and aim at taking into account the current state of knowledge and the latest developments in the field of Alzheimer's Disease testing.

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