Catlab Informa

Bulletin No. 148

Alzheimer's disease: biomarkers in CSF and plasma

Alzheimer's disease is a progressive neurodegenerative disorder that is the most common cause of dementia worldwide. It is characterized by a progressive deterioration of cognitive functions, especially memory, and leads to a loss of functional autonomy and changes in the affected person's behavior and personality.

Alzheimer's disease biomarkers allow for diagnosis in preclinical and mild clinical stages of the disease, and will be essential in the future for monitoring neurological changes in response to drugs that prevent disease progression.

Epidemiology worldwide and in Spain

Alzheimer's currently affects more than 55 million people worldwide, according to data from the World Health Organization (WHO), accounting for 60-70% of dementia cases in developed countries. This figure is estimated to double in the coming years due to the progressive aging of the population.

In Spain, it is estimated that approximately 800,000 people suffer from this disease, especially in older ages. The prevalence increases significantly after the age of 65, affecting more than 40% of those over 85 years old.

Risk factors for Alzheimer's disease

Alzheimer's risk factors can be divided into two large groups: modifiable and non-modifiable.

Modifiable risk factors include physical inactivity, obesity, hypertension, cognitive impairment, high cholesterol levels, diabetes, head injuries, inadequate or poor-quality sleep, and exposure to environmental toxins.

Among the non-modifiable factors, the most important is age. The risk increases exponentially after age 65. Another relevant factor is genetics. There are familial forms of the disease, associated with mutations in genes such as APP, PSEN1, and PSEN2, which cause early-onset Alzheimer's disease. However, these genetic forms are rare (<1%). The late-onset form (>65 years) is the most common and may involve the APOE-Ɛ4 allele, which increases the risk of developing Alzheimer's disease, especially in homozygous individuals.

Pathophysiology of Alzheimer's

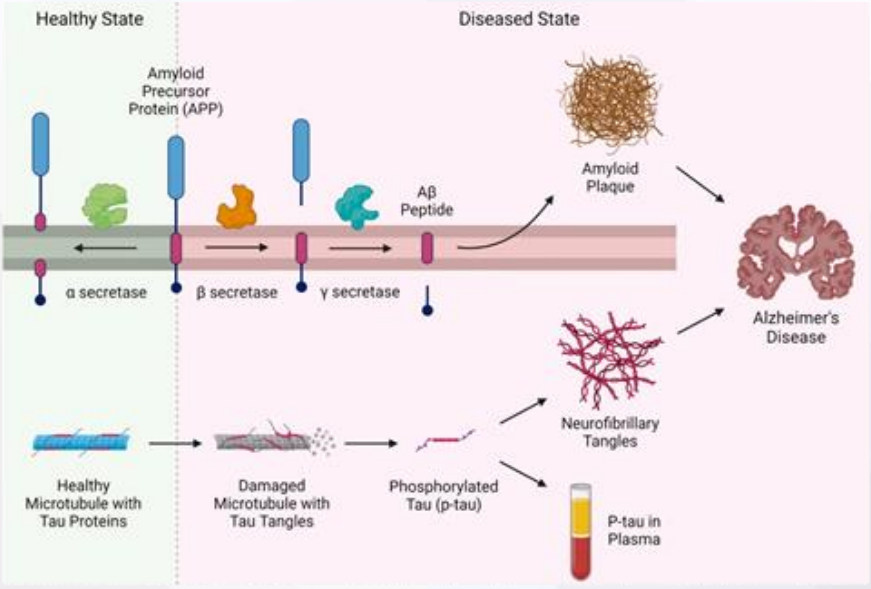
Alzheimer's disease is characterized by two main brain lesions: beta-amyloid plaques and neurofibrillary tangles.

Amyloid- beta plaques are extracellular deposits formed by the accumulation of amyloid -beta peptides (Aβ), especially the insoluble form Aβ 1-42. This protein is derived from the abnormal breakdown of the amyloid precursor protein (APP). The accumulation of Aβ 1-42 is neurotoxic and interferes with synaptic communication, triggers a microglial inflammatory response, and activates oxidative stress processes. This pathway also produces the biologically more abundant peptide Aβ 1-40. These plaques accumulate mainly in the cerebral cortex and hippocampus, areas involved in memory and cognitive functions.

Neurofibrillary tangles form within neurons from hyperphosphorylated Tau protein (pTau). Normally, Tau protein stabilizes intracellular microtubules, but in Alzheimer's disease, Tau protein hyperphosphorylation occurs, resulting in insoluble aggregates that destabilize the neuronal cytoskeleton. The presence of Tau tangles is highly correlated with neuronal degeneration and clinical severity of the disease.

These two alterations trigger a cascade of pathological processes: synaptic dysfunction, neuronal death, neuroinflammation , and progressive brain atrophy. Together, they lead to the loss of cognitive function and the development of clinical symptoms.

In recent decades, a sequential model of the disease, the "amyloid cascade," has been proposed. This model first presents amyloid accumulation, followed by tauopathy and finally clinical neurodegeneration. This model has allowed the establishment of specific biomarkers for each pathophysiological stage of Alzheimer's disease. Furthermore, in advanced age, where many patients may present with mixed dementia, biomarkers allow differentiation between the multiple pathologies present.



Lai R, Li B, Bishnoi R. P-tau217 as a Reliable Blood-Based Marker of Alzheimer's Disease. Biomedicines. 2024; 12(8):1836. https://doi.org/10.3390/biomedicines12081836

Biomarkers for the diagnosis of Alzheimer's disease

At each stage of the “amyloid cascade,” biomarkers present a specific profile and are related to an aspect of the disease's pathophysiology. Currently, CSF biomarkers used for diagnosis include beta -amyloid 1-42 (Aβ42), beta -amyloid 1-40 (Aβ40), the Aβ42/Aβ40 ratio, total Tau protein, and Tau protein phosphorylated at threonine 181 (pTau 181).

Aβ42: Its levels in CSF decrease as the protein aggregates, forming insoluble amyloid deposits. Levels correlate with brain amyloid load as measured by amyloid PET.

Aβ40 and Aβ42/Aβ40 ratio: Aβ40 is found in quantities 10 times higher than Aβ42 and has a protective effect on the formation of amyloid plaque. Its levels do not vary in Alzheimer's disease, but measuring the Aβ42/Aβ40 ratio has a higher diagnostic yield than either biomarker separately. This is because some patients have higher production of beta- amyloid. The Aβ ratio makes it possible to differentiate individuals with normal levels of Aβ40 and decreased levels of Aβ42 from those with decreased levels of both peptides.

Total Tau and Total Tau/Aβ42 Ratio: Elevated levels of total Tau are associated with the presence of neurofibrillary tangles, but their elevation is not specific for Alzheimer's disease. They are also elevated in cases of traumatic brain injury, stroke, and Creutzfeldt -Jakob disease. The total Tau/Aβ42 ratio improves diagnostic performance compared to each biomarker separately.

pTau 181: Increased levels are associated with the presence of neurofibrillary tangles specific to Alzheimer's disease.

In CSF, the combination of low Aβ42 and Aβ42/Aβ40 ratio, and elevated total Tau and pTau 181, has a high diagnostic sensitivity and specificity for differentiating Alzheimer's disease from other dementias.

Plasma biomarkers have been developed in recent years , with the **pTau 217 protein** being the most promising emerging biomarker. Plasma biomarkers enable less invasive, more cost-effective, and earlier diagnoses, improving early access to new disease-modifying therapies.

At Catlab, the biomarker pTau 217 in plasma has recently been incorporated, which has a high diagnostic accuracy in the context of memory loss. It directly correlates with neuronal cell loss and neurofibrillary tangle formation. It strongly correlates with other Alzheimer's disease markers, such as CSF biomarkers, amyloid PET, and tau PET.

In our laboratory, the following cut-off points have been defined for pTau 217 in plasma, with a sensitivity and specificity of 97.5% according to the data from the study by *J Arranz et al* (2024):

> 0.550 pg/mL	Compatible with Alzheimer's disease.
0.550 - 0.129 pg/mL	Gray area.
< 0.129 pg/mL	Not suggestive of Alzheimer's disease.

Patients with values in the gray zone will be candidates for further study of CSF biomarkers, PET-amyloid or PET-Tau to confirm or rule out the diagnosis.

Both CSF and plasma biomarkers are measured at Catlab by chemiluminescence using the Lumipulse® analyzer (Fujirebio). It is important to emphasize the importance of the preanalytical phase of the technique: using tubes with low protein adhesion (usually polypropylene), properly preserving the sample, and avoiding freezing and thawing cycles.

Diagnosis of Alzheimer's disease

The diagnosis of Alzheimer's is based on a combination of clinical elements and complementary tests. A detailed clinical history and a neurological and cognitive examination are required. Instruments such as the MMSE (Mini Mental State examination) or the MoCA (Montreal Cognitive Assessment).

The main imaging biomarkers are based on PET techniques with tracers of Aβ deposits (amyloid PET) or Tau (Tau PET). A brain PET scan can also be performed to assess brain metabolism (FDG PET). MRI can differentiate atrophy associated with Alzheimer's disease from that of other pathologies. Diagnosis can be complemented with biomarkers in cerebrospinal fluid (CSF), currently the most widely used, or in plasma.

The most widely used diagnostic criteria currently are those of the National Institute on Aging – Alzheimer's Association (NIA-AA) of 2018. These define Alzheimer's disease as a biological process identified by both imaging and CSF biomarkers. They use the AT(N) classification system, which, based on the presence or absence of three biomarkers, defines eight biological profiles, four of which are associated with Alzheimer's disease. The presence of these biomarkers can be demonstrated through imaging tests or CSF analysis:

A: Aβ deposition biomarkers: amyloid PET, Aβ 1-42 or Aβ 1-42/Aβ 1-40 ratio in CSF.

B: Tau pathology biomarkers: PET Tau or pTau in CSF.

N: Biomarkers of neurodegeneration/neuronal injury: Total Tau in CSF, PET-FDG or MRI.

AT(N) profiles		
AT-(N)-	Normal Alzheimer's biomarkers	
A+T-(N)-	Pathological change in Alzheimer's	Alzheimer's continuum
A+T+(N)-	Alzheimer's disease	
A+T+(N)+	Alzheimer's disease	
A+T-(N)+	Alzheimer's disease and suspected non-Alzheimer's pathological changes	
A-T+(N)-	Pathological change not related to Alzheimer's.	
AT-(N)+	Pathological change not related to Alzheimer's.	
A-T+(N)+	Pathological change not related to Alzheimer's.	

Treatment

Currently, Alzheimer's disease has no cure. Treatment includes non-pharmacological and pharmacological strategies. Non-pharmacological treatments include cognitive stimulation, physical activity, dietary modifications, and emotional support. Pharmacological treatments include acetylcholinesterase inhibitors (donepezil, rivastigmine, galantamine) and memantine. In recent years, new disease-modifying therapies have emerged, such as the monoclonal antibody lecanemab (approved by the EMA) and donanemab (under study, useful in early stages with low Tau load), which act against beta- amyloid. However, their use is still limited because they present serious adverse effects in patients carrying the E4 allele of the APOE gene.

Mireia Fonolleda Ramboux
FEA Immunology

Mireia Pallarés Borràs
FEA Clinical Analysis

Núria Pacheco
Clinical Analysis Resident

www.catlab.cat

Bibliographic references

1. World Health Organization. Dementia . 2023. Available at: https://www.who.int/news-room/fact-sheets/detail/dementia

2. Spanish Neurology Association. Report on the situation of Alzheimer's disease in Spain. 2022.

3. McKhann GM, et al. The diagnosis of dementia due to Alzheimer's disease: Recommendations from the NIA-AA workgroups. Alzheimers Demented. 2011;7(3):263-269.

4. Jack CR Jr., et al. NIA-AA Research Framework: Towards biological definition of Alzheimer's disease. Alzheimers Demented. 2018;14(4):535-562.

5. Blennow K, Zetterberg H. Biomarkers for Alzheimer's disease: current status and prospects for the future. J Intern Med . 2018;284(6):643-663.

6. Palmqvist S, et al. Discriminative Accuracy of Plasma Phospho-table217 for Alzheimer Disease vs Other Neurodegenerative Disorders . JAMA. 2020;324(8):772-781.

7. Cummings J, et al. Aducanumab : Appropriate Use Recommendations . J Prev Alzheimer's Dis. 2021;8(4):398-410.

8. Arranz J, et al. Diagnostic performance of plasma pTau217, pTau181, Aβ1-42 and Aβ1-40 in LUMIPULSE automated platform for the detection of Alzheimer's disease . Alzheimer's Research & Therapy 2024; 1 6:139.