# Kit biomarker for early detection of Alzheimer's disease

An innovative approach for diagnosis of Alzheimer´s disease based on an important scientific discovery of abnormal tau variants in human platelets.

# The Alz-tau® Biomarker

alztau®

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## 1 Executive Summary

Alzheimer's disease (AD) like other neurodegenerative disorders is a major puzzle for modern medicine. In this context, **it is critical to ascertain the presence of the disease based on reliable, quantitative diagnosis approaches.** To achieve this purpose, Neuroinnovation SpA has developed an innovative and advanced detection method for AD, based on molecular biomarkers.

This technology provides a non-invasive procedure for early detection of AD. The use of this biomarker to identify individuals with AD before clinical symptoms, will be essential for development of potential drugs for early intervention. On the other hand, the determination of peripheral markers of AD pathology can help us to understand the pathophysiology of neurodegeneration in AD.

Years of research carried out by our team have proven that the presence of tau protein present in human platelets exhibits significant differences between Alzheimer's patients and normal controls. There is a close correlation between the presence of this anomalous platelet protein with the level of AD cognitive impairment, as assessed through neuropsychology and more important, a close correlation with respect to neuroimaging (MRI).

We have taken advantage of these differences in order to develop a highly sensitive and efficient tool that allows us to tag the progress of AD in a large population of patients. Four different clinical studies have allowed the validation of the technology, three of which have already been published in scientific journals and one is still being developed.

All four clinical studies conducted showed that the HMW/LMW tau ratio is significantly higher in AD cognitively impaired than in healthy control subjects, and one study showed tau association with specific brain regions atrophy.

Our molecular marker is non-invasive and presents no risk, in comparison with other procedures such as cerebro spinal fluid (CSF) tests that are highly invasive, even though its sensitivity is adequate. Our patented technology is highly innovative because it is based on a high impact scientific discovery from our laboratory.

Considering the need for quantitative markers for this disease, and the total absence of other diagnosis tools, **Alz-tau® technology** if the first biomarker based on tau protein that allows an early detection of AD and a rigorous diagnosis of this disease.

## 2 Alzheimer's and the Tau Protein

The discovery by Alois Alzheimer of neurofibrillary tangles (NFTs) in the brains of patients with neurodegenerative disorder named after him (AD), provided a pivotal impetus for the study of molecular substrates [1]. The major components of NFTs, the paired helical filaments (PHFs), are mainly formed by self-assembly of hyperphosphorylated forms of tau [2]. Since his tau discovery in the mid 70´s, Maccioni´s team has carried out many studies that have improved our understanding of tau hyperphosphorylation, and alterations of the neuronal cytoskeleton; however the precise structural basis for pathological tau self-aggregation remains to be elucidated.

AD is a multifactorial disorder in which protein alterations, oxidative stress, neuroinflammation, immune deregulation, impairment of neuronal-glial communication, and neurotoxic agents appear as major factors triggering neuronal degeneration, the balance among these seems to vary from patient to patient. Although diverse, these factors induce deleterious signalling through different sets of neuronal receptors that converge in the hyperphosphorylation of tau [3].

This raises the question as to precisely what triggers the pathological phosphorylation. On the basis of structural studies, together with the elucidation of the signalling cascades in neurodegeneration, we postulated a hypothesis based on the concept that tau *hyperphosphorylation constitutes a final common pathway in the pathogenesis of AD*, upon which a host of signal mechanisms converge. The low molecular weight tau is the major component of microtubule associated proteins (MAPs) in axons, and plays critical roles in stabilizing microtubules and inducing its own assembly.

One of the most intriguing properties of this brain polypeptide is that, under pathological conditions, tau self-aggregates into PHFs, which turn into NFTs, one of the neuropathological hallmarks of AD and tauopathies. However, today we know that hyperphosphorylated tau or tau oligomers exert the pathological effects, altering the normal interaction patterns of the neuronal cytoskeletal network. Moreover, a link between tau oligomerization and cognitive impairment has been evidenced.

## 3 The Alz-tau® Biomarker

**We have developed an innovative detection method for AD based on the Alz-tau® biomarker** [6, 7, 10]. The presence of heavy (HMW) and light (LMW) tau variants in human platelets and the significant increase in HMW/LMW tau in AD patients is the basis for this technology. Thus, **Alz-tau®** invention is a highly innovative, efficient and non-invasive biomarker for early diagnosis of AD. These findings indicate that the patterns of HMW/LMW platelet tau can be used as a biomarker for AD. It also has implications for the potential development of biomarkers for other tau-based neurodegenerative diseases [4, 6, 7, 10].

Platelet tau ratio was obtained for healthy subjects and AD patients as described by Neumann *et al, 2011*[7]. Densitometric analyses of immunoblots through ImageJ (Wayne Rasband, NIH, USA), show that HMWtau/LMWtau ratio is significantly higher in the AD group in relation to controls (Fig. 3A, B). NFT formation has been identified as a major event involved in neurodegeneration, due to the conversion of either soluble tau (LMWtau) or oligomers (HMWtau) into insoluble filaments. This discovery offers us a tremendous opportunity in the biomarker field, since altered signaling pathways are involved in degenerating neurons in AD.

During more than 40 years there has been a high amount of research on biomarkers for detection of AD, all of them have failed due to erroneous targets. For example, the search for amyloid protein in the blood, saliva and urine have not been successful. The only method being used for accurate detection of AD is based on CSF measurement of tau protein, the correct target, but this method is highly invasive [8,9]. The existence of a reliable and highly efficient biomarker such as **Alz-tau®** is relevant as a diagnosis tool for early detection of AD, but also for the search of new drugs against the disease for early intervention in which the disease may be controlled. The treatments currently available only temporarily alleviate some symptoms but do not cure the pathology.

So far our technology appears as the best solution for diagnosis of AD, considering its usefulness and that there is no other validated method. Today diagnosis for AD is performed by neuropsychology, since neuroimaging by itself does not provide an effective diagnosis. Our biomarker exhibits a sensitivity of 75.7% and a specificity of 73.7% (see Figure 2). Other markers being tested have shown fewer efficacy, with a large number of false positives.

## 4 Experimental Studies & Methodology

Method is based on processing and isolation of platelets from peripheral blood samples obtained from each subject, resuspended in a buffer solution at room temperature and then centrifuged at 1600×g at 4°C, to avoid activation of cells, obtaining the platelet pellet to be lysed in the presence of protease inhibitors. An SDS-PAGE is performed in which multiple samples are subjected to electrophoresis followed by western blots analysis using the **Alz-tau®** or the tau-5 monoclonal antibodies. Immunoreactive bands are detected by using chemioluminescent substrates that reacts very specifically to the tau-binding antibody (see Figure 1). The LMWtau species are those with molecular weight ≤55 kDa., HMWtau are considered the tau oligomers between 75 a 240 kDa.

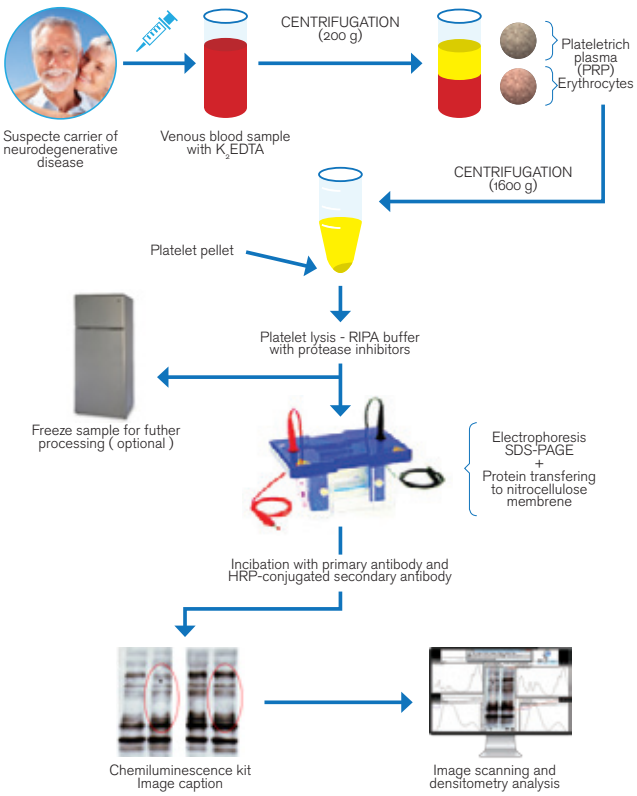



**Figure 1:** Procedure to use the platelet Biomarker. Samples obtained directly from patients are brought to the laboratory, platelets separated and the presence of tau analyzed by western blots by using either tau 5 antibody or **Alz-tau®** (monoclonal we have generated against platelet tau).

## 5 Statistical Analysis & Results

Descriptive and comparative analyses were conducted with the Student's t-test to compare the two groups, and chi-squared test for categorical variables. In addition, the size effects (Cohen's statistics) were determined to assess the group differences. The association between HMW/LMW tau ratio and demographic variables and cognitive scales were evaluated with Pearson's correlation. The analyses were conducted at $p < 0.05$ (two-tailed). Platelet tau was obtained from blood samples of different aged individuals without memory complains. Subjects' ages ranged from 30 to 85 years approx. In the immunoblots analysis, a cut-off point of 1.11 for HMW-tau/LMW-tau ratio displayed a sensitivity of 75.7% and a specificity of 73.7% to discriminate AD and control subjects (Fig. 2). As explained above, of the four clinical studies, we show here only two of them. No correlation between platelet tau ratio and age was found (Table 1). The representative immunoblots of platelet tau tagged with a specific antibody for tau is shown in figure 3A. High molecular weight tau bands can be appreciated, with greater immunoreactivity in patients with AD compared with controls (Fig. 3A).

| | Study 1 | Study 2 | Study 1 and 2 |
|---|---|---|---|
| **Starting date and ending date** | Dec 2007 to Jan 2010 | Mar 2010 to Mar 2013 | --- |
| **Controls Subjects** | 19 | 37 | **56** |
| Male | 5 | 13 | **18** |
| Female | 14 | 24 | **38** |
| Age (avg) | 70 (52-82) | 71 (64-83) | **71 (52-83)** |
| Education level (years) | 11 (4-17) | 13 (6-22) | **12 (4-22)** |
| GDS Controls (see Fig. 5) | 1.15 (1-2) | 1.25 | **1.20** |
| **AD Subjects** | 37 | 41 | **78** |
| Male | 21 | 24 | **45** |
| Female | 26 | 17 | **43** |
| Age (avg) | 76 (61-89) | 73 (60-87) | **75 (60-89)** |
| Education level (years) | 8 (1-17) | 11 (3-20) | **10 (1-20)** |
| GDS AD (see Fig. 5) | 5(4-6) | 4.73 | **4.81** |
| **HMWtau/LMWtau (avg) Controls** | 0.8922 | 1.5270 | **1.2096** |
| **HMWtau/LMWtau (avg) AD** | 2.8766 | 2.0691 | **2.4729** |
| *p value* (t-test) | < 0.0001 | 0.005 | **< 0.0001** |

"avg: Average"

**Table 1:** Demographic results and summary table of two independent studies conducted by International Center for Biomedicine ICC.

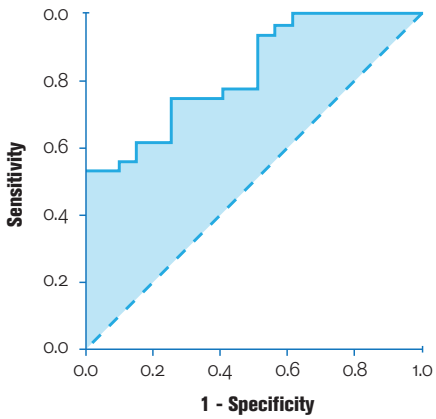
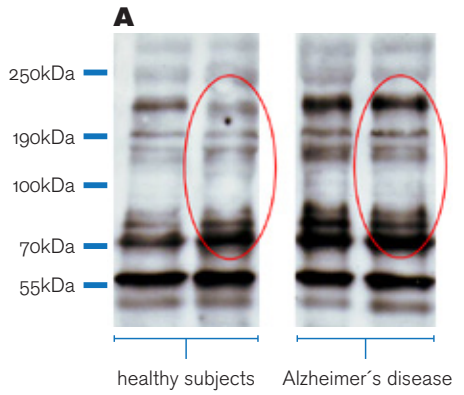



**Figure 2:** ROC curve showing sensitivity and 1-specificity of platelets tau ratio as an AD biomarker. Area under the curve corresponds to 0.824 (IC 95: 0.716–0.932).



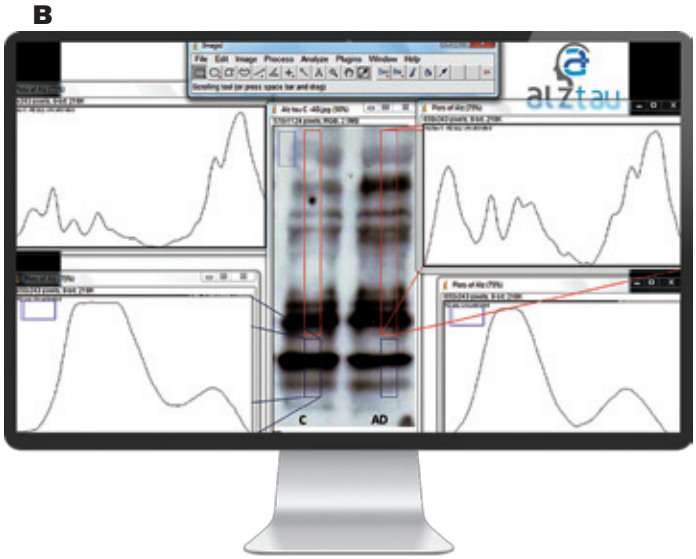




**Figure 3:** **A**) Representative Immunoblots of platelet tau with tau-5 antibody. High molecular weight tau bands (about 80kDa) can be appreciated, with greater immunoreactivity in patients with AD compared with healthy subjects. **B**) Densitometric analysis through of the computer program ImageJ (Wayne Rasband, NIH, USA) for quantifying the density of bands, thus enabling obtaining the relationship between HMWtau versus LMWtau.

Platelet tau ratio (HMWtau/LMWtau) was obtained for controls and AD subjects, through a suitable program ImageJ for determining densitometry of each electrophoretic pattern and analyzed statistically (Table 1 and Fig. 4). For this, two important areas of analysis which correspond to HMWtau (red rectangle) and LMWtau (blue rectangle) are determined. As shown in Figure 3B, when comparing both immunoblot (healthy subjects "C" and AD patient), we see that the graphics blue rectangle does not show major differences compared to the two graphics red rectangles, since the right (AD patient) has a higher density in their bands, compared to healthy subjects (C).The values obtained for each patient, are correlated with neuropsychological batteries, including Global deterioration scale (GDS) (see Table 1 and Fig. 5), Mini-mental State Examination (MMSE), etc.

## 6 Intelectual Property

- Granted patent N° US 9,012,237 B2 "Innovative Blood Platelets Biomarker for Early Diagnosis of Alzheimer's Disease".
- Know how.
- Trademark Alz tau.

## 7 Research


[1] Alzheimer A (1907) Über eine eigenartige Erkrankung der Hirnrinde. *Psychiatr Psych gericht Med.* **64**, 146-148.

[2] Farias G, Cornejo A, Jimenez J, Guzman L, Maccioni RB (2011) Mechanisms of tau self-aggregation and neurotoxicity. Curr *Alzheimer Res* **8**, 608-614.

[3] Morales I, Farias G, Maccioni RB (2010) Neuroimmunomodulation in the pathogenesis of Alzheimer's disease. *Neuroimmunomodulation* **17**, 202-204.

[4] Guzman-Martinez L, Farias GA, Maccioni RB (2012) Emerging noninvasive biomarkers for early detection of Alzheimer's disease. *Arch Med Res* **43**, 663-666.

[5] Guzman-Martinez L, Farias GA, Maccioni RB (2013) Tau Oligomers as Potential Targets for Alzheimer's Diagnosis and Novel Drugs. *Front Neurol* **4**, 167.

[6] Farias G, Perez P, Slachevsky A, Maccioni RB (2012) Platelet tau pattern correlates with cognitive status in Alzheimer's disease. *J Alzheimers Dis* **31**, 65-69.

[7] Neumann K, Farias G, Slachevsky A, Perez P, Maccioni RB (2011) Human platelets tau: a potential peripheral marker for Alzheimer's disease. *J Alzheimers Dis* **25**, 103-109.

[8] Maccioni RB, Lavados M, Maccioni CB, Mendoza-Naranjo A (2004) Biological markers of Alzheimer's disease and mild cognitive impairment. *Curr Alzheimer Res* **1**, 307-314.

[9] **R.B. Maccioni, M.** Lavados, CB Maccioni, G. Farias and P. Fuentes (2006) "Anomalously phosphorylated tau protein and Abeta fragments in the CSF correlates with cognitive impairment in MCI subjects". *Neurobiol Aging* **27**, 237-244

[10] Slachevsky A, Guzmán-Martínez L, Delgado C, Reyes P, Farías GA, Muñoz-Neira C, Bravo E, Farías M, Flores P, Garrido C, Becker JT, López OL, Maccioni RB (2017). Tau Platelets Correlate with Regional Brain Atrophy in Patients with Alzheimer's Disease. *J Alzheimers Dis.* **55**, 1595-1603.


## 8 Links to Related Websites

Further information can be found in the following websites:
**www.neuroinnovation.cl**
**www.neurosciencelab.cl**

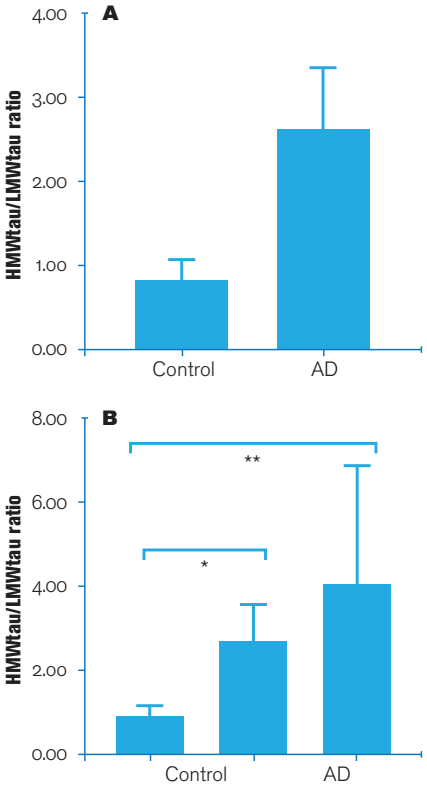



**Figure 4:** **A**) Platelets HMWtau/LMWtau ratio is elevated in AD samples when compared to control samples (p = 0.001). **B**) This difference can also be appreciated when AD subjects are grouped in mild to moderate disease (*p = 0.016) and advanced AD (**p = 0.004). Bars represent 95% confidence intervals.

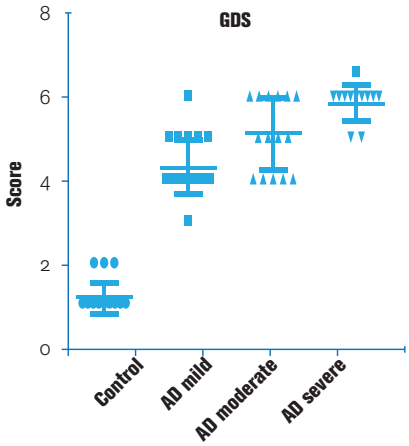



**Figure 5:** Graph of GDS score distribution for each patient participating in the study. A statistically significant difference between healthy subjects and AD and between each stage (mild, moderda and Sevara) disease is observed.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