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BIOMARKERS FOR ALZHEIMER´S DISEASE. WHERE WE STAND AND WHERE WE ARE HEADED

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Abstract Alzheimer disease (AD) is one of the major unresolved health burdens accompanying the increase in life expectancy. The great paradigm shift for this disease has resulted from finding amyloid deposition and neurobrillary degeneration 20 years and 10 years, respectively, prior to onset of the typical clinical memory loss symptoms. The advent of AD biomarkers has enabled a molecular definition of AD, making the clinical definition almost dispensable. Various types of AD biomarkers are available in our country. Each biomarker reflects a particular process and stage of the disease. Although costs restrict their use, the biomarker analysis may be justified in certain clinical scenarios, such as an early onset or an atypical presentation of the disease. Today, the usefulness of biomarkers in AD clinical research is beyond question. Furthermore, the introduction of biomarkers into medical practice has led to significant changes in therapeutic interventions, even in the absence of disease-modifying drugs.

Key words: Alzheimer disease, biomarkers, preclinical, neuroimaging, molecular diagnostic techniques

Resumen ***Biomarcadores de enfermedad de Alzheimer. Dónde estamos y hacia dónde vamos.*** La enfermedad de Alzheimer (EA) es uno de los mayores flagelos aún no resueltos que acompañan al aumento de la expectativa de vida. El gran cambio de paradigma en los últimos años fue consecuencia de descubrir que el depósito amiloideo se presenta hasta 20 años antes, y la degeneración neurofibrilar hasta 10 años antes, de que aparezca la sintomatología clínica típica de pérdida de memoria. La aparición de los biomarcadores permitió reestructurar el concepto de la EA, intentándose llegar a una definición molecular de la misma casi prescindiendo de la emblemática clínica. Existen distintos tipos de biomarcadores de EA disponibles en nuestro país. Cada uno nos habla de un proceso y un momento distinto de la enfermedad. Aunque su uso clínico aún se encuentra restringido por cuestiones de costos, existen escenarios particulares en donde sí se justifica, casi siempre en relación a presentaciones clínicas atípicas o de comienzo muy temprano. Sin embargo, hoy en día ya nadie discute que son imprescindibles en investigaciones clínicas sobre EA. La incorporación de biomarcadores en la práctica médica ha generado cambios significativos en la intervención terapéutica de los pacientes, incluso en un contexto en el que todavía no hay medicamentos modificadores de la enfermedad.

Palabras clave: biomarcadores de la enfermedad de Alzheimer, etapas preclínicas, neuroimágenes, diagnóstico molecular

The Alzheimer disease (AD) is one of the main unresolved health burdens accompanying the increase in life expectancy and has become a crucial public health problem[1]. One out of every two individuals over the age of 80 will develop the disease with the ensuing impact on their family and society[2].

The main pathophysiological mechanism underlying AD involves increase in Aβ peptide species, ultimately leading to extracellular amyloid deposits and neurofibrillary degeneration, secondary to intraneuronal abnormal tau protein hyperphosphorylation. In 2012, Bateman et al.[3] showed that amyloid deposits and neurofibrillary degeneration were present 20 and 10 years before the onset of memory decline, respectively.

As a result of these findings, the course of AD was divided into three stages –presymtomatic, mild cognitive impairment, and dementia– with the presymtomatic phase currently attracting major research efforts on AD pathophysiology, treatment, and prevention.

Prior to the introduction of biomarkers, the clinical diagnosis (NINCDS-ADRDA criteria) was limited to possible or probable AD, given that diagnosis was only confirmed in *post-mortem* brain tissue histopathology [4].

Biomarkers are defined as objective, quantifiable parameters which allow *in vivo* assessment of pathophysiological disease traits. Current biomarkers for AD include: 1) $A\beta_{1-42}$, total tau and phosphorylated tau assay in cerebrospinal fluid (CSF); 2) structural neuroimaging studies

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such as brain MRI and hippocampal volume analysis; 3) functional neuroimaging of metabolic activity such as 18F-fluorodesoxyglucose (FDG) PET and protein-identifying neuroimaging such as amyloid PET and tau PET.

Based on the application of these specific biomarkers, the US *National Institute of Aging and Alzheimer's Association* revised the accepted diagnostic criteria for AD[5-8], which led to two important changes. First, the use of biomarkers allowed a formal separation of the different disease stages to include mild cognitive impairment, thus providing greater sensitivity and specificity to the detection of early AD. Second, the application of biomarker results allowed to achieve higher diagnostic certainty, in relation to the underlying neuropathological changes present in AD.

In 2018, a new biomarker-based biological classification, the A/T/N (Amyloid/Tau/ Neurodegeneration) system, was published[9], in which "A" refers to the presence of β-amyloid biomarker (detected on amyloid PET or assaying CSF $A\beta_{42}$ level); "T" refers to the value of a tau biomarker (measured in CSF phospho-tau assay, or on tau PET); and "N" refers to biomarkers of neurodegeneration or neuronal injury (evaluated on [^{18}F]-fluorodeoxyglucose-PET, structural MRI, or measuring total tau in CSF). This classification allows a pathophysiological categorization and a clearer prediction of patient outcome[9].

The introduction of biomarker results has profoundly influenced AD diagnosis, prognosis and treatment, since it allows the detection of very early stages in individuals presenting mild AD symptoms without dementia (prodromal AD), or even at pre-symptomatic stages.

Thus, with pharmacological treatments becoming available for very early stages of the AD, the major challenge becomes finding simpler biomarkers. We hope that in the not too distant future the value of their potential application extends to all clinical scenarios for which they may prove useful.

Alzheimer´s disease biomarkers in fluids

A better understanding of the disease mechanisms involved in AD has allowed the development of different types of fluid biomarkers. CSF has become a primary viable source, given its close contact with the CNS at the extracellular compartment level. However, because of the invasive nature of the spinal tap, patients are reluctant to undergo testing, and repeated sampling is poorly tolerated, which is why biomarkers in blood are now being investigated and validated[10].

Three key CSF biomarkers have been included in several guidelines and research manuals. These are: β-amyloid 42 ($A\beta_{1-42}$), total tau (t-tau) and threonine18-phosphorylated tau (p-tau). Altered CSF levels of any of these are recognized diagnostic evidence of underlying brain disease compatible with AD[8]. Added prognostic value has also been reported for these molecules as evidence of disease progression in individuals who remain cognitively intact[8] as well as in individuals with mild cognitive impairment[6]. Also, $A\beta_{1-42}$ levels may help to distinguish AD from other clinical conditions such as frontotemporal dementia.

In recent years, new biomarkers have been described as related to other pathophysiological aspects such as vascular dysfunction, neuronal and synaptic integrity and neuroinflammation, to name a few. In this regard, neurofilament-light chain (NfL), an intermediate filament of the neuronal cytoskeleton, which is abundant in axons, has been recognized as a marker of neuronal damage, increasing in both CSF and blood as a result of different neurodegenerative diseases[11-14]. Although not specific for AD, NfL blood levels could be useful for screening purposes. When high NfL levels are detected, subjects could then be tested for known AD biomarkers, namely $A\beta_{1-42}$, t-tau or p-tau in CSF, or PET PiB.

Other biomarkers, such as neurogranine (a marker of synapse dysfunction) or markers of inflammation likeT-REM2 and YKL-40, require more exhaustive validation before they are included in the panel of accepted AD biomarkers[12].

Aside from their role in diagnosis, biomarkers could soon become indispensable tools for the development of future AD therapies. Currently, their use in clinical trials improves the classification of participants according to the underlying disease, allows staging the disease more precisely and also allows a better and earlier evaluation of treatment response[13].

Prior to implementation, new biomarkers will require validation for different conditions or stages and different applications such as screening, diagnosis, treatment monitoring, among others[15].

Alzheimer´s disease biomarkers in neuroimaging

Morphological imaging, using both magnetic resonance (MRI) and computed tomography (CT), as well as molecular methods like positron emission tomography (PET) and single photon emission tomography (SPECT), play an important role in early diagnosis and in non-invasive *in vivo* follow up of patients with neurodegenerative syndromes. Brain deposits of the abnormal proteins Aβ and tau can be detected using PET and neuronal dysfunction measured by analyzing brain glucose metabolism (FDG). Cerebral perfusion evaluated by SPECT shows good correlation with metabolic changes.

AD is the most prevalent neurodegenerative disease and presents a particular molecular and structural neuroimaging profile, in which cortical and extraneuronal amyloid deposits precede the emergence of clinical symptoms

by 20 years. PET using amyloid-labeling tracers (^{11}C-PIB, ^{18}F-Flutemetamol and ^{18}FAV45, among others), represents a sensitive tool for early detection of abnormal brain deposits in the areas most often affected, namely the bilateral frontal and parietal lobes, the lateral temporal cortex and striatal regions. Although detection of amyloid implies greater disease risk, or increased diagnostic certainty, it is important to note that amyloid deposits are present in up to 30% of cognitively normal individuals.

The tau protein is found in neurofibrillary tangles at intraneuronal level, and its hyperphosphorylated forms are specific to AD. On PET imaging, different tracers still under development can be detected with varying degrees of sensitivity and specificity. Flortaucipir (^{18F}AV1451) has been tested in our country in the ADNI-Arg cohort of patients with clinical diagnosis of AD followed for 5 years (Alzheimer Disease Neuroimaging Initiative – Argentina); the study showed higher concentration in the mesial temporal lobe. Neuronal dysfunction explored using ^{18}FDG-PET, a marker of neurodegeneration, showed bilateral areas of hypometabolism in the temporal lobe, the precuneus, the posterior cingulated cortex, and the parietal lobe. Altogether, these findings tend to show the following chronological sequence of tracer uptake during preclinical phases: first amyloid deposition, then tau deposition, and later neuronal dysfunction[8]. As an example, Fig. 1 shows images of brain PET scans illustrating the biomarker findings characteristic of the ATN classification.

Structural brain MRI reveals the presence of reduced hippocampal volume, as well as area-specific cortical atrophy (parahippocampal gyrus, amygdala, superior, medial and inferior temporal gyri, superior parietal lobe and posterior cingulate cortex). Both are considered biomarkers of neurodegeneration generally appearing later than others, that is, during symptomatic stages of the disease. A specific software is used to measure findings compared to quantitative values of standardized digital atlas images, which are available in most local imaging centers.

The functional imaging technique most recently incorporated is the *resting state* MRI (rs-fMR), in which BOLD signal detection generates resting state images (without any type of activity). This allows "connections" between different areas to be studied by correlating temporal neuronal activity between different areas of the brain cortex. The areas of the greatest synchronicity or correlation represent closely linked neuronal networks. To date, rs-fMRI results have shown different connectivity patterns in AD patients compared to normal controls, as well as in patients with mild cognitive impairment and even at preclinical stages of the disease[16].

In summary, a series of sequential and concurrent events need to be taken into consideration, both spatially and chronologically, which include detection of Aβ42 and phospho-Tau, volumetric analysis, hypometabolic areas and connectivity disruption. Imaging techniques provide information on different "stages or phases" of the AD continuum, generating new opportunities for eventual treatment interventions.

Clinical utility of biomarkers in Alzheimer´s disease

Based on all of the above, it seems clear that the biomarkers have improved the diagnosis of AD beyond clinical findings, and have shown that the preclinical stages of the disease may in fact last much longer than the symptomatic ones[3, 8, 9, 13, 15]. As an example, Fig. 2 shows A/T/N results in the Argentine ADNI cohort (normal controls, n = 14; early mild cognitive impairment patients, n =10; late mild cognitive impairment patients n =1 3, and patients with dementia of the Alzheimer type, n = 12[17]).

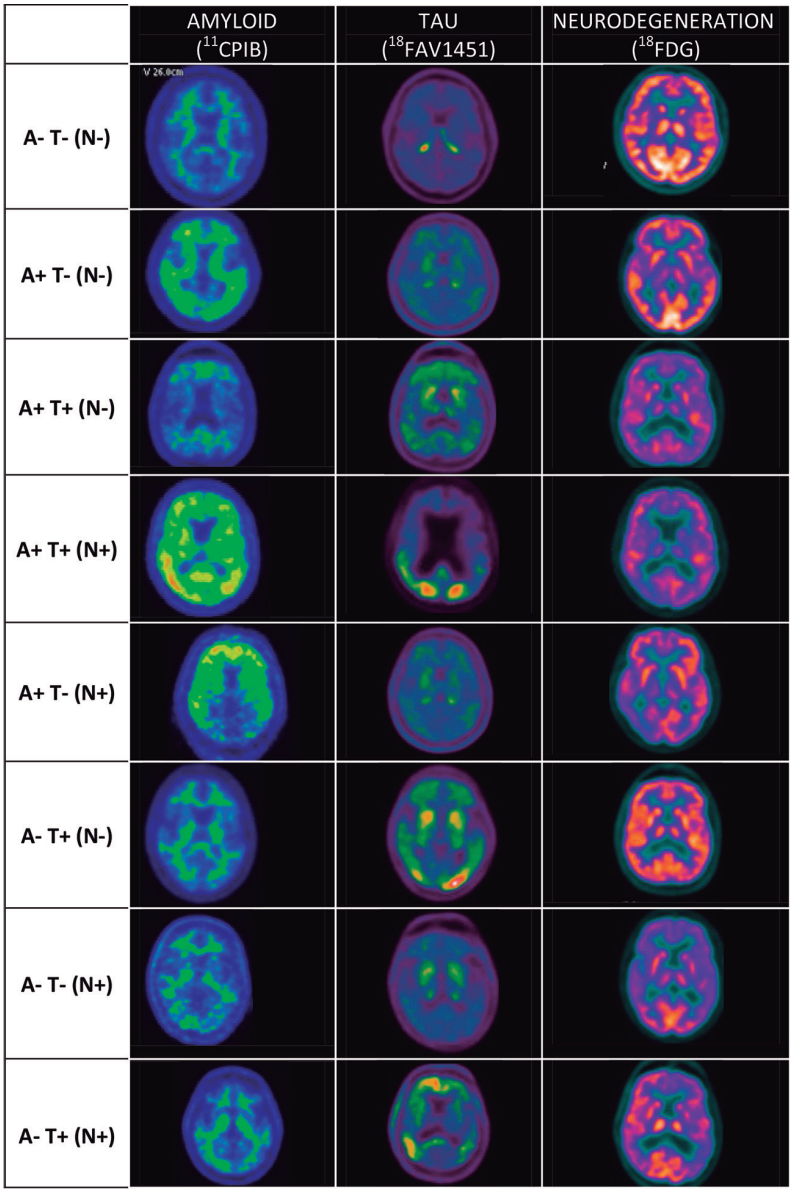
Today, the value of this classification in clinical trials and clinical research is beyond question. However, numerous limitations hinder its application in routine clinical practice, in particular for developing countries in Latin-American, mainly due to financial constraints, limited experience and validation, and restricted clinical access for most of the region. A recent survey has shown however, that it is nevertheless already being used in Argentina, Brazil, Colombia, Chile, Mexico and Uruguay, among other countries[18].

If no disease-modifying drug is available, what is the point of applying biomarkers in clinical practice? Several papers have recommended their use in specific situations[19]. There is general agreement that in early-onset disease (under 65 years of age) as well as in atypical forms of AD, which often also present before 65, biomarkers could be useful. Less anosognosia is present in this age group, which is more concerned on diagnosis and still at very productive stages of life. Diagnostic confirmation would allow making life-changing decisions and improve differential diagnosis with other radically different types of disease. Finally, genetic implications may exist for other family members, (i.e. autosomal dominant forms of AD). One could also add groups of patients in whom AD is suspected, but who present pre-morbid psychiatric conditions, which increase significantly the diagnostic difficulty.

Although clinical syndromes exist with significant overlapping regarding amyloid findings (high pretest probability, i.e. for posterior cortical atrophy), the opposite occurs in other clinical conditions (pretest likelihood is low), hence the value of biomarkers[19].

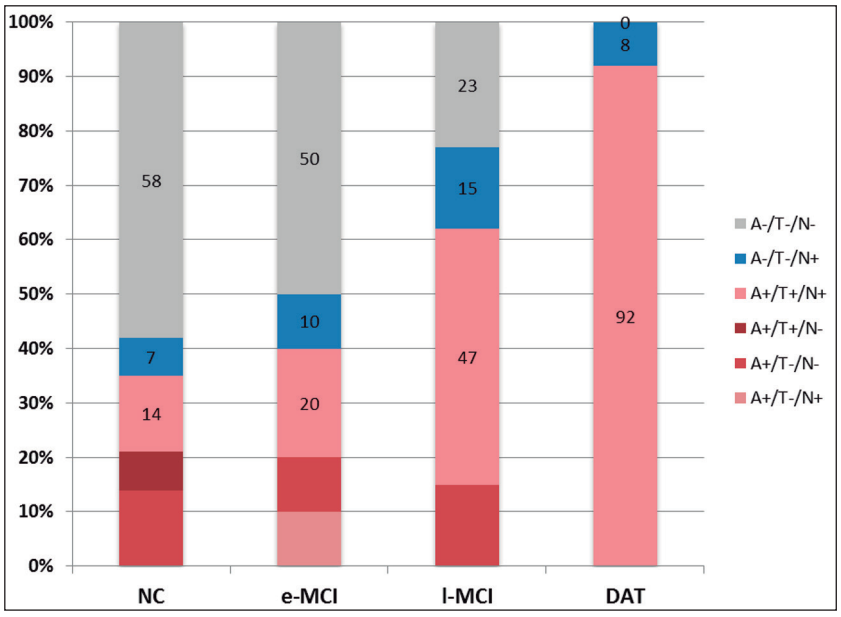
The findings of the IDEAS protocol, conducted on 18 000 imaging studies in US centers to evaluate the clinical utility of amyloid PET results, have recently been published[20]. Briefly, the study showed that the participating physicians modified prescriptions or changed their clinical management in 60% of the patients based on imaging results. This shows that vis-à-vis the diagnostic uncertainty

Fig. 1.– ATN classification based on brain PET (amyloid, tau and FDG), FLENI Neuroimaging database



- A-T-(N-) PET images of amyloid and tau do not show tracer uptake. Normal metabolism on FDG PET. Images correspond to normal subjects
- A+T-(N-) PET image is positive for amyloid, cortical tracer uptake is visible. Tau PET is negative and metabolism normal on FDG. These images correspond to the initial stage of cerebral amyloidosis in pre-symptomatic AD
- A+T+(N-) PET image is positive for amyloid and tau, cortical uptake is seen for both. PET FDG shows normal metabolism. These images correspond to the second stage of pre-symptomatic AD with amyloid and tau deposits
- A+T+(N+) This case shows cortical amyloid and tau deposits and posterior hypometabolism on FDG PET. Typical images in AD patients
- A+T-(N+) This panel shows anterior cortical positivity on amyloid PET, Tau PET is negative y and there is posterior hypometabolism mainly on the right side on FDG PET. This case is compatible with AD associated to non-AD pathology
- A-T+(N-) No amyloid deposits are observed. PET tau shows posterior cortical tau deposits predominantly on the left side and normal FDG-PET. This is observed in degenerative disease other than AD
- A-T-(N+) Amyloid PET and tau are negative. FDG PET shows anterior hypometabolism compatible with non-AD degenerative pathology
- A-T+(N+) Amyloid PET is negative but tau PET is positive in the frontal and posterior cortex. FDG-PET shows left anterior and posterior hypometabolism compatible with non-AD pathology

Fig. 2.– ATN Classification of the Argentine ADNI cohort at FLENI



ADNI: Alzheimer Disease Neuroimaging Initiative; NC = normal control; e-MDI = early mild cognitive impairment; l-MCI = late mild cognitive impairment; DAT = dementia Alzheimer type; A /T /N Amyloid, Tau; Neurodegeneration

% of patients with each ATN subtype in the Argentine ADNI- cohort (*Alzheimer Disease Neuroimaging Initiative* - Argentina)

in AD, biomarker results exert significant influence on daily medical practice.

Discussion

AD had previously been defined based on Alois Alzheimer's original description of neuropathological findings in autopsy material. The diagnosis was based on the detailed presence, density and distribution of the characteristic lesions, namely extracellular amyloid plaque and intraneuronal neurofibrillary degeneration. These criteria were later refined and quantified in an attempt to include other lesions that usually coexist in the aging brain and affect cognition.

The discovery of biomarkers which can report *in vivo* on the presence of these deposits has radically changed neurodegeneration diagnosis. However, as is common in biomedicine, technical advances come together with probably even more relevant conceptual changes. In the case of AD, this has led to a new definition of the disease, separating it from dementia as a single clinical finding, which can develop during the course of several other concurrent diseases. Diagnosis today is determined by detection of amyloid, T-tau and P-tau. It is not known whether these abnormal proteins actually cause the disease, but they are nevertheless their defining feature. Protein deposits make AD a unique and specific neurodegenerative disorder, separating it from other conditions causing dementia. This differentiation is key, since it allows examination of chronological events leading to clinically evident effects on cognition as a continuum, which includes intact subjects amenable to potential treatment strategies. In the not too distant future, taxonomic consequences resulting from biomarker use are foreseeable, linking the nosology to an underlying molecular abnormality, which in turn, may become a potential objective for targeted therapies.

Conflict of interest: None to declare

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