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New perspectives in the search for reliable biomarkers in alzheimer disease

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ABSTRACT – *Background and Objectives:* The search for accurate biomarkers in Alzheimer Disease (AD), on of the most devastating neurodegenerative diseases, remains essential to enable an early prognosis and diagnosis of the disease and to provide more efficient therapeutic strategies.

A wide variety of potential biomarkers are has been identified by neuroimaging techniques and by the analysis of fluid samples, such as cerebrospinal fluid (CSF) or blood. Recently, a growing number of studies are focused on the discovery of reliable blood-based biomarkers in blood, especially in the prodromal stage of AD, which can predict the conversion of asymptomatic cases to AD demented cases.

In this review, the latest challenges in the search for accurate biomarkers of AD is revised, in particular, an update in blood-based biomarkers is described in depth.

Conclusions: Finally, the close link among AD and other neurodegenerative diseases is discussed, mainly based on the last discovered mutation, the chromosome 9 open reading frame 72, *C9ORF72*.

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Challenges in the discovery of potential biomarkers in Alzheimer Disease

To date there are no effective therapies to preserve normal brain function in potential future Alzheimer's disease (AD) patients. The absence of reliable biomarkers to identify cognitive normal individuals that will become early-stage AD patients support this fact. Therefore, this overlap between demented and non-demented population has limited the diagnostic accuracy of the current known biomarkers for AD. Due to the fact that neurological processes that finally result in dementia are assumed to be actively long before the first symptoms appear, the availability of diagnostic biomarkers could make a preclinical diagnostic testing and an early treatment of AD possible.

A great variety of biomarkers of AD has been described using different approaches (Table 1). As Figure 1 shows, different strategies can be studied along AD progression for a better understanding of the disease and to enable an accurate identification of potential AD biomarkers. Currently, the most studied methods to identify markers of mild cognitive impairment (MCI) and AD are neuroimaging and molecular techniques based on cerebrospinal fluid (CSF). A systematic search on PubMed and Scopus databases was performed to find the most relevant studies and review papers. The key words used for this purpose were: "Alzheimer's disease", "Mild Cognitive Impairment", "biomarkers", "blood", "neuroimaging" and "neurodegenerative diseases" in various combinations. The articles selected were published in English from 2004 to 2015.

Neuroimaging is a non-invasive technique which monitors brain regions and allows the subsequent identification and quantification of diagnostic and candidate biomarkers of dementia progression[1]. Magnetic resonance imaging (MRI) is the most widely used neuroimaging technique to investigate brain changes and neurodegeneration. Several longitudinal studies using MRI have been conducted with positive results[2,3]. After follow-up assessments, these studies supported that MRI is a valuable biomarker to predict early conversion to dementia in patients with MCI. In particular, a maximum value of 1.61 for the occipital cortex N-acetylaspartate / creatine ratio was useful to predict dementia at 100% sensitivity and 75% specificity, yielding a positive predictive value of 83% and a negative predictive value of 100%[2]. The same ratio in the posteromedial bilateral parietal cortex could enable a predicted conversion rate to probable AD with a sensitivity of 74.1% and a specificity of 83.7%[3]. Furthermore, MRI is capable of measuring both regional and global brain atrophy, determining the extent of the brain degeneration in patients with dementia[4,5]. Based on this, several groups have proposed the rate of hippocampal atrophy in MCI patients as a predictor for the conversion to AD[6,7]. Other measurements by MRI, like cortical thickness, have been performed in amnestic MCI (aMCI) patients, which have the highest conversion rate to AD[8]. However, these measurements alone could not distinguish among aMCI subtypes and controls. White matter (WM) and grey matter (GM) have also been studied using MRI, which showed lower volumes of WM and GM in different brain regions in MCI patients who converted to AD[9]. A more sensitive to microscope WM changes technique is diffusion tension imaging (DTI). Nir *et al*. (2013) showed widespread diffusivity disruptions associated with neuropsychological and cognitive deficits in specific tracts that passed through the temporal lobe and posterior brain regions in AD and MCI patients[10]. Despite MRI is widely used for AD, the ac-

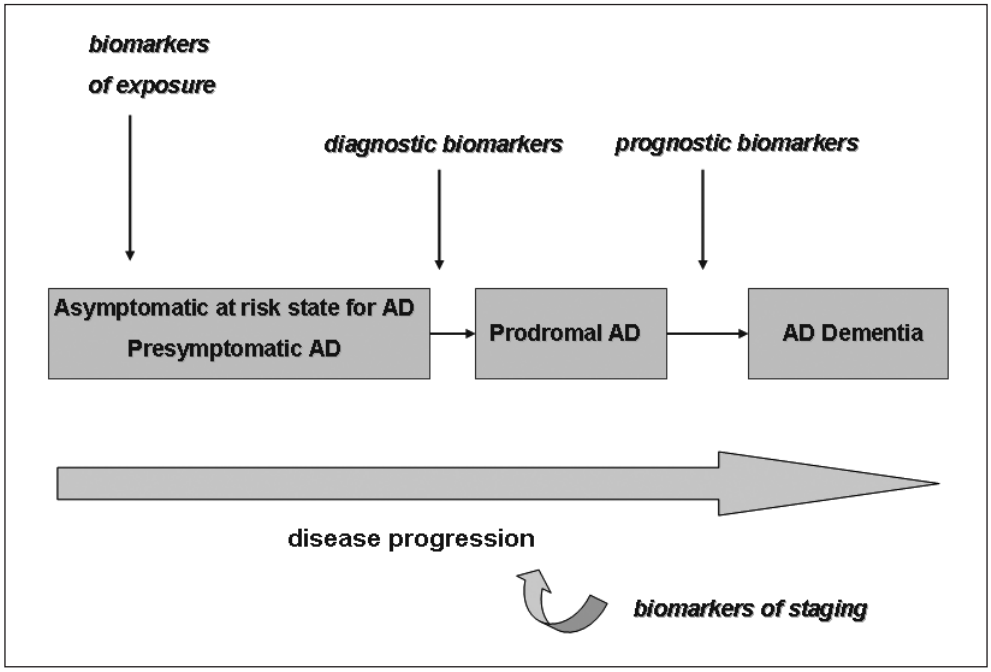

Figure 1. Overview of the main categories of biomarkers that can be identified along AD progression. Biomarkers of exposure can be indicative of disease risk factors, while biomarkers of disease are useful in the screening (prognostic biomarkers), in the early stages of the disease (diagnostic biomarkers) and in the monitoring of disease progression.

curacy of MRI as biomarker of early AD generally reaches an accuracy of 80%[11], what encourages the search of better biomarkers and more accurate diagnostic tools. Several studies demonstrated that the accuracy to predict conversion from MCI to AD in MRI was enhanced when combined with positron emission tomography (PET)[12,13], where the best brain region for MRI and PET was the temporal cortex[13].

Currently, PET is one of the most sensitive tests for and early AD detection comparing to other biomarkers, such as CSF measures of Aβ-42 or Aβ-42/tau[14,15]. However, its high cost hampers its regular clinical use. PET can determine the presence or activity of proteins, enzymes and metabolic pathways involved in dementia[16], and it has been used for prediction of progression from MCI to AD showing promising results. Particularly, ^{11}C-Pittsburgh compound B positron emission tomography (^{11}C-PIB-PET) may play a role in stratifying patients with MCI into risk levels for developing AD, yielding a 83.3% to 100% sensitivity and a 41.1% to 100% specificity for predicting conversion to AD[17]. In addition, in a recent study, fluorodeoxyglucose PET (FDG-PET) images were used to investigate MCI to AD conversion at different prodromal stages[18]. The results showed that MCI to AD conversion can be predicted as early as 24 months prior to conversion. Another molecular imaging technique suggested as a predictor of conversion to dementia is proton magnetic resonance spectroscopy (^{1}H-MRS)[19]. In particular, five metabolite ratios were calculated in four different brain regions in MCI patients, but only mI/CR ratio

Table 1
Relevant biomarkers of AD and MCI. The main biomarkers of AD, MCI and conversion to AD are schematized. Potential neuroimaging techniques that can improve the accuracy to predict conversion to AD are considered as well as potential CSF, urine, blood and genetic biomarkers of AD.

	Useful biomarkers and techniques for early detection of AD and MCI	Relevant findings	Predictive capacity for AD and MCI
Neuroimaging techniques	Magnetic resonance imaging (MRI)	regional and global brain atrophy	Predictive capacity suitable to determine the extent of brain degeneration in patients with dementia (4, 5)
		hippocampal atrophy	suitable to predict conversion to AD (6, 7)
		cortical thickness in amnestic MCI patients	suitable to predict conversion to AD (8)
		volumes of white and gray matter	suitable to predict conversion to AD (9)
		in combination with morphometric variables	suitable to predict conversion to AD (20, 22)
		support vector machines analysis to the whole gray matter	suitable to predict conversion to AD (22)
	Diffusion tension imaging (DTI)	white matter changes	associations with neuropsychological and cognitive deficits in temporal lobe and posterior brain regions in AD and MCI patiens (10)
	positron emission tomography (PET) and MRI	changes in temporal cortex	enhanced accuracy to predict conversion to AD (12,13, 16)
	11C-Pittsburgh compound B positron emission tomography (11C-PIB-PET)	beta-amyloid accumulation	suitable to predict conversion to AD (17)
	fluorodeoxyglucose PET (FDG-PET)	neuropsychological test scores	suitable to predict conversion to AD (24 months prior to conversion) (18)
	proton magnetic resonance spectroscopy (1H-MRS)	cortical area of right parietal lobe in MCI	suitable to predict conversion to AD (sensitivity 70% and specificity 85%) (19)
CSF biomarkers	amyloid β peptide 42 (Aβ-42)	decreased Aβ-42 levels in MCI patients at an early stage	suitable to predict conversion to AD (27)
		decreased Aβ-42 levels in AD patients compared to MCI and other dementias' patients	trustworthy marker for distinguishing among MCI, AD and other dementias (31, 32)
	tau protein	increased total and hyperphosphorilated tau (P-tau) at a later stage	suitable to predict conversion to AD (27, 29)
		Aβ-42/P-tau ratio	suitable to predict conversion to AD (sensitivity 88% and specificity 90%) (27)
		hyperphosphorilated tau (P-tau)	suitable diagnostic biomarker for progression of MCI, less adequate to distinguish AD from others dementias (33)
	NrCAM, YKL-40, chromogranin A, carnosinase I amyloid precursor protein, neuronal pentraxin receptor, NrCAM and chromogranin A	identified by mass spectrometry (LC-MS/MS) and re-evaluated by enzyme-linked immunosorbent assays (ELISA)	suitable to distinguish groups with mild, very mild cognitive impairment or no dementia (34)
		identified by multiple reaction monitoring (MRM) assay, a targeted-proteomic approach	potential disease progression markers (35)

Table 1
Relevant biomarkers of AD and MCI. The main biomarkers of AD, MCI and conversion to AD are schematized. Potential neuroimaging techniques that can improve the accuracy to predict conversion to AD are considered as well as potential CSF, urine, blood and genetic biomarkers of AD (continuation).

	Useful biomarkers and techniques for early detection of AD and MCI	Relevant findings	Predictive capacity for AD and MCI
Blood biomarkers	plasma amyloid β levels	decreased Aβ-42 / Aβ-40 ratio	risk factor to MCI conversion to AD (45)
		decreased levels of Aβ-42 and Aβ-40	associated with the risk of incident AD and dementia (51) and suitable in normal cognitive status conversion to MCI or AD (54, 55)
		associations between low plasma Aβ-42 levels and Aβ-42 / Aβ-40 ratio	associated with higher risk of incident AD and dementia (51)
		unaltered Aβ plasma levels	lack of correlation between plasma Aβ levels and plasma homocysteine (50) and Aβ-42 levels in CSF and plasma (57, 58)
		combination of plasma Aβ-42 and tau levels, monitored by immunomagnetic reduction assays (IMR)	suitable to predict conversion to AD (sensitivity 80% and specificity 82%) (59)
		plasma Aβ-42 levels and CSF p-tau181 / Aβ-42 ratio	strongest correlations in MCI patients (60)
		increased levels of Aβ-40 and Aβ-42	strong correlations with oxidized lipoprotein receptor-related protein-1 (sLRP), CSF tau / Aβ-42 ratios and reductions in MMSE scores, risk of MCI and AD (81)
	serum tau levels	detection of tau post-translational modifications (PTMs)	correlation with cognitive function (62)
	transcriptomic and lipidomic sequencing	detection of miRNA and serum biomarkers for inflammation, homocysteine and cholesterol metabolism and brain specific proteins	useful tool to predict MCI conversion to AD (45, 49, 50, 63-65, 70)
	calmodulin	increased calmodulin plasma levels	reliable biochemical marker in early stages of AD and in the discrimination from other types of dementia (sensibility 87% and specificity 82%) (66)
	phospholipase A2	decreased phospholipase A2 activity	risk marker for AD in subjects with MCI (67)
	sphingomyelins and ceramides	increased levels of Serum sphingomyelins and ceramides	useful in prediction of incident impairment in asymptomatic individuals (68)
		increased long plasma ceramides	predictive of cognitive decline and hippocampal volume loss (69)
	afamin and immunoglobulin heavy constant mu (IGHM)	identified by isobaric tag (iTRAQ) and proteomic methods, decreased afamin and increased IGHM levels	candidate biomarkers for AD and the predementia condition of MCI (73)
Genetic biomarkers	mutations in presenilins PSEN1 and PSEN2	genes for amyloid precursor protein (APP)	related to familial AD (prevalence around 0,1%) (45)
	ε4 allele of APOE gene	one ε4 allele triples the risk of AD, two ε4 alleles increase the risk 15 times	risk factor for AD (45)
	phospholipase D3 (PLD3)	doubles the risk for sporadic AD, an impairment of PLD3 function could lead to the aberrant APP processing	risk factor for sporadic AD (45)
	GGGGCC hexanucleotide repeat expansion intronic to chromosome 9 open reading frame	detected in two early-onset AD patients and in Caucasian families (>30 repeats)	involved in familial AD and other neurodegenerative diseaes (80, 81)

(myo-inositol/creatine ratio) in the cortical area of right parietal lobe in MCI subjects predicted the conversion to AD with sensitivity 70% and specificity 85%.

Recent studies suggest the combination of data for an accurate diagnose and prediction of dementia progression. A longitudinal study evaluated a multivariate method combining morphometric variables in patients with MCI, AD and controls[20]. This study suggested an index based on temporal evolution of brain degeneration using MRI, combined with other longitudinal information could be a reliable method to identify preclinical AD patients, as well as to predict conversion from MCI to AD. Other studies have supported the combination of data from neuroimaging to obtain more accurate diagnostic and predictive tools[21,22]. Particularly, a study combined data from neuroimaging for tracking dementia progression by proposing a method based on support vector machines. The results obtained showed a higher prediction for progression from MCI to AD, and a better differentiation between AD and healthy patients when considering the brain as a whole, rather than separate brain regions[22].

During the last decade, neuroimaging has been shown as a potential tool for diagnosis and prediction of AD; however most of the AD biomarkers described are measured in CSF. Although CSF biomarkers combined can optimally discriminate AD patients from controls, as well as prognosticate conversion from MCI to AD, they are not suitable for distinguishing AD from other dementias[23]. Over the past two decades, three CSF biomarkers have been widely studied: amyloid β peptide 42 (Aβ-42), total tau protein (T-tau) and hyperphosphorilated tau protein (P-tau), which have been shown to be reliable markers for early diagnosis of AD and prediction of disease progression[24,25].

Many studies have shown that AD pathophysiological processes are characterized by decreased amyloid concentrations, increased tau concentrations or increased tau/amyloid concentrations[16,26-28]. As an example, a 4-year follow-up study showed that all MCI patients with levels of the three CSF markers abnormally altered at baseline developed AD dementia within one year[26]. Interestingly, Buchhave *et al*. (2012) found decreased Aβ-42 levels in MCI patients at an early stage, before conversion to AD dementia. In contrast, levels of T-tau and P-tau were found increased at a later stage[27,29]. Moreover, Aβ-42/P-tau ratio predicted the conversion to AD dementia within 9.2 years with a sensitivity and specificity of 0.88 and 0.90, respectively[27]. Other studies have also determined Aβ-42 as an early marker, followed by tau proteins[28,30]. Aβ-42 has also been suggested as a trustworthy marker for distinguishing among MCI, AD and other dementias[31,32]. In this sense, lower CSF Aβ-42 levels were found in AD patients compared to those measured in MCI subjects and with other dementias. Regarding P-tau separately, a meta-analysis of 51 studies based on MCI and AD patients concluded that CSF P-tau levels were a precise diagnostic biomarker for MCI and AD, as well as for progression of MCI. Conversely, CSF P-tau levels were less adequate in distinguishing AD from other dementias[33].

In addition, accuracy of these CSF biomarkers can be improved when combining with other CSF proteins, as Perrin *et al.* (2011) demonstrated. After a CSF proteomic study, they suggested four novel CSF markers for MCI and AD (NrCAM, YKL-40, chromogranin A, carnosinase I) that enhanced diagnostic accuracy of Aβ-42 and P-tau for distinguishing groups with mild, very mild cognitive impairment or no dementia[34]. A recent study also identified changes in several CSF proteins between controls and AD subjects, and sug-

gested four proteins as potential progression biomarkers (amyloid precursor protein, neuronal pentraxin receptor, NrCAM and chromogranin A)[35].

Although the current CSF biomarkers can be considered accurate markers for diagnosis and prediction of AD (Table 1), there are still large variations in biomarker measurements between studies, and between and within laboratories[36]. Both CSF and neuroimaging biomarkers have been proved to have valuable diagnostic and prognostic capacity in AD. Nevertheless, the combination of them, together with cumulative information from clinical examination, could enhance their accuracy in detecting MCI and AD patients in early stages as well as the conversion to AD that will allow an effective therapy before the latest stage[37]. Hu *et al.* (2010) proposed the use of a multi-analyze profiling to identify novel candidate biomarkers, included in the RBM Human DiscoveryMAP™ panel, which could help to improve the accuracy of the established CSF markers[38]. The combination of CSF markers and cognitive tests, such as Mini-Mental State Examination (MMSE) and the clock drawing, has been also suggested, as it showed to be significantly better than these methods alone for prediction of conversion from MCI to AD[39]. Several studies assessed the predictive accuracy for the diagnosis and conversion to AD when analyzing CSF markers together with MRI and/or neuropsychological and functional measures (NMs)[40-43]. The results determined that combination of selected MRI, CSF and/or NM features outperformed a single modality of these features. Moreover, combination of CSF, imaging, genetic and cognitive markers and other methods has been performed to measure both the temporal evolution of AD[29] and predict more accurately the conversion from MCI to AD[44] (Table 1).

Although the established biomarkers of AD from CSF and neuroimaging are precise, their clinical application has limitations due to their invasiveness or high cost. This supports the need to find other easily available and accessible biomarkers that make a diagnostic of early AD possible, and therefore, the possibility to identify those susceptible individuals in order to apply an effective therapy before the onset of the disease.

The search for reliable blood-based biomarkers

The neurodegeneration presented in AD is mainly characterized by the deposition of senile plaques, composed of amyloid beta peptide and neurofibrillary tangles of hyperphosphorylated tau protein, especially in the hypocampus, amygdala and frontal cortex. Dealing with the search of putative biomarkers of AD, an AD prodromal phase, identified as MCI can be precisely the useful time-point to test reliable biomarkers that can enable an early and accurate prognosis and diagnosis of the disease. The complexity of the pathogenesis of AD, which is not fully understood to date, is caused by a synergy of risk factors and the combination of current well-characterized biomarkers, from neuroimaging, genetic to fluid biomarkers (Table 1), can improve prognostic and diagnostic accuracy[45].

Albeit neuroimaging tools have provided a reliable early detection and differentiation of AD, they still remain as a quite expensive diagnostic tool in many hospitals and clinical centers. Conversely, the detection of CSF, blood (plasma or serum) and urine biomarkers could be also useful for the disease characterization, even at preclinical stage. Regarding urine biomarkers, several biochemical markers have been described as potential bio-

markers for dementia, such as the level of urinary 3-hydroxypropyl mercapturic acid (3-HPMA)/creatinine (Cre)[46] and the level of urinary Alzheimer-associated neuronal thread protein (AD7c-NTP), which may be an important biomarker for an early diagnosis of MCI[47]. Particularly, decreased levels in 3-HPMA/Cre in urine correlated with increase in Aβ40/42 in plasma in demented subjects[46], while increased levels of AD7c-NTP were found in AD and MCI patients[47]. Nevertheless, the minimally invasive nature, possible follow-up of patients, low risk and cost and the possibility of screening healthy population make the blood the first choice to analyze putative biomarkers. In spite of the low sensitivity and specificity of blood biomarkers, the invasive sample collection by lumbar puncture, which is needed in the analysis of CSF samples, is less preferred than a wider range of blood-based biomarkers study to identify reliable biomarkers of prodromal AD and AD dementia[45,48]. Interestingly, blood-based proteomic search of biomarkers in heterogeneous populations of individuals supports an alteration of the blood proteome in AD patients[49].

Reliable biomarkers of prodromal AD and AD dementia are essential for early AD detection at preclinical stages. Since it is expected that the number of demented people will exponentially increased in the next years and taking into consideration the lack of an effective therapeutic approach for AD, the detection of these reliable biomarkers could enable the treatment of asymptomatic patients before the degeneration progresses severely. At this step, the standardization of blood-based biomarker studies for the improvement of diagnosis, treatment and care of AD patients has become the ultimate goal of the Standards for Alzheimer's Research in Blood biomarkers (STAR-B) working group[48].

The advantages of blood tissue in the search of reliable AD biomarkers compensate for the limited detection of potential biomarkers closely related to brain pathogenesis through the blood brain barrier and the inevitably dilution of brain derived proteins and metabolites in this tissue[50]. Among the biomarkers studied in blood (plasma or serum), Aβ is the most studied one, especially in plasma samples (Table 1). Abnormal production and aggregation of Aβ isoforms is one of the earliest pathophysiological hallmarks that take place in the brain, and they begin several decades before the onset of clinical symptoms, triggering synaptic loss, neuronal death and clinical dementia. As a consequence, the monitoring of the amyloid processes in asymptomatic subjects could be useful to select those at the prodromal stage that will progress to AD[51].

Although it remains under debate whether plasma Aβ levels could be considered a reliable biomarker because of the controversial published results, recent studies point out to a decreased Aβ-42 / Aβ-40 ratio as a risk factor to MCI conversion to AD, while other studies have suggested that increased Aβ-40 and Aβ-42 plasma levels, simultaneously or separately, could play a role as putative biomarkers of AD[45,50,52,53]. In particular, decreased levels of Aβ-42 or decreased Aβ-42 / Aβ-40 ratio in aging could indicate a conversion from a normal cognitive status to MCI or AD[54,55]. Similarly, unaltered Aβ plasma levels have also been found when comparing AD patients and control cases[50,56]. In addition, plasma Aβ levels have been tested in different studies of correlation. As an example, plasma Aβ levels did not correlate with CSF Aβ levels. A multiplex immunoassay analysis in two independent cohorts of patients showed unaltered levels of plasma Aβ in incipient AD and a lack of correlation of Aβ-42 levels in CSF and plasma[57,58]. Howe-

ver, positive although no significant correlations were found between plasma Aβ levels and plasma homocysteine[50]. More recently, the sensitivity and specificity of plasma Aβ-42 levels were significantly improved to 0.8 and 0.82 respectively, when combined plasma Aβ-42 and tau protein levels and monitored them with the use of immunomagnetic reduction assays (IMR). This ultrahigh sensitivity technology could make possible the low detection limits for amyloids and tau protein (1-10 pg/ml). This improvement in the detection of very low protein levels could enable not only the differentiation of healthy cases and AD patients, in both prodromal and dementia phases, but it also allowed the identification of the group of MCI cases due to AD[59].

One of the largest prospective studies of plasma Aβ levels and the risk of incident of AD disease and dementia is the Framingham Heart Study, which included more than two thousand participants with a long period of follow-up. The main results obtained in this study suggested that lower plasma Aβ-42 and Aβ-40 levels preceded and were associated with the risk of incident AD and dementia. In particular, low plasma Aβ-42 levels were associated with higher risk of incident AD and dementia, and significant associations between low plasma Aβ-42 levels and Aβ-42 / Aβ-40 ratio were also found associated with higher risk of incident AD and dementia[51]. In spite of the limitations of the study, this work reinforced the potential role of plasma Aβ levels as a useful biomarker for preclinical AD and dementia. In accordance with Framingham Heart Study, a previous longitudinal study based on 2,454 patient plasma samples from the Alzheimer's Disease Neuroimaging Initiative study found relatively strongest correlations between plasma Aβ-42 levels and CSF p-tau$_{181}$ / Aβ-42 ratio in MCI patients, pointing out to the use of plasma Aβ as a potential biomarker[60]. In relation to plasma Aβ levels, it has been also shown that an increase in oxidized lipoprotein receptor-related protein-1 (sLRP) could lead to elevated levels of Aβ-40 and Aβ-42, which re-entered the brain favoring the risk of MCI and AD. In fact, high levels of oxidize sLRP and free plasma Aβ-40 and Aβ-42 correlated significantly with CSF tau / Aβ-42 ratios and reductions in MMSE scores[61].

As above mentioned, other relevant biomarkers in blood samples are serum or plasma tau levels (Table 1). The main handicap in this case remains in the increased levels of tau in other pathologies, such as ischemic stroke or traumatic brain injury, while in AD or MCI, tau levels are difficult to detect. However, a recent and more sensitive immunoassay methodology can detect both normal and phosphorylated tau, suggesting that serum tau levels could be useful in the identification of AD[62]. Furthermore, recent studies support the potential nature of AD biomarker of tau since a fragment of tau in serum correlated with cognitive function in a small clinical study. In connection with this result, the existence of plasma post-translational modifications (PTMs), called neo-epitopes, are considered chemical modifications that can prompt proteomic diversity. Albeit protein PTMs can be reversible depending on the nature of modification, the identification and better understanding of PTMs are essential to study the cellular and molecular mechanisms involved under physiological or pathological conditions. Therefore, in relation to AD disease, PTM-based biomarker could provide useful information about the disease progression. The induction of neuronal death in AD, in which tau is truncated in a caspase dependent mechanism, can generate pathological truncated protein species, which could become staging biomarkers that could enable the monitoring of disease progression[62].

Novel blood-based biomarkers for AD (Table 1), such as circulating microRNA and

a wide variety of plasma proteins and lipids suggested by transcriptomic and lipidomic sequencing, support the use of peripheral blood for unbiased screening to detect significant preclinical alterations in AD patients, which are reflected in the periphery that can be easily and minimally invasive analyzed[49]. In this sense, the combination of several markers can significantly improve the diagnostic accuracy. Panels of serum biomarkers for inflammation, homocysteine and cholesterol metabolism and brain specific proteins have been evaluated, yielding high accuracy, close to 90%, to differentiate AD patients from control cases and they also provided a useful tool to predict MCI patients that later converted to AD patients[50]. As an example, some identified plasma biomarkers are α2-macroglobulin, complement factor H, homocysteine, cholesterol, E4 isoform of apolipoprotein E, F2-isoprostanes, Aβ autoantibodies, apolipoprotein A1, clusterin/apolipoprotein-J, isoprostane or glycogen synthase kinase (GSK-3β)[45,63-65]. Regarding calmodulin, this potential plasma biomarker was found significantly upregulated in MCI and AD patients with a sensitivity of 0.87 and a specificity of 0.82, suggesting its possible role in the identification of early stages in AD and in the discrimination from other types of dementia[66]. Reduced phospholipase A_2 activity has also been suggested as a risk marker for AD in subjects with MCI[67]. Similarly, sphingomyelins and ceramides could be predictive of memory impairment and they could be useful for the ongoing AD pathology and progression in asymptomatic cases[68]. Interestingly, very long plasma ceramides were found altered in MCI, and it has been suggested that they could predict memory and right hippocampal volume loss among subjects with MCI[69].

Moreover, 18 signaling proteins were identified in plasma with a 90% accuracy to distinguish patients who had MCI and progressed to AD 2-6 years later. These signaling proteins were related to changes in the periphery, the central nervous system or both that were relatively specific to AD and took place in the first stages of disease process[70]. Similar studies dealing with protein-based multiplex biomarker data from control and AD patients showed that serum protein-based biomarkers improved their diagnostic accuracy when they were combined with clinical information, such as age, sex, education and *APOE* status[71]. A more recent study have identified a panel of 10 plasma proteins associated with neuroimaging measures of the disease that can predict disease conversion from MCI to AD within a year of blood sampling[72]. Another recent study based on isobaric tag (iTRAQ) and proteomic methods, identified 30 plasma proteins, such as afamin and immunoglobulin heavy constant mu (IGHM), that could be potential biomarkers for MCI and AD[73].

The possibility to identify in blood an Alzheimer's biomarker phenotype could favor the diagnosis of early AD. The study not only of the serum or plasma proteome but also of the relationships among different signaling proteins and intercellular communication factors could pave the way to new approaches for the search of AD biomarkers suitable for an early prognosis and diagnosis of the disease, and therefore for novel therapeutic strategies.

Common insights in AD and other neurodegenerative diseases

Regarding other neurodegenerative disorders, there is an important clinical need to identify and establish accurate biomarkers for the classification of neurodegenerative dementias, including AD, Parkinson disease (PD), frontotemporal dementia (FTD) and Amyotrophic Lateral Sclerosis (ALS), in which several pathological pathways lead-

ing to neurodegeneration are overlapped. A frequent pathological characteristic implicated in these disorders is the accumulation and aggregation of abnormal or misfolded proteins (amyloid-β in AD, α-synuclein in PD, and TDP-43 in FTD and ALS)[74]. Moreover, common processes that modulate neurodegeneration also include aberrant regulation of apoptosis, uncontrolled activation of autophagy, mytochondria dysfunction and oxidative DNA damage[74].

Concerning genetics, the GGGGCC hexanucleotide repeat expansion intronic to chromosome 9 open reading frame 72 (*C9ORF72*) was first identified as a common genetic cause of ALS and FTD[75,76]. Nevertheless, recent studies about *C9ORF72* have shown this expansion involved in diverse molecular mechanisms in other dementias, especially in AD and PD, confirming the pathological interrelationship between these diseases[77-79]. In particular, two early-onset AD patients were found to harbour *C9ORF72* expansions in a study regarding FTD genes[80]. Another study concerning Caucasian families showed *C9ORF72* expansions (> 30 repeats) at a rate of 0,76% in AD cases versus zero in controls, supporting the notion that large *C9ORF72* expansions have a considerable role in neurodegenerative diseases including AD[81]. In contrast, the allele frequency of *C9ORF72* repeats was estimated in ALS, frontotemporal lobar degeneration (FTLD), AD and PD; however, this expansion was only commonly found in ALS and FTLD, but not in AD or PD[82]. In accordance to these results, no pathogenic expansions (< 30 repeats) of *C9ORF72* were found in either AD patients or controls[83]. In the same study, as regards PD, it was suggested the intermediate (≥ 7 repeats) repeat allele in *C9ORF72* as a risk factor for PD.

Despite the fact that *C9ORF72* pathological repeats are not frequently found in AD or PD, their presence in some cases supports the simultaneity of diverse clinical and pathological features between several neurodegenerative diseases. On the other hand, their low frequency in AD and PD, compared to it in ALS and FTD, could be a useful tool for making a more precise diagnosis between these dementias.

Given the pathological and clinical overlapping in diverse neurodegenerative disorders, it is crucial to find potential and trustworthy biomarkers that allow developing an accurate classifying method for an early diagnosis of neurodegenerative diseases, which could make an early treatment for patients possible. Regarding AD, the identified biomarkers obtained from CSF and neuroimaging have reached clinical applications and their significant limitations in the disease stage and dementia identification can be accurately improved when they are combined in tandem with blood-based biomarkers. Future studies based on improved methodological approaches will provide a better understanding of the neurodegenerative progression of the disease and they could undoubtedly promote more promising and effective therapeutic strategies for AD.

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Potential conflict of interests

The authors declare that they have no competing interests.

Abbreviations

Alzheimer-associated neuronal thread protein (AD7c-NTP); Alzheimer Disease (AD); amnestic MCI (aMCI); amyloid β peptide (A β); Amyotrophic Lateral Sclerosis (ALS); ^{11}C-Pittsburgh compound B positron emission tomography (^{11}C-PIB-PET); cerebrospinal fluid (CSF); diffusion tension imaging (DTI); fluorodeoxyglucose PET (FDG-PET); frontotemporal dementia (FTD); frontotemporal lobar degeneration (FTLD); glycogen synthase kinase (GSK-3β); grey matter (GM); hexanucleotide repeat expansion intronic to chromosome 9 open reading frame 72 (*C9ORF72*); 3-hydroxypropyl mercapturic acid (3-HPMA)/ creatinine (Cre); immunomagnetic reduction assays (IMR); isobaric tag (iTRAQ); magnetic resonance imaging (MRI); Mild Cognitive Impairment (MCI); Mini-Mental State Examination (MMSE); neuropsychological and functional measures (NMs); oxidized lipoprotein receptor-related protein-1 (sLRP); Parkinson Disease (PD); positron emission tomography (PET); post-translational modifications (PTMs); proton magnetic resonance spectroscopy (^{1}H-MRS); white matter (WM).

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