Introduction

Dementia affects about 50 million people worldwide, with a burden expected to tripli cate by the year 2050 (1). According to the WHO, dementia is a “major global health problem”, with a high social and economic impact (2). Different diseases can cause dementia, but Alzheimer’s disease (AD) is the most prevalent form and is characterized by short term memory loss and increasing neurocognitive impairment causing severe disability. It occurs more frequently in the elderly (over 65 years), but approximately 5–10% of patients show the first symptoms at a younger age.

A progressive neurodegenerative process takes place in AD, with a preclinical course that can last decades. At neuropathological level, it is characterized by progressive aggregation of the 42-amino-acid fragment of amyloid-beta (Aβ42) into extracellular plaques and accumulation of intraneuronal neurofibrillary tangles formed by hyperphosphorylated Tau protein. Emerging evidence shows involvement in the pathophysiological cascade of other molecular pathways as brain metabolic dysregulation, protein misfolding, microglia alteration and neuroinflammation, synaptic dysfunction and neurodegeneration with neuronal loss.

In the last years, the hope to find a cure for this disease has been frustrated by failure of several trials using molecules against beta-amyloid, thus raising the question of whether the peptide(s) is the right target (3).

One of the most important issues is about time and accuracy of diagnosis. As for any other disease, early diagnosis and exact definition of the type of neurodegenerative process are crucial factors. Traditionally, the diagnosis was obtained based on clinical symptoms, type of progression, neuroimaging results, cognitive deficit evaluation and hematological tests, aimed to rule out comorbid conditions. However, AD is a complex neurodegenerative process clinically heterogeneous and post-mortem re-evaluation of previous case series revealed that misdiagnosis is not a rare event. Moreover, the clinical picture can be misleading (4), so that a definite diagnosis requires neuropathological confirmation at autopsy.

The need for a biological marker that could increase the accuracy of the diagnosis has led to deep interest in the research for a biological signature of AD. The

Abstract: The significant advances in understanding the pathophysiology of Alzheimer’s disease (AD) has led to development of biomarkers that can trace the disease at the biological level since the very early phase. PET ligands and cerebrospinal fluid (CSF) core biomarkers were developed and validated, gradually becoming reference tools for a precision-medicine-based diagnosis and therapy. Besides CSF core biomarkers (total Tau, phosphorylated Tau and beta amyloid peptides) other CSF molecules have been investigated that could improve the detection of disease or can be used as a predictor of cognitive decline. However, the cost and invasiveness of these methods are major limitations for use as first-line tools. Blood-based biomarkers can greatly improve the access to the detection of at-risk populations and evaluation of possible biological benefit of treatment over time. The new highly sensitive assays that have been recently developed can make this target feasible opening a new scenario in dementia prevention and therapy.

Keywords: Alzheimer’s disease (AD); biomarkers; early diagnosis; humans; precision medicine

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initial focus was on the Tau protein and beta-amyloid peptides in cerebrospinal fluid (CSF). Over the last years, radioligands for PET imaging were developed along with diagnostic assays for quantification of these peptides in CSF. The use of biomarkers gradually spread from research to clinical practice (5-7) for AD diagnosis, even in the preclinical stage (8), and to sub-classify the type of cognitive decline (9).

However, CSF sampling represents an obstacle to the large use of the biological characterization of the disease. In addition, at least from a theoretical point of view, biomarkers should help clinicians not just for diagnosis, but for other purposes as population screening, prognostic evaluation, the definition of the biological progression of disease and quantification of efficacy after treatment. In this perspective, it would be extremely useful to perform the assays on other biofluids instead of CSF (e.g., saliva or blood).

The present review will focus only on biomarkers of AD, but the research is extremely active in evaluating other biomarkers that can help the differential diagnosis between neurodegenerative diseases (Parkinson’s disease, frontotemporal dementia, Creutzfeldt-Jacob disease, etc.).

Biomarkers for AD in the CSF

Extracellular space of the brain is in close contact with the CSF, and thus modifications of physiology of the brain tissue can be seen in CSF which is used in several neurological situations requiring biological definition. Since AD pathology is brain-specific, CSF is an obvious biological sample where biomarkers for AD could be investigated.

Beta-amyloid accumulation and neurofibrillary tangles of Tau protein are the two main and well known neuropathologic characteristics of AD. During the past 25 years, three core CSF biomarkers have been defined and validated internationally as diagnostic tools for AD using enzyme-linked immunosorbent assay (ELISAs): the β-amyloid peptide 1-42 (Aβ42), total tau (T-Tau) and phosphorylated TAU protein at position 181 (P-Tau). Combining these three CSF biomarkers the diagnostic validity for AD significantly increases, reaching a combined sensitivity and specificity of 85–90%, even in subjects at the very early stage of disease (defined as mild cognitive impairment) (10). AD patients have decreased concentrations of Aβ42 in CSF compared to normal controls, whereas T-Tau and P-Tau are increased. Olsson et al. (11), in a meta-analysis comprising 15,699 patients with AD and 13018 controls, have calculated that the core biomarkers can differentiate AD from normal controls very efficiently: CSF T-tau (average ratio, 2.54; 95% CI, 2.44–2.64; P<0.0001), P-tau (average ratio, 1.88; 95% CI, 1.79–1.97; P<0.0001), and Aβ42 (average ratio, 0.56; 95% CI, 0.55–0.58; P<0.0001). A strong discrimination between cohorts with mild cognitive impairment due to AD and those with stable mild cognitive impairment was also shown (average ratio 0.67 for CSF Aβ42, 1.72 for P-tau, and 1.76 for T-tau).

The decrease of Aβ42 is thought to reflect the sequestration of beta-amyloid in senile plaques in the brain. Studies on neuropathological specimens after autopsy (12) or after biopsy (13) and in vivo evaluation by brain amyloid PET imaging (14) found an inverse association between the CSF Aβ42 levels and the brain amyloid load. Other different-length β amyloid peptides are present in the CSF that can be quantified and become useful for other reasons. Aβ40, which is the most abundant form present in CSF, does not change in concentration in AD and is not associated with any pathological hallmark, but can compensate spurious variations of Aβ42 determinations. The Aβ42/Aβ40 ratio shows a better discriminant value as compared to Aβ42 alone (15,16). In general, the Aβ42/Aβ40 ratio can be seen as an indicator of beta-amyloid protein misfolding and accumulation.

Tau protein serves as a bridge from microtubules to plasma membrane for facilitating formation and stabilization of microtubule network. It is mainly expressed in neurons as a 758 amino acids protein, but multiple isoforms exist, ranging between 316 to 758 aa in length. Microtubules are involved in maintaining the cell shape and are important for axonal transport. Total Tau concentration is increased in CSF as expression of neuronal or axonal damage. It is increased in AD patients, but also other conditions as Creutzfeldt-Jacob disease (CJD) (17), brain trauma or stroke (18). Therefore, T-Tau is not AD-specific, but it is an expression of brain degeneration or damage. On the other hand, P-Tau, which indicates hyperphosphorylated Tau protein, consists of multiple protein isomers. An increased concentration of CSF P-Tau is correlated with the formation of neurofibrillary tangles in the brain (19,20).

The combination of core CSF biomarkers are accepted as biological predictors of MCI conversion and allow defining the disease according to the new AT(N) classification of AD (9), where the Aβ42 levels define the “A” (amyloid) for plaque pathology, the P-Tau concentration defines the “T” (tangle pathology) and the “N” (neurodegeneration) is
associated with the T-tau CSF levels.

From the methodological point of view, Aβ42 can be measured by antibody-dependent techniques such as the widely used ELISA, as well as antibody-independent techniques such as mass spectrometry (MS) (21). The use of core biomarkers in the clinical setting has been hindered by high measurement variability. Both preanalytical handling of samples and analytical problems contribute to high intralaboratory and interlaboratory imprecision (22,23). To reduce this problem of manual immunoassays, multiplex assays or (semi)automated platforms are being developed (24-26), that are commercially available.

Axonal degeneration is evaluated not only by T-tau quantification but also by levels of CSF neurofilament light chain (NF-L) (27). NF-L is a neuronal cytoplasmic protein highly expressed in large caliber myelinated axons. Its levels increase in CSF proportionally to the degree of axonal damage in a variety of neurological disorders, including inflammatory, neurodegenerative, traumatic and cerebrovascular diseases. CSF NF-L concentration, quantified by ELISA, is increased in AD, especially when the disease is rapidly progressing (28), but other dementia conditions show even higher levels such as Frontotemporal Dementia (FTD) and vascular dementia (29). Since NF-L is not disease-specific, is not used in the differential diagnosis of AD, but could be a potentially useful biomarker to quantify treatment efficacy.

Synaptic degeneration is an early event in AD, that can be observed long before symptom onset (30,31), thus making synaptic biomarkers relevant for enabling early diagnosis. Furthermore, neuropathological studies have shown that synaptic pathology appears to be more related to cognitive dysfunction than presence of plaques and tangles (32,33).

Neurogranin (NGRN) is a protein secreted by neuronal cells that is highly expressed in the cortex, hippocampus, and amygdala, and it is particularly concentrated at the dendritic spines (34). NGRN can be quantified in CSF (35), and its levels are increased in AD (36), more recent data show higher CSF NGRN levels in AD and MCI patients compared to cognitively unimpaired elderly subjects (37-40). Other studies suggest that increased CSF concentrations may be specific for AD (41-43).

In a prospective study, Tarawneh et al. (44) analyzed the possible correlations between baseline CSF NGRN levels and future cognitive decline in patients with symptomatic AD and cognitively normal controls over time. The population study comprised 302 individuals, 95 patients with AD and 207 controls. The CSF NGRN levels differentiated patients with early symptomatic AD from controls with comparable diagnostic utility to the other CSF biomarkers [mean area under the ROC curve, 0.71 (0.03); 95% CI, 0.64–0.77]. The levels of NGRN in CSF correlated with brain atrophy in AD, and with amyloid load in preclinical AD. The CSF neurogranin levels predicted future cognitive impairment in controls and rates of cognitive decline in patients with symptomatic AD over time. In a recent metanalysis collecting 16 studies (45), CSF NGRN is confirmed as a good marker for AD and could be associated to other existing biomarkers to comprise a more accurate diagnostic a prognostic biological panel.

Microglial activation. Neuroinflammation is a common pathophysiological mechanism in neurodegenerative diseases. Multiple microglial functions participate in AD and other neurodegenerative diseases (46). The TREM2 protein or Triggering Receptor Expressed on Myeloid cells 2 is a receptor of the innate immune system expressed on the surface of microglia, which is involved in regulating phagocytosis, removal of apoptotic neurons and inhibition of proinflammatory response (47,48). In humans the presence of rare homozygous loss-of-function mutations in TREM2 gene cause a severe form of dementia (FTD-like) associated with bone cystic lesions known as Nasu-Hakola disease (49,50). Heterozygous missense mutations have been recently described to significantly increase the risk of AD, as well as that of other neurodegenerative diseases (FTD; Parkinson’s disease), with an odds ratio similar to that of carrying an APOE ε4 allele (51,52). Besides, heterozygous TREM2 mutation carriers display increased density of amyloid plaques and neurofibrillary tangles and show upregulated proinflammatory cytokine levels and downregulated protective markers (53). The TREM2 receptor undergoes proteolytic processing, releasing its ectodomain into the extracellular space as a soluble variant (sTREM2) that can be quantified in human plasma and CSF (54,55). Concentrations of sTREM2 in CSF are found increased in the early symptomatic stages of AD (56-58).

CSF YKL-40 (also known as CHI3L1, Hcgp-39) has recently been proposed as a neuroinflammatory biomarker (59). YKL-40 that is found in CSF is mostly produced in reactive astrocytes and its concentrations are increased in AD and healthy subjects during late middle age (60-62). Previous cross-sectional analyses have not detected differences in CSF YKL-40 levels between APOE ε4 carriers and noncarriers (61-64). However, in a prospective study in middle-aged subjects with normal cognitive
condition a steeper increase of CSF YKL-40 level was observed associated with aging in APOE ε4 carriers relative to noncarriers (62).

In conclusion, recent data show that the combined use of CSF biomarkers could be used as risk predictor. In a cohort of 770 individuals with normal cognition, mild cognitive impairment and AD, Bos et al. (65) investigated the association of CSF NGRN, NF-L, YKL-40, and T-tau with Aβ42 status (Aβ− vs. Aβ+), clinical diagnosis, presence of APOE ε4, baseline cognition and change in cognition over time. They found that NGRN and T-tau distinguished between Aβ+ from Aβ− individuals in each clinical group, whereas NF-L and YKL-40 were associated with Aβ+ in nondemented individuals only. APOE ε4 carrierness did not influence NF-L, NGRN, and YKL-40 in Aβ+ individuals. NF-L was the best predictor of cognitive decline in Aβ+ individuals across the cognitive spectrum.

**Blood biomarkers**

The abovementioned progress in PET and CSF biomarker analyses have the potential to increase the precision of the diagnostic and prognostic process for AD. On the other hand, these methods have substantial limitations that exclude their use as first-line diagnostic tools.

These problems could be solved by the use of blood-based biomarkers (66), but the identification of potential blood-based biomarkers for CNS diseases presents several challenges. Blood is a more complex system compared with CSF, molecules from CNS cannot freely cross the blood-brain barrier and evidence for peripheral manifestations of AD is limited (67). In addition, concentrations of CSF biomarkers are much lower in blood and comorbidity (e.g., liver disease) can act as a confounder, by affecting protein profiles or biomarkers levels. Nevertheless, a great effort in finding a possible peripheral biomarker has been made and in 2016 the Alzheimer’s Precision Medicine Initiative (APMI) gathered an interdisciplinary expert working group to evaluate the work done and the possible solutions.

Among the conventional AD biomarkers, the Aβ42/Aβ40 ratio was recognized as a potential screening or diagnostic index. Most early studies on plasma Aβ42, Aβ40 and Aβ42/Aβ40 ratio, using ELISA methods, found no differences or only minor differences between AD and control groups (68–70). In 2016, Janelidze et al. (71), studied a cohort of 719 individuals using SIMOA (Single-Molecule Array), a novel ultrasensitive immunoassay technique. They found weak, but significant correlations between plasma and CSF levels for both Aβ42 and Aβ40, and negative correlations between plasma Aβ42 and neocortical amyloid load obtained by amyloid PET imaging. Using a different fully automated immunoassay (Elecys, Roche Diagnostic) on a large cohort, Palmqvist et al. (72) recently showed that plasma Aβ42 and Aβ40 can predict Aβ status with an AUC of 0.80 (95% CI, 0.77–0.83). When adding APOE status, the AUC increased significantly to 0.85 (95% CI, 0.82–0.88). Only modest improvements were seen if Aβ42, Aβ40 and APOE were considered together with plasma Tau (AUC, 0.86; 95% CI, 0.83–0.88) or Tau and NF-L (AUC, 0.87; 95% CI, 0.84–0.89).

An alternative very sensitive technique for detecting Aβ peptides in plasma involves immunoprecipitation and mass spectrometry (73). Nakamura and colleagues (74) evaluated amyloid precursor protein (APP)669–711/ Aβ42 and Aβ40/ Aβ42 ratios and their composites, for predicting individual brain amyloid-β or amyloid-β status determined by amyloid-PET imaging and tested using two independent data sets. Both data sets (373 subjects, overall) included cognitively normal individuals, individuals with mild cognitive impairment and individuals with AD. The composite biomarker exhibited very high AUCs in both data sets (discovery, 0.967, n=121 and validation, 0.941, n=111) with an accuracy approximately equal to 90% using amyloid PET with PIB tracer as a standard.

More recently, Schindler et al. (75) evaluated 158 mostly cognitively normal individuals using immunoprecipitation and liquid chromatography-MS, observing that plasma Aβ42/Aβ40 had high correspondence with amyloid PET status and CSF p-tau181/Aβ42. The combination of plasma Aβ42/Aβ40, age, and APOE ε4 status had very high correspondence with amyloid PET (AUC 0.94, 95% CI, 0.90–0.97). Individuals with a negative amyloid PET scan at baseline and positive plasma Aβ42/Aβ40 ratio (<0.1218) had a 15-fold greater risk of conversion to amyloid PET-positive compared to individuals with a negative ratio.

Other plasma biomarkers include axonal protein neurofilament light (NF-L). Plasma levels of NF-L can be quantified using the ultrasensitive Simoa technique and they are positively associated with CSF concentrations suggesting that serum levels reflect CNS pathophysiology (76). A study on familial AD (FAD) showed that plasma NF-L levels were increased not only in patients with symptomatic FAD, but also in pre-symptomatic mutation carriers, with the levels that correlated with the estimated time of onset of the disease (77).
Patients with AD from the ADNI cohort show a marked increase of plasma NF-L with an AUC value of 0.87, whilst MCI subjects with positive amyloid PET scans have the highest plasma NF-L levels (78). High NF-L plasma levels predicted faster cognitive decline and faster rate of brain atrophy. In a subsequent recent longitudinal study on 1,583 subjects (comprising cognitive normal individuals, MCI and AD) from the ADNI cohort, Mattsson et al. found that blood levels of the NF-L rose over time, and the rate of rising paralleled established cerebrospinal fluid and imaging markers [AT(N) classification] and advancing cognitive decline (79). The work, the first to follow such a large group of people with the common, late-onset form of AD, strengthens the case for blood NF-L as a noninvasive biomarker associated with neurodegeneration in patients with AD and may be useful to monitor effects in trials of disease-modifying drugs.

However, high plasma NF-L is not specific for AD, but is found in several different disorders of CNS including HIV, FTD, progressive supranuclear palsy and cortico-basal syndrome.

Other plasma biomarkers with less clear clinical usefulness are Tau (80) and BACE1 levels (81). Additional research will tell us whether they will be confirmed or dismissed.

**Conclusions**

Substantial progress in search for a biological characterization of AD has been made over the last decade, shifting the diagnosis toward a precision medicine concept that is crucial for the field of neurodegenerative diseases.

Some CSF biomarkers can be considered now as routine clinical tools, which contribute to obtaining a more accurate diagnosis in a very early phase of disease. The research on blood-based biomarkers in AD and other neurodegenerative diseases is now changing the scenario. New technical advances, such as MS quantification or automated ultrasensitive immunoassay, can detect with high accuracy specific protein profiles. Besides, the use of blood instead of CSF makes possible to have a cost-effective procedure that can be used for screening and diagnostic purpose. The development and implementation of a multistep diagnostic approach, starting with a blood test in primary care, will perhaps facilitate access to confirmatory, more expensive, diagnostic test (CSF sampling, PET imaging) and provide a path for substantial improvement in diagnosis and treatment of AD.

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**Footnote**

**Conflicts of Interest:** C.G. reports being a consultant for Roche Diagnostics.

**Ethical Statement:** The author is accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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