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Review Article

Biomarkers of Alzheimer's Disease: From Central Nervous System to Periphery?

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Alzheimer's Disease (AD) is the most frequent form of dementia and represents one of the main causes of disability among older subjects. Up to now, the diagnosis of AD has been made according to clinical criteria. However, the use of such criteria does not allow an early diagnosis, as pathological alterations may be apparent many years before the clear-cut clinical picture. An early diagnosis is even more valuable to develop new treatments, potentially interfering with the pathogenetic process. During the last decade, several neuroimaging and cerebrospinal fluid (CSF) parameters have been introduced to allow an early and accurate detection of AD patients, and, recently, they have been included among research criteria for AD diagnosis. However, their use in clinical practice suffers from limitations both in accuracy and availability. The increasing amount of knowledge about peripheral biomarkers will possibly allow the future identification of reliable and easily available diagnostic tests.

1. Introduction

Autopsy data show that neuropathological features of AD are associated with subtle cognitive changes among nondemented subjects, thus suggesting the presence of a "preclinical Alzheimer's disease" [1, 2]. Moreover "*in vivo*" data based on amyloid PET ligands suggest that accumulation of neuropathologic damage lasts about 20 years before clear-cut clinical manifestations of the disease [3].

Currently, the diagnosis of AD is made according to clinical criteria by the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS)-Alzheimer's Disease and Related Disorders Association (ADRDA) [4], which have limitations in terms of sensitivity and specificity and, above all, do not allow an early diagnosis of the disease.

In the last few years an extensive search for sensitive and specific biochemical and neuroradiological biomarkers of AD has been performed. Such markers would also help to better identify Mild Cognitive Impairment (MCI) patients at higher risk of conversion to AD. Based on the results of such studies, research criteria for AD diagnosis have been proposed which include neuroimaging and cerebrospinal fluid (CSF) parameters [5].

The main aim of such efforts is to diagnose AD when neurological damage might still be reversible. This would be highly valuable in the research setting, as treatments, which are currently under study to potentially interfere with the pathogenetic process of the disease, will probably show their full efficacy only if administered during the prodromal, or even preclinical, phase of AD. Consistently with this idea, the first immunization trial against beta-amyloid in full-blown AD patients showed that, although the treatment was effective in reducing neuropathological changes, it was not able to modify the clinical course of the disease [6].

Therefore, while subtle cognitive changes are highly prevalent among older subjects [7] and neuroradiological and CSF biomarkers, as discussed below, are not optimal diagnostic tools in term of availability, accuracy, and invasiveness (as in the case of lumbar puncture), the identification of reliable and easily accessible peripheral biomarkers will be of great interest in the setting of AD.

2. Neuroradiological Markers

Among neuroimaging parameters, the presence of atrophy, detected with MRI in specific areas of the brain, has been proposed as an early manifestation of AD [8]. Indeed, a good correlation seems to exist between hippocampus atrophy and the extension of neuropathological lesions in AD brains at autopsy [9]. It has been reported also that a visual determination of medial temporal lobe atrophy is a reliable instrument to discriminate AD and MCI from normal controls and to predict AD conversion in MCI patients [10]. On the other hand, other studies have reported that cross-sectional measurements of brain atrophy have limited diagnostic accuracy among older subjects [11]. This is consistent with data that show an overlap between AD- and ageing-associated atrophy in hippocampus and entorhinal cortex [12].

Among nuclear medicine techniques, the evaluation of cerebral blood flow by SPECT (Single Photon Emission Computed Tomography) has a well-established sensitivity in identifying AD, showing hypoperfusion of temporoparietal regions and posterior cingulus [13]. Brain metabolism evaluation by PET (Positron Emission Tomography) with fluor-deoxyglucose has shown an even greater sensitivity and a greater spatial resolution, allowing the study of smaller areas of the brain, such as the hippocampus, of great clinical interest in AD [14]. Several studies have demonstrated that PET has a sensitivity of more than 90%, even in the early phases of the disease, suggesting that it may be able to differentiate AD from age-related cognitive impairment [15]. Moreover, PET proved to be a reliable tool for the identification of MCI patients bound to become AD, with a diagnostic accuracy which has been proposed to be better than SPECT and MRI in a recent meta-analysis [16]. On the other hand, a recent study found no evidence that FDG-PET is more sensitive than MRI to quantify the degeneration present in preclinical and mild AD, in specific brain regions [17]. The authors of this paper suggest that hippocampal volume, measured by MRI, is probably the best trade-off between accuracy and convenience for detection of early AD.

In the last few years some "*in vivo*" PET ligands for AD lesions have been identified, such as [(11)C]PIB (Pittsburgh Compound B) that binds specifically beta-amyloid, and [(18)F]FDDNP (2-(1-{6-[(2-[F-18]fluoroethyl) (methyl) amino]-2-naphthyl} ethylidene)malononitrile) that binds both neurofibrillary tangles and beta-amyloid plaques. Their ability to differentiate AD patients from control subjects has been demonstrated [18, 19]. Longitudinal studies have established that the pathological changes identified with these molecules may occur in preclinical stages of the disease and may be detected earlier than atrophic changes and hypometabolism recognized by FDG-PET [20]. On the other hand, PIB deposition has been shown in about 20% of normal elderly subjects as well [21] and seems to proceed at the same rate both in cognitively intact and in cognitively impaired subjects [22]. These data were confirmed in a Japanese study, which found no difference in PIB retention pattern among very mild, mild, and moderate AD. An AD-like pattern of PIB deposition was also found in 48% of MCI and 18% of healthy control subjects [23]. Nevertheless, higher PIB binding has been related to progression to very mild dementia, independently of age, in 23 out of 159 not cognitively impaired subjects, confirming that PIB retention must not be considered a benign process [24]. Moreover, both PIB and FDDNP retention have been found to correlate with different cognitive domains in AD, MCI, and cognitively normal subjects. In particular an increased FDDNP binding was specifically associated with episodic memory impairment, while increased PIB retention was associated with a broader range of cognitive impairment [25].

3. CSF Biomarkers

CSF biomolecular markers of AD have been extensively investigated in recent years. Among them, an increased concentration of total and hyperphosphorylated tau protein and a reduction of amyloid β peptide Aβ42 have been reported in CSF of AD patients [26], with the combination of the two markers capable of further improving the diagnostic accuracy to a sensitivity and specificity of nearly 90% [27]. CSF markers accurately predict the risk of AD conversion in MCI patients, after a 5-year follow-up [28, 29]. Other authors have shown a strong relation between CSF Aβ42 and PET with 11C-PIB during prodromal and early phases of AD, demonstrating that CSF measurements actually parallel the neuropathological changes that occur inside the brain [30].

Despite their diagnostic accuracy, determination of CSF Aβ42 and tau levels has several limitations: circadian variability of their concentration [31], lack of standardization (use of different techniques or different protocols among different laboratories) [26], inadequacy of these markers to accurately discriminate between AD and other types of dementia, such as vascular and Lewy body dementia [27]. Moreover laboratories able to conduct such determinations are not widespread, and lumbar puncture is a relatively invasive procedure. A large-scale multicenter study, aimed at evaluating CSF Aβ42 and total and hyperphosphorylated tau-protein as predictors of AD in MCI patients, found that these CSF biomarkers are able to identify incipient AD with satisfactory accuracy (sensitivity 83%; specificity 72%; negative predictive value 88%; positive predictive value 62%), but with inferior power than what is reported by single-center studies, because of a great intersite assay variability [32]. The accompanying editorial suggests that the use of CSF markers, although advised in a research setting, should not yet be included in clinical practice [33].

Other CSF surrogate markers of brain amyloid deposition have been proposed. Like Amyloid Precursor-Protein (APP), Amyloid Precursor like Protein (APLP) undergoes a metabolic processing by secretases. Higher levels of APLP-1-derived peptides have been identified in CSF of AD patients, both in familiar and in sporadic forms [34].

Moreover, lower levels of Sortilin-related receptor (SORL1, also known as SorLA or LR11) have been identified in CSF of AD patients compared with cognitively normal controls and have been proposed as a diagnostic biomarker

for AD [35]. During the last few years, SORL1 has been identified as a facilitative factor of intraneuronal APP redistribution inside the Golgi, increasing its processing in the nonamyloidogenic pathway, while SORL1 deficit has been associated to an increased production of Aβ fragment [36]. In neuropathologic AD samples a reduction of SORL1 protein, compared to controls, was observed [37], that was inversely related to amyloid plaques and neurofibrillary tangles [38].

Finally, some studies have focused on products of oxidative stress to discriminate AD from control subjects (see also below): higher CSF and plasma isoprostanes level may represent a marker of oxidative damage in AD [39] and MCI [40] subjects, compared to controls. Other studies have identified lower level of antioxidants, in particular superoxide dismutase (SOD), in CSF of subjects with neurodegenerative diseases, including AD [41].

4. Peripheral Biomarkers

Lately, several Authors have directed their efforts in identifying AD biomarkers in plasma or serum, but the results are still inconclusive. A longitudinal study has found that high plasma levels of Aβ42 were associated with an increased risk of developing AD in subjects without dementia. Besides, conversion to AD was associated with a decrease of plasma Aβ42 levels and of plasma Aβ42/Aβ40 ratio [42]. This biphasic trend might be interpreted as follows: higher levels of Aβ42 are linked to an increased risk, while its subsequent decline might reflect compartmentalization of the peptide in the brain. This interpretation is consistent with a recent study that found an increased risk of dementia after 5 years in subjects with high plasma levels of Aβ42 [43]. On the other hand, a previous study found an increased risk of transition from cognitive normality to MCI or dementia in subjects with low plasma Aβ42/Aβ40 ratio [44]. Moreover, a longitudinal study showed that low plasma levels of Aβ42 and Aβ42 in AD patients were significantly associated with a more rapid functional and cognitive decline [45].

Other putative plasma biomarkers include molecules involved in the inflammatory response. In particular, higher level of soluble CD40 (sCD40) is found in plasma of AD patients compared with age-matched controls [46] and is able to predict the risk of conversion to AD in a sample of MCI patients [47]. Moreover, the expression of CD40 cognate ligand, CD40L, is upregulated in AD patients and is associated with an increased cognitive decline over the following 2 years [48]. These data are consistent with autopsy and animal studies that show an enhanced expression of CD40 and CD40L on astrocytes of AD brains [49].

In agreement with the involvement of inflammation in the pathogenesis of AD, recently an algorithm based on the values of several serum proteins, many of whom are related to inflammation, demonstrated 80% sensitivity and 91% specificity in discriminating AD from controls; the addition of gender, age, education, and ApoE status to the prediction algorithm increased sensitivity and specificity to 94% and 84%, respectively [50]. In a previous study with a similar approach a different set of plasma signaling proteins was identified, which was able to correctly differentiate AD from controls (cognitively normal and other dementia) with 90% sensitivity and 88% specificity; moreover the same algorithm showed 91% sensitivity and 72% specificity in predicting AD development in a small sample of MCI subjects after 2–6 years [51]. Interestingly, the panel of 18 proteins which allowed dementia prediction in the cited study were involved in hematopoiesis and inflammation, leading the authors to hypothesize an impairment in macrophage function in AD subjects, possibly related to a decreased Aβ clearance from brain [52].

Other authors have studied the influence of Brain-Derived Neurotrophic Factor (BDNF), a potential neuroprotective agent, on neuron survival and function, and found that its level is significantly higher in serum of AD and MCI patients, compared with healthy subjects, independently of disease severity, treatment with antidepressant or cholinesterase inhibitors [53]. These data are partially consistent with another study showing an increase of BDNF concentration in mild AD, compared with controls (the result being interpreted as a compensatory mechanism), with a subsequent decline in later stages of the disease [54]. On the contrary, another research has found decreased BDNF levels in MCI subjects, compared with cognitively normal controls [55].

A different approach is the search for possible AD biomarkers in peripheral cells, based on the hypothesis that modifications of signal transduction, oxidative metabolism or APP metabolism that are present in neurons, may be found in peripheral tissues as well [56].

Peripheral tissues would constitute an easier model to study the pathogenesis of AD and to identify biomarkers of the disease. Until now, several peripheral tissues have been employed in AD research, including peripheral blood mononuclear cells (PBMCs), platelets, and fibroblasts. Each model has advantages and disadvantages, with fibroblasts being particularly useful, due to high stability under physiological and pharmacological stress [57]. Unlike PBMCs and platelets, fibroblasts behavior becomes independent from circulating molecules as soon as the cells are propagated *in vitro*. On the other hand, fibroblasts will age in culture, making it more difficult to interpret the results obtained with this model. Moreover, PBMCs and platelets are more accessible and may be a better model when techniques such as cytofluorimetry are employed.

One possible peripheral cell AD biomarker is represented by the PKC intracellular signaling system. In cerebral tissues of AD patients, PKC protein level, activity, and intracellular translocation are altered compared to control brain tissues [58]. In fibroblasts of AD patients, a reduced PKC activity has been described [59]. Moreover, inflammatory stimuli, such as bradykinin (BK), determine in fibroblasts of AD patients a PKC-mediated phosphorylation of extracellular signal-regulated kinases (ERKs) 1/2, which is not detected in fibroblasts of age-matched healthy controls [60]. A phospho-ERK1/phospho-ERK2 index, before and after BK stimulation, has been proposed as AD biomarker, being able to discriminate not only between AD and healthy subjects,

but also between AD and non-AD dementia [61]. This index was validated in a sample which included autopsy-confirmed cases, demonstrating higher sensitivity and specificity for diagnosing AD compared with clinical criteria, especially within the first 4 years from the onset of the disease [62]. Adding to the validity of this result, another study has observed increased levels of phosphorylated ERK1/2 in CSF of patients with neurodegenerative conditions (AD, frontotemporal dementia, and MCI), suggesting that these kinases are released into CSF in parallel with tau and phospho-tau proteins [63].

In a different research line, a conformational modification of p53 protein, associated with an alteration of its transcriptional activity, has been described in skin fibroblasts isolated from AD patients. This protein misfolding, which can be induced in non-AD fibroblasts by low concentrations of Aβ peptide [64], results in an increased resistance of the cells to p53-mediated apoptosis; therefore, its involvement in the early phases of amyloid deposition has been hypothesized and its possible use as a biomarker of early AD proposed [65]. The same authors have developed a cytofluorimetric test on PBMC that quantitatively evaluates the amount of altered p53 present within the cell. Such test has a sensitivity and a specificity comparable to routine CSF biomarkers in identifying AD, but only in patients under 70 years of age. In 70+ subjects, the amount of conformationally altered p53 increases, independently of the presence of AD; however, older AD patients still display increased amount of altered p53 compared to age-matched healthy controls [66]. Moreover the same mutant form of p53 was found to predict MCI conversion to AD after two years with good specificity and satisfactory sensitivity [67].

More recently, the same Authors have described an increase of membrane CD44 expression in lymphocytes of patients with AD, in comparison with healthy subjects. CD44 is an adhesion molecule involved in the immune response even inside the central nervous system, and its increase seems to parallel the rise of unfolded p53 in AD lymphocytes [68].

Another research approach is related to the study of APP metabolism in platelets, based on data showing functional similarities between platelets and neurons. In particular, it has been shown that platelets isolated from AD patients have a different ratio of APP isoforms, with a lower amount of high molecular weight APP, compared to cognitively intact subjects. The "APP ratio" of high and low molecular weight isoforms is able to accurately discriminate between AD patients and normal controls [69] and to predict poor cognitive prognosis in MCI subjects at 2-year follow-up [70]. This test was found to be highly reproducible, with the main limitation being its sensitivity to pharmacological treatments (e.g., cholinesterase inhibitors, antiplatelet agents) [71].

Finally, several studies have shown increased markers of oxidative stress in brain from AD and MCI patients, compared to controls [72, 73]. Oxidative stress can result from diminished levels of antioxidants, even if reactive oxygen species levels are unchanged. A significant decrease of superoxide dismutase (SOD) has been observed in AD and MCI patients, compared with controls, both in plasma [74] and in specific brain areas [75]. In a separate study, the authors found not only a decrease of SOD and glutathione levels, but also an increase of lipid peroxidation markers in serum of AD patients, compared to an age-matched control group [76]. However, such studies, although adding useful information on the pathogenetic process of the disease, do not seem to provide results specific enough to justify their use as diagnostic tools.

5. Conclusions

During the last several years, our knowledge about possible biomarkers of AD has increased, paralleling the development of new therapeutic approaches. CSF and neuroimaging biomarkers seem to be the most promising; however limitations regarding their reliability, diffusion, as well as costs, still remain. In this perspective, the availability of peripheral biomarkers, less invasive, more readily accessible, and possibly cheaper, would be of great value. Results in this field are promising, and some of these biomarkers might become available in the clinical setting soon.

However, due to the multifactorial nature of AD pathogenesis, it seems unlikely that a single marker may prove to be the ultimate diagnostic tool. More likely, a combination of peripheral biomarkers, along with extensive clinical and neuropsychological assessment, might be able to suspect cases of prodromal AD, among the vast number of subjects with subjective or mild cognitive impairment, to be classified as "high risk" and to be subjected to more invasive and/or expensive procedures of functional neuroimaging and CSF analysis. More research is needed to validate this approach.

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