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Emerging Noninvasive Biomarkers for Early Detection of Alzheimer's Disease

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Alzheimer's disease (AD) diagnosis still depends on the triad of clinical, imaging and neuropsychological testing. The development of accurate, easy to use and inexpensive biological markers for AD is a long-standing aspiration for researchers and the medical community. Here we describe some of the recent advances in the field of biomarkers, both in cerebrospinal fluid (CSF) and in peripheral blood.

Key Words: Alzheimer's disease, Peripheral Marker, Biomarkers, Platelet tau, Tau protein and peripheral blood.

Introduction

Tau protein is a microtubule-associated protein stabilizing microtubules normally found on axonal microtubules and plays a role in regulation as well as tracking for axonal transport (1–3). Furthermore, tau is important in maintaining neuronal polarity and stabilization of a particular architecture in the differentiated neuron. It has also been shown that the activity of tau is crucial for morphogenesis of growth cones in brain neurons whose structure also involves local networks of actin filaments and it has been suggested that tau has a role in promoting axonal growth (4). Dysfunction of this protein is a pathological hallmark of many neurodegenerative diseases of the central nervous system (5), which include Alzheimer's disease (AD), frontotemporal dementia with Parkinson syndrome linked to chromosome 17 (FTDP-17), Pick's disease, corticobasal degeneration and progressive supranuclear palsy (6,7). These various neurodegenerative diseases are referred to as tauopathies (8).

AD is a progressive neurodegenerative brain disorder, being responsible for most cases of dementia, particularly in the advanced age population and is considered an important public health problem worldwide. According to Ferri et al., in 2001 60.1% of persons who had some degree of dementia lived in developing countries, with an increase of about 5 percentage points in 20 years (9). On the other hand, it is considered that the criteria raised by the DSM IV may underestimate the prevalence of dementias, particularly in developing countries (10). In the World Alzheimer's Report (2010), Alzheimer's Disease International estimated that there were 35.6 million people living with dementia worldwide in 2010, increasing to 65.7 million by 2030 and 115.4 million by 2050. Nearly two-thirds live in low and middle income countries, where the sharpest increases in numbers are set to occur. The total estimates about the world costs of dementia are US$604 billion in 2010 (Alzheimer's Disease International, World Alzheimer's Report 2010, The Global Economic Impact of Dementia). Today, despite the importance of AD as the primary cause of neurodegenerative dementia and current advances in knowledge of clinical and pathophysiological aspects, the definitive diagnosis of AD is still based on histological and pathological features similar to those described by Alzheimer (11). An inverse relationship between the number of neurofibrillary tangles (NFTs) and the survival of neurons in the hippocampus has been found, suggesting that neuronal loss is closely related to the onset of NFTs (12).

Despite all the advances in modern medicine, in most cases AD can only be diagnosed through neuropsychological studies, neuroimaging and clinical data of patients. The clinical diagnosis of AD only allows us to speak of probable or possible AD (13) with a sensitivity of 93% and

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specificity of ~55%. Furthermore, diagnosis becomes far more difficult in early and unusual presentations of the disease.

Considering the technological tools and the advancement of current knowledge, a necessity has been created for the development of accurate biochemical and imaging tests that support the diagnosis (14,15). In this regard the diagnostic criteria for AD proposed in 2007 (14) consider the usefulness of genetic studies in AD, which would allow a definitive diagnosis based on the demonstration of mutations in any of the three genes responsible for autosomal dominant disease: the gene for amyloid precursor protein (APP) on chromosome 21, presenilin 1 (chromosome 14) and presenilin 2 (chromosome 1).

For the most prevalent sporadic cases, the need for a biological marker of AD has proven to be urgent, both for diagnosis and for monitoring of the disease (16,17). Among the advantages that such markers may bring, we emphasize the possibility of early and even preclinical diagnosis of the disease (18) with the subsequent correct treatment of AD by the medical team. Biological markers can also be used to monitor drug response in phase 2 clinical trials (18,19). The most recent criteria for AD (14,20) propose the use of markers for diagnosis of AD, including neuroimaging tools with volumetric and functional studies and the use of biochemical markers of body fluids including measurement of key proteins of the neuropathology (Aβ, tau and hyperphosphorylated tau isoforms) in cerebrospinal fluid (CSF) (18,21).

Biomarkers in Alzheimer's Disease

A biomarker is defined as an indicator of the presence or extent of disease and is directly associated with its clinical manifestations and prognosis (17,20). Biomarkers for cognitive impairment and dementia have been an issue in recent years. The value of a variety of these markers has been evaluated and discussed (22). Scientists from various countries have focused their efforts on discovering these biomarkers. Within these, we have studied markers in CSF as levels of beta amyloid peptide, total tau protein and phosphorylated tau at various residues (p-tau) (23). Reports highlighted the increase of hyperphosphorylated tau in CSF of AD patients and the close correlation between these, p-tau levels in CSF and cognitive impairment (21,23). Recently, our group reported changes in redox active iron in the CSF of AD patients and we postulated that monitoring these alterations may be an appropriate sensor for assessing cognitive impairment (23).

Biomarkers in the CSF

CSF is in intimate contact with nerve tissue, and there is an important exchange of various substances between neural environment and CSF. For this reason, several groups have studied the levels and modifications of various proteins and substances involved in the pathogenesis of AD and have tried to validate their role as biomarkers (16,17,21).

Considering a possible etiopathogenic role of beta amyloid protein, levels of this protein—especially the Aβ fraction (1-42)—has been thoroughly analyzed in CSF of AD subjects, and a decrease to $<50\%$ of its normal value in CSF has been consistently observed, which means that this marker has a sensitivity of 78% and a specificity of 81–83% for diagnosis of AD (15). Moreover, it has been found that low levels of this peptide can predict the onset of cognitive decline in older women without dementia (24). Regarding tau protein, levels of p-tau have proven very useful, for example, p-tau at threonine 181 allows the differentiation of AD patients from control subjects and patients with dementia with Lewy bodies, being a better marker of AD when compared to Aβ (1-42) and total tau (25). Studies of Maccioni et al. show increases of hyperphosphorylated tau in AD patients, whereas only the subpopulation of subjects with mild cognitive impairment (MCI) with higher cognitive impairment showed abnormal increases in this tau variant (21). During preclinical stages of AD, it has been proposed that CSF Aβ levels may be a useful marker of asymptomatic brain amyloidosis, whereas CSF tau and p-tau evaluations are better correlated with later stages of synaptic dysfunction and early neurodegeneration (18,19).

Peripheral Markers

The search for reliable, noninvasive, and inexpensive biomarkers has been an important drive for biomarker research. Among these peripheral markers are the ApoE polymorphism, inflammatory markers, alterations of p53 protein, and amyloid precursor peptide platelet (18,26,27). Platelets have been postulated as a peripheral marker for

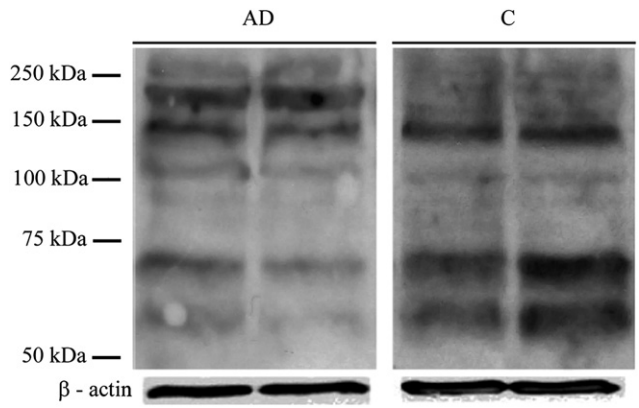

Figure 1. Western blot of platelet tau forms with Tau-5 antibody (1:1000). High molecular weight forms of tau (>80 kDa) are present in Alzheimer's disease (AD) subjects as well as control (C) subjects. However, the most important fraction of tau migrates at high molecular weight (100 kDa–250 kDa) in AD samples when compared to control subjects. β-actin as load control at the bottom (16). This method has led to a potential biomarker for early detection of AD (17).

Table 1. Comparison between several biomarkers for Alzheimer's disease, currently available, according to their sensitivity and specificity

Biomarker	Sensitivity (%)	Specificity (%)	Sample type	Reference
CSF amyloid-β	78	81−83	Cerebrospinal fluid	Wiltfang et al., 2007 (15)
CSF tau	66.7−100	66.7−77.8	Cerebrospinal fluid	Ewers et al., 2007 (29)
Alterations of p53 protein	90	77	Blood sample	Lanni et al., 2008 (30)
ApoE ε4	65	68	Blood sample	Mayeux et al., 1998 (31)
Platelet tau	75.7	79.7	Blood sample	Farías et al., 2012 (17)

AD because they carry 95% of circulating amyloid-β protein precursor (AβPP). The ratio of 130 and 110 kDa forms of AβPP is modified in AD and has been postulated to correlate with the presence of AD; therefore, evaluations of AβPP have been suggested as a reliable noninvasive disease biomarker as well as a sensor for treatment response (16,17). However, it is important to consider that the changes in plasma concentration of Aβ (1-40) are nonspecific for AD and are closely related to age (28).

Platelet Tau as a Biomarker

A pioneer and revolutionary method of detecting Alzheimer's disease at early stages, based on platelet tau detection, was developed in our laboratories. We analyzed platelets of AD patients, evaluating whether tau protein was expressed in these cells because, to date, there has been no scientific information available in the literature. Initial studies with antibodies recognizing tau protein showed the presence of this protein in immunoblots of extracts of platelets obtained from peripheral blood in both AD patients and in healthy young (18−25 years of age) subjects. The same studies draw the attention to the presence of tau immunoreactive bands migrating at much higher molecular weights than expected under denaturing and reducing electrophoresis conditions (SDS-PAGE). These high molecular weight forms of tau that appear to be oligomeric forms of the protein are increased in AD patients when compared to healthy elderly subjects (Figure 1); therefore, platelet tau has been postulated as a biomarker for AD (17). Later on, studies demonstrated a close correlation between the degree of platelet tau modification and the level of cognitive impairment measured by neuropsychological tests in AD subjects, whereas correlation with AβPP platelets was inconsistent during these analyses (18). This new biomarker has a sensitivity of 75.7% and a specificity of 79.7% (17) (Table 1), values that enable it to be an excellent biomarker for AD as well as being able to track the progress of the disease.

In conclusion, despite multiple advances in the field of biomarkers, we still do not have an accurate, widely available laboratory test that can be used for AD detection. New AD criteria have imposed the challenge of developing new biomarkers that are able to detect the disease even at the preclinical stage. Although there is important evidence to support the reliability of some biomarkers—particularly in CSF—there is still the need to define the optimal test and more importantly to advance in the development of a noninvasive biomarker that may allow not only diagnosis but also monitoring of the disease.

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