

## Review Article

# Biochemical Markers for Alzheimer's and Parkinson's Disease

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## Abstract

Many biomarkers are currently being searched in neurodegenerative diseases like Alzheimer's disease and Parkinson's disease. These diseases share some common pathophysiological mechanisms and the presence of protein aggregates. Measurements of those proteins -like tau, amyloid-beta peptide and  $\alpha$ -synuclein-, as well other mediators of neurodegeneration in cerebrospinal fluid and peripheral fluids have been explored as biomarkers. Here we summarize some of the available data on biochemical markers for neurodegenerative diseases, considering advantages and drawbacks of each marker and method of analysis.

**Keywords:** Biomarkers, Alzheimer's Disease, Parkinson's Disease, Protein misfolding diseases

## Abbreviations

AD: Alzheimer's Disease; PD: Parkinson's Disease; FTD: Frontotemporal Dementia; CBD: Corticobasal Degeneration; PSP: Progressive Supranuclear Palsy; MSA: Multiple System Atrophy; LBD: Lewy Body Dementia; PMDs: Protein Misfolding Diseases;  $\alpha$ -syn:  $\alpha$ -synuclein; CSF: Cerebrospinal Fluid; A $\beta$ : Amyloid-beta Peptide; p-tau: Phosphorylated tau; A $\beta$ PP: A $\beta$  Precursor Protein; BBB: Blood-brain Barrier; miRNA: Micro RNA; mRNA: Messenger RNA; SPT: Serine Palmitoyltransferase; Sp 1: Specificity Protein 1; NINDCs: Non-inflammatory Neurological Disease Controls; EGF: Epidermal Growth Factor; CNS: Central Nervous System; TNF- $\alpha$ : Tumor Necrosis Factor- $\alpha$ ; IL-1 $\beta$ : Interleukin-1 $\beta$ ; IL-6: Interleukin-6; INF- $\gamma$ : Interferon- $\gamma$ ; PGE2: Prostaglandin E2; TGF $\beta$ : Transforming Growth Factor  $\beta$ ; IL-8: Interleukine 8

## Background

As world population grows older, age related neurodegenerative diseases like Alzheimer's disease (AD) and Parkinson's disease (PD) have become important public health concerns. Many other age-related conditions also share some combination of their most striking clinical features (i.e. dementia and Parkinsonism), so differentiation between some diagnoses may be very difficult in individual patients.

Among differential diagnoses for AD and PD we can mention many forms of frontotemporal dementia (FTD), corticobasal degeneration (CBD) progressive supranuclear palsy (PSP), multiple system atrophy (MSA) and Lewy body dementia (LBD). All of the above also share the presence of protein aggregates on neuropathology, so they have been termed as protein misfolding diseases (PMDs) [1] and since tau or  $\alpha$ -synuclein ( $\alpha$ -syn) can be found frequently in protein aggregates, many of these diseases have been grouped as tauopathies i.e. AD, many forms of FTD, CBD and PSP [2-4] or as synucleinopathies i.e. PD, LBD and MSA [5]. Anyhow, is clear that multiple interactions exist between proteins in PMDs, so many proteins are affected in each PMD and conversely, most of the proteins described as responsible for PMDs show some degree of modification in each isolated PMD [5].

Many pathological processes appear to be related upstream in the process of protein aggregates generation, including misbalances in inflammatory responses, cell signaling and redox state. Even with all of our advances in the study of neurodegenerative processes and models of disease we have been unable to generate consensus on the true role of protein aggregates, so we still are not sure what is the true importance of protein deposition for PMDs and we don't know if there is a common pathway or generator of the pathological process (like neuroinflammation) in some or all of these diseases, and if there is a common pathway of neurodegenerative process, we don't fully understand what is the defining feature that initiates the deposition of one protein over the other and the neurodegenerative process of some neuronal populations over the next [6-9].

Since one of the main problems in the diagnosis of chronic neurodegenerative diseases is their late onset of symptoms many years after the disease has begun there is a big gap between initiation of pathologic processes and development of clinical symptoms. This is due to the brain extensive ability to compensate functions. This adaptive characteristic generates the problem that in most cases diagnosis is only possible late, when there is an advanced neurodegenerative process, so any present or future treatment will lose its effectiveness. Thereby, the development of suitable test able to identify diseases early, when symptoms are not so obvious has become an essential task for neurodegenerative diseases studies [10].

The lack of certainty on the pathophysiology of PMDs doesn't deny the fact that we have made many interesting discoveries on the biochemical and histopathological features that characterizes each form of PMD. Furthermore many groups have made efforts to follow up some of the changes in key biological markers a protein, cytokine, RNA or other and use them as biological marker for any disease they are interested on [7,9,11].

A biomarker can be defined as an indicator of the presence or extent of a disease in particular and is directly associated with clinical manifestations and prognosis [12,13]. They can be measured and evaluated as indicators of normal biological processes, pathogenic

processes or pharmacological responses to therapeutic interventions. Currently, due to the limited availability and reliability of markers for PMDs scientists from several countries have focused on the discovery of these biomarkers [11].

The most important problem in the search for new biochemical markers of PMDs is the lack of standardization of sampling collection, handling and storage, as well as, the procedures that are performed for analysis -i.e. sample processing by western blot, ELISA, micro array, RT-q PCR or other. All of the above makes very difficult to achieve consistent results.

Since cerebrospinal fluid (CSF) is in close proximity with neural structures and is constantly exchanging different substances including all sorts of proteins -like the ones forming extracellular accumulates in PMDs, many research groups have focused on analyses of markers that may be present in CSF. Among those we can mention studies on levels of amyloid-beta peptide ( $A\beta$ ), total tau protein, phosphorylated tau (p-tau) and  $\alpha$ -syn for AD, PD and other PMDs [14]. Research has highlighted the increased hyperphosphorylated tau in the CSF of patients with AD and the close correlation between the levels of p-tau in CSF and cognitive impairment [14,15].

### **$A\beta$ , tau and $\alpha$ -synas biomarkers for AD and PD**

$A\beta$  is one of the most prominent and well characterized aggregated proteins in neurodegenerative diseases, since it was initially reported by Glenner and Wong [16] it has become a cornerstone leading to a new front for research for the study of AD.

The amyloid precursor protein ( $A\beta$ PP) may be subject to the  $\beta$ -secretase pathway where two peptides may result,  $A\beta$  (1-40) and  $A\beta$  (1-42), however the  $A\beta$  (1-42) isoform has shown to be more toxic, it aggregates faster than  $A\beta$ 40 and it is the main component of amyloid plaques in the extracellular space [17,18]. On the other hand,  $\alpha$ -secretase acts in the non-amyloidogenic pathway, which doesn't lead to the  $A\beta$  formation [19]. Since  $A\beta$  deposition can be found in many neurodegenerative diseases, such as PD, AD [20], and LBD [21], it's been one of the most studied biomarker candidates.

Cerebrospinal fluid (CSF) is the main source for sampling  $A\beta$  and tau *in vivo* [4]. CSF is in intimate contact with nerve tissue, and there is an important exchange of substances with neural environment. For this reason, various groups have focused in modifications of various proteins and substances related to the pathogenesis of AD and PD [12,15,22,23] Considering a possible pathogenic role of  $A\beta$ , there have been lots of analyses of this protein. Measures of  $A\beta$  levels with immune assays in AD patients demonstrate a typical decrease in CSF's  $A\beta$  (1-42) levels, This sole analysis has a sensitivity of 78% and a specificity of 81-83% for the diagnosis of AD [4,24]. The reduction in  $A\beta$  has been attributed to aggregation and sequestration of  $A\beta$  (1-42) in brain senile plaques [25]. In AD there is also an increase in tau protein levels over time that has been attributed to neuronal degeneration and release of this intracellular protein [15]. As for other PMDs, studies in PD patients shows that tau levels seem unchanged and only  $A\beta$  (1-42) shows a decrease below base lines [20].

Previous studies demonstrate that even though  $A\beta$  (1-42) measurements in CSF have sufficient accuracy over 85%- for discriminating between AD and control subjects [17,26]. The capacity of this biomarker for early differential diagnosis is still to low, because

of a neuropathological overlap between AD and other PMDs like PD. Anyways  $A\beta$  (1-42) levels in CSF appear to be lower in AD patients than in PD [20,27].

Moreover, Siderowf *et al.* (2010), concluded that lower levels of CSF  $A\beta$  (1-42) were associated with cognitive decline in PD. Evaluating the relationship between  $A\beta$  (1-42), total tau and p-tau levels in CSF, and the change in cognition measured with Mattis Dementia Rating Scale (DRS-2), they showed that tau and p-tau didn't have any significant relationship with cognitive decline, however, when evaluating  $A\beta$  (1-42) they found that those with levels  $\leq 192$  pg/mL had a 6.1 points increase in annual decline compared to those above that level [25].

Low  $A\beta$  levels in CSF can predict the onset of cognitive decline in older women without dementia [28]. With respect to PD, studies by Bekris *et al.* 2015, found that  $A\beta$  levels are abnormal in the CSF of patients with PD. The objective of this exploratory study was to determine whether genetic variation within the  $A\beta$ PP is correlated with the levels of CSF  $A\beta$  (1-42) in PD. They studied single nucleotide polymorphisms (SNPs) from 19 regulatory regions of the nine genes ( $A\beta$ PP, *ADAM10*, *BACE1*, *BACE2*, *PSEN1*, *PSEN2*, *PEN2*, *NCSTN* and *APH1B*) involved in the cleavage of  $A\beta$ PP. They observed a significant correlation with  $A\beta$  (1-42) levels CSF in PD for two SNPs ( $A\beta$ PP rs466448 and *APH1B* rs2068143). In addition, the researchers suggest that SNP  $A\beta$ PP and SNP *APH1B* marginally associated with PD CSF  $A\beta$  (1-42) levels in non-carriers of *APOE* $\epsilon$ 4 [29].

Studies focused on tau protein in CSF, showed that p-tau levels can be very useful, for example, p-tau phosphorylated at threonine 181 can discriminate between AD patients and control subjects and in patients with dementia with Lewy bodies. P-tau proved to be a better biomarker for AD compared to  $A\beta$  (1-42), and total tau [30]. Studies Maccioni *et al.* show increased hyperphosphorylated tau in AD patients, whereas only the subpopulation of patients with mild cognitive impairment (MCI) with greater cognitive impairment -which could be considered as preclinical AD showed abnormal increases in this tau variant [15]. During preclinical stages of AD, it is proposed that CSF  $A\beta$  levels may be a useful marker of asymptomatic cerebral amyloidosis, while evaluations of CSF tau and p-tau are best correlated with the later stages of synaptic dysfunction and early neurodegeneration [31,32].

Although CSF has proven to be a reliable source for biomarkers research, these kinds of analyses are not suitable for day-to-day diagnosis in clinical practice since lumbar puncture is considered too invasive as a routine diagnostic procedure. Thus, the search for less invasive and inexpensive diagnostic tools has driven researchers to explore peripheral biological fluids such as blood and saliva.

Plasma  $A\beta$  has been examined as a peripheral biomarker. However, the pool of circulating  $A\beta$  in plasma comes not only from brain tissue transported across the blood-brain barrier (BBB), but also from peripheral tissues and organs. This could be troublesome since  $A\beta$  measurements might not reflect the dynamics of senile plaque formation in the brain [18]. Platelets are an important peripheral reservoir for  $A\beta$  generation since they express  $A\beta$ PP. Furthermore platelets express the enzymatic mechanism necessary to process and release  $A\beta$ . Also, platelets can modify the soluble  $A\beta$  oligomers into  $A\beta$  (1-42). Gowert *et al.* (2014) have recently shown that  $A\beta$  improves

**Table 1:** Summary of biomarkers for Alzheimer's disease and Parkinson's disease.

Biomarker	Sample type	AD	PD	Reference
CSF amyloid- $\beta$	CSF	↓	↓	(4, 15, 24, 25, 29)
CSF tau	CSF	↑	↑	(4, 15, 31, 109)
Platelet A $\beta$ PP (130/110kDa)	Blood	↓	NA	(33-36)
Platelet tau (HMW/LMW)	Blood	↑	NA	(4, 12, 22)
$\alpha$ -syn and DJ1	CSF	NA	$\alpha$ -syn ↓; DJ1 ↑	(38)
$\alpha$ -syn and DJ1	Saliva	NA	$\alpha$ -syn ↓; DJ1 ↑	(40)

Note: ↑: increase, ↓: decreases, NA: Not Applicable

platelets activation and generation of reactive species of oxygen, thus they concluded that platelets may have a role in cerebral amyloid angiopathy [33]. Modifications in platelets A $\beta$ PP has been described in AD since an altered proportion of the different forms A $\beta$ PP can be found in western blot with a decreased ratio high molecular weight (130kDa) to lower molecular weight (106 and 110kDa) forms of A $\beta$ PP in AD patients compared to control groups [34,35]. However, it is important to consider that the changes in plasma concentration of A $\beta$  (1-40) are nonspecific for AD and are closely related to age [36].

Tau has also been described in platelets. Studies by Fariás *et al.* 2012 propose a new biomarker for AD, based on the detection of tau protein in platelets with specific antibodies. Some immunoreactive bands that have a higher molecular weight and appear to be oligomeric forms of the protein are increased in AD patients compared to healthy elderly subjects [12,22]. Later studies showed a close correlation between the degree of tau modification in platelets and the level of cognitive impairment measured by neuropsychological tests in subjects with AD and has been proposed as an AD biomarker with sensitivity of 75.7% and a specificity of 79.7% [4].

But plasma and platelets are not the only source for biomarkers in blood, Sotolongo-Grau *et al.* 2014, evaluated A $\beta$  bounded in blood cells and searched a correlation between these A $\beta$  levels and 45 regions of interest in the brain using magnetic resonance imaging. Surprisingly enough, the cell bounded A $\beta$  (1-40) in blood was correlated with the volume of the left hippocampus while plasma A $\beta$  showed no correlation with any of the studied brain regions [37].

Based on the research on A $\beta$  and tau in CSF as AD biomarkers some groups have proposed a similar strategy for the search of PD biomarkers. Recent findings showed leading evidence for high specificity and sensitivity of  $\alpha$ -syn and DJ-1 measurements in CSF in PD. Hong *et al.* (2010), evaluated 117 PD patients and 132 healthy controls. They found that levels of both proteins are lower in PD than in healthy controls [38]. Little is known about the exact biochemical role of DJ-1 in PD, however a study have shown evidence that DJ-1 eliminates the hydrogen peroxide, protecting the cells in oxidative stress. This is particularly interesting since, mutations on DJ-1 (L166P) are the cause of one familial form of PD (PARK7) [39].

Peripheral  $\alpha$ -syn and DJ-1 have also been studied as peripheral PD biomarkers. It is described that the submandibular gland is intimately linked with synucleopathies since this gland is affected by histopathological changes typical of PD [21]. In this context, Devic *et al.* 2011 measured  $\alpha$ -syn and DJ-1 in saliva, a really accessible and non-invasive fluid. For this study saliva samples were obtain from 24 PD patients and 25 healthy controls and. Even though results

did not reach significance, they showed a trend towards decreased levels of  $\alpha$ -syn levels and increased DJ-1 in PD patients. Additionally, they look for a correlation between these proteins and motor scores of the Unified Parkinson's disease Rating Scale (UPDRS), searching for a relation between these proteins and motor decline. Only  $\alpha$ -syn levels showed a trend with UPDRS, but it didn't reach significance. Nonetheless, this study leads to an important hope for biomarkers on PD, as salivary fluid it's an ideal source for high-throughput assays [40].

### MicroRNAs as biomarkers for PMDs

MicroRNAs (miRNA) are small RNA fragments from about 22 nucleotides length that regulates posttranscriptional processes by pairing with messenger RNA (mRNA).

miRNA expression varies depending on the physiological conditions of the cell, therefore that the study of circulating miRNA is a clue of what happens at the intracellular level in normal and pathological situations.

In their study, Geekiyanage and Chan (2011) showed that certain miRNAs related to modulation of ceramides levels-i.e. miR-137, -181c, -9, -29a/b-are down regulated in patients with AD, increasing the expression of the two subunits of serine palmitoyltransferase (SPT). mRNA levels did not differ in relation to control subjects, demonstrating that these are regulated post-transcriptionally, SPT1 by miR-137/-181c and SPT2 by -9, -29a/b, postulating these miRNAs as potential markers. A high level of ceramides in the brain has been associated with sporadic AD. In addition, it was demonstrated that down regulated miR-9 and miR-29 family members control BACE 1 in sporadic AD patients, inducing A $\beta$  accumulation [41].

Sheinerman *et al.* (2012) took plasma from AD and MCI patients and control and identified several miRNA pairs (miR-128/miR-491-5p, miR-132/miR-491-5p, miR-323-3p/miR-491-5p, miR-134/miR-370, miR-323-3p/miR-370 and miR-382/miR-370). They conclude that although these miRNA pairs did not allow distinguishing between AD and MCI, they could be characteristics of neurodegenerative processes [42].

Leidinger *et al.* (2013) Identified 12 miRNAs in blood samples, that together, allowed to differentiate AD patients from control whit an accuracy of 93%, specificity of 95% and a sensitivity of 92%. These miRNAs can even differentiate between AD and other neurodegenerative diseases [43].

Transcription factor Sp1 (specificity protein 1) can control the expression of several protein associated with AD, including A $\beta$ PP and tau. There is an inverse relationship between Sp1 mRNA and

miRNA 29b levels in peripheral blood mononuclear cells [44]. Sp1 and its regulatory hsa-miR-29b are deregulated in AD patients, may be causing abnormal expression of genes that are involved in illness.

A study observes the profile of circulating miRNA in serum and CSF of AD patients compared to non-inflammatory (NINDCs) and inflammatory neurological disease controls and patients with FTD. At the beginning, 84 miRNA were identified but it was demonstrated that only miR-125b levels are decreased in serum from patients with AD as compared with NINDC and distinguish between AD and NINDCs with an accuracy of 82% [45]. This result confirms a previous report for miR-125b [46], in which miR-125b was also postulated as a good candidate as a biomarker. miR-125b is brain-enriched and probably is involved in neurodegenerative processes [47].

In other study six miRNA that were increased in AD patients compared to control i.e. miR-98-5p, miR-885-5p, miR-483-3p, miR-342-3p, miR-191-5p, and miR-let-7d-5p- were identified. Within these six miRNA, it was found that miR-342-3p has the highest sensitivity and specificity and so, may serve as a novel, noninvasive biomarker for AD [48].

miRNAs have also been studied as a biomarker for PD; analysis from PD patient's blood revealed that three miRNA (miR-1, miR-22\*, miR-29a) were down regulated in non-treated patients compared to control. A second group of three miRNA (miR-16-2\*, miR-26-2\*, miR-30a) presented a 50% increase in their relative expression between treated PD patients and control. In addition, miR-16-2\*, miR-26-2\* showed an increase in treated compared to non-treated patients [49].

Khoo *et al.* (2012) identified several circulating miRNA and their results were analyzed by two different strategies. k-TSP1 (miR1826/miR450b-3p), miR-626 and miR-505 attained the highest predictive power of 91% sensitivity, 100% specificity, 100% positive predicted value. However, in the replication set reached 88% negative predicted value [50].

In other work, plasma from PD patients and control was used and analyzed by microarray and qRT-PCR. A significant increase for miR-331-5 was found in PD patient's plasma versus control subjects. Moreover, bioinformatics analysis predicted that miR-331-5 could be involved in gene regulation implicated in PD. No other previously published miRNA was found [50].

Another report explored the alterations at the expression level of serum miRNAs in 10 idiopathic PD patients, 10 PD patients carrying LRRK2 G2019S mutation, and 10 controls by using RT-qPCR and miRNA arrays. Four statistically significant miRNAs were downregulated in either LRRK2 or idiopathic PD (miR-29a, miR-29c, miR-19a, and miR-19b). They validate the study, with another sample set, and confirmed the association of downregulated levels of miR-29c, miR-29a, and miR-19b in idiopathic PD [51].

### Inflammatory biomarkers

The inflammatory process is associated to the development of several neurodegenerative diseases. This process has cellular and molecular immune components such as microglial cells, cytokines and complement; they are main agents involved in the neuroinflammation, and act as inflammatory mediators. These proinflammatory

mediators are either produced locally within the Central nervous system (CNS) or recruited from the peripheral circulatory system following disruption of the BBB. Based in this context, it is possible that the peripheral concentrations of inflammatory proteins may thus reflect changes in neuroinflammation in the CNS.

This in turn, leads to the activation of the glial cells, such as microglia and astrocytes. Neuroinflammatory mechanisms probably also contribute to the cascade of events leading to neuronal degeneration [52].

The identification and validation of molecules involved in this process could be a good strategy for finding new biomarkers. Usually, the inflammation does not trigger neurodegenerative process, but there is evidence that this constant inflammatory process leads to chronic activation of astrocytes and microglial cells contributing to disease progression [53].

Neuroinflammation characterized by microglial activation serves as an engine driving PD progression. In substantia nigra, many endogenous and exogenous factors activate microglia and produce neuroinflammatory factors [54], such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-1 $\beta$  (IL-1 $\beta$ ), interleukin-6 (IL-6), interferon- $\gamma$  (INF- $\gamma$ ) and prostaglandin E2 (PGE2), which cause progressive neurodegeneration in dopaminergic neurons, and play an important role in the pathogenesis of PD [55,56]. After that, dead neurons release many substances to extracellular space including iron, aggregated  $\alpha$ -synuclein and neuromelanin, inducing neuroinflammation, because all these components can trigger the activation of adjacent microglial cells [57], propagating progressive degeneration of dopaminergic neurons and deterioration of motor symptoms of PD [56]. In this way, the inflammation produced by microglial activation contributes to the cascade events leading to neuronal degeneration in PD [52].

Studies by Song group describes that the levels of hs-CRP (high-sensitivity C-reactive protein) and fibrinogen are significantly higher in groups of PD patients, more than those observed in a control patients. These findings are consistent with those reported previously and support the hypothesis that neuroinflammatory reactions are involved in the degenerative processes observed in PD [58,59]. Studies of biological fluids like serum or CSF also support a role for neuroinflammatory processes in Parkinson's disease. Specifically, an increase in TNF $\alpha$ , interleukin 1 $\beta$ , interleukin 6 and osteopontin (a member of the integrins family) has been reported in the CSF of patients with PD. The inflammatory changes are detectable during the course of the disease before the death of the patients and are associated with the progression of the disease [52].

In the other hand, studies by group of Lindqvist, in serum and CSF, show that PD patients had a significant increase in levels of IL-6 [60]. It is of some interest, therefore, that increased levels of IL-1b, IL-6 and TNF $\alpha$  have been found in the basal ganglia and CSF of PD patients [58], and the increase in TNF $\alpha$ , was particularly dramatic, being 366% in tissue and 432% in CSF [58].

Cognitive impairment is a very common non-motor symptom in PD patients, thus two major types of patients can be distinguished: the patients without cognitive impairment or dementia and PD patients with cognitive impairment or dementia. It may be very difficult to

diagnose these cases, therefore, identify a biomarker for severity of cognitive impairment becomes a necessity [56].

Also, PD patients with a high incidence of cognitive impairment (which mainly involves the cognitive domains of vocabulary memory, abstraction, visual-spatial and executive function and language), show elevated levels of IL-6 in CSF [56,60]. In this way it's possible to assume that IL-6 could be potential biomarkers for cognitive impairment in PD patients. Studies by Yu's group, show that demented PD patients have significantly higher level of C-reactive protein in CSF than non-demented PD and in the other hand, IL-6 level in CSF of cognitive impaired PD group is not only prominently enhanced comparing with the group of PD without cognitive impairment, but also has a strikingly negative correlation with MoCA score, indicating that IL-6 may be a potential neuroinflammatory biomarker for the development and severity of cognitive impairment in PD patients [56]. This may have important clinical utility for improving diagnostic accuracy, allowing better prognostication and earlier access to potential disease-modifying therapies [61].

In AD -the most common neurodegenerative disorder two characteristic lesions can be observed: extracellular amyloid aggregates containing A $\beta$  and intracellular neurofibrillary tangles of hyperphosphorylated tau protein. The relationship between misfolding and aggregation of these two proteins and inflammation is based on the hypothesis that the complex nature of plaques and tangles stimulates a chronic inflammatory reaction to scavenge these wastes [62].

The presence of inflammatory process in AD brain has led to the discovery of increases in levels of various inflammatory mediators in cerebrospinal fluid (CSF). Further, abnormalities in immune-related molecules and functions have also been described in peripheral blood [63]. To date, studies of inflammatory biomarkers, in CSF or peripheral blood, of AD patients have also yielded inconclusive results.

It has been demonstrated that the lymphocyte biology is altered in AD. In blood samples from patients with AD, it's possible to observe alterations in intracellular calcium [64] and there is impaired release of IL-1 $\beta$  and IL-6 in response to stimulation with lipopolysaccharide (LPS). Furthermore there is a worsening of this process with disease progression [65]. In another study, LPS-stimulation resulted in exaggerated release of IL-1 $\beta$ , TNF- $\alpha$ , IL-6, and IL-10 [66].

Peskind measured levels of s100B, a protein secreted by astrocytes and directly linked to the inflammatory response, in the CSF of AD patients and healthy controls and found significantly elevated levels of s100B in CSF of mild or moderate AD patients, but declines to normal levels in more advanced stages of the disease [67]. S100B induces release of IL-6, which is one of components involved in the neuroinflammatory process [63]. IL-6 is a pleiotropic cytokine that mediates immune responses and inflammatory reactions affecting CNS cell growth and differentiation [68]. Also, IL-6 immunoreactivity is found within extracellular A $\beta$  deposits [69]. Despite this, Mrak group have collected information about brain tissue levels of IL-6 that are not increased in AD, although increased levels of IL-6 mRNA have been reported in AD brain [63]. Cerebrospinal fluid IL-6 levels have revealed contradictory results unchanged [70,71] and increased

[72,73]. One study found a correlation between CSF levels of IL-6 and tau levels in AD patients [63], but serum levels of IL-6 have reported controversial results, like increased [74] or normal [66].

Transforming growth factor  $\beta$  (TGF $\beta$ ) is an important astrocytes derived cytokine that manifests both pro-inflammatory and anti-inflammatory properties. CSF levels of TGF $\beta$  were found to be increased in AD in one study [75] but decreased in other [76].

IL-1 is an immunoregulatory cytokine that is overexpressed within affected cerebral cortical regions of the AD brain [68]. Studies of IL-1 show opposite results, so it's like two groups found significant increases in AD patients [77] and others found no increase [78].

The pro-inflammatory cytokine TNF $\alpha$  has synergistic effects with IL-1 in inflammatory processes [63]. TNF $\alpha$  is an important mediator of systemic inflammation, activating the central innate immune response [79]. Studies in CSF of AD patients are conflicting since one group found elevated CSF TNF $\alpha$  levels [80] but a second group did not [81]. On the other hand, although most studies report an increase in the serum TNF $\alpha$  levels of patients suffering from neurodegeneration [82-84], one study reported TNF $\alpha$  attenuation in early-onset AD and late-onset AD patients [85].

Many studies have examined the correlation between TNF $\alpha$  levels and age. Production of TNF $\alpha$  is reported to be significantly higher in the elderly than in younger healthy volunteers, and results showed a significant positive correlation with age [74]. Another research by Angelopoulos et al. found a significant correlation between IL-6 and TNF $\alpha$  levels and age [86], and Holmes *et al.* 2011 demonstrated that elevated serum TNF $\alpha$  and IL-6 levels were associated with an approximately 2-fold increase in Neuropsychiatric Inventory scores and an increased frequency of adverse neuropsychiatric symptoms, independent of delirium [82]. Those studies suggested that acute and chronic systemic inflammation, which is associated with an increase in serum TNF $\alpha$ , is associated with an increased cognitive decline in AD [84].

Other component of the inflammatory process is interleukin 8 (IL-8), but the role of the IL-8 in AD progression is not well understood. IL-8 probably represents an additional recruitment mechanism for the migration of microglia to A $\beta$  deposits, followed by a subsequent and persistent activation of microglial cells, favoring the neuroinflammatory context [87]. It is also known that IL-8 receptor has been localized in dystrophic neurites, suggesting that IL-8 mediates glial interactions with neurons and thereby contributes to neuronal damage [88]. Zhang *et al.* showed that levels of IL-8 in CSF of AD patients was significantly increased compared to healthy controls [89], whereas plasma levels of IL-8 in late-onset AD and vascular dementia did not differ from controls [90].

Currently, there is not a profile of inflammatory markers in CSF or plasma that may serve in the diagnosis of AD. In this context, Ray *et al.* performed combined multivariate analysis of plasma signaling and inflammatory proteins and found 18 plasma proteins that may identify AD patients and predict future AD with high accuracy in mild cognitive impairment (MCI) patients [91]. Another research group, Martins *et al.*, found that a set of 18 markers in blood had sensitivity and specificity of more than 80 % for distinguishing patients with Alzheimer's disease from healthy controls [92].

This is very relevant because all of these results could support the clinical diagnosis. Furthermore, it is important to consider that the incorporation of plasma biomarkers yielded high sensitivity with improved specificity, supporting their usefulness as a screening tool in the search of biological diagnosis of AD [93]. However, it will be very important for future clinical applications to find markers capable of differentiating AD from other dementias [62].

### Others biomarkers for AD

Alteration in retina and visual defects are related to MCI and AD [94]. In addition, variations in visuospatial perception can be detected at first stage of AD [95].

Neurons need highly amounts of energy that is generated by mitochondria. Mitochondrial DNA is resistant to degradation because of its characteristic closed circular form, thus it can be present in CSF as a marker of neurodegeneration. It was demonstrated that low concentration mitochondrial DNA can be found in CSF of presymptomatic subjects who will develop AD, even before any change in A $\beta$  or tau [96].

Ubiquitin is a protein involved in degradation of other proteins; it interacts with lysine residues and signals for degradations by proteases. In AD patients, PHF are ubiquitinated and ubiquitin conjugated protein can exist into the cell indefinitely [97]. It was demonstrated that increased levels of free and conjugated ubiquitin are found in CSF of patients with MCI progressing to AD [98]. Other group demonstrated that ubiquitin level were significantly higher in AD patients and found that a positive correlation between ubiquitin, tau and apolipoprotein E $\epsilon$  4 genotype exists while there is a negative correlation with A $\beta$ 42 [99].

Plasma of AD patients has abnormal levels of phospholipids. Diminished level was observed also in people who will develop MCI and AD within 2-3 years -over 90% accuracy. That could be reflecting cell membrane integrity and consequently early neurodegeneration of preclinical AD [100].

### Others biomarkers for PD

DJ-1 was a protein firstly identified in familial PD [101]. It is a multifunctional protein that plays an important role principally in oxidative stress. A study performed using whole blood from PD patients and healthy control found significant difference between DJ-1 isoforms levels, using 2D-electrophoresis, immunoblotting techniques and mass spectroscopy analysis. Although it was confirmed that not differences exists between total DJ-1 levels from PD patients and control, some isoforms in whole blood samples with 4-hydroxy-2-nonenal modifications were significantly increased and related to both PD diagnosis and PD severity [102].

Results from studies in CSF are not very consistent but one showed decreased level of DJ-1 in PD patients compared to control with a sensitivity of 90% and specificity of 70% [38].

Urate circulates at high concentrations in humans and also represents the principal end product of purine metabolism. It has antioxidant characteristics and thus protects against oxidative damage. Lower levels of uric acid could indicate increased risk and severity for motor symptoms of PD [103,104].

People with PD may develop cognitive impairment. Immunoassays from plasma samples, allowed identifying eleven proteins associated with cognitive performance, of which the most significant was epidermal growth factor (EGF). Suggesting that plasma EGF may be a biomarker for progression to cognitive impairment in PD [105].

Dihydroxyphenylacetic acid a neuronal metabolite of catecholamines metabolism- is diminished in patients with recent onset of Parkinsonism and may be used as a biomarker separating these patients from controls with 100% sensitivity and 89% specificity in [106].

Lower ApoA1 levels can be found in symptomatic PD patients and in asymptomatic individuals with physiological reductions in dopamine transporter density consistent with prodromal PD. It has been suggested that increasing plasma ApoA1 level in small amounts could reduce the risk of developing PD [107].

The Lewy body formation is due to the deficient protein degradation. Lysosomal hydrolases like GCase have been measured in CSF from PD patients and in other neurodegenerative disorders including LBD. The activity of GCase is reduced in PD and LBD patients in contrast to controls [108]. Therefore, the lysosomal dysfunction represents a good target to search for biomarkers.

### Concluding remarks

AD and PD are currently recognized as the most common neurodegenerative diseases. Many efforts have been made to understand the pathophysiological mechanisms that underlie both diseases. Even though they appear as dissimilar conditions i.e. PD predominantly a motor disease and AD predominantly a cognitive disease- both are considered as PMDs and as such, share a lot of common mechanisms, including regulation of processes by miRNAs, generation of inflammatory signals, misfolding and aggregation of proteins. Our search for biomarkers must consider those markers that account for common neurodegenerative pathways from those that are specific for any PMD. Even if a perfect biomarker is not currently available we can advance in the definition of biomarkers profile that characterizes any neurodegenerative disease. Anyhow we must recognize that in order to validate any present or future biomarker is crucial to standardize the conditions for sample collection and processing. CSF is the most validated source for PMDs biomarkers' considering it reflects biochemical changes occurring in the CNS, but applicability of these biomarkers is limited because lumbar puncture is considered an invasive collection method [62]. On the other hand, blood samples are much easier to obtain, but concentrations of most potential neuronal biomarkers are several fold lower in blood than in CSF and there is a lot of "contamination" by plasma proteins that are not related to neuropathological processes. Given the multiplicity of pathophysiological processes implicated in PMDs, a combination of biomarkers related to different mechanisms might increase diagnostic accuracy and validity of any biomarker [62].

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