Journal of Parkinsonism and Restless Legs Syndrome

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REVIEW

Biomarkers of Parkinson's disease: recent insights, current challenges, and future prospects

This article was published in the following Dove Press journal:
Journal of Parkinsonism and Restless Legs Syndrome
22 February 2016
Number of times this article has been viewed

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Abstract: A biomarker represents a tool possibly helping physicians in predicting onset, diagnosis, and progression of a disease as well as evaluating the response to disease-modifying treatments. Currently, there is no biomarker fulfilling all such ideal criteria for Parkinson's disease (PD). In this article, we have critically reviewed the literature searching for the most reliable and reproducible clinical, biochemical, and imaging biomarkers for prodromal phase, diagnosis, and progression of PD. Different comprehensive batteries of biomarkers have been proposed as a sensitive approach to predict the onset of PD during the prodromal phase. There is a discussion about the redefinition of the clinical diagnosis of PD, including clinical biomarkers as non-motor symptoms; however, on the other hand, we have also observed that imaging biomarkers support the differential diagnosis from other causes of parkinsonism. Various clinical (eg, freezing of gait or cognitive impairment), biochemical (eg, epidermal growth factor, insulin-like growth factor 1, uric acid, etc), and imaging (eg, functional magnetic resonance imaging, voxel-based morphometry, etc) biomarkers may help envisaging disease progression of PD. To conclude, given the lack of a single biomarker that could track the entire course of the disease, our challenge is to find the best combinations of biomarkers for the different stages of the disease.

Keywords: biomarkers, Parkinson's disease, progression, motor, imaging , staging, non motor

Introduction

The National Institutes of Health defines a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention."[1] In the field of neurodegenerative diseases, a biomarker represents a tool possibly helping physicians in predicting onset, diagnosis, and progression of a disease as well as evaluating the response to disease-modifying treatments (DMT).

Parkinson's disease (PD) is the second most common neurodegenerative disease after Alzheimer's disease, affecting up to 1 million Americans over age 65 and up to 10 million individuals worldwide.[2] DMT as well as a cure for PD have yet to come. Current research is focusing on finding reliable biomarkers for PD which, ideally, should be sensitive, reproducible, inexpensive, noninvasive, and thoroughly validated. How can biomarkers really help us to tackle PD? The aim of this review is to find an answer to such a question by summarizing the current knowledge on clinical, imaging, and biochemical biomarkers for prodromal phase, diagnosis, and disease progression of PD (Tables 1–3).

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http://dx.doi.org/10.2147/JPRLS.S85488

Table 1 Biomarkers for PD prodromal phase

Biomarker	Comments	References
Clinical biomarkers		
Non-motor symptoms (NMS)	Family history for either PD or tremor	4
	Lack of smoking history	4,5
	More frequent NMS 2 years before diagnosis:	3
	anhedonia	3
	apathy	3
	memory complaints	3
	inattention	3
	More frequent NMS 2–10 years before diagnosis:	3
	smell loss	3,10–12
	mood disturbances	3
	excessive sweating	3
	fatigue	3
	pain	3
	other factors: tremor, balance impairments, constipation, hypotension, erectile dysfunction, urinary dysfunction, dizziness, fatigue, depression, and anxiety.	6–9
	More frequent NMS more than 10 years before diagnosis:	3
	constipation	3,9
	dream-enacting behavior	3,10–12
	frequent nightmares	3,10–12
	excessive daytime sleepiness	3
	postprandial fullness	3
	other factor: tremor	9
Biochemical biomarkers		
Metabolic factors	Cholesterol may be either a protective or a risk factor for PD	14,15
Neurotrophic factors	High IGF-1	16,17
	Low vitamin D	18
Oxidative stress	Protective role of higher uric acid levels and of gout in the development of PD	19–23
Imaging biomarkers		
Nuclear imaging	DaT imaging (DaTscan) is used to detect nigrostriatal degeneration; if combined with multiple factors (hyposmia, male sex, and constipation), the probability of detecting subjects with a nigrostriatal deficit increases up to 40%.	24–26
Transcranial sonography (TCS)	Substantia nigra hyperechogenicity has been proposed as a prodromal biomarker of PD at both 3- and 5-year follow-up.	27,28
	Sensitivity and specificity of PD conversion can be significantly increased when TCS is associated with nuclear imaging or applied in *LRRK2* mutation carriers.	29–31
Magnetic resonance imaging (MRI)	Functional MRI shows reorganization of corticostriatal circuits in *LRRK2* mutation carriers	32

Note: DaTscan signifies dopamine transporter imaging technique.
Abbreviations: PD, Parkinson's disease; IGF, insulin-like growth factor.

Methods

We searched the published data in English language from inception to November 2015 on PubMed. Our keywords included "Parkinson's disease", "biomarkers", "imaging", "clinical", "serum", "plasma", and "cerebrospinal fluid or CSF". Totally, we have retrieved 1,601 papers. Out of these, however, we considered only 141 papers for this review after excluding preclinical studies, reviews, duplicated data, and papers not specifically focused on PD.

Prodromal phase

Clinical biomarkers

Several retrospective studies have examined the frequency and timeline of clinical biomarkers prior to the onset of overt PD motor symptoms. In the ONSET PD (onset of non-motor symptoms in PD) study, the occurrence of non-motor symptoms (NMS) prior to diagnosis was surveyed in 109 PD patients with an estimation of the time span between onset of NMS and motor symptoms (within 2 years; more than 2 but less than 10 years; and more than 10 years). The most common NMS reported to occur more frequently during the 2-year premotor period were anhedonia, apathy, memory complaints, and inattention. NMS occurring more frequently between 2 and 10 years before motor onset were smell loss and mood disturbances, taste loss, excessive sweating, fatigue, and pain. NMS occurring usually more than 10 years before motor onset were constipation, dream-enacting behavior and frequent nightmares (both suggestive of rapid eye movement [REM]

Table 2 Biomarkers for PD diagnosis

Biomarkers	Comments	References
Clinical biomarkers		
Non-motor symptoms (NMS)	Frequent NMS at diagnosis: psychiatric disturbances, fatigue, attention/memory problems	34
	Sleep symptoms (RBD and insomnia) associated with worse cognitive performances	35
	Taste/smelling and difficulties in sexual activities more prevalent in men	36
	Role of "red flags" in differential diagnosis with multiple system atrophy (MSA)	41
Biochemical biomarkers		
Metabolic factors	Increased homocysteine and its metabolites (ie, methylmalonate or cystatin C)	42–44
Neurotrophic factors	Low BDNF	46
	High IGF-1	16,48,49
	Low vitamin D	50,51
	GH response to arginine test may be helpful in the differential diagnosis with MSA	47
Neuroinflammation	PDGFB and prolactin are possible biomarkers for PD	52,53
Oxidative stress	Bilirubin and ceruloplasmin have been variably associated with PD	54–57
	Lower levels of uric acid in PD patients compared to healthy controls	58,59
Iron and other metals	Iron and manganese have been variably associated with PD	62,63
α-Synuclein	It is still debated if plasmatic levels of α-synuclein are similar or higher in PD, as compared to healthy controls; different methods to detect α-synuclein in plasma possibly account for such divergent results.	64–67
CSF biomarkers	Altered Aβ peptides	68–70
	Decreased α-synuclein	71,72
	Higher tau protein	74
	Higher DJ-1	75
	Metabolomic studies	79,80
Imaging biomarkers		
Nuclear imaging	Evaluation of the presynaptic dopaminergic function by means of either 6-[18F] fluoro-L-dopa or VMAT 2 or DAT availability measurement can be helpful in differentiating degenerative parkinsonism from essential tremor and secondary parkinsonism, but it is not helpful in the differential diagnosis with atypical parkinsonism.	81,82
	Cardiac [^{123}I] metaiodobenzylguanidine (MIBG) scintigraphy may also be useful for the differential diagnosis with MSA.	83
	DAT availability might be different between motor subtypes in relation to different disease severity; striatal dopamine depletion may also contribute to both cognitive (ie, executive tasks) and nonmotor symptoms (ie, anxiety) in de novo PD patients.	87–89
Magnetic resonance imaging (MRI)	Neuromelanin decrease (whose paramagnetic properties result in high signal on specific T1-weighted sequences) in the substantia nigra and locus coeruleus may differentiate between PD patients and healthy controls.	84
	Functional MRI can discriminate among PD patients with TD and non-TD phenotype and healthy controls.	85
	MRI shows positive correlation between TD phenotype and transverse relaxation rate (reflecting iron load) within the putamen, caudate, and thalamus.	86

Abbreviations: BDNF, brain-derived neurotrophic factor; IGF, insulin-like growth factor; CSF, cerebrospinal fluid; PD, Parkinson's disease; PDGFB, platelet-derived growth factor subunit B; TD, tremor dominant; RBD, REM-sleep behavior disorders; REM, rapid eye movement; GH, growth hormone; Aβ, amyloid beta.

sleep behavior disorders, RBD), excessive daytime sleepiness, and postprandial fullness. Interestingly, constipation, frequent nightmares, and dream-enacting behavior were found to associate together across all premotor time spans.[3]

A recent systematic meta-analysis showed that positive familial history for either PD or tremor, constipation, and lack of smoking history were strongly associated with later development of PD.[4] In accordance with these data, a survey among 116 de novo PD patients and 232 controls showed that current smokers were less likely to have PD, while former smokers were more likely to have PD, as compared to never-smokers, further suggesting that quitting smoking may be considered an early NMS of PD.[5]

Further, in agreement with other data,[6–8] a recent population-based study demonstrated that at 5 years before diagnosis, patients who went on to develop PD had a higher incidence of tremor, balance impairments, constipation, hypotension, erectile dysfunction, urinary dysfunction, dizziness, fatigue, depression, and anxiety compared with controls. At 10 years before diagnosis of PD, the incidence of tremor and constipation was higher in those who went on to develop PD than in controls.[9] According to prospective

Table 3 Biomarkers for PD progression

Biomarker	Comments	References
Clinical biomarkers		
Non-motor symptoms (NMS)	NMS tend to remain stable during the early phase of disease.	97
	Several clinical biomarkers herald worsening of cognition, as the presence of MCI, apathy, subjective memory complaints, and visual hallucinations.	98–103
Motor symptoms	TD variant has a slower disease progression rate, less cognitive decline, and lower incidence of neuropsychiatric complications such as visual hallucinations and depression, when compared to the non-TD phenotype.	37,91
	Freezing of gait may represent a biomarker for development of cognitive impairment and dementia.	93–96
Biochemical biomarkers		
Metabolic factors	The overall cardiovascular risk has been associated with axial motor impairment.	105
Neurotrophic factors	Serum BDNF levels have been associated with the severity of PD motor symptoms and cognitive symptoms.	45,106
	Serum epidermal growth factor (EGF) levels have been associated with cognitive deficits within 2 years since diagnosis.	107,108
	IGF-1 levels have been associated with both motor and cognitive symptoms.	48,49
	Vitamin D levels were negatively associated with motor disability, cognitive functions, and psychiatric symptoms.	50,109
Oxidative stress	Coenzyme Q10 levels are inversely associated with motor symptoms.	113
	Higher bilirubin levels were associated with worse motor symptoms.	54
	Higher UA levels were associated with less motor and non-motor burden.	114–123
Iron and other metals	Higher iron levels have been associated with the presence of RBD, poor quality of sleep, and depression.	125–127
	Serum manganese and copper have been associated with depressive symptoms.	127
CSF biomarkers	Conflicting results have been reported when analyzing the relationship between α-synuclein and motor severity of PD.	71,72,128,129
	Higher phosphorylated-tau protein has been related with greater cognitive decline.	130,131
	CSF Aβ peptides have also been studied in relation to cognitive functions in early PD, but conflicting results have been reported.	68,69
Imaging biomarkers		
Magnetic resonance imaging (MRI)	Functional MRI shows resting-state functional connectivity changes in relation to cognitive decline	132,133
	Gray matter volume analysis suggest that the caudate volume loss may contribute to cognitive decline while changes in thalamic volume may have relevance to tremor severity.	135
	A positive correlation between Mini-Mental State Examination scores and cortical thickness in the anterior temporal, dorsolateral prefrontal, posterior cingulate, temporal fusiform, and occipito-temporal cortex has been detected supporting the usefulness of cortical thickness measurement in assessing disease stage and cognitive impairment in patients with PD.	136,137
	Iron content in basal ganglia, assessed as MRI relaxation rate R2*, has been proposed as a biomarker of PD progression in PD.	138

Abbreviations: CSF, cerebrospinal fluid; IGF-1, insulin-like growth factor-1; PD, Parkinson's disease; TD, tremor dominant; MCI, mild cognitive impairment; BDNF, brain-derived neurotrophic factor; UA, uric acid; RBD, REM-sleep behavior disorders; REM, rapid eye movement; Aβ, amyloid beta, R2*, effective transverse relaxation rate.

studies, RBD and impaired olfaction are, by far, associated with the highest PD risk.[10–12]

The occurrence of NMS during the premotor phase reflects the widespread neurochemical and neuroanatomical changes that occur throughout the course of PD, with involvement of not only the dopaminergic nigrostriatal system but also serotonergic and noradrenergic brain stem areas, cholinergic frontal and brain stem regions.[13] However, each of these clinical biomarkers has poor predictive value when considered individually. Thus, ideally, screening strategies should include a combination of clinical and imaging as well as biochemical biomarkers to increase the likelihood of diagnosing as many individuals (developing PD) as possible (see "Biochemical biomarkers").

Biochemical biomarkers

Metabolic factors

Conflicting data suggest that increase in total cholesterol may be either a protective or a risk factor for PD.[14,15] Indeed, the association with motor symptoms may be explained in light of the white matter damage associated with metabolic factors, although a direct effect on neurodegenerative process has been hypothesized.

Neurotrophic factors

Insulin-like growth factor 1 (IGF-1) has been shown to provide effective protection against the loss of dopaminergic neurons in cellular and animal models of PD. Preliminary studies found that IGF-1 levels were directly associated with the burden of motor dysfunction in subjects at risk of PD.[16,17]

Vitamin D may exert a neuroprotective activity, although the exact mechanism is not fully understood. Accordingly, a 30-year longitudinal Finnish study showed that individuals with higher serum vitamin D concentrations have a 30% reduced risk of PD.[18]

Neuroinflammation

Neuroinflammation indeed play a role in enhancing neurodegenerative processes. However, there are no studies on single markers of neuroinflammation.

Oxidative stress

Oxidative stress plays a significant role in PD pathogenesis. Uric acid (UA) has antioxidant and iron scavenger features, possibly providing natural neuroprotection against PD. Evidence suggests a protective role of higher UA levels[19–21]and of gout in the development of PD.[22,23]

CSF biomarkers

To our knowledge, no study has investigated possible cerebrospinal fluid (CSF) biomarkers for prodromal phase of PD to date.

Imaging biomarkers

It is unrealistic to expect that a single biomarker will fulfill all the criteria for a reliable prodromal marker of PD. Indeed, using a combination of tools merging clinical and imaging biomarkers is the most likely rational approach. Different techniques have been used to visualize striatal or nigrostriatal denervation with nuclear imaging (both positron emission tomography [PET] and single-photon emission computed tomography [SPECT]) or the hyperechogenicity of the substantia nigra (SN) with transcranial sonography (TCS), while functional magnetic resonance imaging (fMRI) has been used to visualize abnormalities in corticostriatal circuits.

The Parkinson-Associated Risk Syndrome (PARS) study applied a two-stage screening strategy for PD consisting of olfactory testing followed by dopamine transporter (DaT) imaging (DaTscan) to detect nigrostriatal degeneration.[24,25] Among the 5,000 individuals screened with the 40-item University of Pennsylvania Smell Identification Test (UPSIT), 669 were at or below the 15th percentile based on age and sex, indicating hyposmia.[25] Among those, 303 (203 hyposmic and 100 normosmic) were subjected to DaTscan, and nigrostriatal dysfunction was detected in 11% of the hyposmic subjects compared with 1% of the normosmic subjects. Combining multiple factors (hyposmia, male sex, and constipation) increased the probability of detecting subjects with a nigrostriatal deficit, up to 40%.[26]

TCS is an imaging technique based on reflection and scattering of ultrasound waves at interfaces with diverse acoustic impedance depicting the brain as the mesencephalon and the basal ganglia. The alteration in SN signal, seen as an area of increased signal intensity and extent (termed hyperechogenicity), has been proposed as an easy and noninvasive biomarker of PD.[27,28] The Prospective Validation of Risk factors for the development of Parkinson Syndromes (PRIPS) study showed that enlarged SN hyperechogenicity is a good biomarker of PD development at both 3-[27] and 5-year follow-up.[28] However, another prospective study showed low sensitivity and specificity of TCS in predicting conversion to PD in patients affected by idiopathic RBD when used alone.[29] The Sleep Innsbruck Barcelona group showed that the combination of ioflupane (^{123}I) single photon emission computed tomography ([^{123}I]-FP-CIT SPECT) and TCS can better predict conversion to synucleinopathy in idiopathic RBD.[30]

Healthy carriers of the G2019S *LRRK2* mutation display a markedly increased, age-dependent risk of developing PD and, thus, represent an ideal enriched cohort to test the feasibility of biomarkers' screening approaches. Both TCS and fMRI show specific alterations in these individuals prior to PD diagnosis.[31,32]

Biomarkers for diagnosis

Clinical biomarkers

Currently, diagnosis of PD is based on the presence and combination of cardinal motor signs.[33] However, increasing evidence suggests a major role for a complex spectrum of NMS in the characterization of PD since the onset, possibly helpful in the process of redefinition of PD diagnosis.[12]

The PRIAMO (PaRkInson And non-Motor symptOms) study first showed that early, drug-naïve patients complained more frequently about psychiatric disturbances (66%), fatigue (52%), and attention/memory problems (48%).[34] Another study showed that both RBD and insomnia were associated with lower scores on several cognitive tests in early, drug-naïve patients possibly suggesting that sleep symptoms might be considered an early marker of dementia.[35] A survey examining the prevalence of NMS at onset in 200 PD patients and 93 healthy controls showed specific sex-related differences in the

spectrum of NMS with men with PD complaining of problems having sex and taste/smelling difficulties significantly more frequently than women with PD.[36]

From a purely motor perspective, it is possible to distinguish two main distinct clinical subtypes in consideration of the predominant motor features, a tremor dominant (TD) and an akinetic-rigid type (ART), and one mixed phenotype, not presenting one prevailing motor feature.[37] In keeping with the increasing role of NMS, more recent approaches provide classification of PD patients at diagnosis based on both motor and non-motor features.[38] A study performing a cluster analysis using data assessing both motor and NMS with a data-driven approach in 100 newly diagnosed untreated PD patients identified four distinct groups of patients, namely (1) benign pure motor, (2) benign mixed motor–non-motor, (3) non-motor dominant, and (4) motor dominant, suggesting the presence of a cross talk between motor and NMS in defining PD phenotype at diagnosis. As a matter of fact, the identification of such subtypes may have important implications for generating pathogenetic hypotheses and therapeutic strategies.[39]

Newer and even more recent approach for PD subtyping encompasses clinical and genetic data.[40] A population-based modeling study proposed such a model which correctly distinguished PD patients from healthy controls in the Parkinson's Progression Markers Initiative with high sensitivity and specificity.[40] Further application of this model to premotor prospective cohorts could facilitate identification of biomarkers and interventions.[40]

Another role for clinical biomarkers of diagnosis is to help differentiating PD from atypical parkinsonism, as multiple system atrophy (MSA). Besides the poor response to levodopa, and the additional presence of pyramidal or cerebellar signs or autonomic failure as major diagnostic criteria, certain other clinical features known as "red flags" or warning signs may raise the clinical suspicion of MSA.[41]

Biochemical biomarkers

Metabolic factors

Homocysteine is a neurotoxic, intermediary product of the methionine cycle, whose concentrations can be influenced by different factors and, in particular, by the genetic background.[42] Interestingly, homocysteine and its metabolites (ie, methylmalonate or cystatin C) have been suggested to be increased in PD, since the time of the diagnosis.[43,44]

Neurotrophic factors

Preclinical evidence shows that the brain-derived neurotrophic factor takes part in the survival and in the activity of striatal dopaminergic neurons. Accordingly, serum BDNF levels have been found to be decreased in PD patients as compared to controls[45] and associated with striatal DaT binding.[46]

Growth hormone (GH) and growth hormone-inhibiting hormone (or somatostatin) pathways have been investigated as diagnostic markers of PD. In particular, the growth hormone response to arginine test may be useful to differentiate PD from MSA.[47]

IGF-1 has been reported to be higher in PD patients than in controls[16,48,49] with one single study suggesting that a cutoff value of 114 ng/mL might correctly differentiate de novo PD patients from controls.[16]

PD patients have lower serum vitamin D levels as compared to controls.[50] Given the fact that seasonal differences in vitamin D levels have been observed,[50] one study suggested that the season of birth is a predictor of PD development.[51]

Neuroinflammation

Platelet-derived growth factor subunit B and prolactin as well as other neuroinflammatory markers have been reported as significantly different between PD patients and controls.[52,53] However, conflicting results have been reported so far, possibly because of differences in methodologies applied for determining these markers and of heterogeneity in populations.

Oxidative stress

Bilirubin levels were reported to be either increased[54] or decreased[55] in PD patients compared to healthy controls. Overall, PD populations that have been described so far are heterogeneous, and these conflicting results might be explained by the presence of confounding factors affecting bilirubin levels and/or by bilirubin variations during the course of the disease.

Decreased serum ceruloplasmin levels might reflect more severe oxidative stress with subsequent neurodegenerative alterations. Accordingly, lower serum ceruloplasmin levels have been associated with younger age at onset of PD,[56] and with more impaired neuroimaging features within the SN.[57]

Several meta-analyses confirm that PD patients, with particular regard to men, present lower UA levels as compared to controls, notwithstanding potential confounding factors (ie, body mass index or comorbidities).[58,59] Interestingly, higher UA levels were associated with the likelihood of having a scan without evidence of dopaminergic deficit in a secondary analysis of the PRECEPT study (recruiting

800 early PD),[60] and with an increased DaT availability among newly diagnosed PD patients, suggesting that neurodegenerative alterations can be tracked based on UA levels right from the early phases of the disease.[61]

Iron and other metals

Each positive variation of 10 μg/dL of serum iron has been associated with 3%–10% reduced relative risk of having PD,[62] suggesting the involvement of iron metabolism in PD pathogenesis.[62] Among other metals, manganese (Mn) has been associated with PD.[63]

α-Synuclein

Lewy bodies, the neuropathological hallmark of PD, are mainly composed of α-synuclein (α-syn), and, thus, different studies have investigated α-syn as a diagnostic biomarker in serum and plasma.[64,65] It is still debated if plasmatic levels of α-syn are similar,[66] or higher in PD, as compared to healthy controls, when there is an increased efflux of the protein to the peripheral blood of these patients.[67] However, utilization of different methods to detect α-syn in plasma possibly account for such divergent results.

CSF biomarkers

CSF amyloid beta peptides are altered from the early phases of PD,[68,69] especially in patients with dementia.[70] Regarding α-syn, the majority of recent studies have shown decreased CSF levels in PD patients, as compared to controls, although this marker apparently cannot distinguish among different synucleopathies.[71–73] In addition, levels of CSF tau protein were higher in PD patients, in particular in the early phases, as compared to controls.[74]

Among other CSF biomarkers, DJ-1 levels were found to increase in early PD patients compared to controls.[75] However, CSF DJ-1 increased levels are not specific and, for instance, they are also raised in MSA.[76] Furthermore, several studies suggest an increase in the number of CSF biomarkers with regard to the status of oxidative balance in PD patients when compared to controls.[77,78]

Different studies have been investigating difference in CSF metabolomics between PD patients and controls. In particular, metabolomics focuses on the quantitative analysis of the small metabolites affected by PD. Preliminary data show that specific combinations of metabolites can differentiate PD patients from healthy controls.[79,80] Overall, these studies provide a significant contribution in the identification of novel biomarkers that enable early diagnosis of the disease, as well as provide knowledge about the metabolic pathways and molecular mechanisms involved.

Imaging biomarkers

Clinically, there is no indication to perform imaging studies when the patient fulfills PD clinical criteria[33] and there is no diagnostic doubt. On the other hand, imaging may be sometimes helpful in the differential diagnosis with other movement disorders (ie, essential tremor and atypical parkinsonism). However, when it comes to research, imaging biomarkers may be helpful in further delineating the different PD phenotypes at diagnosis.

Evaluation of the presynaptic dopaminergic function by means of either 6-[18F] fluoro-L-dopa or VMAT 2 or DaT availability measurement can be helpful in differentiating degenerative parkinsonism from essential tremor (eg, DaT availability is approved by the Food and Drug Administration for this purpose),[81] and secondary parkinsonism, but it is not helpful in the differential diagnosis with atypical parkinsonism. For this purpose, it may be useful to combine pre- and postsynaptic dopaminergic imaging (with PET tracers for D2-like dopamine receptors), or to use fluorodeoxyglucose-PET which may disclose specific metabolic patterns for the different forms of atypical parkinsonism.[82]

As sympathetic denervation in PD involves postganglionic neurons, and MSA affects the preganglionic neurons, cardiac [^{123}I] metaiodobenzylguanidine scintigraphy may also be useful for the differential diagnosis of these disorders.[83]

Recent studies have suggested that neuromelanin decrease (whose paramagnetic properties result in high signal on specific T1-weighted magnetic resonance imaging [MRI]) in the SN and locus coeruleus of PD patients may differentiate between PD patients and healthy controls with high sensitivity and specificity.[84]

For a research purpose, imaging studies may further contribute in the delineation of different PD phenotypes at diagnosis. A recent fMRI study demonstrated that the subtype-specific functional networks could discriminate among PD patients with TD and non-TD phenotypes and healthy controls.[85] Further supporting different imaging features according to motor phenotype, another MRI study detecting iron load found a positive correlation between TD phenotype and transverse relaxation rate (reflecting iron load) within the putamen, caudate, and thalamus.[86]

Nuclear imaging studies also suggest different patterns of uptake of radiotracers according to motor phenotype. A recent study investigating the role of presynaptic nigrostriatal dopaminergic damage in 51 drug-naïve PD patients with

ART and TD phenotypes found that ART patients presented higher motor scores and lower DaT availability in affected and unaffected putamen, suggesting that DaT availability might be different between motor subtypes in relation to different regimens of disease severity.[87]

Nuclear tracers may also present different patterns in relation to cognitive profile and NMS at PD diagnosis. Recent data suggest that striatal dopamine depletion may contribute to both cognitive (ie, executive tasks)[88] and NMS (ie, anxiety)[89] in de novo PD patients.

Biomarkers of progression

Clinical biomarkers

Identifying those patients at risk of greater progression (eg, with development of cognitive impairment) is of particular importance in order to include patients in clinical trials when DMT are available.

Based on pathological evidence,[90] clinical studies showed that the TD variant has a slower disease progression, less cognitive decline, and lower incidence of neuropsychiatric complications such as visual hallucinations and depression, when compared to the ART group.[37,91]

Although dopaminergic treatment may mask disease progression, reported evidence suggests that several symptoms become severe with disease duration. However, little is known about the anatomical progression over the body segments of extrapyramidal signs in PD. Furthermore, a significant unmet need is the availability of instruments to detect disease progression, even in the early phase and on dopaminergic medications.[92]

Motor symptoms, as freezing of gait, may represent as a biomarker of development of cognitive impairment and dementia.[93,94] Indeed, gait is not merely considered a pure motor task as evidence shows the influence of specific cognitive profiles on gait parameters suggesting the existence of a complex interplay between gait and cognition.[95,96]

NMS generally tend to remain stable during the early phase of disease, with only a few of them being affected by dopaminergic therapy and, specifically, by the use of dopamine agonists (ie, reduction in depression and increase in weight).[97,98] However, several clinical biomarkers have shown to herald worsening of cognition in PD patients, as the presence of mild cognitive impairment,[99,100] apathy,[101] subjective memory complaints,[102] and visual hallucinations.[103] Interestingly, a recent study suggested that voluptuary habits might also affect the burden of NMS and, for instance, smoking might be associated with sleep disturbances and sexual difficulties.[104]

Biochemical biomarkers

Metabolic factors

The overall cardiovascular risk has been associated with the rate of accrual of axial motor impairment,[105] possibly by determining white matter changes.

Neurotrophic factors

Brain-derived neurotrophic factor serum levels were found to be associated with the severity of PD motor symptoms and cognitive symptoms.[45,106] Overall, these findings suggest that brain-derived neurotrophic factor serum levels may represent a biomarker not only of motor and cognitive dysfunction in PD but also of the effects of treatments on these outcomes.

Epidermal growth factor acts as a neurotrophic factor on dopaminergic nigrostriatal neurons in animal models of PD. Confirming previous data,[107] Pellecchia et al[108] showed the association between levels of epidermal growth factor, measured at the time of PD diagnosis and cognitive deficits within 2-year follow-up, with particular regard to executive dysfunctions.

Intriguingly, Picillo et al[48] suggested that IGF-1 levels might be associated with those motor symptoms predominantly responsive to dopaminergic treatment, as compared to the whole UPDRS-III score, since IGF-1 seems to act specifically on dopaminergic cells. With regard to NMS, IGF-1 levels were positively associated with cognitive functions and, in particular, with executive and memory tasks.[49]

Vitamin D levels were negatively associated with motor disability,[50,109] with cognitive functions (in particular with executive and memory tasks), and with psychiatric symptoms.

Neuroinflammation

Several neuroinflammatory factors were associated with motor and NMS in PD.[110–112] However, these results are based on small samples and need to be replicated in different population in order to test their reliability.

Oxidative stress

Coenzyme Q10 is a powerful antioxidant whose levels are inversely associated with motor symptoms of PD, thus supporting its therapeutic use.[113]

Higher bilirubin levels were associated with worse motor symptoms at the time of PD diagnosis in a cohort of 75 de novo patients, but with better ones after 2-year follow-up, suggesting that higher bilirubin levels might depict a more pronounced neurodegenerative process, but are possibly related to improved outcomes over time.[54]

Higher UA levels were associated with slower motor progression and with a reduced risk of requiring levodopa treatment, in a secondary analysis of the DATATOP study.[114] The latter result has been confirmed in different studies conducted on de novo or advanced PD populations.[115–117] It is worth noting that UA levels might be particularly lower in those subjects with predominant axial features, as compared to TD patients, suggesting a more severe disease course.[118] Accordingly, higher UA levels have been associated with 80% reduced likelihood of wearing-off[119] and with lower total score of the NMS questionnaire. In detail, UA levels have been associated with specific NMS, such as attention/memory, depression/anxiety, and cardiovascular.[117,120,121] The association with the impairment of attention/memory domains seems particularly promising since confirmed by different studies exploring cognitive function with comprehensive neuropsychological batteries.[122,123] Furthermore, a recent Phase 2 trial on the use of inosine in PD, a precursor of UA, showed a possible association between increased UA levels and mood disorders assessed with the Geriatric Depression Scale.[124]

Iron and other metals

Iron metabolism has been associated with the presence of RBD and, more in general, with poor quality of sleep in PD patients.[125,126] Furthermore, there is preliminary evidence of higher iron levels in PD patients with depression.[127]

Manganese and copper have also been associated with depressive symptoms of PD, but these findings need to be confirmed by further studies on larger samples.[127]

CSF biomarkers

Conflicting results have been reported when analyzing the possible relationship between α-syn and motor severity of PD.[71,128,129] In a secondary analysis of the DATATOP study, lower baseline CSF α-syn levels predicted a better preservation of cognitive functions in early PD patients after 8-year-follow-up.[72]

Another analysis of the DATATOP study found an association between higher phosphorylated-tau protein and greater decline in cognitive functions but slower motor progression.[130,131] Thus, it is clear that the role of tau protein is largely unknown, and, so far there are only association studies, not providing possible explanations on the importance of tau species in PD pathogenesis.

CSF amyloid beta peptides have also been studied in relation to cognitive functions in early PD, but conflicting results have been reported.[68,69]

Imaging biomarkers

Once the overt disease has become manifested, imaging biomarkers of prodromal or diagnosis of PD (ie, TCS, DaT imaging, etc) do not reliably feature clinical disease progression and, thus, are not considered useful biomarkers of progression. Thus, other imaging markers are currently being tested and validated as biomarkers of disease progression and many other in this regard are in the pipeline.

A study evaluating fMRI whole-brain resting-state functional connectivity changes in relation to cognitive decline found a decrease in functional connectivity in PD patients independent of aging effects and directly related to cognitive decline.[132] These findings confirm previous data,[133] and support the role of reduced functional connectivity in cognitive decline and dementia of PD.[132]

Previous evidence showed a resting state functional connectivity disruption of "executive attention" and visual neural networks in association with the presence of freezing of gait in PD.[134] Indeed, this data further strengthened the relationship between gait and cognition in PD.[134]

PD pathology leads to volumetric changes in the brain. A study investigated the pattern of gray matter changes according to disease progression in 89 patients with PD and detected decreased gray matter volume in the fronto-temporo-parietal areas and the bilateral caudate and increased gray matter volume in the bilateral limbic/paralimbic areas, medial globus pallidus/putamen, and the right occipital cortex as compared with healthy controls. This study suggested that the caudate volume loss may contribute to cognitive decline, while changes in thalamic volume may have relevance to tremor severity.[135]

Supporting previous findings,[136] a recent study found a positive correlation between Mini-Mental State Examination scores and cortical thickness in the anterior temporal, dorsolateral prefrontal, posterior cingulate, temporal fusiform, and occipito-temporal cortex, thus supporting the usefulness of cortical thickness measurement in assessing disease stage and cognitive impairment in patients with PD.[137]

Even more recently, changes in iron content in basal ganglia, assessed as MRI relaxation rate R2*, have been proposed as a biomarker of disease progression in PD, suggesting that R2* MRI could be an interesting tool for individual assessment of neurodegeneration, and also be useful in testing the efficiency of DMT.[138]

Conclusion

In this review, we have summarized the current insights on clinical, imaging, and biochemical biomarkers as markers

for prodromal phase, diagnosis, and disease progression of PD. Indeed, it is unrealistic to expect that there will be a unique biomarker useful to discriminate between PD and other diseases with high sensitivity and specificity as well as to track progression throughout the entire disease course.[139] By combining microscopic (CSF α-syn) and macroscopic (fMRI functional connectivity) observations, as well as Pittsburgh compound B-PET imaging to detect amyloid beta peptides' deposit, Campbell et al[140] provided a first step toward a comprehensive combined biomarkers strategy. In this cross-sectional study, the authors measured soluble CSF α-syn in PD patients without dementia and found that reduced CSF α-syn levels correlated with reduced fMRI sensorimotor connectivity. Indeed, the current challenge as well as the future perspective of development of biomarkers in PD is to find the best combination of biomarkers to track the disease progression since the prodromal stages.

In this context, genetics as well as the possibility to share data through consortia will accelerate the pace of PD biomarker research.[40,141]

Author contributions

MP contributed to conception, organization, and execution of the research project; manuscript writing of the first draft. MM contributed to organization and execution of the research project, manuscript writing of the first draft. ES contributed to execution of the research project, manuscript writing of the first draft. MTP contributed to conception and organization of the research project, review and critique the manuscript. MP, MM, ES, and MTP gave final approval of the version to be published. All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

Disclosure

MP, PB, and MTP were staff at the University of Salerno, Italy. MM and ES were staff at the "Federico II" University of Naples, Italy. The authors did not receive financial support for this research. All expenses were funded by the researchers. The authors report no other conflicts of interest in this work.

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