Neurosci. Bull. February, 2020, 36(2):183–194
https://doi.org/10.1007/s12264-019-00433-1

www.neurosci.cn
www.springer.com/12264

REVIEW

Biomarkers for Parkinson's Disease: How Good Are They?

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Received: 27 July 2019 / Accepted: 17 September 2019 / Published online: 23 October 2019

Abstract Parkinson's disease (PD) is a complex neurodegenerative disorder with no cure in sight. Clinical challenges of the disease include the inability to make a definitive diagnosis at the early stages and difficulties in predicting the disease progression. The unmet demand to identify reliable biomarkers for early diagnosis and management of the disease course of PD has attracted a lot of attention. However, only a few reported candidate biomarkers have been tried in clinical practice at the present time. Studies on PD biomarkers have often overemphasized the discovery of novel identity, whereas efforts to further evaluate such candidates are rare. Therefore, we update the new development of biomarker discovery in PD and discuss the standard process in the evaluation and assessment of the diagnostic or prognostic value of the identified potential PD biomarkers in this review article. Recent developments in combined biomarkers and the current status of clinical trials of biomarkers as outcome measures are also discussed. We believe that the combination of different biomarkers might enhance the specificity and sensitivity over a single measure that might not be sufficient for such a multiplex disease.

Keywords Parkinson's disease · Biomarkers · Combined biomarkers · Clinical trials

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Introduction

Parkinson's disease (PD) is a chronic and progressive neurodegenerative disorder that results from the loss of dopamine neurons within the substantia nigra (SN) and manifests with a broad range of motor and non-motor symptoms [1, 2]. As the second most common neurodegenerative disorder, PD affects 1% of people older than age 60, and 3% at the age of 80 years or older [3]. Although great achievements in the understanding of PD have been made during the over 200-year history of PD research [4], the diagnostic criteria for PD are still based on the identification of only motor symptoms, namely bradykinesia plus rigidity and resting tremor, which occur years after the neurodegenerative process has started [2]. Moreover, even when the new diagnostic criteria are correctly applied, the misdiagnosis rate is still high (16%–20% by movement disorder experts) due to substantial clinical overlap among parkinsonian disorders [5]. Delayed diagnosis and misdiagnosis militate against the therapeutic benefits of disease-modifying therapies. Therefore, there is an urgent need to make an effort to discover and identify reliable and accurate biomarkers for PD.

In 1998, the National Institutes of Health Biomarkers Definitions Working Group defined a biomarker as "a measurable indicator of some biological state or condition that is objectively measured and evaluated to examine normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" [6]. Biomarkers can be classified according to their functional characteristics: susceptibility risk biomarkers representing the potential for developing PD [7]; diagnostic biomarkers are used to confirm the presence of PD; and prognostic biomarkers indicating disease progression, treatment-associated changes, or disease recurrence [8].

For over 20 years, given the urgency of early diagnosis and effective disease-modifying treatments of PD, great efforts have been made to discover diagnostic and prognostic biomarkers [9]. A robust and accurate diagnostic biomarker can help recognize PD before the motor symptoms appear or when the motor or non-motor signs are inadequate to make the clinical diagnosis. It can also be used to make a differential diagnosis between PD and other neurological disorders, especially differentiating idiopathic PD from other forms of parkinsonism. It would be preferable if the biomarker could be verified in neuropathologically demonstrated cases of PD [10]. In general, biomarkers include physiological measurements, bodily fluid or tissue examinations, genetic or metabolic data, imaging measures, and even rating scales or survey measures that could represent candidate biomarkers [6].

Although various studies have been conducted in discovering potential PD biomarkers, only a few biomarkers have been translated into clinical practice [11]. PD biomarker studies have often overemphasized the discovery of novel potential biomarkers, whereas efforts to further evaluate such candidates are rare [12]. In this review, we summarize the evaluation measures of biomarkers and provide an update on the discovery of promising candidates in the diagnosis and prognosis of PD. Some recent developments on combined biomarkers and the current status of clinical trials using biomarkers as outcome measures are also discussed.

Process for the Evaluation of PD Biomarkers

Concerning the lack of a coherent pipeline connecting biomarker discovery with well-established methods for validation, it is important to establish standard protocols for the method of biomarker evaluation. Many studies on biomarkers have suggested pipelines for evaluating and validating novel biomarkers [8, 13]. In 2010, the National Academy of Sciences of America recommended a framework for the evaluation of biomarkers at the request of the Food and Drug Administration, with critical components of analytical validity, qualification, and utilization [14]. The framework provides guidelines for the previously non-uniform process in the evaluation of biomarkers.

Analytical Validation

Analytical validation can be employed to evaluate the assays and measurement performance characteristics of potential biomarkers, determining the accuracy and repeatability of biomarkers [14]. As an assessment of a biomarker test, analytical validation includes the detection approaches and the discrimination of the biomarkers.

Establishing appropriate and standard approaches is crucial to biomarker discovery studies, which can ensure the quality and reproducibility of results, especially when comparing across multiple laboratories and clinical settings [15]. Studies of biomarker discovery usually start by establishing an accessible and confirmed PD patient cohort according to clinical diagnostic criteria with specialists. A meta-analysis of longitudinally-followed participants with autopsy-confirmed diagnoses shows that a good correlation between clinical diagnosis and neuropathological diagnosis can be established if a specialist follows international diagnostic criteria, and continues follow-up to correct any initial diagnostic error [10]. It is equally important to set up a standard sample collection process, and storage procedures for samples such as cerebrospinal fluid (CSF) and blood. In addition, harmonization of access to biobank samples streamlines the process of PD biomarker discovery [11]. Overall, standard metrics exist for all the processes from biomarker discovery to validation.

As an important evaluation index in characterizing the performance of a diagnostic biomarker, discrimination indicates the capability of a test to distinguish between disease and control, or those with and without an outcome of interest. When it comes to the discrimination of a diagnostic biomarker of PD, the ability to distinguish PD from healthy controls (HCs) is usually not enough. The inclusion of non-PD neurological disease controls (NDCs), especially parkinsonian syndrome, is critical in biomarker validation studies [11]. Sensitivity and specificity are most commonly regarded in the discrimination analysis. Here, the receiver operating curve (ROC) is a graphical plot to illustrate the diagnostic ability of a binary classifier system by plotting the true-positive rate (sensitivity) against the false-positive rate (1-specificity) at a range of cutoff points [13]. Every possible cutoff point of a test result corresponds to the resulting sensitivity and specificity. The area under the ROC curve (AUC) is the most widely used measure of testing discrimination, and can directly compare the diagnostic accuracy of biomarkers. A perfectly discriminating test would have an AUC of 1. Currently, a particular AUC value of 0.8 is considered a "good" performance of a test. Many PD biomarker studies have evaluated the performance characteristics of biomarkers with relatively small sample sizes, and these approaches may not yield clinically useful AUC characteristics. However, in some cases, a higher AUC value may not be so helpful either, since the clinician may only care about a single cutoff point selected from the curve to meet special demands, whereas the AUC refers to the entire curve.

Qualification

Qualifications can furnish available evidence on associations between biomarkers and disease states or clinical outcomes. One of the most important components in qualification is to evaluate the prognostic value of the biomarker-disease relationship [14]. Prognostic biomarkers as indicators of disease progression or treatment-associated changes that must be conducted by prospective or cohort studies, allow for causal inferences to be made since biomarkers and measurements of PD occur simultaneously [16]. Follow-up work in the prospective studies always takes years or decades, especially in PD because of its chronic course. On the contrary, cross-sectional studies enroll a population of interest and collect data on the characteristics of interest almost at the same time, but biomarkers found by cross-sectional studies cannot reveal a prognostic or predictive value [17].

When assessing the relevance for predicting future events, the calibration of biomarkers should also be considered. Calibration is used to estimate probabilities that closely correspond with the outcomes in reality. A simple way of presenting the calibration value is by plotting observed *versus* predicted results [8]. Under some circumstances, biomarkers may improve the accuracy of a test through an improvement in calibration without altering the AUC.

Utilization

Utilization is a contextual analysis of the available evidence about the risks and benefits associated with the use of biomarkers [14]. The safety and efficacy of biomarkers should be weighed against their risk of failure to determine a range of proper performance for each specific biomarker [8]. Cost-effectiveness analysis is a key tool in considering the utilization of biomarkers, and can represent the outcome probabilities and assign values to particular outcomes [8]. A great deal of research has been done on how to conduct such studies [18], although definitive estimates of costs can be made only after the measurement of clinical outcomes.

Recent Advances in PD Biomarkers

Clinical Biomarkers

It is widely accepted that before the classical motor symptoms occur, subtle motor dysfunction or non-motor symptoms may already appear. Non-motor symptoms such as rapid eye movement sleep behavior disorder (RBD), hyposmia, constipation, and mood disorders are referred to as promising biomarkers in the detection of prodromal PD [19]. RBD as the most common and best-characterized parasomnia in PD, is assumed to be an original symptom of progressive neurodegeneration. Numerous studies have revealed that RBD can serve as an anticipatory biomarker of prodromal PD and other synucleinopathies. RBD is strongly associated with PD with a 45% risk of developing neurodegeneration at 5 years and a 76% risk at 10 years, according to a 7-year follow-up study [20]. Aiming to assess the diagnostic accuracy of the prodromal criteria introduced by the International Parkinson and Movement Disorder Society in 2015, one RBD cohort study found that 39.7% of individuals with RBD converted to PD/dementia with Lewy bodies. The prodromal criteria had 81.3% sensitivity and 67.9% specificity for conversion to PD in RBD cohorts at 4-year follow-up [21].

Data on the prevalence of olfactory dysfunction in PD range from 45% to 90% [22]. There is good evidence that most PD patients develop olfactory dysfunction 4–6 years before the motor impairment occurs [23]. However, the specificity of olfactory dysfunction is lower than RBD and motor markers [24]. This could be because olfactory dysfunction is also common in other synucleinopathies and in older adults. Combined assessment of olfactory dysfunction, motor asymmetry, and a typical finding at ultrasound (midbrain hyper-echogenicity) can improve diagnostic accuracy in early PD [25]. Since the specificity of these non-motor symptoms is generally not sufficient for the early diagnosis of PD, constructing other objective measures and auxiliary methods in combination with the results of non-motor signs would enhance the predictive value of the clinical biomarkers for PD.

Imaging Biomarkers

An increasing number of imaging tests are promising for indicating early changes in PD patients and as an independent measure of disease progression, with the character of less susceptibility to the effects of subjectivity, medication, and placebo [26]. Among the imaging studies on PD, neuroimaging of the dopamine system has received the most attention. Dopamine transporter single-photon emission computed tomography (DAT-SPECT) and fluorodopa positron emission tomography (F-DOPA PET) have been used to detect neurochemical changes in the dopamine system [27]. The majority of studies on DAT-SPECT imaging of PD patients have shown a high accuracy of diagnostic performance with a sensitivity of 79%–100% and specificity of 80%–100% [27, 28]. Various SPECT radiotracers for imaging DAT also have been used to evaluate the severity of disease and differentiate PD from other forms of parkinsonism [29]. In a 4-year clinical follow-up study, the combination of hyposmia and DAT deficit revealed by DAT-SPECT is able to identify the risk of PD onset, with a 5% reduction in DAT binding annually [30]. Similar to studies with DAT-SPECT, F-DOPA

PET imaging recognizes decreased F-DOPA uptake in the caudate and putamen of PD patients [31]. Reduced F-DOPA uptake has been reported to occur contralateral to the hypokinesia-rigidity symptoms and is correlated with its severity [quantified by the Unified Parkinson's Disease Rating Scale (UPDRS)].

Advanced magnetic resonance imaging (MRI) techniques, including several specific sequences and high field-strength scanners, have shown promise in the early diagnosis of PD and monitoring disease progression [32, 33]. Neuromelanin MRI (NM-MRI) is a novel technique that reflects the loss of neurons containing neuromelanin, and the signal intensity of the SN is greatly reduced on NM-MRI in PD patients [34]. Nigrosome-1 (N1) indicates an area of high signal intensity in the dorsal part of the SN and is visualized as a "swallow-tail" sign on high-resolution susceptibility-weighted imaging (SWI) [35]. Calloni *et al.* have assessed the loss of N1 on multiple-echo SWI of 126 PD patients, 30 with non-PD parkinsonism, and 24 HCs [36]. They found that the sensitivity and specificity of N1 in discriminating PD from controls is 96.43% and 85.00%, whereas N1 does not play a leading role in the differentiation of PD from non-PD parkinsonism, with a low level of specificity [36]. While promising, the utility of MRI in early diagnosis and monitoring the course of PD remains to be defined.

Biofluid-Based Biomarkers

α-Synuclein

Misfolded and aggregated α-synuclein is the major protein component of Lewy bodies, and is thought to be the pathological hallmark of PD [37]. Genetic mutations and post-translational modifications of the α-synuclein protein, such as phosphorylation, ubiquitination, and oxidization, participate in the process of protein misfolding [7]. Since α-synuclein is both genetically and pathologically associated with PD and can be detected in biofluids, it has become a widely-used approach in PD biomarker studies [37, 38]. Rapid progress has been made in the identification and validation of α-synuclein species as biomarkers for PD in recent years.

Studies from different laboratories on total α-synuclein in the CSF have produced consistent results with high analytical precision and inter-laboratory correlation, collectively showing that total CSF α-synuclein is significantly lower in PD patients than in HCs and patients with NDCs [38–41]. Other α-synuclein species such as oligomeric α-synuclein and phosphorylated α-synuclein have also been evaluated as potential biomarkers for PD [42–44]. However, none of these alone has a satisfactory diagnostic accuracy in distinguishing PD patients from controls [7]. A meta-analysis of data from 34 studies on total CSF, as well as oligomeric and phosphorylated α-synuclein in patients with PD, NDCs, or other forms of parkinsonism, and HCs has been reported. The results revealed that the sensitivity and specificity of total α-synuclein for distinguishing PD from controls are 0.72 and 0.65, respectively, and the sensitivity and specificity of oligomeric α-synuclein are 0.71 and 0.64 [45]. In longitudinal studies, no significant change of total α-synuclein in CSF has been found in patients with early PD and HCs over 6–24 months [46, 47]. A study assessing the combined α-synuclein species showed that the ratio of CSF oligomeric α-synuclein to total α-synuclein, together with phosphorylated α-synuclein and neurodegenerative biomarkers improves the diagnostic performance of oligomeric α-synuclein alone, with an AUC of 0.86%, sensitivity 79%, and specificity 67% [42]. For these reasons, the majority view is that a single species of CSF α-synuclein cannot be regarded as a reliable biomarker for PD, while the combination of α-synuclein species, or with other CSF biomarkers may furnish encouraging results.

α-Synuclein aggregation in the CSF has aroused great interest among researchers in recent years for its remarkable diagnostic accuracy in distinguishing PD patients from controls [48–50]. α-Synuclein seeding aggregation assays including protein-misfolding cyclic amplification and real-time quaking-induced conversion (RT-QuIC) are the main techniques for measuring pathogenic α-synuclein aggregates in biofluids [50]. An RT-QuIC assay study showed α-synuclein aggregation in the CSF of PD patients with a sensitivity of 95% and a specificity of 100% compared to HCs, while patients with Alzheimer's disease (AD), progressive supranuclear palsy, or corticobasal degeneration give negative results for α-synuclein aggregation [48]. A more recent study has detected oligomeric α-synuclein in the CSF of 105 PD patients and 79 HCs from the BioFIND cohort, a North American multicenter study of PD patients with standardized clinical and biospecimen acquisition protocols [17], by independently cross-validating two different platforms of α-synuclein seeding aggregation assays [51]. The AUC value for the diagnosis of PD *vs* HCs reached 0.95 with a sensitivity of 97.1% and a specificity of 92.5%. Given that these assays represent a process central to the pathogenesis of PD, and support the hypothesis that α-synuclein pathology spreads by a "prion-like" mechanism [52], α-synuclein aggregation assays in CSF may have the potential to be a surrogate for the presence of α-synuclein pathology. As for whether α-synuclein aggregation can be considered acceptable for its introduction into clinical practice as a biomarker for PD, larger cohorts of patients with pathological confirmation of PD and ongoing longitudinal assessment of prodromal individuals are needed.

α-Synuclein can be detected in blood due to its high level of expression and production by red blood cells (RBCs). Since blood is more easily accessible than CSF in

clinical circumstances, a body of studies has assessed the levels of α-synuclein species in RBC, serum, and plasma as a candidate biomarker of PD [37]. However, these studies have yielded inconsistent results for the changes in the α-synuclein level in the blood of PD patients compared to HCs [53–55]. This could be due to the easy contamination of RBC and different approaches to sample collection and determination across studies.

Extracellular Vesicles

Extracellular vesicles (EVs) are a subset of small membranous vesicles derived from the endosomes and released into biofluids by almost all kinds of tissue, including the central nervous system (CNS) [56]. They can be categorized into exosomes (50 nm–150 nm), microvesicles (100 nm–1000 nm), and apoptotic bodies (> 1000 nm) [57]. EVs harbor a cargo of proteins and nucleic acids that are likely to indicate pathogenic processes in CNS. Therefore, CNS-derived EVs may hold promise for biomarker discovery in PD.

Increasing evidence has demonstrated that EVs mediate the transfer and transport of toxic α-synuclein between cells, suggesting a pivotal mechanism underlying the spread of α-synuclein aggregates and the acceleration of pathology in PD [58]. It has been reported that exosomes derived from the CNS occur in the blood and the level of α-synuclein from CNS-derived exosomes in plasma is significantly higher in PD patients, but the performance of plasma exosomal α-synuclein is only moderate (AUC 0.654, sensitivity 70.1%, specificity 52.9%) [59]. Recent findings on α-synuclein from EVs in plasma are consistent with these results and also lack sufficient diagnostic performance [60, 61]. Apart from α-synuclein, changes in other proteins or nucleic acids such as microRNAs have also been detected [58, 62, 63]. Proteomic analysis of urinary EVs has shown that the combination of SNAP23 and calbindin attains a diagnostic performance at an AUC of 0.86 with 77% sensitivity and 85% specificity [64]. It would be worth evaluating candidate biomarkers in EVs using a larger clinical PD cohort and investigating panels of combined biomarkers in EVs to enhance the accuracy of diagnosis.

MicroRNAs

Recent studies have demonstrated that microRNAs are involved in the regulation of PD-related genes and alterations of certain microRNAs possibly relevant to either disease onset or disease progression [65, 66]. MicroRNAs have been considered as potential biomarkers for the early detection of PD as well as monitoring the progression of the pathology for their characteristics of detectability and stable expression in biofluids [67]. Altered expression levels of microRNAs in patients with PD *versus* controls have been widely reported. A recent meta-analysis has shown hsa-miR-221-3p, hsa-miR-214-3p, and hsa-miR-29c-3p to be significantly differentially expressed in the blood of PD patients [68]. Yang and colleagues measured the level of hsa-miR-105-5p in the plasma of 319 PD patients, 305 patients with NDCs, and 273 HCs and found that it was significantly higher in PD patients than in HCs (0.163 ± 0.018 *vs* 0.065 ± 0.011, $P < 0.001$), or patients with NDCs (0.163 ± 0.018 *vs* 0.047 ± 0.007, $P < 0.001$). The discriminative values (AUC) in differentiating PD from HCs, essential tremor (ET), and AD are 0.768, 0.786, and 0.787, respectively [69]. A comprehensive analysis of the microRNA combinations of hsa-miR-335-5p/hsa-miR-3613-3p (95% CI, 0.87–0.94), hsa-miR-3355p/hsa-miR-6865-3p (95% CI, 0.87–0.93), and miR-335-5p/miR-3613-3p/miR-6865-3p (95% CI, 0.87–0.94) showed that they are closely related to a high degree of discriminatory accuracy (AUC 0.9–1.0) [70]. Although several microRNAs have been demonstrated to be relevant to the onset and progression of PD, and their combination could present a relatively high diagnostic accuracy for PD, more effort is needed to further evaluate the potential of microRNAs and other small molecules as candidate biomarkers before application in clinical practice.

Inflammation-Related Biomarkers

Mounting evidence supports the role of inflammation as a measurable driving force of PD pathology. Neuroinflammation is associated with abnormally activated microglia and altered levels of inflammatory mediators in the brains of PD patients. Many studies have shown that CSF and plasma levels of inflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-4, IL-6, and IL-10 are significantly higher in PD than in HCs [71, 72]. On account of the expression levels of inflammatory cytokines that may not be specifically elevated in PD, combinations of inflammatory cytokines with other candidate biomarkers have been investigated to help the early diagnosis and detection of PD progression [73, 74]. Edison *et al.* identified a panel of inflammatory factors with α-synuclein in serum and CSF that can be measured with stable results regardless of sample collection time; they distinguish between PD and HCs with 82% sensitivity and 83% specificity, and monitor inflammation as disease progresses [74]. In addition, several T cell-mediated immunity-related proteins in the peripheral blood have been discovered and verified as potential biomarkers of PD. Lymphocyte activation gene-3 (sLAG-3), an important marker of helper T cell activity, has been shown to be a candidate novel biomarker for PD with an AUC of 0.82 (serum sLAG-3) in differentiating PD from HCs [75].

Recent investigations suggest an important role of gut-derived inflammation through the microbiota-gut-brain axis in the pathogenesis of PD [76]. Gut inflammation may interact with microbiota changes and facilitate the expression and aggregation of α-synuclein from gut to the brain *via* the vagus nerve [76, 77]. Studies have revealed differences in gut microbiota and microbiota metabolism between early-stage PD patients and controls [78]. The gut microbiome has also been shown to be associated with the severity of constipation and motor phenotype [78]. Pardo *et al.* found increased expression of the bacterial endotoxin-specific ligand TLR4, CD^{3+} T cells, and cytokines in colonic biopsies from PD patients [77]. These alterations of gut microbiota and gut inflammation-related indices may have the potential to be early diagnostic biomarkers for PD and deserve further investigation.

Combinations of Biomarkers

An increasing number of studies have revealed that a combination of biomarkers can improve the diagnostic accuracy of individual biomarkers. Combined biomarkers might be able to predict the motor progression or cognitive impairment of PD. Even though various types of biomarkers have been involved in the analysis of combined biomarkers, α-synuclein is still of the most concern (Table 1). It is recommended to use the combined novel biomarkers with existing clinical predictors rather than expecting a biomarker to simply substitute for clinical assessment. The use of biomarkers in combination should span multiple modes (for example, clinical, biochemical, and imaging-based biomarkers) to maximize their utility. And yet, very little research has been conducted to evaluate the performance of combined novel imaging or biofluid-based biomarkers with clinical assessment. The challenge is to establish a methodology to unite these biomarkers of disparate type and strength into merged criteria. Mollenhauer *et al.* have explored a panel of multi-modal progression biomarkers for PD in a longitudinal cohort [47]. After 24 months covering non-motor symptoms, cognitive function, and REM sleep behavior disorder by polysomnography, voxel-based morphometry of the brain by MRI, and CSF markers (including total α-synuclein, amyloid beta 1-42 (Aβ42), total and phosphorylated tau protein, and neurofilament light chain proteins), they found that biomarkers with relative worsening included the sleep and imaging measures, whereas the cognitive measures and selected biofluid-based biomarkers were not significantly altered in PD compared to HCs [47]. A cohort study constructed a predictive model by the composition of multivariate measures including age, UPSIT (University of Pennsylvania Smell Inventory Test), RBDSQ (Rapid Eye Movement Sleep Behaviour Disorder Screening Questionnaire), CSF Aβ42, and caudate uptake on DAT imaging, and found that the five variables in combination showed the strongest associations with cognitive impairment, allowing prediction of cognitive impairment at 2 years in PD patients (0.80, 0.74–0.87; $P = 0.0003$ compared to age alone) [95]. Since none of the candidate biomarkers so far has provided an accurate and early diagnosis of such a complex disease, our vision for the future is that a combination of different kinds of biomarkers may solve this dilemma.

Conclusions

With an increasing number of novel candidate biomarkers, it is of great importance to establish a standard evaluation measure of PD biomarkers, which may help to connect the discovery to the validation of candidate biomarkers. Processes in the evaluation of PD biomarkers mainly consist of analytical validation, quality control, and utilization, in which standardization of approaches, discrimination value, follow-up work in cohort studies, calibration, and cost-effectiveness analysis should be taken into consideration.

Current biomarkers mainly focus on the symptomatic evaluation of PD, specific neuroimaging changes, and biochemical measurements of biofluids. Some of these candidate biomarkers have relatively high diagnostic performance or predictive value for PD and may have the potential to be applied in clinical practice after a standard evaluation. Biomarkers may address many of the critical issues in clinical trials, such as the selection of appropriate participants and the assessment of treatment effects. To date, biomarkers have been applied in several clinical trials for PD, and most of them are used as surrogate outcomes for investigating the biological efficacy of a treatment (Table 2). For instance, total α-synuclein has been proposed as a surrogate biomarker in assessing the efficacy of drugs targeting α-synuclein in phase 1–2 clinical trials. In spite of this, biomarkers with the function of identifying a population by confirming the clinical diagnosis are still rarely used. Therefore, to improve the diagnostic accuracy of individual biomarkers, an increasing number of studies have focused on developing panels of combined biomarkers or predictive models involving a combination of biomarkers. We believe that exploration for biomarkers will continue. Relative to looking for novel biomarkers, more effort should be made in systematically proceeding to the long process of confirming clinical validity and utility in future studies. As they are validated, more reliable biomarkers should begin to deliver on their full potential, with opportunities for clinical trials, personalized treatments, and primary or secondary prevention of PD.

Table 1 Overview of combined diagnostic and prognostic biomarkers.

Combined biomarkers	Diagnostic value	Prognostic value		Sample size	Study
		Motor progression	Cognitive impairment		
Biofluid-based biomarkers					
Oligomeric α-synuclein/total α-synuclein in CSF	NA	Correlation with UPDRS motor ($r = 0.41$, $P < 0.01$)	NA	121 early PD patients from the DATATOP cohort	Majbour *et al.* [79]
Oligomeric α-synuclein/total α-synuclein, phosphorylated α-synuclein, and phosphorylated tau in CSF	AUC 0.86, sensitivity 79%, specificity 67%	NA	NA	46 PD patients and 48 HC	Majbour *et al.* [42]
Total tau/total α-synuclein in CSF	AUC 0.83, sensitivity 70%, specificity 88% (PD *vs* AD)	NA	NA	78 PD patients and 20 AD patients	Førland *et al.* [80]
Phosphorylated tau/α-synuclein and TNF-α in CSF	AUC 0.91, sensitivity 92.9%, specificity 75%	NA	NA	40 PD patients and 40 HC	Delgado-Alvarado *et al.* [81]
NFL, FABP3, and Aβ42 in CSF	AUC 0.87 (NFL/Aβ42)	NA	Higher NFL ($P = 0.0005$), lower Aβ42 ($P = 0.00053$), and higher FABP3 ($P = 0.0037$) conferred high hazard ratios for PDD	104 PD patients and 30 HC	Bäckström *et al.* [82]
FABP3 with phosphorylated tau and total α-synuclein in CSF	AUC 0.96 (PDD *vs* AD)	NA	NA	54 PD, 20 PDD, and 48 AD patients	Chiasserini *et al.* [83]
Total tau, phosphorylated tau, α-synuclein, Aβ42, NFL, MCP-1, and YKL-40 in CSF	AUC 0.95 (PD *vs* non-PD parkinsonism)	NA	NA	31 PD and 94 non-PD parkinsonism patients	Magdalinou *et al.* [84]
DJ-1, total tau and phosphorylated tau	AUC 0.92, sensitivity 82%, specificity 81% (PD *vs* MSA)	NA	NA	43 PD and 23 MSA patients	Herbert *et al.* [85]
Total α-synuclein, Aβ42, GCase, β-hex, and cathepsin D	AUC 0.83, sensitivity 84%, specificity 75%	NA	NA	79 PD patients and 61 HCs from the BioFIND cohort	Parnetti *et al.* [86]
Serum TNF-α and CSF α-synuclein	AUC 0.75, sensitivity 82%, and specificity 83%	NA	NA	12 PD patients and 6 HCs at 11 time-points across 24 h	Eidson *et al.* [74]
IFN-γ, IL-10, and TNF-α in serum	AUC 0.87, sensitivity 83%, specificity 80%	Correlation with postural instability ($P < 0.001$)	Correlation with cognitive impairment ($P < 0.001$)	72 PD patients and 56 controls	Rathnayake *et al.* [87]
Nurr1, TNF-α, IL-1β, IL-4, IL-6, and IL-10 in PBMCs	AUC 0.71	NA	NA	312 PD patients, 318 HCs, and 332 NDC patients	Le *et al.* [73]
MiR-19a, miR-19b, miR-24, miR-30c, miR-34b, miR-133b, and miR-205 in CSF	AUC 0.98	NA	NA	28 PD patients and 28 controls	Marques *et al.* [88]

Table 1 continued

Combined biomarkers	Diagnostic value	Prognostic value		Sample size	Study
		Motor progression	Cognitive impairment		
Imaging-based biomarkers					
NM-MRI and N1 imaging	AUC 0.935, sensitivity 0.85, specificity 0.92 (PD *vs* ET)	NA	NA	68 PD and 25 ET patients	Jin *et al.* [89]
DAT-SPECT, DTI, and sMRI	NA	Correlation with the UPDRS ($P < 0.001$)	NA	205 PD patients and 105 controls at 1-year follow-up	Lorio *et al.* [90]
NM-MRI and DAT-SPECT	AUC 0.99, sensitivity 93%, specificity 100% (PD *vs* non-PD parkinsonism)	NA	NA	30 PD and 19 non-PD parkinsonism patients	Matsusue *et al.* [91]
Multiple models of combined biomarkers					
Plasma oligomer α-synuclein and multiple ESWAN imaging markers	AUC 0.827, sensitivity 0.8, specificity 0.8	NA	NA	60 PD patients and 30 HCs	Chen *et al.* [92]
PIGD score, caudate DAT imaging, and CSF Aβ42	NA	Accuracy (AUC 0.684, 95% CI 0.628–0.740) in prediction of later development of FOG	NA	393 patients with newly diagnosed PD without FOG at 4 years of follow-up	Kim *et al.* [93]
Age, CSF oligomeric/total α-synuclein, and β-glucocerebrosidase activity	AUC 0.87, sensitivity 82%, specificity 71%	NA	NA	71 PD and 45 NDC patients	Parnetti *et al.* [94]
Age, UPSIT, RBDSQ, CSF Aβ42, and caudate DAT imaging	NA	Accuracy of prediction of cognitive impairment (AUC 0.8)	NA	390 PD patients at 2 years of follow-up	Schrag *et al.* [95]

PD, Parkinson's disease; HC, healthy controls; AD, Alzheimer's disease; PDD, PD dementia; ET, essential tremor; CSF, cerebrospinal fluid; AUC, area under the curve; UPDRS, Unified Parkinson's Disease Rating Scale; NA, not analyzed; DATATOP, Deprenyl and Tocopherol Antioxidative Therapy for Parkinsonism study; NFL, neurofilament light chain protein; FABP3, fatty-acid-binding protein 3; PBMCs, peripheral blood mononuclear cells; NM-MRI, neuromelanin-sensitive magnetic resonance imaging; N1, nigrosome-1; DAT-SPECT, dopamine-transporter SPECT; DTI, diffusion tensor imaging; sMRI, structural magnetic resonance imaging; ESWAN, multiple enhanced T2 star-weighted angiography; PIGD, postural instability gait difficulty; FOG, freezing of gait; UPSIT, smell identification test; RBDSQ, REM sleep behavior disorder screening questionnaire.

Table 2 Clinical trials using biomarkers as outcome measures

Study title	Biomarkers	Participants	Phase	Clinical Trials. gov identifier
The Swedish BioFINDER 2 Study	Aβ42, tau, and phosphorylated tau	1505	Not applicable	NCT03174938
KM-819 for patients with PD	KM-819, oligomeric α-synuclein, total tau, phosphorylated tau	88	1	NCT03022799
Evaluation of a multimodal neuroimaging method for diagnosis in PD	Combination of biomarkers in multimodal imaging	60	1	NCT02428816
Development of a novel 18F-DTBZ PET imaging as a biomarker to monitor neurodegeneration of PARK6 and PARK8 parkinsonism	18F-DTBZ PET imaging	49	2	NCT01759888
Single ascending dose study of MEDI1341 in healthy volunteers	Total α-synuclein	40	1	NCT03272165
Effect of undenatured cysteine-rich whey protein isolate (HMS 90®) in patients with PD	Plasma glutathione, urinary 8-hydroxydeoxyguanosine, and urinary total antioxidant status	38	4	NCT01662414
Florbetapir F18-PET imaging of beta-amyloid in PD patients	Aβ, tau, and phosphorylated tau	31	2	NCT00857532
Ambroxol in disease modification in PD	GCase	20	2	NCT02941822
Modulation of gut microbiota by Rifaximin in PD patients	Blood biomarkers of neuroinflammation and exosomal α-synuclein	20	2	NCT03958708

BioFINDER, biomarkers for identifying neurodegenerative disorders early and reliably; KM-819, Fas-associated factor 1 inhibitor; DTBZ, dihydrotetrabenazine; PET, positron emission tomography; GCase, β-glucocerebrosidase.

Acknowledgements This review was supported by the National Natural Science Foundation of China (81430021 and 81771521) and the Key Research and Development Plan of the Science and Technology Department of Liaoning Province, China (2018225051).

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