Biomarkers of Parkinson's disease in perspective of early diagnosis and translation of neurotrophic therapies

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Abstract

Parkinson's disease (PD) is a common neurodegenerative disorder characterized by progressive loss of dopamine neurons and aberrant deposits of alpha-synuclein (a-syn) in the brain. The symptomatic treatment is started after the onset of motor manifestations in a late stage of the disease. Preclinical studies show promising results of disease-modifying neuroprotective or even neurorestorative therapies with neurotrophic factors (NTFs). Three NTFs have entered phase I-II clinical trials with inconclusive outcomes. This is not surprising since the preclinical evidence is from acute early-stage disease models but the clinical trials included advanced PD patients. In order to conclude the value of NTF therapies, clinical studies should be performed in early-stage patients with prodromal symptoms, i.e. before motor manifestations. In this review, we summarize currently available diagnostic and prognostic biomarkers that could help identify at-risk patients benefiting from NTF therapies. Focus is on biochemical and imaging biomarkers, but also other modalities are discussed. Neuroimaging is the most important diagnostic tool today, but a-syn imaging is not yet viable. Modern techniques allow measuring various forms of a-syn in cerebrospinal fluid, blood, saliva and skin. Digital biomarkers and artificial intelligence offer new means for early diagnosis and longitudinal follow-up of degenerative brain diseases.

Introduction

Ample pre-clinical evidence points to neurotrophic factors (NTFs) as possible disease modifying therapies of Parkinson's disease (PD). Glial cell-line derived neurotrophic factor (GDNF), neurturin (NTR) and cerebral dopamine neurotrophic factor (CDNF) convincingly demonstrate protection and restoration of nigrostriatal dopamine neurons by infusion of the NTFs or delivery of the corresponding genes into brain tissue in various experimental PD models [1]. GDNF, NRT and CDNF have also been studied in a limited number of phase I-II clinical studies but the outcomes have remained inconclusive [2]. GDNF protein and gene therapies show rather large interindividual variability among subjects which may at least partly mirror the status of the underlying disease pathology. In general, the subjects with advanced disease gain less benefit of the therapy when compared to subjects with shorter disease history.

Diagnosis of PD is based on the onset of motor symptoms which occur several years after the neurodegenerative process has started [3]. According to guidelines by Movement Disorders Society (MDS), the recognition of prodromal or early-phase PD relies on careful clinical observation and use of several biomarkers [4]. However, none of the biomarkers is specific or reliable enough to be used alone to predict the risk of PD, make an early diagnosis or follow the progression of the disease [5-7]. A reliable and discriminating prodromal biomarker would provide a temporal window during which putative disease-modifying therapies could be administered to halt or slow down the neuronal loss.

Several ongoing multicenter biomarker studies look for potential biomarkers that are collected longitudinally from a large cohort of early-stage PD patients and healthy controls using standardized data acquisition protocols [8, 9]. As an example, a Parkinson Progression Marker Initiative (PPMI) data-based study on

the progression of PD shows promising results to monitor both motor and non-motor markers [10]. The currently available biomarker candidates can be grouped into biochemical biomarkers, imaging biomarkers and other biomarker modalities.

Biochemical biomarkers

Biochemical biomarkers are collected from body fluids, such as the cerebrospinal fluid (CSF), blood, saliva and urine, or tissue biopsies such as skin, and analyzed using molecular biology techniques [10-12]. Indeed, such biomarkers are easily accessible without risky procedures, but sensitivity and specificity issues might limit their utility [13]. In this respect CSF and blood biomarkers reflecting the pathophysiology of PD, such as alpha-synuclein (a-syn) species, lysosomal enzymes, neurofilaments and markers of beta-amyloid (Aβ) and tau pathology are obvious candidates [9, 14]. There is only a limited number of studies with focus on serum or plasma biochemistry during experimental PD [15].

Cerebrospinal fluid

Seeding aggregation assays, such as protein-misfolding cyclic amplification (PMCA) and real-time quaking-induced conversion (RT-QuIC), detect misfolded proteins prone to aggregation by exploiting their prion-like behavior [16]. In PD, the total concentration of a-syn in the CSF seems to be lower, whereas the CSF levels of a-syn oligomers and phosphorylated a-syn are elevated as compared with healthy controls [9, 17]. These measures can serve as useful biomarkers for the early detection of Lewy body disorders and distinguishing PD from Alzheimer's disease [18].

The activity of various lysosomal enzymes is changed in the CSF of PD patients as compared to healthy controls [9]. Mutations in *GBA1* gene cause changes in beta-glucocerebrosidase activity and levels of glycosphingolipids in various body fluids which underlines development of GBA-associated PD and can be measured to diagnose this subtype of the disease [19]. The diagnostic accuracy can be improved when combining several lysosomal enzyme activity measurements, and further increased when Aβ, tau and a-syn pathology markers are added to the model [20, 21]. Defining the subtype of prodromal PD may guide future neurorestorative therapies [22].

Reduced levels of circulating cell-free mitochondrial DNA measured from the CSF of PD patients have also been proposed as a potential biomarker for the disease onset and progression, but several factors such as concomitant treatments and comorbidities are likely to confound the reliability of the readout [23, 24].

Peripheral body fluids

Plasma levels of a-syn species, neurofilaments, glial fibrillary acidic protein (GFAP), pTau181 and inflammatory cytokines have been studied as prospective biomarkers of PD [5]. Increased quantities of total a-syn, but no change in oligomeric and phosphorylated species of a-syn, have been measured in plasma from PD patients [25]. Extracellular vesicles isolated from blood and carrying a-syn oligomers and phosphorylated a-syn may bring new possibilities for the early diagnosis of PD [26]. Blood beta-synuclein may also have relevance since, unlike a-syn, it is not present in the blood of healthy individuals [27]. Additionally, the concentration of different a-syn species and DJ-1 in saliva have been proposed as potential easily accessible biomarkers [28]. However, the correlation between salivary a-syn and the presence or severity of PD have remained controversial.

Neurofilaments are abundant structural proteins exclusively expressed in nerve fibers. Upon axonal damage different subunits of neurofilaments are released to the interstitial space in the central nervous system (CNS) [29]. Neurofilament light chain (NfL) shows promise for the differential diagnosis of parkinsonian syndromes discriminating idiopathic PD from progressive supranuclear palsy, multiple system atrophy and corticobasal degeneration [9, 29]. In a recent study, plasma levels of both NfL and GFAP correlated with motor symptoms of PD making them promising prognostic biomarker candidates [5]. Another PPMI study suggests serum uric acid as a potential prodromal marker of PD [30].

Neuroinflammation and increased endoplasmic reticulum (ER) stress are implicated in the pathogenesis of

PD making serum immune and ER stress marker profiles potential tools for the follow-up of the disease state [31, 32]. However, their specificity to PD can be questioned as inflammatory cytokine levels and ER stress are elevated in a wide range of conditions [32]. Nevertheless, peripheral signs of inflammation should be considered for the biomarker battery of PD [31].

Reduced trophic factor support may underly early pathological steps in neurodegenerative diseases including PD. A meta-analysis by Rahmani et al. [33] suggested reduced serum levels of brain-derived neurotrophic factor (BDNF) in PD patients as compared to healthy controls and showed that BDNF downregulation is associated with progressive motor symptoms. Thus, we should not forget NTFs as potential diagnostic biomarkers of PD [32, 34].

Tissue samples

Histological examination of phosphorylated a-syn in different peripheral tissue biopsies of PD cases has been subject of increasing interest [12, 14]. Detection of phosphorylated a-syn aggregates in the autonomic nerve fibers of salivary glands and skin shown promise as an early histological biomarker [35, 36]. However, the finding is not specific for PD as similar deposits can be observed in other alpha-synucleinopathies, too [35]. Similarly, gastrointestinal biopsy could represent an accessible strategy to assess a-syn neuropathology in PD patients [37].

Peripheral tissue miRNAs may be of significance as hsa-miR-1260a from PD patients nasal and buccal swabs was significantly increased as compared with healthy controls [38]. Interestingly, non-coding miRNAs, miR-134 and miR-141 downregulated CDNF levels in an experimental setting calling for clinical studies on the biomarker properties of these miRNAs [34].

Imaging biomarkers

Brain imaging techniques are widely used diagnostic tools for PD [39]. Multimodal imaging allows the visualization of structural and functional changes in the brain and can reveal neuroanatomical and pathophysiological mechanisms underlying the disease processes [40]. As imaging modalities are non-invasive and can be used repeatedly, they are well suited for longitudinal investigations of, for example, the integrity of nigrostriatal dopamine system [41].

Magnetic resonance imaging

Structural magnetic resonance imaging (MRI) is used to assess regional tissue abnormalities and atrophy [42]. Neuromelanin-sensitive MRI of the substantia nigra (SN) and locus coeruleus has shown promising diagnostic accuracy in differentiating both idiopathic and monogenic forms of PD from healthy controls [43, 44]. Likewise, longitudinal changes in MRI index (R2*) of midbrain iron content are associated with declining motor function in early-stage PD, and thus, may serve as a biomarker of disease progression [45].

Diffusion tensor MRI (diffusion tensor imaging, DTI) measures the magnitude and direction of water molecule flow in the brain tissue allowing indirect assessment of microstructural integrity and white matter tract injury in the brain [46]. High-resolution DTI seems to be useful in distinguishing PD patients from healthy controls based on characteristic readouts in the SN and olfactory structures, but in early-stage PD results have been less conclusive and need further validation. DTI readouts of the corpus callosum, putamen, midbrain and cerebellum, however, may be useful to distinguish atypical parkinsonism from PD.

Functional MRI (fMRI) monitors brain activity by measuring cerebral oxygen-rich blood flow. Significantly reduced functional connectivity in resting-state fMRI have been reported within the basal ganglia circuits of patients with early-stage PD [43]. Indeed, it is a promising indicator of early dysfunctions and may help to identify patients at risk of developing PD [47, 48].

Single-photon emission computed tomography

Single-photon emission computed tomography (SPECT) tracers, such as ^{123}I-ioflupane (^{123}I-FP-CIT) or ^{123}I-β-CIT, bind selectively to presynaptic dopamine transporter (DAT) and can be used to measure the density

of nigrostriatal nerve terminals. The outcome of a meta-analysis suggested that DAT-SPECT is useful in the diagnosis of early PD and in differentiating PD from essential tremor and vascular parkinsonism [46]. DAT-SPECT can also be utilized to follow disease progression because striatal binding seems to correlate to different stages of disease severity [49]. Importantly, ^{123}I-β-CIT SPECT-CT has shown translational potential as it can be used to visualize dopaminergic degeneration in experimental animal models of PD [50].^{123}I-IBZM is an example of a radiotracer that binds to dopamine D2 receptors and can be used for SPECT imaging of postsynaptic striatal neurons [51].

Positron emission tomography

Positron emission tomography (PET) is a versatile imaging modality that utilizes radioactive ligands to gauge the integrity of the dopamine system, cerebral glucose metabolism, pathological Aβ and tau protein accumulation and neuroinflammation [46]. 6-^{18}F-fluoro-L-dopa (^{18}F-dopa) is a presynaptic PET tracer that is converted to^{18}F-dopamine by aromatic L-amino acid decarboxylase (AADC). Thus, ^{18}F-dopa measures the activity of AADC and provides an indirect estimation of the nigrostriatal dopamine storage pools.

In line with DAT-SPECT findings, striatal PET imaging of presynaptic DAT, using for example ^{18}F-FP-CIT or^{11}C-methylphenidate tracers, has found reduced uptake in the putamen and SN in PD and atypical parkinsonism [46]. As compared with DAT-PET, DAT-SPECT or AADC-PET, PET imaging of VMAT-2 using ^{11}C-dihydrotetrabenazine (^{11}C-DTBZ) seems to be less prone to compensatory changes. Thus, decreased striatal VMAT-2 binding more reliably reflects the nigrostriatal degeneration in PD. This has been confirmed in experimental settings, too [52].

Animal models indicate that lesion of locus coeruleus, the main noradrenergic nucleus in the brain, may be important for the pathogenesis of non-motor symptoms of PD [53]. PET ligand (S,S)-^{11}C-2-(α-(2-methoxyphenoxy)benzyl)morpholine (^{11}C-MeNER) labels noradrenaline transporter, and several studies in PD patients show reduced noradrenergic innervation in the brain supporting the view that ^{11}C-MeNER can be used as one imaging biomarker for non-motor symptoms of PD.

PD causes functional changes in multiple neuronal networks. These changes are reflected by a specific pattern of abnormal glucose metabolism in resting-state ^{18}F-fluorodeoxyglucose (^{18}F-FDG) PET referred to as PD-related pattern (PDRP), or a distinct PD-related cognitive pattern (PDCP) [54]. PDRP can be used to discriminate between idiopathic PD, atypical parkinsonian syndrome and healthy controls. PDRP and PDCP also show promise as biomarkers to follow the progression of the disease.

PET ligands can be used to assess the degree of neuroinflammation in the brain [55]. ^{11}C-(R)-PK11195 binds to mitochondrial translocator protein (formally a peripheral benzodiazepine receptor), the upregulation of which is indicative of augmented microglial activation. ^{11}C-(R)-PK11195 uptake correlates with various aspects of PD pathology in the brain and can be used in combination with other markers to support the diagnosis.

A major limitation in the development of valid imaging biomarkers for PD is the incapacity for direct a-syn imaging *in vivo* . Considerable efforts are underway to develop a-syn-specific radiotracers for PET imaging [56]. Such tracer would allow for tracking the degree and location of a-syn pathology over time and monitoring efficacy of a-syn targeting therapies.

In addition to CNS imaging also peripheral imaging modalities warrant attention as the initial pathological a-syn inclusions appear in the peripheral autonomic and enteric nervous systems even decades prior to the diagnosis of PD [57]. For example, loss of sympathetic and parasympathetic nerve terminals can be visualized using ^{18}F-dopamine and ^{11}C-donepezil PET imaging, and radiological techniques can reveal dysmotility and prolonged transit time through the gastrointestinal tract in PD patients.

Collectively, brain imaging modalities seem to comprise the most promising biomarker candidates in PD because they provide a direct approach to measure specific neurofunctional properties and the same methodology can be applied both to experimental animals and humans [57]. Even though imaging modalities assure relatively accurate conclusions, they are expensive, may entail harmful radiation and are available only in

specific centers limiting their usefulness in standard diagnostics [58]. Thus, they may not be feasible to be used in screening purposes in large populations. Imaging, however, may prove to be the most useful approach in confirming the diagnosis triggered by more accessible screening methods.

Other biomarker modalities

Transcranial sonography shows that enlarged areas of the SN echogenicity is a characteristic and stabile feature in idiopathic PD [46]. Thus, increased echogenicity of the SN seems to serve as a convenient and inexpensive biomarker candidate for the early diagnosis of idiopathic PD and differentiating it from atypical parkinsonian syndromes.

Electroencephalography (EEG) can be used to identify biomarker candidates for PD. Elevated spectral beta power within basal ganglia is implicated in PD, and recent advances in EEG-based analytic approaches to quantify oscillatory beta band synchrony seem promising [59]. Lower background rhythm frequency and increased relative power in delta and theta bands in resting-state EEG hold potential as predictive biomarkers for cognitive deterioration in PD [60]. In a prospective study, marked EEG slowing during rapid eye movement (REM) sleep together with increased relative powers in delta and theta frequencies were predictive biomarkers for patients who later develop dementia [60, 61]. EEG readouts are very well suited for machine and deep learning based techniques that may assist in objective screening and staging of PD as discussed in a recent review [62]. Despite being an inexpensive and easily accessible approach, the predictive and translational value of EEG biomarkers still needs to be confirmed.

Digital biomarkers (or technology-based objective measures) form a rapidly emerging field of research to improve the longitudinal tracking of neurodegenerative diseases in clinical care and research [63]. Digital biomarkers refer to the use of inbuilt sensors in portable (e.g. smartphone), wearable (e.g. smartwatch or ring) or implantable devices allowing for active or passive data collection on biological (e.g. blood glucose), physiological (e.g. heart rate or body temperature) or functional (e.g. motor activities, speech or facial expressions) parameters [64]. By their nature, digital biomarkers are unbiased and offer an opportunity to collect dense datasets during everyday life. Real-world data together with sophisticated artificial intelligence (AI) -based algorithms can detect even slight changes in daily life which could hardly be detected in a clinical setting. This could help predicting PD before the onset of classical symptoms or estimating the course of the disease, thus allowing for more personalized care. The diagnostic utility of digital biomarkers, however, needs further validation.

Concluding remarks

Despite the multiple biomarker candidates for PD and the ongoing intensive research efforts, there is no single definitive biomarker with sufficient accuracy or reproducibility that could be used in clinical practice to diagnose PD, predict the onset of the disease or indicate response to therapeutic interventions in clinical trials [64]. The use of a combination of biomarkers, however, could detect multiple pathological aspects of the disease and result in improved diagnostic accuracy. Background risk factors (genetic, demographic and environmental), combined with typical prodromal symptoms and different biochemical and imaging biomarkers can be used in tandem to improve the predictive diagnosis of PD [9, 58]. AI-algorithms may be of great value to define the diagnosis early on if these markers can be properly validated [11, 65, 66].

More accurate disease subtyping would contribute to the development of translational disease models and design of successful clinical trials with stratified inclusion criteria. The failed attempts to find neuroprotective strategies for PD may stem from the reductionist approach in the conducted clinical trials which have paid little attention to the variability of the disease at the individual level [67]. Development of digital biomarkers may help to address many of the current diagnostic shortcomings in an economical fashion. They would allow an objective approach to continuously track fluctuations in motor and non-motor symptoms during patients' daily life. The resulting rich real-world datasets may prove to be highly predictive in assessing clinical improvement in PD studies and permit personalized therapeutic adaptations [68].

Ideally, preclinical drug development would already be accompanied with a reliable and accessible biomarker

that could be followed through the whole process from animal models to clinical trials and regular patient monitoring. This kind of biomarker would help to predict the effects of an intervention in a patient population based on preclinical tests in animal models, and thus, increase the likelihood of successful clinical translation.

Figure: Summary of biomarker candidates for Parkinson's disease and matrices from which they are being analyzed. Aβ, beta-amyloid; a-syn, alpha-synuclein; GFAP, glial fibrillary acidic protein; ER, endoplasmic reticulum; NTFs, neurotrophic factors; miRNA, microRNA; MRI, magnetic resonance imaging; PET, positron emission tomography; SPECT, single-photon emission computed tomography. Figure created with BioRender.com.

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