The Role of Biomarkers in Parkinson's Disease

In many neurodegenerative diseases, the search for biomarkers has been driven by an extensive investigation and characterisation of the disease itself, as well as diseased tissue. In Parkinson's disease, PD, the examination of post-mortem brain tissue has led to the identification of relevant molecular pathways and genes that have allowed for targeted therapies, development of animal models, and new drug delivery systems. These targeted strategies have identified many biomarker candidates that are being actively evaluated for their potential as different types of PD biomarkers. The following article will discuss these biomarkers in depth.

Parkinson's disease is a progressive, neurodegenerative condition, phenotypically characterised by akinesia, resting tremor and muscular rigidity. This "classical" clinical expression is a consequence of complex pathophysiological processes in the substantia nigra (SN), leading to intraneural accumulation of alpha-synuclein (a-syn), thus forming the so-called "Lewy bodies" and eventually degeneration and loss of dopaminergic neurons. However, it appears that this pathological process occurs long before clinical expression, as nearly 50-60% of dopaminergic neurons are destroyed within SN before the appearance of motor symptoms.

In addition to typical motor symptoms, there are several non-motor symptoms such as are constipation, depression, lack of smell sense and rapid eye movement sleep behaviour disorders (RBD) frequently present in Parkinson's disease, before the onset of the classical motor symptoms. Whereas the first three symptoms are sensitive yet not specific to PD, RBD is now accepted as the most specific phenotype of the PD premotor phase with an associated risk of more than 80% of patients converting to PD, or dementia with Lewy bodies (DLB) or less frequently into multiple system atrophy (MSA).[1]

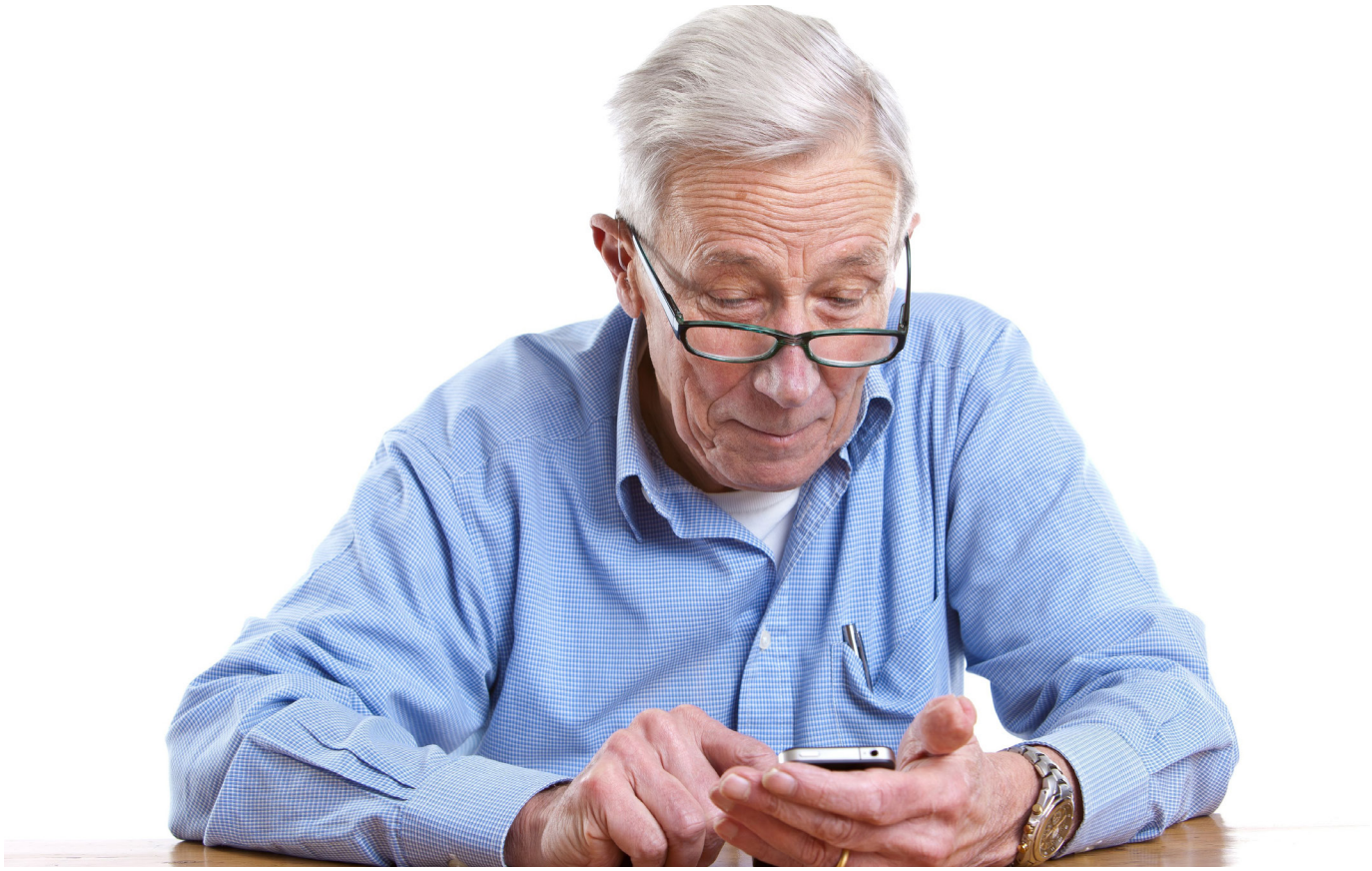
The average latency between onset of RBD and occurrence of parkinsonian motor symptoms is 12–14 years[2], making the premotor period quite long. The pathogenic process that causes Parkinson's disease is presumed to be underway during the premotor phase, involving regions of the peripheral and central nervous system in addition to the dopaminergic neurons of the SN[3]. Thus, this prodromal period provides a pivotal temporal window during which disease-modifying therapy could be administered to prevent or delay the development and progression of disease[4].

An emerging picture is one of a vicious cycle in which a-syn aggregation and mitochondrial dysfunction exacerbate each other, which could explain why these cellular changes are observed together in degenerating neurons in PD. As a result of oxidative stress, disruption of Ca homeostasis, abnormal kinase activity and interaction with misfolded a-syn accumulation, neuro-inflammation associated with T-cell infiltration and glial cell activation is becoming the salient feature of Parkinson's disease[6]. Neuroinflammation is playing the vital role in degeneration of dopaminergic neurons[7], which might be of great importance in development of new therapies for PD.

Clinical diagnosis of PD in daily practice is often based on physician's experience and impression rather than stringent use of standard clinical diagnostic criteria such as the UK Parkinson's Disease Society Brain Bank (UKPDSBB) criteria or recently published MDS diagnostic criteria. In fact, diagnosis of PD in the early stage has been problematic, as nearly one-quarter of patients are wrongly diagnosed, even in specialised centres. The most common misclassifications in clinicopathological series are MSA, progressive supranuclear palsy (PSP) and, less frequently, corticobasal degeneration. In clinically-based studies, common errors relate to essential tremor, drug-induced parkinsonism and vascular parkinsonism[8]. Source data verification in various multicentre clinical trials has shown low concordance of existing PD diagnosis with requested criteria. The translation process from physician-based diagnosis of PD into scientific diagnostic criteria used in multicentre clinical studies in PD is difficult, often impossible. Moreover, the diagnostic accuracy at first visit is only slightly above 80%, as shown by a meta-analysis of eleven studies assessing a UKPDSBB-based clinical diagnosis against post-mortem pathological examination as the gold standard[9]. Such findings highlight the need for diagnostic tests and biomarkers to enhance diagnostic confidence in early disease, or to eventually diagnose PD in its prodromal stages[10]. The suitable biomarker would allow treatment with putative neuroprotective agents to begin long before the significant and irreversible loss of neurons, and would enable the assessment of disease modification in individuals receiving treatment[11].

It is unclear why candidate drugs that successfully demonstrate therapeutic effects in animal models or drug discovery fail to show disease-modifying effects in clinical trials. To overcome this hurdle, patients with homogeneous pathologies should be detected as early as possible. The Biomarkers Definitions Working Group[12] has defined 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". PD biomarkers can be categorised as genetic, biochemical and imaging. The utility of either single or group biomarkers are limited, but when combined and considered collectively, biomarkers for PD may be more useful.

Genetic biomarkers: Mutations on SNCA, LRRK2, or VPS35 are responsible for development of autosomal dominant forms of PD. Autosomal recessive PD with early onset and complex phenotypes that include parkinsonism have been assigned mutation on another



PARK loci. There is increasing knowledge of other genes (including GBA, GCH1, ADH1C, and TBP) that contribute to an increased risk for the sporadic form of the PD. In fact, glucocerebrosidase (GBA) heterozygous mutation is the most prevalent genetic biomarker, affecting 5–10% PD population. As genetic PD is still rare, accounting for only 2–3% of all PD populations, genetic tests are not part of the standard diagnostic process.

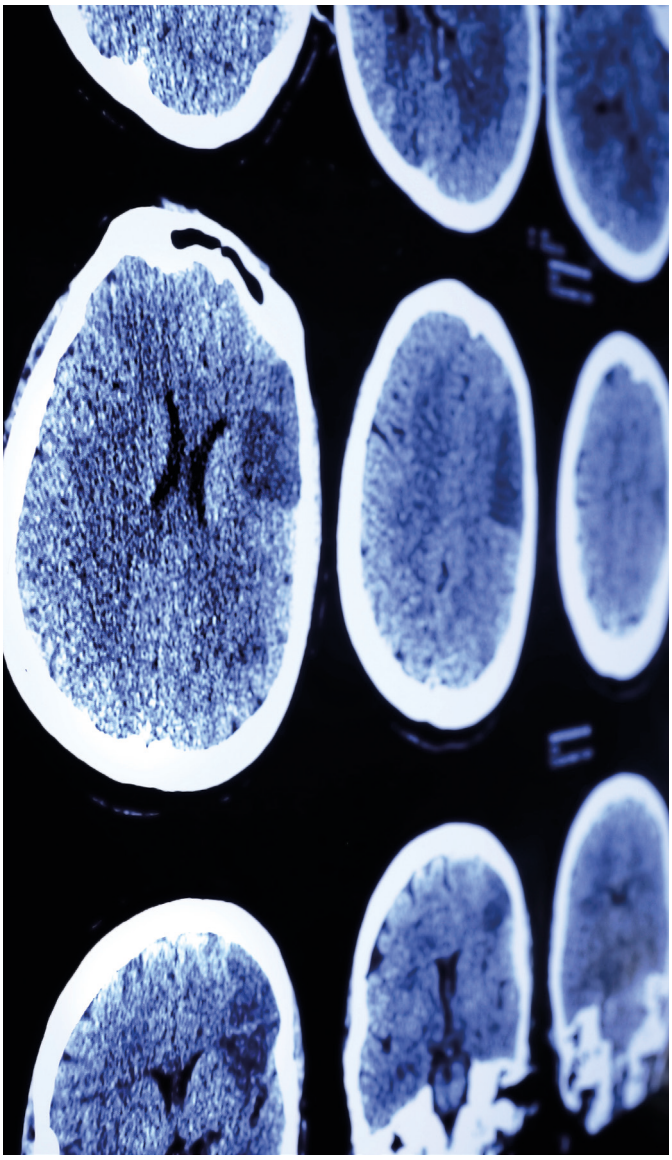
Biochemical biomarkers. There have been numerous attempts to identify specific and sensitive PD biomarkers in the body fluids and biopsy tissues. Blood, CSF and saliva have all been extensively investigated. Studies of a-syn in CSF showed conflicting results, although data has shown that PD patients have significantly lower a-syn levels. However, because a-syn and other proteins are present in the blood, erythrocytes and thrombocytes, even minor blood contamination may profoundly affect the results of CSF analysis. According to one research group, CSF samples should not contain more than 10 erythrocytes per microlitre CSF[13], or 500 erythrocytes per microlitre CSF according to a European recommendation[14]. In saliva, a-syn was lower in PD patients compared to controls and this was inversely correlated with the change in UPDRS score[15]. Alpha-synuclein has also been found in the colonic mucosa before the emergence of PD clinical symptoms[16], and additionally, published data showing gut microbiota in subjects with PD might be another potential biomarker for diagnosis of premotor PD[17]. Development of new, powerful tools – so-called 'omics' techniques — such as proteomics, metabolomics and transcriptomics in PD biomarker research will certainly make significant progress shortly.

Neuroimaging biomarkers have been widely used in visualisation of striatal dopaminergic depletion of neurons. Dopaminergic PET scan is sensitive in identifying dopamine deficiency, even during the preclinical disease, and it is potentially useful in quantifying disease progression. However, there are a number of challenges associated with neuroimaging biomarkers, such as: interpretation of results may be affected by compensatory changes resulting from disease and pharmacological intervention, and dopaminergic PET is expensive, and needs specialised infrastructure and expert analysis.

^{123}I-ioflupane single-photon emission CT (SPECT) (also known as DaTscan) is a more widely available and less expensive tool, which is already approved for routine clinical use. It can be used to differentiate between Parkinson's disease and other diseases that manifest as PD, but are not associated with presynaptic nigrostriatal terminal dysfunction. Both dopaminergic PET and SPECT are useful adjuncts, but have shown limited correlation with clinical measures in therapeutic trials.
FDG-PET in PD is helpful in differential diagnosis of parkinsonism and may be helpful in the assessment of disease progression. However, it is less specific than dopaminergic PET and it may be affected by compensatory changes or drug treatment.

Structural MRI is more widely available than PET or SPECT and it is useful in differential diagnosis to identify symptomatic parkinsonism. Newly developed MRI techniques can reveal specific changes in the basal ganglia (i.e. iron accumulation at SN during PD progression), whereas diffusion-weighted imaging, volumetric imaging, automated subcortical volume segmentation and multimodal imaging have been explored to enhance diagnostic accuracy for Parkinson's disease versus other types of degenerative parkinsonism.

Transcranial ultrasound (TCUS) has been used to demonstrate increased echogenicity in the midbrain of patients with PD, as a result of the increased nigral iron content in this region. Although TCUS can be useful in the detection of premotor PD and differentiations against other akinetic-rigid syndrome, the hyperechogenicity does not seem to increase with disease progression. TCUS is cost-effective and has shown promise as a possible imaging biomarker in PD, but it is very dependent on



operator skill, it is not specific and requires an adequate temporal acoustic bone window for good imaging.

Apart from dopaminergic biomarkers, there are a few non-dopaminergic biomarkers useful in diagnosis of PD. Loss of cardiac sympathetic innervation can be documented in PD by decreased uptake of the sympathetic marker, ^{123}I-metaiodobenzylguanidine (MIBG), in cardiac SPECT. Moreover, this marker contributes to the differential diagnosis between PD and other forms of parkinsonism such as MSA or DLB. MIBG is the only biomarker specifically addressed in the recently published Movement Disorders Society criteria for diagnosis of PD[18]. Uptake of ^{123}I-MIBG in myocardial scintigraphy is often reported as a heart-to-mediastinum (H/M) ratio of count densities, whereas washout rate index may also be assessed using early and delayed images. MIBG should be considered in the light of the entire clinical presentation because various cardiovascular morbidities, latent cardiac disorder and medications may damage the postganglionic sympathetic neurons, leading to false positive findings. Additionally, ^{123}I-MIBG H/M ratios may also decrease with age and show gender-specific variations, making it essential to use well-matched subgroups in clinical investigations.

Neuroinflammation markers of activated microglia, such as ^{11}C-PBR028-PET have been tested with varying success. Small sample sizes and lack of autopsy-verified diagnosis have limited the value of results. A viable application of this technique is in monitoring therapeutic responses in clinical trials.

Conclusion

Regardless of critical need, there is neither a fully validated diagnostic nor prognostic PD biomarker available for clinical studies and drug development. It seems that functional imaging, regardless of several limitations is still representing the best available tools to study PD progression in disease-modifying clinical studies. We believe that new methods like a-syn accumulation assessment or combination of markers will provide greater reliability in the forthcoming years.

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