CSF and blood biomarkers for Parkinson's disease

Lucilla Parnetti, Lorenzo Gaetani*, Paolo Eusebi, Silvia Paciotti, Oskar Hansson, Omar El-Agnaf, Brit Mollenhauer, Kaj Blennow, Paolo Calabresi*

In the management of Parkinson's disease, reliable diagnostic and prognostic biomarkers are urgently needed. The diagnosis of Parkinson's disease mostly relies on clinical symptoms, which hampers the detection of the earliest phases of the disease—the time at which treatment with forthcoming disease-modifying drugs could have the greatest therapeutic effect. Reliable prognostic markers could help in predicting the response to treatments. Evidence suggests potential diagnostic and prognostic value of CSF and blood biomarkers closely reflecting the pathophysiology of Parkinson's disease, such as α-synuclein species, lysosomal enzymes, markers of amyloid and tau pathology, and neurofilament light chain. A combination of multiple CSF biomarkers has emerged as an accurate diagnostic and prognostic model. With respect to early diagnosis, the measurement of CSF α-synuclein aggregates is providing encouraging preliminary results. Blood α-synuclein species and neurofilament light chain are also under investigation because they would provide a non-invasive tool, both for early and differential diagnosis of Parkinson's disease versus atypical parkinsonian disorders, and for disease monitoring. In view of adopting CSF and blood biomarkers for improving Parkinson's disease diagnostic and prognostic accuracy, further validation in large independent cohorts is needed.

Lancet Neurol 2019; 18: 573–86

Published **Online**
April 10, 2019
http://dx.doi.org/10.1016/S1474-4422(19)30024-9

*Contributed equally

Section of Neurology, Laboratory of Clinical Neurochemistry, Department of Medicine (Prof L Parnetti MD, S Paciotti PhD), **Section of Neurology, Department of Medicine** (L Gaetani MD, P Eusebi PhD, Prof P Calabresi MD), **and Section of Physiology and Biochemistry, Department of Experimental Medicine** (S Paciotti), **University of Perugia, Perugia, Italy; Clinical Memory Research Unit, Department of Clinical Sciences Malmö, Lund University, Malmö, Sweden** (Prof O Hansson MD); **Memory Clinic, Skåne University Hospital, Malmö, Sweden** (Prof O Hansson); **Neurological Disorders Research Center, Qatar Biomedical Research Institute, Hamad Bin Khalifa University, Education City, Doha, Qatar** (Prof O El-Agnaf PhD); **Paracelsus-Elena-Klinik, Kassel, Germany** (Prof B Mollenhauer MD); **University Medical Center, Department of Neurology, Göttingen, Germany** (Prof B Mollenhauer); **Institute of Neuroscience and Physiology, Department of Psychiatry and Neurochemistry, The Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden** (Prof K Blennow MD); **Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden** (Prof K Blennow); **and IRCCS Fondazione Santa Lucia, Rome, Italy** (Prof P Calabresi)

Correspondence to:
Prof Lucilla Parnetti, Section of Neurology, Laboratory of Clinical Neurochemistry, Department of Medicine, University of Perugia, 06132 Perugia, Italy
lucilla.parnetti@unipg.it

Introduction

In the past three decades, increased quality of health care has led to higher life expectancy and, as a consequence, to increased incidence and prevalence of age-related neurodegenerative diseases.[1] In view of having effective disease-modifying treatments, early diagnosis represents a priority, together with the need to predict the disease course. For these reasons, diagnostic and prognostic biomarkers are necessary in the field of neurodegeneration (panel 1). Alzheimer's disease, the commonest neurodegenerative disorder, provides an example of the usefulness and application of CSF biomarkers for diagnosis, independent of clinical stage.[4]

Progress has been made in identifying both neuroimaging and biofluid biomarkers for Parkinson's disease, the second most common primary neurodegenerative disorder of the CNS.[5] The proximity of CSF to the CNS makes this biofluid the ideal source for diagnostic markers of ongoing pathological processes, although it is not a good matrix for monitoring drug effects or other variables over time, because of the need for repeated lumbar punctures. In this context, blood samples are a more accessible source of biomarkers.

The diagnostic criteria for Parkinson's disease allow for identification of only manifested disease, which occurs years after the neurodegenerative process has started.[6] Moreover, even when criteria are correctly applied, the frequency of misdiagnosis is high due to substantial clinical overlap among parkinsonian disorders.[7] PET and SPECT imaging are available to detect reduced density of dopaminergic nerve terminals in the basal ganglia. Even though these techniques are very sensitive, they are not specific for Parkinson's disease, are costly, and involve radiation exposure. Thus, biomarkers for early identification of patients with Parkinson's disease are needed.

Parkinson's disease is a clinically heterogeneous disease with varied progression patterns. Older age at diagnosis, symmetrical motor involvement, cognitive impairment, and a longer disease duration at the time of diagnosis have been reported as predictive factors for motor progression,[8] while right-sided bradykinesia, low level of education, self-reported cognitive complaints at the time of diagnosis, and presence of rapid eye movement (REM) sleep behaviour disorder are often observed in patients who will go on to have cognitive decline.[9] The availability of objective fluid biomarkers specifically associated with motor or cognitive trajectories of Parkinson's disease subtypes could allow reliable prediction of clinical outcomes.

Over the past 5 years, research on biofluid biomarkers in Parkinson's disease has markedly expanded, and several systematic reviews and meta-analyses on this topic have been published.[10–13] In this Review, updated evidence about CSF and blood biomarkers for Parkinson's disease diagnosis and prognosis is reported. With respect to diagnostic and prognostic markers, those closely related to the main pathological processes taking place in Parkinson's disease are the focus of this Review. Also, the potential usefulness of biomarkers as measures of target engagement for drugs in clinical trials is discussed. According to the relevance of pathophysiological mechanisms, we will first discuss markers of synucleinopathy, followed by markers of lysosomal dysfunction, amyloid pathology, tauopathy, and finally of axonal damage.

Diagnostic biomarkers for Parkinson's disease

α-Synuclein species

α-Synuclein misfolding has a central role in the development of Parkinson's disease and other synuclein aggregation disorders.[6] Genetic mutations and post-translational modifications of the protein, such as phosphorylation, ubiquitination, nitration, truncation, and oxidisation, could have a role in facilitating protein misfolding (figure).[14] Several investigations into biomarker identification and validation have therefore focused on the measurement of total α-synuclein species first in CSF, then in blood. Specific α-synuclein species (ie, oligomeric α-synuclein, phosphorylated α-synuclein at residue Ser129, and pro-aggregating forms of α-synuclein in CSF and blood) have been considered as potential diagnostic biomarkers for Parkinson's disease.

***Panel 1*: Glossary of terms related to biomarkers**

A biomarker is a defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or responses to an exposure or intervention, including therapeutic interventions.[2]

- A **susceptibility risk biomarker** indicates the potential for developing a disease or medical condition in an individual who does not currently have clinically apparent disease or the medical condition.
- A **diagnostic biomarker** is used to detect or confirm the presence of a disease or condition of interest, or to identify individuals with a subtype of the disease.
- A **monitoring biomarker** is measured serially for assessing the status of a disease or medical condition, or for evidence of exposure to (or effect of) a medical product or an environmental agent.
- A **prognostic biomarker** is used to identify the likelihood of a clinical event, disease recurrence, or progression in patients who have the disease or medical condition of interest.
- A **predictive biomarker** is used to identify individuals who are more likely than individuals without the biomarker to have a favourable or unfavourable effect from exposure to a medical product or an environmental agent.
- A **pharmacodynamic or response biomarker** is used to show that a biological response has occurred in an individual who has been exposed to a medical product or an environmental agent.
- A **safety biomarker** is measured before or after an exposure to a medical product or an environmental agent to indicate the likelihood, presence, or extent of toxicity as an adverse effect.
- **Clinical utility** refers to the conclusion that a given use of a biomarker will lead to a net improvement in health outcome or provide useful information about diagnosis, treatment, management, or prevention of a disease. Clinical utility includes the range of possible benefits or risks to individuals and populations. In neurodegenerative diseases, clinical utility can be shown by means of phase 4 (prospective diagnostic accuracy) and phase 5 (disease burden reduction) studies.[3]
- **Clinical validation** is a process to establish that the test acceptably identifies, measures, or predicts the concept of interest. In the context of neurodegenerative diseases, the clinical validation of a biomarker requires phase 2 (clinical assay development for a specific disease pathology) and phase 3 (retrospective studies using longitudinal data available in repositories) studies.[3]

CSF total α-synuclein

α-Synuclein can be measured in CSF by means of immunoassays such as ELISA, electrochemiluminescence, and xMAP technology (panel 2). Different immunoassays for CSF total α-synuclein have been compared in different laboratories, showing high analytical precision, excellent inter-laboratory correlation, and good inter-assay correlation. Although absolute values were different between the tested assays, measurements were in line with those of immunoprecipitation mass spectrometry.[15] These results support the analytical validity of immunoassays for measuring CSF total α-synuclein. Four meta-analyses assessed the potential diagnostic value of CSF total α-synuclein in Parkinson's disease, with largely concordant results (appendix).[11,16–18] Indeed, in all of the meta-analyses, it was concluded that CSF total α-synuclein is lower in patients with Parkinson's disease compared with healthy controls and other neurological controls,[11,16–18] and the publications from the past 2 years are in line with these findings.[19–21] However, when considering CSF total α-synuclein as a potential diagnostic biomarker for Parkinson's disease, several limitations hamper its clinical use. CSF total α-synuclein values vary greatly among studies, which could be explained by the enrolment of patients with Parkinson's disease with different characteristics in terms of age, disease stage, and use of medications, as well as of different control populations, which range from healthy controls to patients who are cognitively impaired due to other neurological diseases.[22] Pre-analytical and analytical factors, such as blood contamination, which have been shown to significantly increase CSF total α-synuclein, are important sources of heterogeneity.[23] Additionally, other methodological processing differences and the use of different immunoassays could limit the comparability between studies. In the immunoassay comparison study,[15] different methods gave different CSF total α-synuclein absolute concentrations, thus showing the need to generate common reference materials to harmonise results. Also, a round robin comparison across 18 different laboratories using the same assay showed variability in absolute concentrations of total α-synuclein.[24] Moreover, data from meta-analyses show an unsatisfactory diagnostic accuracy of CSF total α-synuclein in distinguishing patients with Parkinson's disease from controls, with a pooled sensitivity between 88% (95% CI 84–91) and 78% (62–88), and a specificity between 40% (35–45) and 57% (36–76; appendix).[11,17] Changes towards low CSF total α-synuclein have been found in other synuclein aggregation disorders, such as multiple system atrophy and dementia with Lewy bodies, and in other neuro-degenerative diseases not belonging to synucleinopathies, such as progressive supranuclear palsy, corticobasal syn-drome, and frontotemporal dementia. All of these diseases show CSF total α-synuclein values largely overlapping with those from patients with Parkinson's disease.[25–27] Several meta-analyses found no difference in CSF total α-synuclein between Parkinson's disease and dementia with Lewy bodies,[11,16–18] and only one meta-analysis reported a difference between Parkinson's disease and multiple system atrophy, with multiple system atrophy showing lower CSF total α-synuclein compared with Parkinson's disease.[18] No difference has been found in CSF total α-synuclein between Parkinson's disease and corticobasal syndrome.[11] On the contrary, a trend towards higher CSF total α-synuclein values in progressive supranuclear palsy compared with Parkinson's disease has been found,[16,18] but was not confirmed by the most recent of the meta-analyses cited.[11] Similar to progressive supranuclear palsy, but to a larger extent, in Alzheimer's disease and Creutzfeldt-Jakob disease, a significant increase in CSF total α-synuclein has been reported compared with controls.[28] Therefore, CSF total α-synuclein could serve as a marker of synucleinopathy, when its CSF concentrations decrease, as well as an unspecific marker of synaptic damage, when its CSF concentrations increase.[28] In line with this hypothesis,

See Online for appendix

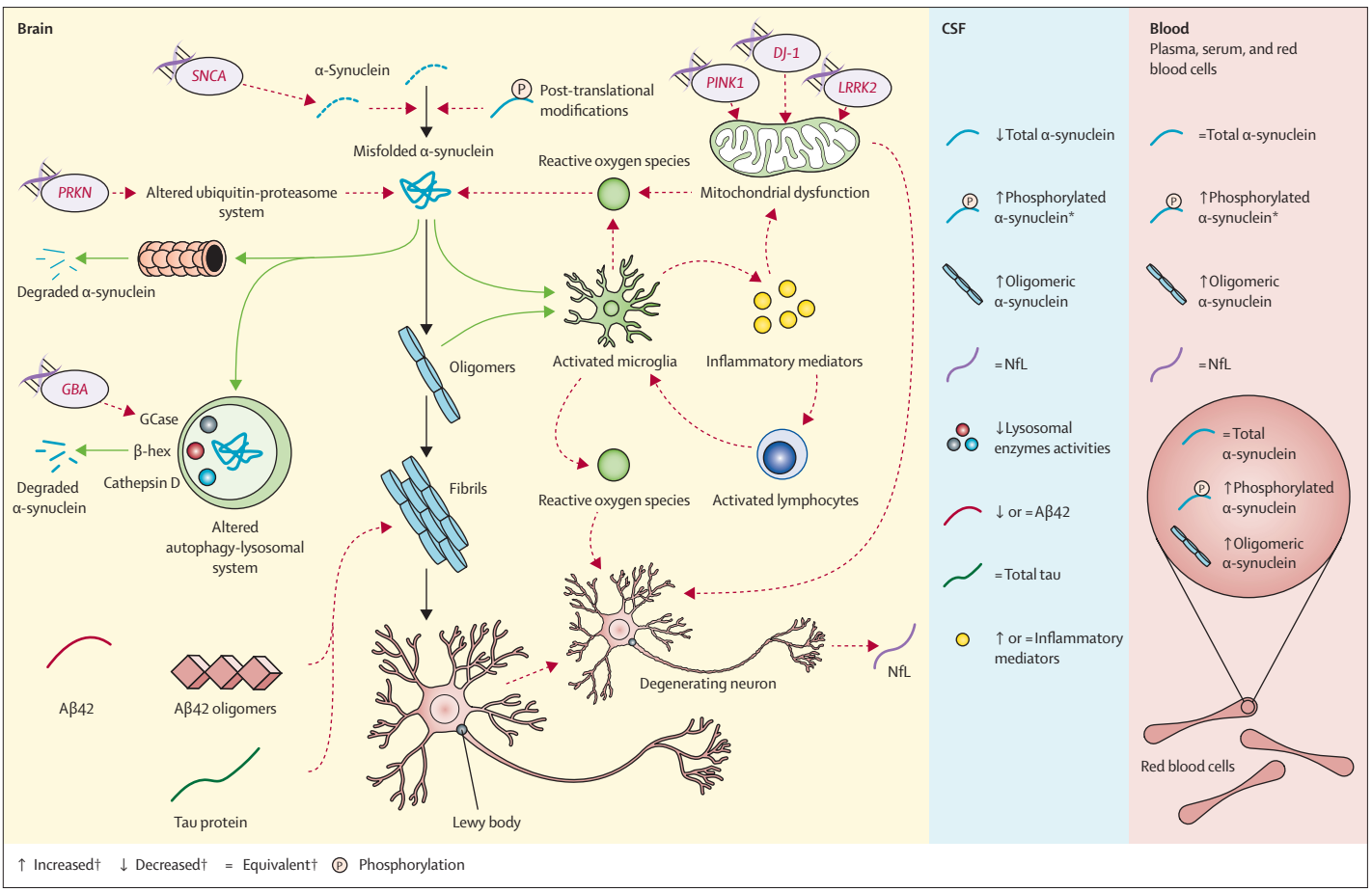

Figure: **Main pathophysiological mechanisms in a patient with Parkinson's disease and the corresponding biomarkers under investigation**
Misfolding of α-synuclein is facilitated by several factors, including genetic mutations and post-translational modifications of the protein (eg, phosphorylation of α-synuclein). Misfolded α-synuclein could still be eliminated via the ubiquitin-proteasome and the autophagy-lysosomal systems. However, when these two systems are impaired and the misfolding process overcomes clearance activity, misfolded α-synuclein tends to organise into soluble oligomers and, finally, into insoluble fibrils, which represent the core of intraneuronal Lewy bodies and Lewy neurites. Mitochondrial dysfunction, possibly triggered by genetically driven loss of function of *PINK1, DJ-1*, or *LRRK2*, can exacerbate the α-synuclein aggregation cascade and, together with Lewy bodies, lead to neuronal degeneration. In parallel with these processes, α-synuclein aggregation boosts a microglial reaction that, through inflammatory mediators, attracts peripheral immune cells within the CNS. Activated lymphocytes in turn lead to persistent and dysfunctional microglial activation, which could facilitate both mitochondrial dysfunction and neuronal degeneration. Finally, the coexistence of amyloid and tau pathology could facilitate the process of α-synuclein aggregation. As a consequence of these pathogenic pathways, in patients with Parkinson's disease there is a particular CSF and blood biochemical profile, schematically depicted in the figure. The solid black line represents the cascade of α-synuclein from misfolded protein up to Lewy body formation. Green lines represent those mechanisms counteracting this cascade, while dotted red lines represent abnormal cellular mechanisms facilitating this cascade as well as other pathological processes (eg, neuroinflammation, mitochondrial dysfunction, neurodegeneration). Aβ42=amyloid β peptide 1–42. *GBA*=β-glucocerebrosidase. GCase=β-glucocerebrosidase. *LRRK2*=leucine-rich repeat kinase protein-2. NfL=neurofilament light chain. *PINK1*=PTEN-induced putative kinase protein 1. *PRKN*=parkin. β-hex=β-hexosaminidase. *SNCA*=α-synuclein. *Unconfirmed. †Compared with controls.

evidence seems to suggest that patients with Parkinson's disease with higher CSF total α-synuclein (ie, with more evident ongoing neurodegeneration) might have a faster decline in motor progression than patients with Parkinson's disease with lower concentrations.[29] The dual implications of changes in concentration can raise the complexity in the interpretation of CSF total α-synuclein concentration. Patients with Parkinson's disease with an aggressive disease course could have CSF total α-synuclein similar to healthy controls as a result of decreased concentrations (due to α-synuclein sequestration because of aggregation) counterbalanced by increased release (due to neurodegeneration). It is worth noting, however, that in longitudinal studies, no significant change in CSF total α-synuclein has been found in Parkinson's disease versus healthy controls over 12 to 24 months.[19,30] In conclusion, CSF total α-synuclein per se does not seem to be a reliable diagnostic marker for Parkinson's disease, while the study of other more pathophysiologically specific species of α-synuclein, and the combination of total α-synuclein with other CSF biomarkers could provide promising results.

CSF oligomeric α-synuclein

The oligomerisation of α-synuclein precedes its aggregation into mature amyloid fibrils in Lewy bodies. In-vitro and in-vivo studies provided strong evidence that oligomeric α-synuclein might play a primary role in Parkinson's disease pathophysiology.[31] CSF oligomeric α-synuclein has been consistently found at higher concentrations in

Panel 2: **Glossary of techniques for biomarker measurement**

- An **ELISA** is an immunoassay that allows for the measurement of an analyte, usually by means of a pair of antibodies (sandwich ELISA). The binding of both antibodies to the analyte generates a fluorescent or colorimetric signal, which is finally quantified as a measure of the analyte's concentration.
- **Electrochemiluminescence** is an immunoassay with higher sensitivity than ELISA. It relies on the binding of specific antibodies to the analyte within electron-enriched wells, with the subsequent generation of an electrochemiluminescent signal.
- **xMAP technology** allows for the measurement of multiple analytes in a liquid suspension. Antibodies of the immunoassays are linked to colour-coded beads (microspheres), which are then read in a compact analyser. By means of multiple lasers, the analyser reads multiplex assay results by reporting the reactions occurring on each microsphere type.
- **Protein-misfolding Cyclic Amplification (PMCA) and Real-Time Quaking-Induced Conversion (RT-QuIC)** are high-sensitivity protein amplification assays, which enable the detection of misfolded protein aggregates in biological samples, currently used for the diagnosis of prion disease. Similar to PCR, PMCA and RT-QuIC are amplification cyclic reactions done through shaking. During each cycle, the protein aggregates contained in the biological sample grow at the expense of a substrate, resulting in an exponential increase of the protein aggregates, which is monitored using a specific fluorophore.
- The **HANdai Amyloid Burst Inducer (HANABI) technique** has been proposed as an alternative to PMCA and RT-QuIC methods to measure pro-aggregating proteins in biofluids. It induces amyloid fibril formation by sonication and an incubation cycle with real-time monitoring of a fluorescent signal. HANABI shortens the time required for aggregate formation, thus resulting in a faster assay compared with PMCA and RT-QuIC. It is under investigation as a technique to measure pro-aggregating forms of α-synuclein.
- **Single-molecule array technology** is based on single-molecule sandwich immunoassays and simultaneous counting of singulated capture microbeads. This technique has a 1000-times higher sensitivity compared with ELISA, which has allowed measurement of low concentrations of analytes, such as those of the light subunit of neurofilament in blood.

patients with Parkinson's disease compared with controls,[11,18] even though diagnostic accuracy is unsatisfactory, with a pooled sensitivity of 71% and specificity of 64%.[11] The ratio of CSF oligomeric α-synuclein to total α-synuclein improves diagnostic performance of oligomeric α-synuclein alone, with an area under the curve (AUC) up to 0·78 (sensitivity 82%, specificity 64%),[32] which is still unsatisfactory for potential use in clinical practice. Additionally, no data are available on CSF oligomeric α-synuclein performance in discriminating Parkinson's disease from dementia with Lewy bodies, multiple system atrophy, progressive supranuclear palsy, and corticobasal syndrome.

CSF phosphorylated α-synuclein

Phosphorylated α-synuclein is one of the main disease-associated forms of α-synuclein, and this modification accounts for more than 90% of α-synuclein found in Lewy bodies.[33] Only a few studies have focused on CSF phosphorylated α-synuclein as a diagnostic marker, finding increased concentrations in patients with Parkinson's disease compared with controls and those with progressive supranuclear palsy.[11] Similar to oligomeric α-synuclein, the diagnostic accuracy of phosphorylated α-synuclein increases when considered together with other α-synuclein species and neurodegenerative biomarkers (ratio of oligomeric α-synuclein to total α-synuclein, together with phosphorylated α-synuclein and phosphorylated tau protein: AUC 0·86, sensitivity 79%, specificity 67%).[34] These studies on phosphorylated α-synuclein still lack validation in independent laboratories. Furthermore, phosphorylated α-synuclein is found at very low concentrations in the CSF and many assays cannot reliably measure its quantities.

CSF α-synuclein aggregates

Seeding aggregation assays, such as Protein-Misfolding Cyclic Amplification (PMCA) and Real-Time Quaking-Induced Conversion (RT-QuIC; panel 2), are based on detection of misfolded proteins prone to aggregation and exploit their prion-like behaviour. These techniques have shown to be able to detect and measure α-synuclein pathogenic aggregates in biofluids.[35] The application of RT-QuIC showed remarkable diagnostic accuracies in distinguishing patients with neuropathologically confirmed Parkinson's disease (sensitivity 95%) and dementia with Lewy bodies (sensitivity 92%) from controls (specificity 100% for both comparisons), with none of the patients with neuropathologically confirmed progressive supranuclear palsy, corticobasal degeneration, or Alzheimer's disease being positive for α-synuclein aggregates.[36] These results were replicated in a larger independent cohort, in which patients with Parkinson's disease were discriminated from patients with other neurological disorders including non-synucleinopathy neurodegenerative diseases.[37] In the same study, CSF samples from patients with dementia with Lewy bodies were recognised with a sensitivity of 100% and CSF samples from patients with multiple system atrophy were recognised with a sensitivity of 80%. Furthermore, a significant negative correlation between α-synuclein aggregation rate and Hoehn and Yahr (H&Y) stages was found, pointing out the possibility to monitor the disease progression by measuring α-synuclein aggregates in CSF.[37] In 2018, Groveman and colleagues[38] did RT-QuIC on CSF samples from patients with Parkinson's disease, dementia with Lewy bodies, and non-synucleinopathy controls (including patients with Alzheimer's disease and healthy individuals). In patients with Parkinson's disease and dementia with Lewy bodies, 93% of individuals had positive RT-QuIC responses whereas none of the non-synucleinopathy control individuals had positive RT-QuIC responses, resulting in a specificity of 100%.[38]

Because α-synuclein aggregation is an early phenomenon in synucleinopathies, the detection of aggregates could help in early diagnosis. Shahnawaz and colleagues[37] presented two individuals, formerly classified as controls,

who were clinically diagnosed with Parkinson's disease 1 and 4 years after sample collection. At baseline, these patients were positive to PMCA, indicating the ability of this assay to identify patients even at the preclinical stage.

Taken together, these results suggest that measurement of CSF α-synuclein aggregates is a promising diagnostic marker for synucleinopathies. The encouraging results obtained when applying these approaches for diagnosis of prion disease on more accessible tissues (eg, olfactory mucosa)[39] lead us to hypothesise the possibility of using other biological fluids (eg, saliva, plasma, serum, or urine) for the detection of aggregated α-synuclein in patients with Parkinson's disease. However, neither PMCA nor RT-QuIC can discriminate between different synucleinopathies. More in-depth investigations on α-synuclein aggregation kinetics, the structure of α-synuclein fibrillary aggregates, and further studies using larger series and independent cohorts are required to support CSF α-synuclein aggregates as potential diagnostic marker for synucleinopathies and to validate the potential usefulness of these techniques in a clinical setting for early and differential diagnosis of Parkinson's disease. Another similar technique, called HANdai Amyloid Burst Inducer, has been proposed to detect fibril formation in biofluids.[40] This method amplifies protein aggregates more efficiently, shortening the time to do the assay compared with PMCA and RT-QuIC (panel 2). The technique is under investigation in patients with Parkinson's disease for α-synuclein aggregate measurement.

α-Synuclein species in the blood

α-Synuclein is largely expressed outside the CNS, and the protein can be measured in blood.[41] However, its quantities in the blood are strongly influenced by red blood cell (RBC) contamination and haemolysis, even more than in CSF. Indeed, RBCs are the major source (>99%) of α-synuclein in blood and their abundance and fragility make it possible that even low RBC contamination could result in a substantial increase of α-synuclein in serum or plasma.[23] For this reason, amounts of intracellular RBC α-synuclein have been studied as an alternative measure (appendix).

Serum and plasma quantities of total α-synuclein have been reported to be either higher, lower, or not significantly different in patients with Parkinson's disease compared with controls.[42–49] These results, together with the issue concerning RBC contamination, limit the utility of plasma or serum total α-synuclein measurement for diagnostic purposes in patients with Parkinson's disease. Similarly, conflicting data have been obtained on RBC total α-synuclein.[50,51]

Studies on blood oligomeric α-synuclein have provided concordant results, showing increased quantities in patients with Parkinson's disease both in serum,[52] and in RBCs,[51,53,54] with serum values characterised by remarkable diagnostic accuracy (sensitivity 75%, specificity 100%). This diagnostic performance obtained in a small cohort of patients,[52] needs confirmation in larger populations. In a single study,[51] the combination of RBC oligomeric α-synuclein with RBC total α-synuclein, RBC hetero-aggregates of α-synuclein with amyloid β peptide 1–42 (Aβ42), and RBC total tau protein and phosphorylated tau has been reported to have an excellent diagnostic accuracy (AUC 0·98) in distinguishing patients with Parkinson's disease from controls.

Similar to oligomeric α-synuclein, plasma phosphorylated α-synuclein is higher in patients with Parkinson's disease compared with controls (AUC 0·71).[43] In this context, it is worth noting that RBC measurement of a panel of post-translational modified forms of α-synuclein (including Tyr125 phosphorylated α-synuclein, nitrated α-synuclein, glycated α-synuclein, and SUMOylated α-synuclein) showed a high discriminatory power in distinguishing patients with Parkinson's disease from controls (AUC 0·84).[55]

Lysosomal enzymes

The process leading to accumulation of aggregated α-synuclein has been associated with the impairment of the autophagy-lysosomal pathway, which represents one of the main routes for the intracellular degradation of α-synuclein (figure).[56] Following this pathophysiological model, a few studies have investigated the use of CSF lysosomal enzyme activities for the diagnosis of synuclein aggregation disorders.[32,57,58] A significant decrease of β-glucocerebrosidase (GCase) activity in patients with Parkinson's disease compared with controls has been repeatedly found.[59,60] In a 2017 study, activities of GCase, cathepsin D, and β-hexosaminidase were assessed in CSF samples of 79 patients with Parkinson's disease and 61 healthy controls from the BioFIND cohort (panel 3).[59] This study showed a significant reduction of GCase (–28% in Parkinson's disease *vs* controls) and cathepsin D (–21% in Parkinson's disease *vs* controls) activity in patients with Parkinson's disease; a similar trend was also observed for β-hexosaminidase activity (–9% in Parkinson's disease *vs* controls). In this cohort, 13% of patients with Parkinson's disease and 5% of healthy controls were carriers of mutations in the GCase coding gene (*GBA*). Although GCase activity was lower in carriers versus non-carriers (–27%), the overall decrease was present independent of *GBA* mutation carrier status (–25% in non-carrier patients with Parkinson's disease *vs* non-carrier controls). Receiver Operating Characteristic curve analysis showed a suboptimal diagnostic accuracy of GCase (sensitivity 67%, specificity 77%) and cathepsin D (sensitivity 61%, specificity 77%).[59] The diagnostic performance improved when combining the panel of all of the measured lysosomal enzymes activities (sensitivity 71%, specificity 85%) and further increased when amyloid, tau, and α-synuclein pathology markers were added to the model.[59]

Panel 3: **Key initiatives in the field of biofluid biomarker research in Parkinson's disease**

De Novo Parkinson (DeNoPa) study

The DeNoPa study is a single-centre, prospective, longitudinal investigation on a cohort of patients with early stage de novo Parkinson's disease who were drug naive. The study involves deep clinical phenotyping, genetics, fluid biomarkers (including CSF and blood), neuropsychological, polysomnography, and ancillary investigations of non-motor signs at baseline and biannual follow-up.[61] Launched in 2009, enrolment ended in 2012 and included 159 patients with Parkinsons's disease, 110 matched healthy controls and 50 patients with rapid eye movement sleep behaviour disorder (ie, prodromal Parkinsons's disease).

Parkinson's Progression Marker Initiative (PPMI)

The PPMI is an observational, international, multicentre study designed to identify Parkinson's disease progression biomarkers in a cohort of patients with early stage Parkinson's disease who are drug naive, healthy controls, prodromal individuals, and genetically affected and unaffected individuals. The study encompasses clinical, imaging, and genetic investigations and the collection, analysis, and storage of biological samples, including a longitudinal collection of blood, CSF, and urine.[62] Started in 2010, recruitment completed in 2013, leading to the inclusion of 424 patients with de novo Parkinson's disease and 196 healthy controls. Over the years, other cohorts have been added to this initiative: (1) the SWEDD (Scans without Evidence of Dopaminergic Deficit) cohort; (2) the Prodromal Parkinson's disease cohort; and (3) the genetic cohort and registry.

Biomarkers For Identifying Neurodegenerative Disorders Early and Reliably (BioFINDER) study

BioFINDER is a prospective and longitudinal Swedish multicentre study that has consecutively enrolled, from 2010 to 2015, 500 patients with mild cognitive impairment (including patients who later developed Parkinson's disease dementia) and 350 healthy controls. Since 2008, the study has recruited 350 patients with parkinsonian disorders (including Parkinson's disease) and 450 patients with dementia (including Parkinson's disease dementia). Study participants are followed up with longitudinal neurological, psychiatric, and cognitive assessments and collection of CSF and blood samples.[29]

Fox Investigation for New Discovery of Biomarkers in Parkinson's Disease (BioFIND) study

BioFIND is a cross-sectional, observational, North American multicentre study of patients with moderate to advanced Parkinson's disease and healthy controls evaluated with standardised clinical and biospecimen acquisition protocols.[63] Started in 2012, recruitment ended in 2015 with 119 patients with Parkinson's disease and 96 healthy controls.

Systemic Synuclein Sampling Study (S4)

The S4 study is an observational clinical study to better understand the progression of Parkinson's disease by identifying the best biofluids and tissues for measuring α-synuclein protein outside of the brain as a potential biomarker in people with Parkinson's disease. S4 has enrolled individuals with Parkinson's disease and control volunteers across six clinical sites in North America.[64]

For more on the **DeNoPa study** see http://www.denopa.de/

For more on the **PPMI** see https://www.ppmi-info.org/

For more on **BioFINDER** see http://biofinder.se/

For more on **BioFIND** see https://www.michaeljfox.org/page.html?biofind-clinical-study

For more on the **S4 study** see https://www.michaeljfox.org/page.html?s4

Classic Alzheimer's disease biomarkers

Together with Lewy bodies, amyloid plaques and neurofibrillary tangles can coexist in Parkinson's disease brains,[65] and both amyloid and tau pathology can dynamically interact with the α-synuclein misfolding process (figure). A trend towards lower CSF Aβ42 in patients with Parkinson's disease compared with controls has been found in some investigations,[66–69] but not confirmed in other reports,[29,32,70] with a low diagnostic accuracy (AUC 0·64).[71] By contrast, CSF Aβ42 tends to be higher in patients with Parkinson's disease dementia than in patients with Alzheimer's disease, with a good discriminative power (AUC 0·81).[72,73] Also, both patients with Parkinson's disease and Parkinson's disease dementia have been found to have higher CSF Aβ42 compared with patients with dementia with Lewy bodies, even if with poor diagnostic accuracy (AUC 0·64 for Parkinson's disease dementia *vs* dementia with Lewy bodies).[72,74,75] Studies on CSF total tau and phosphorylated tau have not shown a distinctive Parkinson's disease profile, with findings of both lower and not significantly different values in Parkinson's disease compared with controls and other parkinsonian disorders.[19,27,29,32,60,66,69,70] Although CSF Alzheimer's disease classic biomarkers alone do not have a specific role for Parkinson's disease diagnosis, they might contribute to improve diagnostic accuracy when considered together with other markers.

Neurofilament light chain

Following axonal injury or degeneration, different subunits of neurofilaments are released in the interstitial space within the CNS.[76] Large myelinated axons have a high content of neurofilaments in their axoplasm and, for this reason, an increase of their CSF and blood concentration is considered a sensitive marker of white matter axonal degeneration, a process that is not typical for Parkinson's disease, at least in its earliest stages. By contrast, in atypical parkinsonian disorders, such as progressive supranuclear palsy, multiple system atrophy, and corticobasal syndrome, a remarkable involvement of myelinated axons occurs.[77] Among neurofilaments, CSF concentration of the light subunit (NfL) has been extensively studied as a biomarker in various neurological diseases, with ultrasensitive techniques (ie, single-molecule array) developed in the past 10 years that have allowed for a reliable measurement of this protein also in blood (panel 2).

In patients with Parkinson's disease, no strong evidence for a difference in CSF and blood NfL concentrations has been found, compared with controls. Conversely, CSF NfL concentration has been shown to be higher in patients with atypical parkinsonian disorders compared with those with Parkinson's disease, with excellent accuracies in discriminating Parkinson's disease from progressive supranuclear palsy (AUC 0·97, sensitivity 93%, specificity 95%), multiple system atrophy (AUC 0·95, sensitivity 89%, specificity 93%), and corticobasal syndrome (AUC 0·96, sensitivity 100%, specificity 93%).[26,27,78,79] Because blood NfL has a diagnostic accuracy similar to CSF NfL, it might represent a valid tool for clinicians in differential diagnosis between Parkinson's disease and atypical parkinsonian disorders (appendix).[78] Many of the reported findings come from longitudinal cohorts that were retrospectively evaluated, which increases the accuracy of clinical diagnoses. However, studies done on large cohorts of neuropathologically confirmed Parkinson's disease and atypical parkinsonian disorders are required to support the accuracy of CSF and blood

NfL as biomarkers for the differential diagnosis between these diseases.

Combinations of multiple biomarkers

Over the past 10 years, several studies have shown that a combination of multiple biomarkers, reflecting different pathophysiological mechanisms, can reach remarkable diagnostic accuracies in Parkinson's disease. Most relevant diagnostic findings have been obtained with the combination of CSF α-synuclein species, classic Alzheimer's disease biomarkers, and NfL (table 1). The ratio of NfL to Aβ42 in the CSF can discriminate patients with Parkinson's disease from patients with progressive supranuclear palsy with a good accuracy (AUC 0·82, sensitivity 100%, specificity 68%), which further improved after 1 year of follow-up (AUC 0·93, sensitivity 89%, specificity 93%).[26] Moreover, when considering CSF NfL, Aβ42, phosphorylated tau, total tau, and total α-synuclein together, discrimination between Parkinson's disease and other parkinsonian disorders (ie, progressive supranuclear palsy, multiple system atrophy, and corticobasal syndrome) could reach an even better accuracy (AUC 0·93, sensitivity 85%, specificity 92%).[85] Similar levels of accuracy in discriminating patients with Parkinson's disease from controls have been reported also for the combinations of ratio of CSF total tau and Aβ42, CSF total α-synuclein and total tau, ratio of CSF phosphorylated tau and total α-synuclein, and ratio of CSF oligomeric α-synuclein and total α-synuclein together with CSF phosphorylated α-synuclein and phosphorylated tau (table 1).[34,71,83,88] CSF total α-synuclein, Aβ42, and a panel of lysosomal enzymes including GCase showed a good diagnostic accuracy (AUC 0·83, sensitivity 84%, specificity 75%) in samples from the BioFIND cohort (panel 3).[59] In a previous study,[60] the combination of GCase activity, ratio of oligomeric α-synuclein to total α-synuclein, and older age improved the diagnostic performance in discriminating patients with Parkinson's disease from patients affected by other neurological disorders (sensitivity 82%, specificity 71%).

A combination of classic CSF Alzheimer's disease biomarkers with total α-synuclein and heart-type fatty acid binding protein (FABP3; a polyunsaturated fatty acid that could interact with α-synuclein) improved the discrimination of Parkinson's disease dementia, Alzheimer's disease, and dementia with Lewy bodies. In particular, CSF FABP3 and phosphorylated tau showed high accuracy for the differential diagnosis between dementia with Lewy bodies and Alzheimer's disease (AUC 0·92, sensitivity 95%, specificity 76%), whereas a combination of CSF phosphorylated tau, FABP3, and total α-synuclein could distinguish Parkinson's disease dementia from Alzheimer's disease (AUC 0·96, sensitivity 100%, specificity 88%; table 1).[84] The performance of combined CSF biomarkers can be further improved by including demographical and clinical variables. Re-analysing data from the PPMI cohort (panel 3) CSF total α-synuclein, olfactory function, age, and gender achieved a high discriminative power between patients with Parkinson's disease and healthy controls (AUC 0·93, sensitivity 90%, specificity 80%).[90] Magdalinou and colleagues[27] found that a panel of nine biomarkers (comprising CSF total α-synuclein, Aβ42, total tau, phosphorylated tau, YKL-40, NfL, monocyte chemotactic protein-1, soluble amyloid precursor protein α and β), together with disease duration and H&Y stage could differentiate patients with Parkinson's disease from patients with progressive supranuclear palsy, multiple system atrophy, and corticobasal syndrome with excellent accuracy (AUC 0·95, sensitivity 91%, specificity 91%).

These results suggest that a biomarker-based diagnosis of Parkinson's disease requires the measurement of multiple CSF biomarkers, necessary to discriminate among several parkinsonian disorders, which might have confounding clinical presentations.

Prognostic biomarkers for Parkinson's disease

Prediction of motor progression

Progression of motor disability in patients with Parkinson's disease has been associated with α-synuclein species concentrations in CSF and blood, with ambiguous results. Hall and colleagues[29] found that CSF total α-synuclein is positively associated with H&Y stage, Unified Parkinson's Disease Rating Scale, Part III (UPDRS-III), and prolonged Timed Up and Go test over 2 years. These findings are in line with a study showing that low CSF total α-synuclein acts as predictor of motor progression (hazard ratio [HR] 1·98, 95% CI 1·13–3·49).[91] Similar results have been found with both the ratio of CSF oligomeric α-synuclein to total α-synuclein,[87] and with plasma total α-synuclein.[92] However, other studies did not find evidence for an association between baseline CSF total α-synuclein and subsequent motor progression.[26,93]

CSF amyloid pathology markers alone do not seem to predict motor progression in patients with Parkinson's disease,[29] while the rate of change of the ratio of phosphorylated tau to Aβ42 has been found to correlate with a faster decline of performance in total UPDRS and UPDRS-III over time.[80] CSF total tau and phosphorylated tau seem to have prognostic value in terms of motor progression. CSF phosphorylated tau has been found to correlate with worsening of motor symptoms as measured by the UPDRS-III score.[29,93] Similarly, longitudinal changes in CSF total tau positively correlated with increases in scores for total UPDRS and UPDRS-III over time.[80]

According to these findings, tau pathology, as assessed by CSF phosphorylated tau, seems to have a role in accelerating motor progression. Neurodegeneration, measured by means of CSF total tau, acts similarly to tau pathology (ie, it accelerates motor progression). However, when axonal loss is measured by means of CSF NfL, it does not have prognostic value in terms of motor progression in patients with Parkinson's disease.[29,93]

	Study	Diagnostic value	Prognostic value	
			Motor progression	Cognitive impairment
Phosphorylated tau to total tau ratio	Zhang et al, 2013[80]	NA	Significant correlation with the rate of change in motor UPDRS (r=–0·12)	NA
Phosphorylated tau to Aβ42 ratio	Zhang et al, 2013[80]	NA	Significant correlation with the rate of change in motor UPDRS (r=–0·14)	NA
Phosphorylated tau to Aβ42 ratio	Liu et al, 2015[81]	NA	NA	Higher phosphorylated tau and Aβ42 predicted subsequent decline in SRT and SDMT
Aβ42 to total tau ratio	Parnetti et al, 2014[60]	PD *vs* control: AUC 0·71, sensitivity 82%, specificity 56%	NA	No significant association with MMSE and MoCA score decrease
Aβ42 to total tau ratio	Schrag et al, 2017[82]	NA	NA	Baseline Aβ42 and total tau associated with MoCA score at 2 years
Total tau to Aβ42 ratio	Constantinides et al, 2017[83]	PD *vs* MSA: AUC 0·82, sensitivity 71%, specificity 93%	NA	NA
NfL to Aβ42 ratio	Bäckström et al, 2015[26]	PD *vs* control: AUC 0·69; PD *vs* PSP: AUC 0·82, sensitivity 100%, specificity 68%; PD *vs* PSP (after 1 year): AUC 0·93	NA	Ratio >1:6·7 HR for PDD development
NfL with FABP3 to Aβ42 ratio	Bäckström et al, 2015[26]	NA	NA	Ratio >2·1:11·8 HR for PDD development
FABP3 with phosphorylated tau and total α-synuclein	Chiasserini et al, 2017[84]	PDD *vs* AD: AUC 0·96, sensitivity 100%, specificity 88%	NA	NA
FABP3 with total tau and total α-synuclein	Chiasserini et al, 2017[84]	PD *vs* DLB: AUC 0·92, sensitivity 80%, specificity 95%	NA	NA
NfL with Aβ42, phosphorylated tau, total tau, and total α-synuclein	Hall et al, 2012[85]	PDD and DLB *vs* AD: AUC 0·90, sensitivity 90%, specificity 81%; PD *vs* PSP, MSA, CBS: AUC 0·93, sensitivity 85%, specificity 92%	NA	NA
Oligomeric α-synuclein to total α-synuclein ratio	Aasly et al, 2014[86]	PD *vs* control: AUC 0·79, sensitivity 65%, specificity 83%	NA	NA
Oligomeric α-synuclein to total α-synuclein ratio	Majbour et al, 2016[87]	PD *vs* control: AUC 0·82, sensitivity 68%, specificity 85%	Correlation between decrease of CSF oligomeric α-synuclein and total α-synuclein and changes in motor UPDRS (r=–0·41) in patients with PIGD-PD	NA
Total α-synuclein with total tau	Heegard et al, 2014[88]	PD *vs* control: AUC 0·80, sensitivity 75%, specificity 93%	NA	NA
Total tau to total α-synuclein ratio	Delgado-Alvarado et al, 2017[71]	PD *vs* control: AUC 0·79	NA	NA
Phosphorylated tau to total α-synuclein ratio	Delgado-Alvarado et al, 2017[71]	PD *vs* control: AUC 0·86	NA	NA
Total tau to total α-synuclein ratio with Aβ42	Delgado-Alvarado et al, 2017[71]	PD *vs* control: AUC 0·65	NA	NA
Phosphorylated tau to total α-synuclein ratio with Aβ42	Delgado-Alvarado et al, 2017[71]	PD *vs* control: AUC 0·82	NA	NA
DJ-1 with total tau and phosphorylated tau	Herbert et al, 2014[89]	PD *vs* MSA: AUC 0·92, sensitivity 82%, specificity 81%	NA	NA
Oligomeric α-synuclein to total α-synuclein ratio with Aβ42 to total tau ratio	Parnetti et al, 2014[60]	PD *vs* control: AUC 0·83, sensitivity 70%, specificity 84%	NA	No significant association with MMSE and MoCA score decrease
GCase with β-hex, cathepsin D, total α-synuclein, and Aβ42	Parnetti et al, 2017[59]	PD *vs* control: AUC 0·83, sensitivity 84%, specificity 75%	NA	NA
Oligomeric α-synuclein to total α-synuclein ratio with phosphorylated α-synuclein and phosphorylated tau	Majbour et al, 2016[34]	PD *vs* control: AUC 0·86, sensitivity 79%, specificity 67%	NA	NA

AD=Alzheimer's disease. AUC=area under the Receiver Operating Characteristic curve. Aβ42=amyloid β peptide 1–42. CBS=corticobasal syndrome. DLB=dementia with Lewy bodies. FABP3=heart-like fatty acid binding protein. GCase=β-glucocerebrosidase. HR=hazard ratio. MMSE=Mini Mental State Examination. MoCA=Montreal Cognitive Assessment. MSA=multiple system atrophy. NA=not assessed. NfL=neurofilament light chain. PD=Parkinson's disease. PDD=Parkinson's disease dementia. PIGD-PD=postural instability and gait disorder dominant Parkinson's disease. PSP=progressive supranuclear palsy. SDMT=Symbol Digit Modalities Test. SRT=Selective Reminding Test. UPDRS=Unified Parkinson's Disease Rating Scale. β-hex=β-hexosaminidase.

Table 1: **Overview of combined CSF diagnostic and prognostic biomarkers**

Prediction of cognitive impairment

CSF classic Alzheimer's disease biomarkers have been investigated as predictors of cognitive impairment in patients with Parkinson's disease. Several studies have provided support that lower baseline CSF concentrations of Aβ42 are associated with worse cognition and might predict cognitive decline in patients with Parkinson's disease, and transition to Parkinson's disease dementia.[26,29,94–97] These findings were consistently observed despite heterogeneous settings, different outcome measures, and different CSF biomarker assays. By contrast, CSF total tau and phosphorylated tau have shown inconsistent findings.[68,94,96,98] The prognostic value of CSF Aβ42 substantially improves when considered together with other CSF biomarkers. In the study by Bäckström and colleagues,[26] CSF Aβ42 of less than 626 ng/L was associated with a hazard ratio (HR) of 2·8 (95% CI 1·4–5·8) for dementia development within 5–9 years of follow-up. In the same study, HR values increased up to 6·7 (95% CI 1·5–30·5) for the CSF ratio of NfL to Aβ42 more than 1, and up to 11·8 (95% CI 3·3–42·1) when considering a CSF NfL and FABP3 to Aβ42 ratio more than 2·1.[26] Additionally, the combination of CSF Aβ42 with clinical features further improves its prognostic value. A multivariate analysis of data from the PPMI cohort (panel 3) showed that age, olfactory dysfunction, CSF ratio of Aβ42 to total tau, and apolipoprotein E status were associated with a decrease of Montreal Cognitive Assessment scores over time.[82] Also, a model including olfactory dysfunction, REM sleep behaviour disorder, CSF Aβ42, and dopamine transporter imaging findings allowed for prediction of cognitive impairment at 2 years with high accuracy (AUC 0·80).[82] Of interest, low CSF Aβ42 has been found to predict early psychosis in patients with Parkinson's disease, being associated with the appearance of illusions or hallucinations within 3–4 years of follow-up.[99]

By contrast with CSF Aβ42, biomarkers of synucleinopathy have shown conflicting results as a predictor of cognitive impairment. Higher CSF total α-synuclein has been associated with worsening of cognitive function, especially on verbal memory and information processing speed over time,[29,100] and low CSF total α-synuclein has been identified as a significant predictor of cognitive decline (HR 2·57, 95% CI 1·38–4·79).[91] However, in other reports, no prognostic effect of CSF total α-synuclein and oligomeric α-synuclein has been found.[26,93,101,102] Such inconsistency could depend on the different characteristics of patients included in studies, as CSF total α-synuclein might increase with progressive disease stages.[93] A study by Wang and colleagues[92] reported a possible association between higher plasma total α-synuclein and lower Montreal Cognitive Assessment scores at 2 years after blood sampling in the Parkinson's Associated Risk Syndrome study, which included hyposmic or dopamine transporter imaging positive individuals not yet diagnosed with Parkinson's disease, but considered at risk for the disease. Further studies on blood α-synuclein species are required to support their potential prognostic value in both prodromal and manifested Parkinson's disease.

***Panel 4*: Status of CSF and blood biomarkers in Parkinson's disease**

- CSF total α-synuclein is lower in patients with Parkinson's disease compared with healthy controls and with neurological controls not affected by synuclein aggregation disorders.[11,16–21] The risk of blood contamination is high.[23] Diagnostic accuracy is low so this biomarker alone should not be considered useful in the diagnosis of Parkinson's disease.[11,17]
- CSF oligomeric α-synuclein and phosphorylated α-synuclein are higher in patients with Parkinson's disease compared with healthy and neurological controls.[11,18] However, further studies are required to support their potential diagnostic value and to overcome analytical issues.
- CSF pro-aggregating forms of α-synuclein have shown promising preliminary results as diagnostic markers in Parkinson's disease.[36–38] Larger studies are required and analytical issues (ie, reproducibility and assay time) have to be addressed.
- Plasma and serum α-synuclein species measurement is at high risk for erythrocyte contamination and dynamics of α-synuclein in this fluid are unknown.[23] Intra-erythrocyte α-synuclein species represent a valid alternative, but their diagnostic and prognostic value must be confirmed in larger studies.[51]
- Lysosomal enzyme activities are reduced in the CSF of patients with Parkinson's disease.[53–55] Together with other biomarkers, their activity measurement can improve diagnostic accuracy in identifying patients with Parkinson's disease.[59]
- Classic Alzheimer's disease biomarkers alone are not helpful in the diagnostic process for Parkinson's disease,[71] but can improve prognostic assessment, with CSF Aβ42 being a valid marker of future cognitive decline and CSF total tau and phosphorylated tau being potential markers of motor progression in Parkinson's disease.[29,80,93]
- CSF and blood neurofilament light chain are biomarkers useful to discriminate Parkinson's disease from progressive supranuclear palsy, multiple system atrophy, and corticobasal syndrome in cases with confounding clinical presentations.[26,27,78,79]
- A combination of multiple CSF (and probably blood) biomarkers reflecting different pathogenic mechanisms taking place during Parkinson's disease (figure), might enable earlier diagnosis and more accurate prognostic assessment in Parkinson's disease (table 1).

Conclusions and future directions

Over the past decade, multicentre key initiatives focused on research into diagnostic and prognostic biomarkers for Parkinson's disease (panel 3) have substantially contributed to improving knowledge and reaching achievements with high clinical potential in this neurodegenerative disease (panel 4). In the field of diagnostic biomarkers, the most promising results have been obtained by studying molecules related to the pathophysiological mechanisms occurring in the disease, such as α-synuclein species, lysosomal enzyme activities, classic Alzheimer's disease biomarkers, and NfL (figure). However, only a few studies have focused on very early disease stages (ie, premotor Parkinson's disease), when biomarkers would be necessary for diagnostic accuracy. It is therefore desirable that longitudinal studies on CSF and blood biomarkers for Parkinson's disease include patients with premotor manifestations, which will allow clarification of both their diagnostic accuracy and predictability on clinical outcome. One of the most important innovations in the diagnostic

	Phase	Outcome	Biofluid	Biomarker	ClinicalTrials.gov identifier	Planned sample size	Trial period
PD01A	1	Secondary	CSF	α-Synuclein	NCT01568099	32	From February, 2012, to May, 2014
Nilotinib	1	Primary	CSF, blood	α-Synuclein, total tau	NCT02281474	12	From November, 2014, to May, 2015
Ambroxol	2	Primary and Secondary	CSF, blood	GCase	NCT02941822	20	From December, 2016, to April, 2018
Nilotinib	2	Secondary	CSF	Homovanillic acid	NCT02954978	75	From November, 2016, to April, 2018
KM-819	1	Secondary	CSF, blood	KM-819, oligomeric α-synuclein, total tau, phosphorylated tau	NCT03022799	88	From October, 2016, to October, 2017
MEDI1341	1	Secondary	CSF, blood	Total α-synuclein	NCT03272165	40	From October, 2017, to September, 2019
Cerebral dopamine neurotrophic factor administered by means of the Renishaw drug delivery system	1/2	Other	CSF, blood	α-Synuclein (various species)	NCT03295786	18	From September, 2017, to November, 2019
Niacin (dietary supplement)	NA	Secondary	CSF	Cytokines	NCT03462680	80	From September, 2016, to September, 2019
Glycerol phenylbutyrate	1	Primary	Blood	α-Synuclein	NCT02046434	40	From February, 2015, to September, 2018
EPI-589	2	Secondary	CSF, blood	Biomarker assessments as measured in CSF, blood and urine	NCT02462603	44	From March, 2016, to December, 2018
Mechanical peripheral stimulation (GONDOLA)	NA	Secondary	Blood	BDNF	NCT02594540	28	From December, 2015, to September, 2016
Osteopathic manipulative medicine	NA	Other	Blood	Blood draws at week 1, 3, and 6 to compare changes in serum biomarkers	NCT02107638	50	From April, 2014, to December, 2021
Droxidopa-carbidopa	4	Secondary	Blood	Catecholamine	NCT03115827	15	From April, 2017, to August, 2018
EPI-743	2	Secondary	Blood	Blood biomarker concentrations	NCT01923584	15	From December, 2015, to June, 2016

BDNF=brain-derived neurotrophic factor. GCase=β-glucocerebrosidase. KM-819=Fas-associated factor 1 inhibitor. NA=not available. PD01=Affitope.

Table 2: **Design of clinical trials using CSF and blood biomarkers as outcome measures**

area relies on the ability to identify forms of α-synuclein prone to aggregation, which represents a revolution in the diagnostics of synucleinopathies. Techniques such as PMCA and RT-QuIC have enabled identification of α-synuclein that is prone to aggregation and dynamically involved in Parkinson's disease pathology. To date, preliminary results are very encouraging, also in the perspective of diagnosing Parkinson's disease in the earliest stages. It is necessary, however, to refine analytical methods, to enlarge sample sizes, and, most importantly, to test these techniques in the very early stages of Parkinson's disease.

It is also worth citing efforts in identifying synucleinopathies in more accessible peripheral tissues such as gastrointestinal mucosae and salivary glands. Thus far, histological differentiation of normal and pathological α-synuclein has been challenging with variable results.[103] The complexity of the pathophysiological processes taking place in the Parkinson's disease brain makes it necessary, as the most reliable diagnostic approach, to measure a panel of markers reflecting different aspects of the disease-related pathways. The highest diagnostic accuracies in identifying patients with Parkinson's disease have been obtained by combining different biomarkers (table 1). These results are quite promising, indicating that we have reliable markers that now need full validation—namely, standardisation of techniques, generation of reference materials, definition of reliable cutoffs—up to the clinical validation (panel 1). Another issue that needs to be clarified is to what extent a panel including several biomarkers could be used in clinical practice. With respect to the differential diagnosis between Parkinson's disease and atypical parkinsonian disorders, further studies are needed on cohorts with neuropathologically verified diagnoses, because clinical manifestations can easily overlap, thus making it difficult to interpret the actual diagnostic value of fluid biomarkers.

Search strategy and selection criteria

We searched PubMed between Jan 1, 2013, and Sept 30, 2018, and references from relevant articles. The search terms "biomarkers", "Parkinson's disease", "cerebrospinal fluid", and "blood" were used. If one of these papers referred to another relevant study, published after Jan 1, 2013, we also included the cited study. For this Review, we selected only studies on biomarkers with a well defined pathophysiological rationale for Parkinson's disease and validated at least in one second independent cohort. We also specified a threshold for small studies of n=10 per diagnostic group. There were no language restrictions. The final reference list was generated on the basis of relevance to the topics covered in this Review. A flow chart on the selection of the studies is available in the appendix.

In the context of prognostic value, fluid biomarkers have shown very encouraging results, especially in defining the cognitive trajectories in Parkinson's disease. Among classic CSF Alzheimer's disease biomarkers, CSF Aβ42 has been shown to reliably predict the risk of cognitive impairment in patients with Parkinson's disease. By contrast, predictability of motor outcome by fluid biomarkers is still unsatisfactory. A potential prognostic role of CSF phosphorylated tau has been proposed, but this finding requires further confirmation in larger cohorts with longer follow-up.

Another important application of CSF and blood biomarkers would be as outcome measures in clinical trials with disease modifying drugs. A largely unmet medical need in Parkinson's disease is represented by the availability of treatments for blocking or slowing the disease progression. The discovery of genetic variants that cause or increase the risk for Parkinson's disease could be used in the search for new targets for potential therapies to be tested in clinical trials.[104] Among others, *SNCA* and *GBA* genetic variants have their molecular counterpart in CSF with α-synuclein species and GCase activity. These strong genetic and pathological links make α-synuclein and GCase the most attractive targets for Parkinson's disease drug development. So far, only a few clinical trials have considered fluid biomarkers as surrogate outcomes for investigating the biological efficacy of a treatment or for exploratory purposes (table 2). Specifically, referring to clinical trials done in the past 5 years, CSF total α-synuclein has been proposed as a surrogate marker of efficacy in patients with Parkinson's disease in phase 1–2 clinical trials targeting α-synuclein. Other molecules used as a surrogate marker were urate, GCase, tau, and homovanillic acid, the main metabolite of dopamine. Finally, in several trials, CSF and blood were used as a source for investigating the mechanism of action of tested drugs (table 2). A biomarker-based strategy would improve the selection of participants, the assessment of efficacy in counteracting one or more pathophysiological mechanisms, and the prognostic effect.

In conclusion, sufficient evidence exists of analytical validity for most CSF and blood biomarkers discussed in this Review, with highest performance accuracy when considering a combination of several biomarkers. What is now needed, more so than looking for new biomarkers, is to systematically proceed to the long process of confirming clinical validity and utility, similar to what has been proposed for Alzheimer's disease.[3] Also, the longitudinal tracking of motor and cognitive trajectories in different—both retrospective and prospective—Parkinson's disease cohorts represents a mandatory need.

Contributors

LP, LG, PE, and SP did the literature search and drafted the manuscript. LG, PE, and SP prepared the figures and the tables. OH, OE-A, BM, KB, and PC reviewed the manuscript. All authors read and approved the final version of the manuscript.

Declaration of interests

LP received research support from Fujirebio. LG received travel grants from Biogen-Idec, Biogen, Novartis, Teva, Genzyme, Almirall, Roche, and Mylan to attend national and international conferences. OH has acquired research support (for their institution) from Roche, GE Healthcare, Biogen, AVID Radiopharmaceuticals, Fujirebio, and Euroimmun. In the past 2 years, he has received consultancy or speaker fees (for their institution) from Biogen, Roche, and Fujirebio. BM has received independent research grants from TEVA-Pharma, Desitin, Boehringer Ingelheim, and GE Healthcare and honoraria for consultancy from Bayer Schering Pharma AG, Roche, AbbVie, TEVA-Pharma, Biogen and for presentations from GlaxoSmithKline, Orion Pharma, TEVA-Pharma and travel costs from TEVA-Pharma. BM is a member of the executive steering committee of the Parkinson Progression Marker Initiative and PI of the Systemic Synuclein Sampling Study of the Michael J Fox Foundation for Parkinson's Research and has received grants from the Deutsche Forschungsgemeinschaft, BMBF, EU (Horizon2020), Parkinson Fonds Deutschland, Deutsche Parkinson Vereinigung, Michael J Fox Foundation for Parkinson's Research, Stifterverband für die deutsche Wissenschaft, and has scientific collaborations with Roche, Bristol-Myers Squibb, Ely Lilly, Covance/BioLegend, and Biogen. KB has served as a consultant or at advisory boards for Alzheon, BioArctic, Biogen, Eli Lilly, Fujirebio Europe, IBL International, Pfizer, and Roche Diagnostics, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg. The aim is to license antibodies generated within research projects. The company was initiated by the University, that also provides the administrative means, and any money generated would go into further research. KB is not employed by the company, and neither has got any equity, salary or similar, and will not profit from this publication. PC has received research support from Bayer Schering, Biogen-Dompé, Boehringer Ingelheim, Eisai, Lundbeck, Merck-Serono, Novartis, Sanofi-Aventis, Sigma-Tau, and UCB Pharma. All other authors declare no competing interests.

Acknowledgments

PC was supported by the Fresco Institute Network Research Program (FI-NRP), Fresco Network of Excellence Italy Sites (09.12.2016). This organisation had no direct input in any aspect of this Review. The work of PE was supported by the Italian Ministry of Health (Grant GR-2013-02357757).

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