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REVIEW

Cerebrospinal fluid biomarkers in parkinsonian conditions: an update and future directions

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ABSTRACT

Parkinsonian diseases comprise a heterogeneous group of neurodegenerative disorders, which show significant clinical and pathological overlap. Accurate diagnosis still largely relies on clinical acumen; pathological diagnosis remains the gold standard. There is an urgent need for biomarkers to diagnose parkinsonian disorders, particularly in the early stages when diagnosis is most difficult. In this review, several of the most promising cerebrospinal fluid candidate markers will be discussed. Their strengths and limitations will be considered together with future developments in the field.

INTRODUCTION

Idiopathic Parkinson's disease (iPD) is a progressive neurological disorder initially described as a clinical entity by James Parkinson and then embellished by Charcot and other nineteenth-century physicians, including Trousseau, Gowers and Erb. It is a clinical construct, based upon the presence of bradykinesia accompanied by at least one other characteristic feature, such as resting tremor, rigidity and impaired postural reflexes.¹ The signs and symptoms are usually asymmetrical at onset and, typically, there is a good response to levodopa treatment.

'Parkinson-plus' or 'atypical parkinsonism' are terms that refer to a heterogeneous group of neurodegenerative disorders that may masquerade particularly in the early stages of the disease as Parkinson's disease (PD).² The 'plus' or 'atypical' descriptor indicates the presence of additional characteristics not usual in patients with iPD, such as early autonomic disturbance and pyramidal signs exhibited by patients with multiple system atrophy (MSA), supranuclear gaze palsy and frontal/dysexecutive syndrome by those with progressive supranuclear palsy (PSP), dystonia and myoclonus in corticobasal degeneration (CBD) and early postural instability and falls by all of them. Another disease that could be classified as an atypical parkinsonian disorder is dementia with Lewy bodies (DLB), where dementia onset is before or within a year of onset of extrapyramidal features. The earlier onset of dementia differentiates DLB from Parkinson's disease dementia (PDD).

Atypical parkinsonian disorders account for less than 10% of all parkinsonism and rarely respond with sustained improvement to levodopa. They usually follow a much more aggressive disease course than iPD and are characterised by atrophy to several different cortical and subcortical networks. Furthermore, atypical parkinsonism has been described in other conditions, such as Alzheimer's disease (AD) and frontotemporal dementia (FTD).

PATHOLOGY

Protein misfolding and aggregation is seen with many neurodegenerative diseases. Based on pathological findings, parkinsonian syndromes are classified into *α-synucleinopathies* (PD, DLB and MSA) and *primary tauopathies* (PSP and CBD). For pathological lesions used in postmortem diagnosis of parkinsonism, see [figure 1](#).

α-Synuclein (*α-Syn*) has been found to be the major constituent of the intracellular aggregates in Lewy bodies and Lewy neurites (pathological hallmark of PD and DLB) and in the glial cytoplasmic inclusions in MSA.^{3–4} The presence of abnormally aggregated tau proteins in the form of neurofibrillary tangles, for example, are diagnostic of PSP.⁵ Tau-positive intracellular inclusions are the neuropathological findings in CBD.⁶ Even though there are also neurofibrillary tangles in AD, Aβ plaques are closely tied to the primary disease process and thus AD is considered to be a *secondary tauopathy*. FTD can also have underlying tau pathology.

There is often some overlap between synucleinopathies and tauopathies (for a review, see ref. 7). Co-occurrence of tau and *α-Syn* pathology has been found in neurons and oligodendrocytes in AD, PD and DLB.⁸ *α-Syn* has complex and dynamic interactions with tau. Each of these two proteins has the tendency to seed the aggregation of the other.⁹ *α-Syn* induces aggregation and polymerisation of tau, which promotes formation of intracellular amyloid-tau inclusions.¹⁰ Similar interactions have been described between *α-Syn* and Aβ pathology.¹¹

GENETICS

Recent advances in genetics have shed light on the underlying pathophysiology because mutations in the gene for each misfolded protein can give rise to an inherited form of a relevant neurodegenerative condition. For example, rare hereditary forms of PD can be caused by mutations affecting the gene coding for *α-Syn* (*SNCA*); *PARK1* (missense) and *PARK4* (duplication, triplication).¹² Furthermore, in both PD and to a lesser extent in MSA, population studies demonstrated an association between disease risk and distinct single-nucleotide polymorphisms in *SNCA*. *DJ-1* (*PARK7*) mutations can lead to rare forms of autosomal-recessive PD, pointing towards mitochondrial damage/oxidative stress pathways driven pathogenesis.¹³ Even though PD is not a 'tauopathy', population studies also showed variants in tau (*MAPT*) gene, particularly the H1 haplotype, as another risk factor for PD (for a review, see ref. 14). Several tauopathies are



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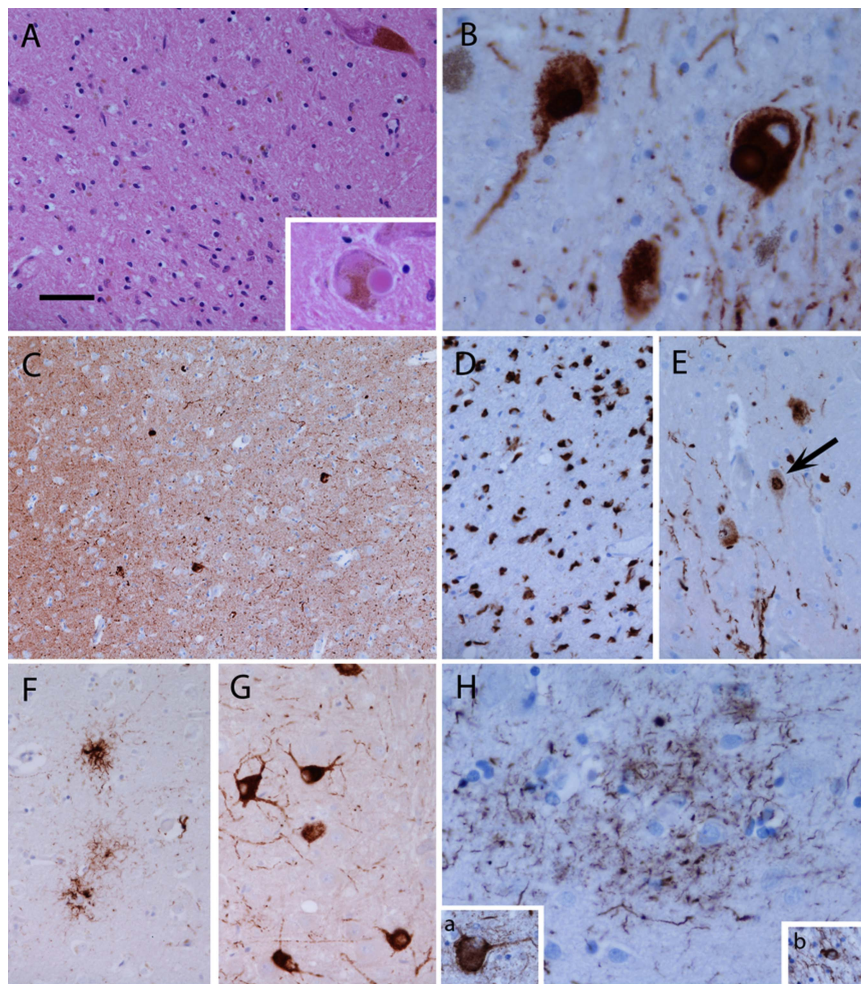


Figure 1 In Parkinson's disease (PD), there is loss of pigmented neurons from the substantia nigra and remaining neurons may be very sparse (A). Lewy bodies can be observed in residual neurons (A, inset) and are highlighted, together with Lewy neuritis, using α -synuclein immunohistochemistry (B). Lewy bodies and Lewy neurites may be present in significant numbers in the neocortex (C, frontal cortex). In multiple system atrophy (MSA), α -synuclein is primarily deposited in the form of glial cytoplasmic inclusions in oligodendrocytes (D, putamen) and may also form inclusions in neuronal cytoplasm and nuclei (arrow) (E, pontine nuclei). In progressive supranuclear palsy tau forms aggregates in neurons and glia, giving rise to tufted astrocytes (F, caudate) and neurofibrillary tangles (G, pontine nuclei). A characteristic feature of corticobasal degeneration (CBD) is the astrocytic plaque, formed from aggregated tau in the distal processes of astrocytes (H, parietal cortex). In CBD, tau also accumulates in neurons in the form of neurofibrillary tangles (H, inset a) and in oligodendrocytes as coiled bodies (H, inset b). (A) Haematoxylin and eosin; (B–D) α -synuclein immunohistochemistry; (F–H) tau immunohistochemistry. Bar in (A) represents 100 μ m in (C); 50 μ m in (A, D–G); 25 μ m in inset A, B and H. Pathological images kindly provided by Dr Janice Holton, Queen Square Brain Bank for Neurological Disorders, London.

associated with variants in *MAPT*, including CBD, FTD linked to chromosome 17 (FTDP-17T) and PSP.¹⁵ The fact that the *MAPT*/tau haplotype also shows an association with PD strongly suggests that the pathogenic cascades in the tauopathies may be related to those in the synucleinopathies.¹⁶

DIAGNOSTIC CHALLENGES

Accurate diagnosis of parkinsonian disorders still relies heavily on clinical acumen, although imaging and ancillary investigations may be helpful in some situations. In one postmortem series, 24% of patients clinically diagnosed with idiopathic PD by a consultant neurologist during life were found to have an alternative diagnosis.¹

CEREBROSPINAL FLUID BIOMARKERS

A biomarker is “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic response to a therapeutic

intervention”.¹⁷ An ‘ideal’ biomarker should be sensitive, reproducible, closely associated with the disease process, non-invasive and inexpensive.

Cerebrospinal fluid (CSF) has more physical contact with the brain than any other fluid and as such represents a potentially reliable biomarker source. Unlike plasma, CSF is not separated from the brain by the tightly regulated blood–brain barrier. Proteins/peptides that may be directly reflective of brain specific activities or disease pathology would most likely diffuse into the CSF. Furthermore, CSF can be tested serially, which makes possible the study of protein changes reflecting the evolving pathology throughout the clinical course of the disease. This is preferable to pathological studies, which only reveal the terminal changes of a disease process that has developed over decades.

HISTORICAL BACKGROUND

CSF has been widely investigated in parkinsonian disorders and is considered to offer the most promising insights into the

disease process. Historically, because of dopaminergic abnormalities in parkinsonism, the first compounds to be tested as potential markers were dopamine and other monoamines and their metabolites. In the 1960s and 1970s, reduced CSF monoamine concentrations (homovanillic acid and 5-hydroxyindoleacetic acid) were found in patients with parkinsonism and dementia.¹⁸ A study conducted at the National Hospital, Queen Square, London, assessed the effect of levodopa treatment in CSF homovanillic acid concentration of PD patients. Before levodopa treatment, homovanillic acid concentration was low in all patients, while after treatment it rose to a level that correlated significantly with the levodopa dose.¹⁹

As these metabolic results were prone to be influenced by a multitude of other factors,⁷ the quest went further to investigate a priori defined compounds, such as α -Syn and tau. These were tested in patients and in healthy controls, looking for differences, patterns and associations. Even though several promising candidates exist, there is still no reliable biomarker.

METHODS

We reviewed the potential use of CSF proteins as biomarkers in parkinsonism, focusing on α -Syn, neuronal injury markers and A β 42. In addition, we briefly reviewed the latest novel markers and the 'omics' approach. We performed a PubMed/Medline search and limited searches to studies reported in English and published after 2006, including antemortem, human, lumbar CSF; all studies included at least one parkinsonian cohort compared with healthy or neurological controls. We combined searches with 'Parkinson's disease', 'progressive supranuclear palsy', 'multiple system atrophy', 'corticobasal syndrome' (CBS), 'corticobasal degeneration', 'Parkinson's disease dementia', 'dementia with Lewy bodies', 'Lewy body dementia', 'parkinsonism', 'synucleinopathies', 'tauopathies', 'neurodegenerative diseases' with 'CSF biomarkers' and specific biomarkers (' α -Syn', 'tau', 'phosphorylated tau', 'A β 42', 'neurofilaments', 'neuronal injury markers', 'inflammatory', 'metabolic' and 'oxidative stress markers'). Further references were found manually

from identified publications. For a review of the earlier literature, not captured using the time limit of our search criteria, see Eller and Williams.²⁰

CSF BIOMARKER CANDIDATES IN PARKINSONISM

A β 42

A β 42 is a 42 amino-acid long, aggregation-prone protein, derived from the proteolytic processing of amyloid precursor protein and is a major component of neuritic plaques in AD. Cognitive impairment and dementia are much more common in parkinsonism than in the general population and have a detrimental effect on quality of life and life expectancy. The link between A β 42 and PD and dementia has been studied extensively (see table 1).

In most studies, A β 42 is significantly reduced in PD compared with controls and is associated with worse cognitive performance.^{21–25} However, other investigations showed no difference between PD and controls.^{26–30}

Compta *et al*³¹ collected CSF from 27 non-demented PD patients and followed them over time. Patients who converted to dementia within 18 months had a significantly lower baseline CSF A β 42 than the patients who remained non-demented.

DLB patients have the lowest CSF levels of A β 42 among the parkinsonian cohorts.^{25 32–34} One study found that almost half of DLB patients had a CSF biomarker profile consistent with AD,³³ which agrees with the knowledge of A β pathology in this disease.^{35–37}

There is evidence that low A β 42, a marker of A β plaque pathology, may predict cognitive decline in patients with PD,³⁸ but other longitudinal studies with larger cohorts are necessary to clarify this further.

α -Syn

α -Syn is a 140 amino-acid long protein that localises to pre-synaptic terminals and is widespread in the brain, comprising 1% of cytosolic protein. In presynaptic terminals, α -Syn is present in close proximity to the synaptic vesicles. The precise

Table 1 CSF A β 42 in parkinsonian disorders

Research groups	Participants	Main findings
Kang <i>et al</i> ²¹	PD n=39 (drug-naïve patients), HC n=63	Decrease in PD vs HC
Compta <i>et al</i> ³¹	Baseline: PD n=27 (non-demented) 18 month follow-up: PD n=16 (non-demented), PD n=11 (dementia converters)	Decrease in dementia converters
Bech <i>et al</i> ³²	PD n=22, PDD n=3, DLB n=11, MSA n=10, PSP n=20, CBD n=3	Decrease in DLB vs other disease groups
Hall <i>et al</i> ²⁶	PD n=90, PDD n=33, DLB n=70, PSP n=45, CBD n=12, MSA n=48, AD n=48, controls n=107	Decrease in AD>DLB+PDD
Schoonenboom <i>et al</i> ³³	DLB n=52, PSP n=20, CBD n=16, AD n=512, FTD n=144, VaD n=34, CJD n=6, controls n=275	Decrease in AD>VaD>DLB>CBD
Parnetti <i>et al</i> ²⁷	PD n=38, DLB n=32, AD n=48, FTD n=31, controls n=32	► Decrease in AD, FTD+DLB vs PD and controls ► No difference between PD and controls
Andersson <i>et al</i> ³⁴	DLB n=47, PDD n=17, AD n=150	Decrease in DLB vs PDD
Shi <i>et al</i> ²²	Discovery cohort: PD n=126, MSA n=32, AD n=50, controls n=137 Validation cohort: PD n=83	Slight decrease in PD and MSA vs controls
Montine <i>et al</i> ²⁸	PD n=41, PDD n=11, AD n=49, HC n=150	Decrease in PDD vs HC
Süssmuth <i>et al</i> ²⁹	PSP-RS n=20, PSP-P n=7, MSA-P n=11, MSA-C n=14, PD n=23, controls n=20	► No difference in parkinsonian syndromes ► Lower in PSP-RS vs PSP-P
Alves <i>et al</i> ²³	PD n=109, AD n=20, HC n=36	Decrease in PD vs HC
Ohrfelt <i>et al</i> ³⁰	PD n=15, DLB n=15, AD n=66, controls n=55	Decrease in AD+DLB vs controls and PD
Compta <i>et al</i> ²⁴	PD n=20, PDD n=20, HC n=15	Decrease in PDD>PD vs HC
Parnetti <i>et al</i> ²⁵	PD n=20, PDD n=8, DLB n=19, AD n=23, HC n=20	Decrease in DLB>PDD>PD

AD, Alzheimer's disease; CBD, corticobasal degeneration; CJD, Creutzfeldt–Jakob disease; CSF, cerebrospinal fluid; DLB, dementia with Lewy bodies; FTD, frontotemporal dementia; HC, healthy controls; MSA, multiple system atrophy; MSA-C, multiple system atrophy cerebellar type; MSA-P, multiple system atrophy parkinsonian type; PD, Parkinson's disease; PDD, Parkinson's disease dementia; PSP, progressive supranuclear palsy; PSP-P, progressive supranuclear palsy–parkinsonism; PSP-RS, progressive supranuclear palsy–Richardson's syndrome; VaD, vascular dementia.

function of α -Syn is obscure, but it is speculated that its main role is in the control of neurotransmitter release.³⁹ Although mostly considered an intracellular protein, α -Syn is capable of transfer between cells leading to a speculation of a prion-like mechanism operating in PD pathology spread.⁴⁰

α -Syn can be modified by truncation, acetylation, phosphorylation, oxidation, nitrosylation, glycation or glycosylation.⁴¹ Lewy bodies are formed mostly of post-translationally modified α -Syn. α -Syn deposition is key in the pathogenesis of synucleinopathies. In vitro, similar to AD, α -Syn fibrillation involves α -Syn oligomerisation followed by oligomer conversion into mature amyloid cultures, which are toxic to cultured neuronal cells.⁴²

Total α -Syn (t- α -Syn)

Inconsistent results were initially reported in parkinsonian conditions with studies demonstrating considerable overlap of t- α -Syn in several neurodegenerative conditions.^{30 43–45} A consensus is now emerging, and the vast majority of recent studies (predominantly using ELISA techniques) have shown a reduction of t- α -Syn levels in PD compared with controls.^{21 22 26 46–55} In addition, there is decreased t- α -Syn in other synucleinopathies, such as MSA and DLB,^{26 47 49 50 51 54} without good discriminatory value between the groups (see table 2).

Mollenhauer *et al* assessed levels of t- α -Syn in patients with synucleinopathies, patients with tauopathies and in neurological controls without neurodegenerative disease, first in a training set and then in a validation set. In the training set, a combination of t- α -Syn, t-tau and age differentiated synucleinopathies from neurological controls and AD with an area under the curve (AUC) of 0.908. Only t- α -Syn levels and not t-tau or A β 42 discriminated PD and MSA from controls with a positive predictive value of 91%.⁵⁴

Parnetti *et al* investigated whether the combination of t-tau, p-tau and t- α -Syn can improve differentiation of PD from DLB, AD, FTD and controls. They found an inverse correlation between t- α -Syn and total tau in all subjects and a lack of specificity of CSF t- α -Syn determination alone as a marker of synucleinopathy (sensitivity 94%, specificity 25%). However, t-tau/t- α -Syn and p-tau/t- α -Syn ratios were identified as possible biomarkers for PD (sensitivity 89%, specificity 61%).²⁷

Shi *et al*²² also showed that a combination of t- α -Syn and p-tau/t-tau could discriminate PD from MSA with a sensitivity of 90% and a specificity of 71%, when blood contaminated samples were excluded. t- α -Syn was decreased in PD and especially in MSA compared with controls.

In most studies, there was no correlation of t- α -Syn with disease duration or disease severity. Interestingly, gender-specific variations were reported in levels of t- α -Syn.⁴⁷ Both Mollenhauer *et al*⁴⁸ and Kang *et al*²¹ studied drug-naïve PD patients and still found reduction in t- α -Syn, so it was proven that this finding was not related to a dopaminergic medication effect. There are several theories why there is reduced t- α -Syn in PD, MSA and DLB. High brain levels of pathological t- α -Syn and low CSF levels may reflect a reduction of 'free' t- α -Syn circulating in the CSF. This could be similar to 'pathological protein trapping' reported for brain A β 42 in AD CSF.⁵⁶

Oligomeric and phosphorylated α -Syn

Tokuda *et al* evaluated soluble α -Syn oligomers as potential early markers of PD and found that both the level of oligomeric α -Syn and the oligomer/t- α -Syn ratio were substantially higher in patients with PD (including those with mild and early-stage disease) compared with healthy controls and patients with non-neurodegenerative neurological conditions. CSF oligomer/

t- α -Syn ratio had a sensitivity of 89.3% and a specificity of 90.6% for PD.⁵⁷ These findings were replicated in two further, independent studies.^{58 59} Both oligomeric and phosphorylated oligomeric forms of α -Syn were detected in postmortem ventricular CSF, which may be useful in distinguishing between PD, DLB and MSA.⁶⁰ The results need to be replicated in larger groups of living patients.

NEURONAL INJURY MARKERS

Tau

Tau is important for the function of axonal microtubules and, as a result, plays an important role in the structural integrity of the neuron and axonal support. When hyperphosphorylated, it has reduced binding affinity for microtubules, causing their malfunction. At the same time, it adopts an abnormal conformation leading to aggregation and inclusion formation.⁶¹

Total and phosphorylated tau (t-tau and p-tau)

In the past, there were inconclusive results when assessing tau levels in CSF of parkinsonian patients (see table 3). In PD, most studies found normal values,^{23 24 25 26 27 29 30} but lower levels were also reported.^{21 22 28} In atypical parkinsonism, high t-tau levels were found in DLB^{25 27 34} and low p-tau/t-tau ratio in MSA and PSP compared with PD.²⁹ However, other investigations found no difference between parkinsonian syndromes.^{22 26 30} In particular, no significant change has been seen in PSP.²⁶ Age, not diagnosis, is thought to be the strongest factor affecting t-tau protein levels.⁵⁴

t-tau and p-tau may prove useful in differentiating AD from PD and can perhaps improve diagnostic accuracy when used in combination with other markers rather than on their own.

Tau isoforms

Imbalances in the homeostasis of tau isoforms with three- (3R-tau) and four- (4R-tau) microtubule-binding repeat domains are important in neurodegenerative disease pathogenesis. In a normal adult brain, there are comparable levels of 3R- and 4R-⁶² but in PSP, CBD and FTDP-17 cases, the neurofibrillary tangles and glial inclusions are predominantly 4R, whereas Pick bodies in FTD are predominantly 3R-tau⁶³ and neurofibrillary tangles in AD contain both 3R- and 4R-tau isoforms.

Luk and colleagues had previously developed antibodies selective for the two isoforms and adapted an immuno-PCR procedure in order to detect the isoforms' miniscule amounts in the CSF. Decrease in 4R-tau isoform was found in PSP and AD compared with CBS, PDD and controls. There was no difference in 3R-tau.⁶⁴

We think that 4R-tau could be used as a marker of disease progression in PSP, but further large samples and longitudinal series are needed.

Truncated tau forms

Borroni and colleagues looked at full-length (55 kDa) and truncated (33 kDa) tau forms in several neurodegenerative diseases. In ratio with the full-length tau forms, the truncated tau forms (33 kDa/55 kDa forms) were substantially reduced in PSP compared with healthy controls (sensitivity 96% and specificity 85% PSP compared with PD/DLB; sensitivity 90% and specificity 76.2% PSP compared with CBD).⁶⁵ These fragments are proteolytic products of tau that were detected by immunoprecipitation techniques, which are more time consuming, less quantitative and more operator-dependent than ELISA techniques.

Findings were reproduced by the same group in another cohort of patients.⁶⁶ However, these results were

Table 2 CSF α -synuclein in parkinsonian disorders

Research groups	Participants	Analyte	Method	Main findings
Van Dijk <i>et al</i> ⁴⁶	PD n=53, HC n=50	t- α -Syn t- α -Syn/ t-protein ratio	TR-FRET	Decrease in both t- α -Syn+t- α -Syn/t-protein ratio levels in PD vs HC
Kang <i>et al</i> 2013 ²¹	PD n=39 (drug-naïve patients), HC n=63	t- α -Syn	ELISA	Decrease in PD vs HC
Wennström <i>et al</i> ⁴⁷	PD n=38, PDD n=22, DLB n=33, AD n=46, HC n=52	t- α -Syn	ELISA	Decrease in PDD > PD > DLB vs AD+HC
Mollenhauer <i>et al</i> ⁴⁸	PD n=78 (de novo, drug-naïve patients), HC n=48	t- α -Syn	ELISA (3rd generation)	Decrease in de novo PD patients vs HC
Hall <i>et al</i> ²⁶	PD n=90, PDD n=33, DLB n=70, PSP n=45, CBD n=12, MSA n=48, AD n=48, controls n=107	t- α -Syn	Bead-based multi-analyte assay (Luminex)	Modest decrease in AD > DLB+PDD > PD +MSA vs controls, AD and PSP
Aerts <i>et al</i> ⁴³	PD n=58, MSA n=47, DLB n=3, VaD n=22, PSP n=10, CBD n=2	t- α -Syn	ELISA	No difference between groups
Tateno <i>et al</i> ⁴⁹	PD n=11, DLB n=6, MSA n=11, AD n=9, controls n=11	t- α -Syn	ELISA	<ul style="list-style-type: none"> ▶ Decrease in PD, DLB, MSA vs AD+controls ▶ No difference among PD, DLB, MSA
Wang <i>et al</i> ⁵⁰	<i>Discovery cohort:</i> PD n=83, MSA n=14, PSD n=30, AD n=25, HC n=51 <i>Validation cohort:</i> PD n=109, MSA n=20, PSP n=22, AD n=50, HC n=71	t- α -Syn p- α -Syn p- α -Syn: t- α -Syn ratio	Bead-based multi-analyte assay (Luminex)	<ul style="list-style-type: none"> ▶ t-α-Syn decrease in PD+MSA vs controls ▶ Increase α-Syn ratio in MSA vs PSP ▶ Increase α-Syn ratio in PD vs controls and PSP
Park <i>et al</i> ⁵⁸	PD (drug-naïve) n=23, controls n=18	t- α -Syn o- α -Syn	Dual ELISA method for simultaneous measurement of t-and o- α -Syn	<ul style="list-style-type: none"> ▶ t-α-Syn: no difference ▶ o-α-Syn: increase in PD
Mollenhauer <i>et al</i> ⁵⁴	<i>Training cohort:</i> PD n=51, DLB n=55, MSA n=29, AD n=62, controls n=76 <i>Validation cohort:</i> PD n=273, DLB n=66, PSP n=8, MSA n=15, NPH n=22, controls n=23	t- α -Syn	ELISA (1st and 2nd generation)	<ul style="list-style-type: none"> ▶ Decrease in PD, DLB, MSA vs AD, NPH, PSP and controls ▶ High degree of concordance in t-α-Syn levels between PD+MSA
Parnetti <i>et al</i> ²⁷	PD n=38, DLB n=32, AD n=48, FTD n=31, controls n=32	t- α -Syn t- α -Syn/t-tau ratio	ELISA	<ul style="list-style-type: none"> ▶ t-α-Syn decrease in all diseased groups (especially DLB/FTD) ▶ Ratio: decrease in PD vs all other diseased groups
Shi <i>et al</i> ²²	<i>Discovery cohort:</i> PD n=126, MSA n=32, AD n=50, controls n=137 <i>Validation cohort:</i> PD n=83	t- α -Syn	Bead-based multi-analyte assay (Luminex)	Decrease in PD vs controls and AD
Tokuda <i>et al</i> ⁵⁷	<i>First cohort (all analytes):</i> PD n=32, controls n=28 <i>Second cohort (o-α-Syn):</i> PD n=25, AD n=35, PSP n=18, controls n=43	t- α -Syn o- α -Syn o- α -Syn: t- α -Syn ratio	ELISA	<ul style="list-style-type: none"> ▶ t-α-Syn: trend towards decrease in PD ▶ o-α-Syn+ratio increase in PD
Hong <i>et al</i> ⁵²	PD n=117, AD n=50, HC n=132	t- α -Syn	Bead-based multi-analyte assay (Luminex)	Decrease in PD vs AD and controls (after omitting samples with high haemoglobin concentration)
Noguchi-Shinohara <i>et al</i> ⁴⁴	DLB n=16, AD n=21	t- α -Syn	ELISA	No difference
Spies <i>et al</i> ⁴⁵	DLB n=40, AD n=131, VaD n=28, FTD n=39	t- α -Syn	ELISA	No difference
Ohrfelt <i>et al</i> ³⁰	PD n=15, DLB n=15, AD n=66, controls n=55	t- α -Syn	ELISA	Decrease in AD, no difference in parkinsonian groups
Mollenhauer <i>et al</i> ⁵³	PD n=8, DLB n=38, AD n=13, CJD n=8, controls n=13	t- α -Syn	ELISA (1st and 2nd generation)	Marginal decrease in LBD and PD vs all other groups
Tokuda <i>et al</i> ⁵⁵	PD n=38, controls n=38	t- α -Syn	ELISA	Decrease in PD vs controls

AD, Alzheimer's disease; CBD, corticobasal degeneration; CJD, Creutzfeldt-Jakob disease; CSF, cerebrospinal fluid; DLB, dementia with Lewy bodies; FTD, frontotemporal dementia; HC, healthy controls; MSA, multiple system atrophy; NPH, normal pressure hydrocephalus; PD, Parkinson's disease; PDD, Parkinson's disease dementia; PSP, progressive supranuclear palsy; TR-FRET, time-resolved Förster's resonance energy transfer; VaD, vascular dementia.

not reproduced by another group, which did not find a reduced tau ratio in an independent cohort of PSP patients,⁶⁷ speculating that the 33/55 kDa bands seen are heavy and light IgG chains. Recent findings of other endogenous tau fragments in CSF suggest that specific assays for these fragments should be developed and evaluated in relation to different tauopathies.⁶⁸

Neurofilament light chain protein (NF-L)

Neurofilaments are major structural elements, whose main role is to maintain the axonal calibre and neuronal shape and size.⁶⁹ They are, thus, critical for the morphological integrity of neurons and for the conduction of nerve impulses along axons. They are composed of three subunits of different molecular weights: light, medium and heavy chain.

Table 3 CSF neuronal injury markers: tau, neurofilament light chain (NF-L) and glial fibrillary acidic protein (GFAP) in parkinsonian disorders

Research groups	Participants	Analyte	Method	Main findings
Kang <i>et al</i> ²¹	PD n=39 (drug-naïve patients), HC n=63	t-tau, p-tau	Bead-based multi-analyte assay (Luminex)	Decrease in t-tau+p-tau in PD vs controls
Luk <i>et al</i> ⁶⁴	PDD n=11, PSP n=44, CBS n=22, AD n=11, controls n=34	3R/4R isoforms	Immuno-PCR (adapted from sandwich ELISAs)	<ul style="list-style-type: none"> ▶ Decrease in 4R-tau in PSP and AD vs controls ▶ Lower 4R-tau in AD vs PDD ▶ -No difference in 3R-tau
Hall <i>et al</i> 2012 ²⁶	PD n=90, PDD n=33, DLB n=70, PSP n=45, CBD n=12, MSA n=48, AD n=48, controls n=107	t-tau, p-tau, NF-L	Bead-based multi-analyte assay (Luminex)	<ul style="list-style-type: none"> ▶ Increased t-and p-tau in AD vs DLB+PDD ▶ NF-L differentiates PD from atypical parkinsonism
Bech <i>et al</i> ³²	PD n=22, PDD n=3, DLB n=11, MSA n=10, PSP n=20, CBD n=3	NF-L	ELISA	<ul style="list-style-type: none"> ▶ Higher NF-L levels in atypical parkinsonian disorders vs PD ▶ No difference between parkinsonian groups
Andersson <i>et al</i> ³⁴	DLB n=47, PDD n=17, AD n=150	t-tau, p-tau	ELISA	Increased t-tau in DLB vs PDD
Shi <i>et al</i> ²²	<i>Discovery cohort:</i> PD n=126, MSA n=32, AD n=50, controls n=137 <i>Validation cohort:</i> PD n=83	t-tau, p-tau	Bead-based multi-analyte assay (Luminex)	<ul style="list-style-type: none"> ▶ -Decrease in PD vs to controls ▶ -Decrease in PD+MSA vs AD
Parnetti <i>et al</i> 2011 ²⁷	PD n=38, DLB n=32, AD n=48, FTD n=31, controls n=32	t-tau, p-tau	ELISA	<ul style="list-style-type: none"> ▶ Increase in AD>FTD>DLB vs PD and controls ▶ No difference between PD and controls
Kuiperij <i>et al</i> ¹⁰²	NA	33/55 kDa tau forms	Immunoprecipitation assay and western blotting	<ul style="list-style-type: none"> ▶ Not able to detect tau form ratio ▶ Suggested that 33/55 kDa bands seen are heavy and light IgG chains
Borroni <i>et al</i> ¹⁰³	PSP n=18, CBS n=16, FTD n=28, controls n=25	33/55 kDa tau forms	Immunoprecipitation assay and western blotting	tau form ratio significantly reduced in PSP vs other groups
Constantinescu <i>et al</i> ⁷¹	PD n=10, MSA n=21, PSP n=14, CBD n=11, HC n=59 (x2 consecutive samples)	NF-L, GFAP	ELISA	<ul style="list-style-type: none"> ▶ NF-L: normal levels in PD, elevated in MSA, PSP+CBD ▶ No statistical significance overtime ▶ GFAP: no difference
Montine <i>et al</i> ²⁸	PD n=41, PDD n=11, AD n=49, HC n=150	t-tau, p-tau	Bead-based multi-analyte assay (Luminex)	<ul style="list-style-type: none"> ▶ t-tau: no difference between parkinsonian groups ▶ p-tau: reduced in PD vs HC
Süssmuth <i>et al</i> ²⁹	PSP-RS n=20, PSP-P n=7, MSA-P n=11, MSA-C n=14, PD n=23, controls n=20	t-tau, p-tau, GFAP	ELISA	<ul style="list-style-type: none"> ▶ p-tau/t-tau ratio lower in PSP and MSA vs PD ▶ GFAP: increase in parkinsonian syndromes (no difference between disease groups)
Alves <i>et al</i> ²³	PD n=109, AD n=20, HC n=36	t-tau, p-tau	ELISA	No difference between PD and controls
Ohrfelt <i>et al</i> ³⁰	PD n=15, DLB n=15, AD n=66, controls n=55	t-tau, p-tau	ELISA	No difference between parkinsonian groups
Compta <i>et al</i> ²⁴	PD n=20, PDD n=20, HC n=15	t-tau, p-tau	ELISA	t- and p- tau: increase in PDD vs PD and controls
Parnetti <i>et al</i> ²⁵	PD n=20, PDD n=8, DLB n=19, AD n=23, HC n=20	t-tau, p-tau	ELISA	<ul style="list-style-type: none"> ▶ t-tau: DLB > PDD > controls ▶ p-tau: no difference between parkinsonian groups
Borroni <i>et al</i> ⁶⁵	PSP n=21, CBS n=20, FTD n=44, AD n=15, PD n=10, DLB n=15, controls n=27	33/55 kDa tau forms	Semiquantitative immunoprecipitation and western blotting	tau forms significantly reduced in PSP vs controls and other neurodegenerative diseases
Brettschneider <i>et al</i> ⁷⁰	PD n=22, MSA n=21, PSP n=21, CBD n=6, controls n=45	NF-H	ELISA	Increased in MSA and PSP vs PD, CBD and controls

AD, Alzheimer's disease; CBD, corticobasal degeneration; CBS, corticobasal syndrome; CSF, cerebrospinal fluid; DLB, dementia with Lewy bodies; FTD, frontotemporal dementia; HC, healthy controls; MSA, multiple system atrophy; MSA-C, multiple system atrophy cerebellar type; MSA-P, multiple system atrophy parkinsonian type; NF_H, neurofilament heavy chain; NF-L, neurofilament light chain; PD, Parkinson's disease; PDD, Parkinson's disease dementia; PSP, progressive supranuclear palsy; PSP-P, progressive supranuclear palsy-parkinsonism; PSP-RS, progressive supranuclear palsy-Richardson's syndrome.

Neurofilament heavy chain (NF-H) forms an important component of the cytoskeleton. Higher CSF levels of NF-H were found in PSP and MSA compared with PD, CBD and neurological controls.⁷⁰

Neurofilament light chain forms the backbone of neurofilaments and can self-assemble. Increased levels in CSF reflect axonal degeneration of large myelinated axons. Recent studies showed consistent results in differentiating PD from atypical parkinsonian conditions^{26 32 71} but not in discriminating between atypical parkinsonian syndromes. Consecutive analyses of CSF showed no increase in NF-L levels with disease progression.⁷¹

NF-L can be useful in the differential diagnosis of PD versus other neurodegenerative conditions as it is very sensitive in detecting more aggressive neuronal death than occurs in PD.

Glial fibrillary acidic protein

Glial fibrillary acidic protein (GFAP) is a protein predominantly expressed in fibrillary astrocytes. Disintegration of astroglial cells postacute brain injury can lead to high CSF GFAP levels. Süssmuth *et al*²⁹ showed that there are increased levels in parkinsonian syndromes compared with controls (patients with other neurological disorders), but there was no difference between diseased groups. However, another group found

similar GFAP levels in parkinsonian syndromes and healthy controls without significant change over time.⁷¹

OTHER CANDIDATE MARKERS

Oxidative stress markers

DJ-1

DJ-1 is a multifunctional protein involved in many processes. It is thought to have a protective role in oxidative stress during neurodegeneration (table 4). As we have already discussed, it has been linked to autosomal-recessive PD. Results on DJ-1 as a CSF biomarker have been inconsistent so far. One study showed decreased levels in PD compared with controls with a sensitivity of 90% and a specificity of 70%,⁵² whereas another showed no difference among parkinsonian syndromes⁷² and the most recent one demonstrated significant increase in MSA compared with PD and controls.⁷³ The diagnostic accuracy for discriminating MSA from PD was improved by combining DJ-1 levels with t-tau and p-tau levels.

8-Hydroxydeoxyguanosine (8-OHdG)

8-OHdG is a marker of oxidation and mitochondrial dysfunction in neurodegeneration and malignancy. CSF 8-OHdG levels were increased in non-demented PD patients compared with

controls and there was a negative correlation with MMSE levels in PDD.⁷⁴

Urate

Urate is an endogenous and most potent antioxidant. Even though there is considerable evidence linking low serum levels of urate to PD,^{75 76} CSF studies have shown inconsistent results. Maetzler *et al*⁷⁷ found increase levels in PD compared with DLB, but Constantinescu *et al*⁷⁸ showed no difference among parkinsonian groups and healthy controls.

Inflammatory markers

Fractalkine

Fractalkine is an inflammatory cytokine that acts as a neurotrophic and antiapoptotic factor in the central nervous system. It was decreased in MSA and could alone differentiate between PD and MSA with a sensitivity of 99% and a specificity of 95%.²² In addition, the fractalkine/Aβ42 ratio was closely associated with disease severity and progression in PD. These results are in need of replication.

Complement C3/factor H ratio

The C3/factor H ratio in CSF was significantly decreased in MSA compared with PD, AD and healthy controls. Increased

Table 4 CSF biomarkers for oxidative stress, inflammation and energy failure in parkinsonian disorders

Research groups	Participants	Analyte	Method	Main findings
Herbert <i>et al</i> ⁷³	PD n=43, MSA n=23, controls n=30	DJ-1	ELISA	<ul style="list-style-type: none"> ▶ Increase in MSA>PD ▶ Significant difference in MSA vs PD, MSA vs controls and PD vs controls
Constantinescu <i>et al</i> ⁷⁸	PD n=6, MSA n=13, PSP n=18, CBD n=6, HC n=18	Urate	Enzymatic method on a modular system	No difference
Wennstrom <i>et al</i> ⁴⁷	PD n=38, PDD n=22, DLB n=33, AD n=46, HC n=52	Neurosin	ELISA	<ul style="list-style-type: none"> ▶ Lowest levels in DLB, but no difference between synucleinopathies ▶ When pooled, synucleinopathies decrease levels vs AD+HC
Goldstein <i>et al</i> ⁶²	PD n=34, MSA n=54, PAF n=20, HC n=38	Dihydroxyphenylactic acid (DOPAC)	Batch alumina extraction followed by liquid chromatography with electrochemical detection	<ul style="list-style-type: none"> ▶ Decrease in PD, MSA and to a lesser degree PAF vs HC ▶ No difference between synucleinopathy groups
Salvesen <i>et al</i> ⁷²	PD n=30, DLB n=17, MSA n=14, PSP n=19	DJ-1	ELISA	No difference among groups
Maetzler <i>et al</i> ⁷⁷	PD n=55, PDD n=20, DLB n=20, controls n=76	Uric acid	ADVIA analyser+photometric methods	Increase in PD vs DLB
Shi <i>et al</i> ²²	<i>Discovery cohort:</i> PD n=126, MSA n=32 <i>Validation cohort:</i> AD n=50, controls n=137 PD n=83	DJ-1 Fractalkine	Bead-based multi-analyte assay (Luminex)	<ul style="list-style-type: none"> ▶ DJ1: decrease in MSA+PD vs controls +AD ▶ Fractalkine: decrease in MSA vs PD, AD+controls
LeWitt <i>et al</i> ⁶¹	PD n=217 (samples collected ×2 occasions) HC n=26	Homovallinic acid/xanthine ratio	Gas chromatography-mass spectrometry	<ul style="list-style-type: none"> ▶ Increased ratio in PD vs HC ▶ Ratio increased further in PD specimens collected up to 2 years later
Wang <i>et al</i> ⁷⁹	PD n=86, MSA n=20, AD n=38 HC n=91	Complement C3/factor H (FH)	Bead-based multi-analyte assay (Luminex)	<ul style="list-style-type: none"> ▶ C3: decrease in MSA vs PD+HC; increase in AD vs all other groups ▶ FH: increase in AD vs PD+HC ▶ C3/FH ratio: decrease in MSA vs all other groups
Maetzler <i>et al</i> ⁶⁰	PD n=38, PDD n=20, DLB n=21 m, controls n=23	Nephrilysin	Fluorometric assay	Decrease in DLB+PDD vs PD+ controls
Hong <i>et al</i> ⁵²	PD n=117, AD n=50, HC n=132	DJ-1	Bead-based multi-analyte assay (Luminex)	<ul style="list-style-type: none"> ▶ Decreased levels in PD vs Controls and AD ▶ No difference between AD+ controls

AD, Alzheimer's disease; CBD, corticobasal degeneration; CSF, cerebrospinal fluid; DLB, dementia with Lewy bodies; HC, healthy controls; MSA, multiple system atrophy; PAF, pure autonomic failure; PD, Parkinson's disease; PDD, Parkinson's disease dementia; PSP, progressive supranuclear palsy.

levels of C3 or factor H, together with decreased levels of A β 42, correlate positively with disease severity and progression in PD.⁷⁹

Neurosin

Neurosin is a protein expressed in human brain tissue, and it is one of several enzymes suggested to cleave α -Syn. A study comparing neurosin levels in synucleinopathies showed lowest levels in DLB, but no difference among DLB, PDD and PD. However, when pooled together, synucleinopathies had significantly lower neurosin levels compared with AD and controls.⁴⁷

Nephrilysin

Nephrilysin is a membrane bound presynaptic protein involved in A β clearance. CSF levels were significantly decreased in DLB and PDD compared with PD and controls, and they correlated well with A β 42 levels in all cohorts.⁸⁰

Catecholamine metabolites

Homovanillic acid (HVA)/xanthine ratio

HVA is the major catabolite of dopamine and has been extensively studied in the past in relation to PD, as described above. Xanthine is the immediate precursor of urate. HVA/xanthine ratio was increased in PD compared with controls and correlated with diseased severity.⁸¹

Dihydroxyphenylactic acid (DOPAC)

Depletion of dopamine (a catecholamine) in basal ganglia is a defining neurochemical characteristic in PD. DOPAC is a neuronal metabolite of catecholamines. It was found to be decreased in PD and MSA compared with healthy controls, but there was no difference between synucleinopathy groups.⁸²

The above compounds may be promising candidate makers, but they need verification in further studies. CSF HVA has been extensively studied in relation to PD and treatment response but still has no definite place in the clinical routine.

Lysosomal dysfunction

Lysosomes are the cell's waste disposal system, and their dysfunction is an early event in PD pathogenesis.⁸³ Patients suffering from Gaucher disease, a rare, autosomal-recessive storage disorder caused by lysosomal enzyme β -glucocerebrosidase (GCase) deficiency,⁸⁴ have an increased risk of parkinsonism,⁸⁵ which appears to be driven by a direct effect of GCase deficiency and lysosomal dysfunction on α -Syn aggregation.⁸⁶

Measuring GCase activity in the CSF could be a useful biomarker in PD. PD⁸⁷ and DLB⁸⁸ patients were found to have significantly reduced GCase activity compared with neurological controls. A recent study showed that the combination of GCase activity, oligomeric/total α -Syn ratio and age discriminates best PD from neurological controls.⁸⁹ However, in a Dutch cohort of de novo PD patients and healthy controls, there was a trend towards a reduction in CSF GCase activity.⁹⁰ The usefulness of GCase as a potential biomarker in parkinsonian conditions needs to be evaluated in future studies that include additional neurodegenerative groups to PD.

'Omics' approaches

The markers already discussed have been hypothesis driven based on pathophysiological studies, which have identified potentially deranged pathways in neurodegenerative diseases. The 'omics' techniques offer an unbiased approach of identifying biochemical pathways that are unexpectedly involved in neurodegeneration. Ultimately, the aim is to generate a list of

candidate markers deserving further targeted studies.⁹¹ The 'omics' approach results in unbiased and systematic measurement of patterns of variations in genes (genomics), RNA (transcriptomics), proteins (proteomics) and small molecules (metabolomics). We have briefly discussed genomics and touched on metabolomics in previous sections, and we will now review proteomics in parkinsonian disorders.

Abdi *et al*⁹² used a multiplex quantitative proteomic platform to find 72 altered proteins in PD compared with healthy controls. Apolipoprotein H and ceruplasmin seemed to differentiate PD from healthy controls and from non-PD patients (AD and DLB). Eight of the proposed proteins were validated using a multianalyte CSF profile and showed good PD discriminatory power compared with AD and healthy controls.⁹³

Using surface-enhanced laser desorption/ionisation time-of-flight mass spectrometry (SELDI-TOF MS), Constantinescu *et al*⁹⁴ found a CSF proteomic profile consisting of four proteins (ubiquitin, β 2-microglobulin and two secretographin 1 fragments), which differentiated PD and healthy controls from atypical parkinsonian patients with an AUC of 0.8. Recently, Ishigami *et al*⁹⁵ were able to differentiate PD from MSA, even at the early stages, using their proteomic pattern (ie, the combined set of many protein peaks), rather than a single peak. Multiple peaks differentiated MSA and PD from control groups, consistent with previous reports that a panel of potential biomarkers is essential to distinguish between disease states.⁹⁶

Another recent study attempted to differentiate PD from PDD patients using proteomic technology. Six proteins were identified, but only serin-protease inhibitor Serpin A1 was verified using biochemical methods. Performing 2-D immunoblots, there was 100% specificity and 58% sensitivity for the test procedure.⁹⁷ Testing CSF obtained from PD, PDD patients and non-demented controls using a gel-free proteomics mass spectrometry approach with isotope-labelled samples (iTRAQ) led to the identification of 16 differentially regulated proteins, which could be potentially diagnostic markers.⁹⁸

While proteomics studies have produced a number of interesting candidate markers, these are still in need of replication and far from being established. It has also become clear that many of the protein expression changes seen so far represent changes that are common to several neurodegenerative diseases. Reliable detection of disease-specific changes most likely depends on the development of more advanced techniques that allow for deeper analyses of the CSF proteome.

Imaging markers

Even though imaging biomarkers are beyond the scope of this review, we would like to point out that combination of CSF and imaging markers can provide increased diagnostic accuracy compared with using either modality alone. For example, Borroni and colleagues used mid-sagittal midbrain-to-pons atrophy in addition to CSF tau fragments levels to increase the discriminative power in identifying PSP from other neurodegenerative conditions.⁶⁶

DISCUSSION

The vast majority of the studies discussed are cross-sectional, retrospective and do not have pathological confirmation. The accuracy of the clinical diagnosis is uncertain, and the contribution of comorbidity to the clinical phenotype is unknown.

There is lack of standardisation both of preanalytical (sampling collection, handling and storage) and analytical (analysis execution/sample processing) factors. For example, CSF contamination by blood can alter study outcomes in α -Syn and DJ-1 assays. In addition, there is lack of assay standardisation;

different assays can give different absolute concentrations of the protein, making it almost impossible to use global reference limits and diagnostic cut-off points.

Furthermore, both disease groups and control groups are heterogeneous. The neurodegenerative groups differ in terms of age, disease duration and severity. The control groups include a very small proportion of healthy controls and are mostly non-neurodegenerative neurological patients. However, some studies include patients with possible neurodegenerative conditions, such as mild cognitive impairment or normal pressure hydrocephalus.

Finally, there is lack of combination of different biomarker modalities, such as imaging and CSF markers.

A very promising study is the Parkinson's Progression Markers Initiative (PPMI), which aims to identify PD progression markers and to better define subsets of PD patients.^{99–100} It is a 5-year, multicentre, longitudinal study of drug-naïve PD patients with early-stage disease, compared with healthy controls. Detailed motor and neuropsychological assessments, DaT-scan and CSF examinations are performed. There is strict standardisation of data acquisition, CSF collection and processing.²¹

SUMMARY POINTS: CSF BIOMARKERS IN PARKINSONISM

- ▶ Aβ₄₂ has a role in predicting cognitive decline in Parkinson's disease (PD)
- ▶ t-α-Syn: most promising marker; differentiates synucleinopathies from other neurodegenerative diseases and controls but is not specific
- ▶ t-tau and p-tau: inconsistent data, can help differentiate PD from AD and can be useful in combination with other markers
- ▶ NF-L: useful in differentiating PD from atypical parkinsonian conditions
- ▶ 4R-tau: possible marker of disease progression in PSP
- ▶ DJ1: potential role in discriminating MSA from PD
- ▶ Oxidative stress/inflammatory/metabolic markers: promising initial results, requiring further validation

FUTURE DEVELOPMENTS FOR THE CSF FIELD IN PARKINSONISM

We think that several hypothesis-driven biomarkers are going to be investigated at the same time using multiplex platforms. The proteomics field is likely to expand and gain in analytical sensitivity, resulting in the identification of more candidate markers, some of which may be unexpected and give new clues on disease mechanisms. There needs to be large, prospective and longitudinal cohorts with serial CSF examinations and pathological confirmation in as many patients as possible. A very important issue to be resolved is the standardisation of protocols and improvement in quality controls in CSF analysis. Finally, like in AD, it will likely be important to combine several CSF markers with other modalities, like imaging.

Accurate diagnosis of parkinsonian conditions should occur as early as possible, before too much irreversible neuronal damage has accumulated. This is essential, especially with the emergence of potential disease-modifying drugs, which must be used to target the correct underlying pathology. There is promising progress in the development of an α-Syn imaging agent, using radio ligands that bind to α-Syn fibrils. This should enable the assessment of the distribution of brain α-Syn during life.¹⁰¹

CONCLUSION

Parkinsonian conditions, like most neurodegenerative diseases, have complex and dynamic interaction of several underlying

pathogenic mechanisms. A combination of biomarkers possibly from different modalities in large, longitudinal cohorts might be required for early diagnosis and accurate disease prognosis.

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