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 Trouseau, 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 fronto/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.1 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
associated with variants in MAPT, including CBD, FTD linked to chromosome 17 (FTDP-17T) and PSP. 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.

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.

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”. 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. 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.

As these metabolic results were prone to be influenced by a multitude of other factors, 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 Aβ42. 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’, ‘taupathies’, ‘neurodegenerative diseases’ with ‘CSF biomarkers’ and specific biomarkers (‘α-Syn’, ‘tau’, ‘phosphorylated tau’, ‘Aβ42’, ‘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.

**CSF BIOMARKER CANDIDATES IN PARKINSONISM**

**Aβ42**

Aβ42 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 Aβ42 and PD and dementia has been studied extensively (see table 1).

In most studies, Aβ42 is significantly reduced in PD compared with controls and is associated with worse cognitive performance. However, other investigations showed no difference between PD and controls.

Compta et al 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 Aβ42 than the patients who remained non-demented.

DLB patients have the lowest CSF levels of Aβ42 among the parkinsonian cohorts. One study found that almost half of DLB patients had a CSF biomarker profile consistent with AD, which agrees with the knowledge of Aβ pathology in this disease.

There is evidence that low Aβ42, a marker of Aβ plaque pathology, may predict cognitive decline in patients with PD, 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 presynaptic 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

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**Table 1** CSF Aβ42 in parkinsonian disorders

| Research groups | Participants | Main findings |
|-----------------|--------------|---------------|
| Kang et al | PD n=39 (drug-naïve patients), HC n=63 | Decrease in PD vs HC |
| Compta et al | Baseline: PD n=27 (non-demented) | Decrease in dementia converters |
| Bech et al | PD n=22, PDD n=3, DLB n=11, MSA n=10, PSP n=20, CBD n=3 | Decrease in D LB vs other disease groups |
| Hall et al | 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+D LB+PDD |
| Schoonenboom et al | DLB n=52, PSP n=20, CBD n=16, AD n=512, FTD n=144, VaD n=34, CID n=6, controls n=275 | Decrease in AD, FTD+DLB vs PD and controls |
| Parnetti et al | PD n=38, DLB n=32, AD n=48, FTD n=31, controls n=32 | No difference between PD and controls |
| Anderson et al | DLB n=47, PDD n=17, AD n=150 | Decrease in DLB vs PDD |
| Shi et al | Discovery cohort: PD n=126, MSA n=32, AD n=50, controls n=137 | Slight decrease in PD and MSA vs controls |
| Montine et al | PD n=81, PDD n=11, AD n=49, HC n=150 | Decrease in PDD vs HC |
| Süßmuth et al | 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 |
| Alves et al | PD n=109, AD n=20, HC n=36 | Lower in PSP-RS vs PSP-P |
| Olfert et al | PD n=15, DLB n=15, AD n=66, controls n=55 | Decrease in PD vs HC |
| Compta et al | PD n=20, PDD n=20, HC n=15 | Decrease in AD+D LB vs controls and PD |
| Parnetti et al | PD n=20, PDD n=8, DLB n=19, AD n=23, HC n=20 | Decrease in PD+D LB vs PD |

AD, Alzheimer’s disease; CBD, corticobasal degeneration; CID, 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; PSP, Parkinson’s disease dementia; PSP-RS, progressive supranuclear palsy; PSP-P, progressive supranuclear palsy–Parkinsonism; PSP-RS, progressive supranuclear palsy–Richmond’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. Although it is known that it is capable of transfer between cells leading to a speculation of a prion-like mechanism operating in PD pathology spread.

α-Syn can be modified by truncation, acetylation, phosphorylation, oxidation, nitrosylation, glycation or glycosylation. 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 bodies.

In vitro, similar to AD, α-Syn fibrillation in patients with PD 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-tau, 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-tau 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.

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.

### Oligomeric and phosphorylated α-Syn

Takoda 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/α-Syn ratio had a sensitivity of 89.3% and a specificity of 90.6% for PD. These findings were replicated in two further, independent studies. 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. The results need to be replicated in larger groups of living patients.

### 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. 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. In addition, there is decreased t-α-Syn in other synucleinopathies, such as MSA and DLB.

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%).

Shi et al 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. Both Mollenhauer et al and Kang et al 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 Aβ42 in AD CSF.

### NEURONAL INJURY MARKERS

### Tau

### 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, but lower levels were also reported. In atypical parkinsonism, high t-tau levels were found in DLB and MSA and PSP compared with PD. However, other investigations found no difference between parkinsonian syndromes. In particular, no significant change has been seen in PSP. Age, not diagnosis, is thought to be the strongest factor affecting t-tau protein levels.

### 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.
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.

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**Table 2 CSF α-synuclein in parkinsonian disorders**

| Research groups | Participants | Analyte | Method | Main findings |
|-----------------|--------------|---------|--------|---------------|
| Van Dijk et al66 | PD n=53, HC n=50 | t-α-Syn/t-α-Syn ratio | TR-FRET | Decrease in both t-α-Syn+t-α-Syn/t-protein ratio levels in PD vs HC |
| Kang et al 201331 | PD n=39 (drug-naïve patients), HC n=63 | t-α-Syn | ELISA | Decrease in PD vs HC |
| Wennström et al37 | 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 et al48 | 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 et al46 | 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 et al43 | 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 et al49 | PD n=11, DLB n=6, MSA n=11, AD n=9, controls n=11 | t-α-Syn | ELISA | ▶ Decrease in PD, DLB, MSA vs AD+ controls |
| ▶ No difference among PD, DLB, MSA |
| Wang et al50 | Discovery cohort: PD n=83, MSA n=14, PSD n=30, AD n=25, HC n=51 | t-α-Syn | Bead-based multi-analyte assay (Luminex) | ▶ t-α-Syn decrease in PD+MSA vs controls |
| Validation cohort: PD n=109, MSA n=20, PSP n=22, AD n=50, HC n=71 | t-α-Syn | t-α-Syn ratio | ▶ Increase α-Syn ratio in MSA vs PSP |
| ▶ Increase α-Syn ratio in PD vs controls and PSP |
| Park et al48 | PD (drug-naïve) n=23, controls n=18 | t-α-Syn | Dual ELISA method for simultaneous measurement of t-α-Syn and o-α-Syn | ▶ t-α-Syn: no difference |
| ▶ o-α-Syn: increase in PD |
| Mollenhauer et al64 | Training cohort: PD n=51, DLB n=55, MSA n=29, AD n=62, controls n=76 | t-α-Syn | ELISA (1st and 2nd generation) | ▶ Decrease in PD, DLB, MSA vs AD, NPH, PSP and controls |
| Validation cohort: PD n=273, DLB n=66, PSP n=8, MSA n=15, NPH n=22, controls n=23 | t-α-Syn | t-α-Syn ratio | ▶ High degree of concordance in t-α-Syn levels between PD+MSA |
| Parnetti et al47 | PD n=38, DLB n=32, AD n=48, FTD n=31, controls n=32 | t-α-Syn | ELISA | ▶ t-α-Syn decrease in all diseased groups (especially DLB/FTD) |
| ▶ Ratio: decrease in PD vs all other diseased groups |
| Shi et al52 | Discovery cohort: PD n=126, MSA n=32, AD n=50, controls n=137 | t-α-Syn | Bead-based multi-analyte assay (Luminex) | Decrease in PD vs controls and AD |
| Validation cohort: PD n=83 | t-α-Syn | t-α-Syn/t-tau ratio | |
| Tokuda et al57 | First cohort (all analytes): PD n=32, controls n=28 | t-α-Syn | ELISA | ▶ t-α-Syn: trend towards decrease in PD |
| Second cohort (α-syn): PD n=25, AD n=35, PSP n=18, controls n=43 | t-α-Syn | t-α-Syn ratio | ▶ o-α-Syn+ratio increase in PD |
| Hong et al62 | 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 et al64 | DLB n=16, AD n=21 | t-α-Syn | ELISA | No difference |
| Spies et al65 | DLB n=40, AD n=131, VaD n=28, FTD n=39 | t-α-Syn | ELISA | No difference |
| Ohrfelt et al60 | 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 et al63 | 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 et al65 | 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 resonance energy transfer; VaD, vascular dementia.
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. Non.

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 but not in discriminating between atypical parkinsonian syndromes. Consecutive analyses of CSF showed no increase in NF-L levels with disease progression.

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.

### Gliarial fibrillary acidic protein

Gliarial fibrillary acidic protein (GFAP) is a protein predominantly expressed in gliarial astrocytes. Disintegration of astroglial cells postacute brain injury can lead to high CSF GFAP levels. 

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 et al23    | PD n=39 (drug-naive 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 et al44     | PDD n=11, PSP n=44, CBS n=22, AD n=11, controls n=34 | 3R/4R isofoms | Immuno-PCR (adapted from sandwich ELISA) | Decrease in 4R-tau in PSP and AD vs controls | Lower 4R-tau in AD vs PDD | No difference in 3R-tau |
| Hall et al 201226 | 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) | Increased t- and p-tau in AD vs DLB+PDD | NF-L differentiates PD from atypical parkinsonism |
| Bech et al32    | PD n=22, PDD n=3, DLB n=11, MSA n=10, PSP n=20, CBD n=3 | NF-L | ELISA | Higher NF-L levels in atypical parkinsonian disorders vs PD |
| Andersson et al34 | DLB n=47, PDD n=17, AD n=150 | t-tau, p-tau | ELISA | Increased t-tau in DLB vs PDD |
| Shi et al22     | Discovery cohort: PD n=126, MSA n=32, AD n=50, controls n=117 | t-tau, p-tau | Bead-based multi-analyte assay (Luminex) | Decreased in PD vs controls | Decrease in PD+MSA vs AD |
| Parnetti et al201127 | PD n=38, DLB n=32, AD n=48, FTD n=31, controls n=32 | t-tau, p-tau | ELISA | Increase in AD+FTD+DLB vs PD and controls | No difference between PD and controls | Not able to detect tau form ratio | Suggested that 33/55 kDa bands seen are heavy and light IgG chains |
| Kuiperij et al102 | NA | 33/55 kDa tau forms | Immunoprecipitation assay and western blotting | Tau form ratio significantly reduced in PSP vs other groups |
| Borrioni et al63 | 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 et al11 | PD n=10, MSA n=21, PSP n=14, CBD n=11, HC n=59 (×2 consecutive samples) | NF-L, GFAP | ELISA | NF-L normal levels in PD, elevated in MSA, PSP+CBD |
| Montine et al28 | PD n=41, PDD n=11, AD n=49, HC n=150 | t-tau, p-tau | Bead-based multi-analyte assay (Luminex) | t-tau: no difference between parkinsonian groups | p-tau: reduced in PD vs HC |
| Süssmuth et al19 | 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 | ELISA | p-tau/t-tau ratio lower in PSP and MSA vs PD | GFAP: increase in parkinsonian syndromes (no difference between disease groups) |
| Alves et al23 | PD n=109, AD n=20, HC n=36 | t-tau, p-tau | ELISA | No difference between PD and controls |
| Ohrfelt et al10 | PD n=15, DLB n=25, AD n=66, controls n=55 | t-tau, p-tau | ELISA | No difference between parkinsonian groups |
| Compta et al24 | 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 et al25 | PD n=20, PDD n=8, DLB n=19, AD n=23, HC n=20 | t-tau, p-tau | ELISA | t-tau: DLB > PDD > controls | p-tau: no difference between parkinsonian groups |
| Borrioni et al65 | 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 et al11 | PD n=22, MSA n=21, PSP n=21, CBD n=6, controls n=48 | 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.
similar GFAP levels in parkinsonian syndromes and healthy controls without significant change over time.\textsuperscript{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%.\textsuperscript{52} whereas another showed no difference among parkinsonian syndromes\textsuperscript{72} and the most recent one demonstrated significant increase in MSA compared with PD and controls.\textsuperscript{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 without significant increase over time.\textsuperscript{71}

The C3/factor H ratio in CSF was significantly decreased in MSA and could alone differentiate between PD and MSA compared with PD, AD and healthy controls. Increased urate was endogenous and most potent antioxidant. Even though there is considerable evidence linking low serum levels of urate to PD,\textsuperscript{75, 76} CSF studies have shown inconsistent results. Maetzler et al\textsuperscript{77} found increased levels in PD compared with DLB, but Constantinescu et al\textsuperscript{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%.\textsuperscript{69} In addition, the fractalkine/Al42 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 urate is an endogenous and most potent antioxidant. Even though there is considerable evidence linking low serum levels of urate to PD,\textsuperscript{75, 76} CSF studies have shown inconsistent results. Maetzler et al\textsuperscript{77} found increased levels in PD compared with DLB, but Constantinescu et al\textsuperscript{78} showed no difference among parkinsonian groups and healthy controls.

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

| Research groups | Participants | Analyte | Method | Main findings |
|-----------------|--------------|---------|--------|---------------|
| Herbert\textsuperscript{73} et al | PD n=43, MSA n=23, controls n=30 | DJ-1 | ELISA | Increase in MSA vs PD |
| Constantinescu\textsuperscript{79} et al | PD n=6, MSA n=13, PSP n=18, CBD n=6, HC n=18 | Urate | Enzymatic method on a modular system | Lowest levels in DLB, but no difference between synucleinopathies |
| Wennstrom\textsuperscript{80} et al | PD n=38, PDD n=22, DLB n=33, AD n=46, HC n=52 | Neurosin | ELISA | When pooled, synucleinopathies decrease levels vs AD+HC |
| Goldstein\textsuperscript{81} et al | PD n=34, MSA n=54, PAF n=20, HC n=38 | Dihydroxyphenylacetic acid (DOPAC) | Batch alumina extraction followed by liquid chromatography with electrochemical detection | Increase in PD vs DLB |
| Salvesen\textsuperscript{82} et al | PD n=30, DLB n=17, MSA n=14, PSP n=19 | DJ-1 | ELISA | Increase in PD vs DLB |
| Maetzler\textsuperscript{83} et al | PD n=55, PDD n=20, DLB n=20, controls n=76 | Uric acid | ADVIA analyser+photometric methods | Increase in PD vs DLB |
| Shi\textsuperscript{84} et al | Discovery cohort: PD n=126, MSA n=32, AD n=50, controls n=137 | DJ-1 | Bead-based multi-analyte assay (Luminex) | DJ1: decrease in MSA+PD vs controls +AD |
| LeWitt\textsuperscript{85} et al | PD n=217 (samples collected ×2 occasions) HC n=26 | Homovallinic acid/xanthine ratio | Gas chromatography-mass spectrometry | Increased ratio in PD vs HC |
| Wang\textsuperscript{86} et al | PD n=86, MSA n=20, AD n=38 HC n=91 | Complement C3/factor H (FH) | Bead-based multi-analyte assay (Luminex) | C3: decrease in MSA vs PD+HC; increase in AD vs all other groups |
| Maetzler\textsuperscript{87} et al | PD n=38, PDD n=20, DLB n=21 m, controls n=23 | Neprilysin | Fluorometric assay | FH: increase in AD vs PD+HC |
| Hong\textsuperscript{88} et al | PD n=117, AD n=50, HC n=132 | DJ-1 | Bead-based multi-analyte assay (Luminex) | C3/FH ratio: decrease in MSA vs all other groups |

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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 Aβ42, 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

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 decreased severity.81

Dihydroxyphenylacetic 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 markers, 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 al92 used a multiplex quantitative proteomic platform to find 72 altered proteins in PD compared with healthy controls. Apolipoprotein H and ceruloplasmin 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 al94 found a CSF proteomic profile consisting of four proteins (ubiquitin, β2-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 al95 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 serpin-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 (TMT) 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. 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.

SUMMARY POINTS: CSF BIOMARKERS IN PARKINSONISM

- α-Syn: most promising marker; differentiates synucleinopathies from other neurodegenerative diseases and controls but is not specific
- α-Syn: consistent 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 is likely to 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.

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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REFERENCES

1. Hughes AJ, Daniel SE, Kilford L, et al. Accuracy of clinical diagnosis of idiopathic Parkinson’s disease: a clinicopathological study of 100 cases. J Neurol Neurosurg Psychiatry 1992;55:181–4.
2. Fahn S. Secondary Parkinsonism. Sci Approaches Clin Neurol 1977:1159–89.
3. Spillantini MG, Schmidt ML, Lee VM-Y, et al. Alpha-synuclein in Lewy bodies. Nature 1997;388:839–40.
4. Gai WP, Power JHT, Blumbergs PC, et al. Multiple-system atrophy: a new α-synuclein disease? Lancet 1998;352:547–8.
5. Litvan I, Haou J, Barrio JI, et al. Validity and reliability of the preliminary NINDS neuropathologic criteria for progressive supranuclear palsy and related disorders. J Neuropathol Exp Neurol 1996;55:97–105.
6. Schneider JA, Watts RL, Gearing M, et al. Corticobasal degeneration: neuropathologic and clinical heterogeneity. Neurology 1997;48:959–69.
7. Constantinou R. Cerebrospinal fluid biomarker candidates for Parkinson’s disorders. Frant Neurosci 2013;3:1–15.
8. Vekrellis K, Xilouri M, Emmanouilidou E, et al. Pathological roles of alpha-synuclein in neurological disorders. Lancet Neurol 2011;10:1015–25.
9. Giasson BI, Duda JE, Quinn SM, et al. Neuronal alpha-synucleinopathy with severe movement disorder in mice expressing ASHT human α-synuclein. Neuroon 2002;34:521–33.
10. Waxman EA, Giasson BI. Induction of intracellal tau aggregation is promoted by α-synuclein seeds and provides novel insights into the hyperphosphorylation of tau. J Neurosci 2011;31:7604–18.
11. Tsigelny IF, Crews L, Desplats P, et al. DJ-1, PINK1, and their effects on mitochondrial pathways. J Neuropathol Exp Neurol 2004;63:657–71.
12. Gottfries CG, Gottfries I, Roos BE. Homovanillic acid and 5-hydroxyindoleacetic acid in the cerebrospinal fluid of patients with Parkinsonism treated with L-dopa. J Neurol Neurosurg Psychiatry 1977;40:251–3.
13. Cookson MR. DJ-1, PINK1, and their effects on mitochondrial pathways. J Neurochem 1997;65:370–7.
14. Curzon G, Godwin-Austen RB, Tomlinson EB, et al. The cerebrospinal fluid α-synuclein in Parkinson disease and multiple system atrophy. Ann Neurol 1997;42:726–32.
15. Gottfries CG, Gottfries I, Roos BE. Homovanillic acid concentration in patients with Parkinsonism treated with L-dopa. J Neurol Neurosurg Psychiatry 1977;40:251–3.
"
Neurodegeneration

24 Compta Y, Martí MJ, Ibarretxe-Bilbao N, et al. Cerebrospinal tau, phospho-tau, and beta-amyloid and neuropsychological functions in Parkinson’s disease. Mov Disord 2009;24:2203–10.

25 Pametti L, Tiraboschi P, Lanari A, et al. Cerebrospinal fluid biomarkers in Parkinson’s disease with dementia and dementia with Lewy bodies. Biol Psychiatry 2008;64:850–5.

26 Hall S, Öhrfelt A, Constantinescu R, et al. Accuracy of a panel of 5 cerebrospinal fluid biomarkers in the differential diagnosis of patients with dementia and/or Parkinsonian disorders. Arch Neurol 2012;69:1445.

27 Pametti L, Chiaseinni D, Bellomo G, et al. Cerebrospinal fluid Tauer-synuclein ratio in patients with dementia and degenerative dementias. Mov Disord 2011;26:1428–35.

28 Montine TJ, Shi M, Quinn JF, et al. CSF Abeta(42) and tau in Parkinson’s disease with cognitive impairment. Mov Disord 2010;25:2682–5.

29 Sussmuth SD, Utterm I, Landwehrmeyer B, et al. Differential pattern of brain-specific CSF proteins tau and amyloid-beta in Parkinsonian syndromes. Mov Disord 2010;25:1183–8.

30 Öhrfelt A, Grognet P, Andreassen N, et al. Cerebrospinal fluid alpha-synuclein in neurodegenerative disorders—a marker of synapse loss? Neurosci Lett 2009;450:332–5.

31 Compta Y, Pereira JB, Iñigo S, et al. Combined dementia-risk biomarkers in Parkinson’s disease: A prospective longitudinal study. Parkinson Relat Disord 2013;19:717–24.

32 Lech S, Hjermdal E, Salvesen L, et al. Amyloid-related biomarkers and axonal damage proteins in parkinsonian syndromes. Parkinsonism Relat Disord 2012;18:69–72.

33 Schoonenboom NS, Reesink FE, Verwey NA, et al. CSF biomarker panel differentiates synucleinopathies (Parkinson Disease, dementia with Lewy bodies, and atypical parkinsonian disorders) from Alzheimer disease. Acta Neuropathol 2011;122:119–29.

34 Amundsen M, Zetterberg H, Minthon L, et al. The cognitive profile and CSF biomarkers in dementia with Lewy bodies and Parkinson’s disease dementia. Int J Geriatr Psychiatry 2011;26:100–5.

35 Jellinger KA, Attanas J. Prevalence and impact of vascular and Alzheimer pathologies in Lewy body disease. Acta Neuropathol 2008;115:427–36.

36 Ballard C, Ziabreva I, Perry R, et al. CSF biomarkers in dementia for differential diagnosis in a large memory clinic cohort. Neurology 2012;78:47–54.

37 Andrénsson M, Zetterberg H, Minthon L, et al. The cognitive profile and CSF biomarkers in dementia with Lewy bodies and Parkinson’s disease dementia. Int J Geriatr Psychiatry 2011;26:100–5.

38 Lech S, Hjermdal E, Salvesen L, et al. Amyloid-related biomarkers and axonal damage proteins in parkinsonian syndromes. Parkinsonism Relat Disord 2012;18:69–72.

39 Schoonenboom NS, Reesink FE, Verwey NA, et al. Cerebrospinal fluid markers for differential dementia diagnosis in a large memory clinic cohort. Neurology 2012;78:47–54.

40 Andrénsson M, Zetterberg H, Minthon L, et al. The cognitive profile and CSF biomarkers in dementia with Lewy bodies and Parkinson’s disease dementia. Int J Geriatr Psychiatry 2011;26:100–5.

41 Öhrfelt A, Zetterberg H, Andersson K, et al. Identification of novel alpha-synuclein isoforms in human brain tissue by using an online nanolC-ESI-FTICR-MS method. Neurochem Res 2011;36:2039–42.

42 Conway KA, Lee S-J, Roche JC, et al. Acceleration of oligomization, not fibrillization, is a shared property of both alpha-synuclein mutations linked to early-onset Parkinson’s disease: implications for pathogenesis and therapy. Proc Natl Acad Sci USA 2000;97:571–6.

43 Aerts MB, Esselin RA, Abdo WF, et al. CSF α-synuclein does not differentiate between parkinsonian disorders. NBA 2012;33:430.e1–430.e13.

44 Noguchi-Shinohara M, Tokuda T, Yoshida M, et al. CSF α-synuclein levels in dementia with Lewy bodies and Alzheimer’s disease. Brain Res 2009;1251:1–6.

45 Spies PE, Melis RJ, Sijben MJ, et al. Cerebrospinal fluid alpha-synuclein does not discriminate between dementia disorders. J Alzheimers Dis 2010;69:209–10.

46 van Dijk KB, Weiss M, Raijmakers A, et al. Reduced α-synuclein levels in cerebrospinal fluid in Parkinson’s disease are unrelated to clinical and imaging measures of disease severity. Eur J Neurol 2013;20:1388–94.

47 Emstrom Moilanen M, Yli H, Hall S, et al. Low CSF Levels of both α-synuclein and the α-synuclein cleaving enzyme neurin in patients with synucleinopathy. PLoS ONE 2013;8:e53250.

48 Mollenhauer B, Trautmann E, Taylor P, et al. Total CSF α-synuclein is lower in de novo Parkinson patients than in healthy subjects. Neurosci Lett 2013;532:44–8.

49 Tateno F, Sakakibara R, Kawai T, et al. Alpha-synuclein in the cerebrospinal fluid differentiates synucleinopathies (Parkinson Disease, dementia with Lewy bodies, multiple system atrophy) from Alzheimer disease. Alzheimer Dis Assoc Disord 2012;26:213–16.

50 Wang Y, Shi M, Kong KA, et al. Phosphorylated α-synuclein in Parkinson’s disease. Sci Transl Med 2012;4:121ra20.

51 Mollenhauer B, Schulz-Schaeffer WF, Schlössmer MG. CSF α-synuclein, tau, and amyloid β in Parkinson’s disease. Authors’ reply. Lancet Neurol 2011;10:681–3.

52 Hong Z, Chung KA, Quinn JF, et al. DJ-1 and α-synuclein in human cerebrospinal fluid as biomarkers of Parkinson’s disease. Brain 2010;133:713–26.

53 Mollenhauer B, Cullen V, Kahn I, et al. Direct quantification of CSF alpha-synuclein by ELISA and first cross-sectional study in patients with neurodegeneration. Exp Neurol 2008;213:315–25.
Goldstein DS, Holmes C, Sharabi Y. Cerebrospinal fluid biomarkers of central catecholamine deficiency in Parkinson’s disease and other synucleinopathies. Brain 2012;135:1900–13.

Pametti L, Castrotto A, Chiasserini D, et al. Cerebrospinal fluid biomarkers in Parkinson’s disease. Nat Rev Neurol 2013;9:131–40.

Brady RO, Kanfer J, Shapiro D. The metabolism of glucocere-brosides. I. Purification and properties of glucocerebrosidase-de­aving enzyme from spleen tissue. J Biol Chem 1965;240:39–43.

Sidransky E, Nalls MA, Aasly JO, et al. Multicenter analysis of glucocerebrosidase mutations in Parkinson’s disease. N Engl J Med 2009;361:1651–61.

Mazzulli JR, Xu YH, Sun Y, et al. Gaucher disease glucocerebrosidase and alpha-synuclein form a bidirectional pathogenic loop in synucleinopathies. Cell 2011;146:37–52.

Pametti L, Balducci C, Pierguidi L, et al. Cerebrospinal fluid beta-glucocerebrosidase activity is reduced in Dementia with Lewy Bodies. Neurobiol Dis 2009;34:484–96.

Pametti L, Chiasserini D, Persichetti E, et al. Cerebrospinal fluid lysosomal enzymes and alpha-synuclein in Parkinson’s disease. Mov Disord 2007;22:1481–4.

Pametti L, Balducci C, Pierguidi L, et al. Cerebrospinal fluid beta-glucocerebrosidase activity is reduced in Dementia with Lewy Bodies. Neurobiol Dis 2009;34:484–96.

Caudle WM, Bammerrl TK, Lin Y, et al. Using ‘omics’ to define pathogenesis and biomarkers of Parkinson’s disease. Expert Rev Neurother 2010;10:925–42.

Abdi F, Quinn JJ, Jankovic J, et al. Detection of biomarkers with a multiplex quantitative proteomic platform in cerebrospinal fluid of patients with neurodegenerative disorders. J Alzheimer’s Dis 2006;9:293–348.

Zhang J, Sokal I, Peskind ER, et al. CSF Multianalyte Profile Distinguishes Alzheimer and Parkinson Diseases. Am J Clin Pathol 2008;129:526–9.

Constantinescu R, Andreasson U, Li S, et al. Proteomic profiling of cerebrospinal fluid in parkinsonian disorders. Parkinsonism Relat Disord 2010;16:545–9.

Ishigami N, Tokuda T, Ikekawa M, et al. Cerebrospinal fluid proteomic patterns discriminate Parkinson’s disease and multiple system atrophy. Mov Disord 2012;27:851–7.

Mattison HA, Stewart T, Zhang J. Applying bioinformatics to proteomics: Is machine learning the answer to biomarker discovery for PD and MSA? Movement Disorders 2012;27:1595–7.

Jesse S, Lehnert S, Jahn O, et al. Differential sialylation of serpin A1 in the early diagnosis of Parkinson’s disease dementia. PLoS One 2012;7:e48783.

Lehnert S, Jesse S, Rist W, et al. iTRAQ and multiple reaction monitoring as proteomic tools for biomarker search in cerebrospinal fluid of patients with Parkinson’s disease dementia. Exp Neurol 2012;234:499–505.

The Lancet N. Biomarker promise for Parkinson’s disease. Lancet Neurol 2010;9:1139.

Parkinson Progression Marker I. The Parkinson Progression Marker Initiative (PPMI). Prog Neurobiol 2011;95:502–57.

Bagchi DP, Yu L, Perlmutter JS, et al. Binding of the radioligand SIL23 to alpha-synuclein fibrils in Parkinson disease brain tissue establishes feasibility and screening approaches for developing a Parkinson disease imaging agent. PLoS ONE 2012;8:e55031.

Kuiper JJ, Verbeek MM, Borroni B. Tau forms in CSF as a reliable biomarker for progressive supranuclear palsy. Neurology 2011;76:1443; author reply 1443.

Borroni B, Malinverno M, Gardoni F, et al. A combination of CSF tau ratio and mid sagittal midbrain-to-pons atrophy for the early diagnosis of progressive supranuclear palsy. J Alzheimer’s Dis 2010;22:195–203.