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REVIEW ARTICLE

Blood based biomarkers for movement disorders

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Movement disorders have been carefully clinically defined, based on clinico-pathological series; however there is often diagnostic and prognostic uncertainty, especially in early stage disease. Blood-based biomarkers for Alzheimer's disease (AD), particularly p-tau181 and p-tau217, may be useful in the movement disorder clinic, especially in identifying corticobasal syndrome due to AD pathology and in identifying Parkinson's disease (PD) patients at high risk for the future development of dementia. Serum or plasma neurofilament light (NfL) may be useful in separating Parkinson's plus syndromes (progressive supranuclear palsy—PSP, multiple system atrophy – MSA, and corticobasal syndrome—CBS) from PD. NfL is also a prognostic biomarker, in that the level of baseline or cross-sectional plasma/serum NfL is associated with a worse prognosis in PD and PSP. The development of protein aggregation assays in cerebrospinal fluid and multiplex assays which can measure 100s-1000s of proteins in blood will provide new tools and insights for movement disorders for clinicians and researchers. The challenge is in efficiently integrating these tools into clinical practice and multi-modal approaches, where biomarkers are combined with clinical, genetic, and imaging data may guide the future use of these technologies.

KEYWORDS
neurofilament light, Parkinson's disease, plasma, progressive supranuclear palsy, serum

1 | INTRODUCTION

Historically, neurodegenerative diseases have been primarily clinically diagnosed conditions, based on careful clinico-pathological correlation, in which neuroimaging, blood, and cerebrospinal fluid (CSF) analyses have played a small part in diagnosis and clinical management. The primary role of these investigations, particularly in Alzheimer's disease (AD) and Parkinson's disease (PD), has been to exclude rare structural or neuro-inflammatory mimics of the primary neurodegenerative condition.[1] However, developments in the application of pathology-based PET imaging in AD CSF biomarkers have led to a shift in the concept of AD from a clinical entity to a biomarker supported clinico-pathological construct, particularly in clinical research and trials where the underlying pathology can be identified pre-symptomatically or at very early disease stages.[2] Importantly, the clinical syndrome of progressive dementia in AD can now be separated from the pathological AD process which can be defined either at post-mortem or using imaging or fluid biomarkers in life. Up until 2010, CSF biomarkers were measured with immunoassays measuring in the ng/ml range, and proteins relevant to brain diseases such as Amyloid A-Beta, tau, and alpha-synuclein were not reliably detected in blood. However, the identification of CSF biomarkers has presaged the development of blood assays. The development of new highly sensitive assays in blood, including meso-scale discovery (MSD), single-molecule array (SIMOA), and immunomagnetic reduction (IMR), have enabled the reliable and accurate determination of AD biomarkers at the femtomolar level in blood. The potential convenience of blood-based biomarkers means that these biomarkers may be rapidly adopted into clinical practice, and the scope and limitations of these assays will need to

be appreciated by clinicians and clinical researchers. The application of these blood-based biomarkers in movement disorders is less well-established, and although we await the development of specific, reproducible blood-based biomarkers for neurodegenerative movement disorders, the current assays that have been established in AD are relevant to common movement disorders and will likely impact practice in the near future.

Movement disorders are a group of clinical disorders relating to dysfunction of the basal ganglia, leading to disorders of increased (hyperkinetic) or decreased (hypokinetic) movement. Movement disorders can be divided into neurodegenerative disorders with a progressive clinical course and well-defined (although importantly often mixed) neuropathology, such as Parkinson's disease (PD), Huntington's disease (HD), multiple system atrophy (MSA), and progressive supranuclear palsy (PSP) and conditions usually without well-defined neuropathology such as dystonia and essential tremor. All of these conditions may be difficult to diagnose in the early disease stages. The development of successful clinical trials requires accurate diagnosis, balanced stratification of trial participants into active treatment and placebo arms, and well-powered measures of disease progression. Well-designed prospective clinical studies with biomarker analysis are needed to validate the use of biomarkers in clinical practice, but it is likely that we will see the increasing use of blood-based biomarkers to increase the accuracy of early diagnosis, predict future progression, determine target engagement and potentially track disease progression in clinical trials.

PD is the most common neurodegenerative movement disorder with a prevalence of around 140/100,000.[3] More benign (essential tremor, dystonic tremor) and more aggressive (progressive supranuclear palsy [PSP], multiple system atrophy [MSA], corticobasal syndrome [CBS]) conditions may be misdiagnosed as PD in the early disease stages.[1] PD itself is heterogeneous with some patients developing rapidly progressive motor symptoms with autonomic involvement and dementia, with other patients having a relatively indolent disease course. When patients present with dementia or develop dementia in the first year of symptoms and develop fluctuating cognition, visuo-spatial impairment with visual hallucinations, and parkinsonism, they may meet criteria for dementia with Lewy bodies (DLB). DLB may be indistinguishable pathologically from PD and DLB, Parkinson's disease dementia (PDD) and PD can be referred to collectively as Lewy body disorders (LBD). Conceptually, biomarker studies can be described analogously to clinical therapeutic trials in which there is an early phase (I/II) in which the biomarker is explored in a well-characterised patient population to provide preliminary evidence of the efficacy of the biomarker in the condition or process of interest and late phase (III) in which the effectiveness of the biomarker test is established in a realistic and well-powered clinical cohort. Like therapeutic clinical trials, late-phase biomarker studies are ideally large, multi-centre, and directly applicable to routine clinical practice, providing good evidence for their cost and clinical effectiveness. This also means that they are likely to be expensive, time consuming, and difficult to execute. Here, I will review the relevant pathology and pathogenesis of neurodegenerative movement disorders, the application of blood amyloid A-beta (Aβ) and AD biomarkers, total tau (t-tau), Phospho-tau (p-tau), and Neurofilament light (NFL) measurement in neurodegenerative movement disorders, and the potential for multimodal biomarker implementation (Table 1). I will also briefly review the development of blood alpha-synuclein, tau and alpha-synuclein protein aggregation assays, and high-throughput multiplex assays, as areas where development is likely in the medium term, although currently not mature as blood-based clinical "tests".

TABLE 1 Emerging blood biomarkers for movement disorders

Blood biomarker	Pathological correlate	Potential relevance to movement disorders	References
Reduced plasma A-beta-42	Alzheimer disease amyloid plaque pathology	Diagnosis of CBS-AD, Prediction of PD-D and DLB	16,25
Reduced plasma A-beta-42/40 ratio	Alzheimer disease amyloid plaque pathology	Diagnosis of CBS-AD, Prediction of PD-D and DLB	16,25
Elevated plasma/serum p-tau-181 and p-tau-217	Alzheimer disease amyloid plaque pathology	Diagnosis of CBS-AD, Prediction of PD-D and DLB	30–32,34
Elevated plasma/serum p-tau-181 and p-tau-217	Alzheimer disease amyloid plaque pathology	Identification of AD co-pathology in PDD and DLB	36
Elevated plasma/serum NFL	Release from damaged neurons	Diagnostic—Separation of PSP, CBS and MSA from PD	20,42
Elevated plasma/serum NFL	Release from damaged neurons	Prognostic—predicting more severe disease course in PD	44,45,51,52
Elevated plasma/serum NFL	Release from damaged neurons	Prognostic—predicting more severe disease course in PSP and MSA	28,47,53,54,73

Abbreviations: AD, Alzheimer's disease; CBS-AD, Corticobasal syndrome due to underlying Alzheimer disease pathology; DLB, dementia with Lewy bodies; MSA, multiple system atrophy; PD, Parkinson;s disease; PD-D, Parkinson's disease with subsequent dementia; PSP, Progressive supranuclear palsy.

2 | PATHOLOGY AND PATHOGENESIS OF NEURODEGENERATIVE MOVEMENT DISORDERS

The neurodegenerative movement disorders have distinct patterns of disease based on the protein deposited and the regional and cellular pattern of disease development. The primary role of specific proteins in the pathogenesis of neurodegenerative movement disorders has been validated through single gene (Mendelian) genetics analysed with neuropathology and driven the search for specific biomarkers. However, very commonly neuropathologies overlap, and "pure" neuropathology may be the exception rather than the rule. PD is characterised by deposition of filamentous insoluble alpha-synuclein as Lewy bodies and Lewy neurites in the brainstem, limbic system, and cortex.[4] Autosomal dominant PD may be caused by coding or copy number variation (duplications or triplications) in the alpha-synuclein gene *SNCA*, and common variation at the *SNCA* locus is a major risk factor for development of sporadic PD.[5] In MSA, filamentous alpha-synuclein inclusions are deposited in oligodendroglia which form glial cytoplasmic inclusions in the cerebellum, brainstem, and basal ganglia. PSP and CBD are tauopathies defined by the deposition of insoluble four repeat tau protein (4R-Tau), containing the alternatively spliced second microtubule binding domain of tau, encoded by exon 10 of the tau gene.[6] Common variation at the *MAPT* locus encoding tau is a major risk factor for PSP and CBD, and rare mutations in *MAPT* segregate with fronto-temporal dementia linked to chromosome 17, which may be clinically and pathologically similar to PSP.[6] In PSP, the pathology predominantly involves the brainstem, basal ganglia, and frontal cortex and is characterised by neuronal tau deposition with the accumulation of tau in the proximal astrocytic process, leading to tufted astrocyte pathology. In CBD, there is fronto-parietal cortical involvement with involvement of the basal ganglia and distal tau accumulation in astrocytes (astrocytic plaques) with prominent tau positive white matter threads. In both PSP and CBD, the 4R-Tau predominant isoform pattern differs from AD, in which all six isoforms of the alternatively spliced tau gene are deposited. These differences in protein deposition and regional/cellular involvement suggest that specific biomarkers may be detected in CSF or blood that reflect the underlying disease pathology.

Recent work has established that there may be a more precise definition of protein neuropathology. Firstly, proteins can be defined by the seeding properties of the abnormal protein deposits in these diseases. Work over the last 10 years has shown that protein extracted from post-mortem brain samples has seeding activity, leading to formation of protein aggregates in recipient cells or animal models.[7,8] These newly formed protein aggregates have specific morphologies, which in animal models may closely recapitulate the donor pathology.[7] Secondly, cryo-EM studies have shown that the abnormal filaments in different diseases have specific morphologies. The tau filaments of AD, PSP, CBD, and chronic traumatic encephalopathy have different fold patterns, associated with different protofilaments and non-proteinaceous material and define these conditions as different diseases.[9] This suggests that, beyond differences in protein isoforms and post-translational modification, there are specific conformational and seeding properties related to neurodegenerative disease proteins which, in addition to having a role in disease pathogenesis, may ultimately be assayed in diagnostic tests to establish the likely underlying pathology in life.

In addition, it appears that biomarker proteins in CSF and blood do not just represent passive leakage of the hallmark protein released from dysfunctional or dying neurons and glia. These proteins are processed both with proteolysis and editing of post-translational modification, and the protein epitope or fragment levels measured in blood and CSF represent a dynamic equilibrium between active secretion, processing in the extracellular space and uptake, and peripheral production and processing by cells such as microglia and macrophages.

With respect to the development of diagnostic biomarkers, the final clinical diagnosis of PD, MSA, and PSP has a high correlation with post-mortem diagnosis, particularly when patients are followed up with careful longitudinal assessment in specialist movement disorder clinic.[10] For corticobasal syndrome (CBS), the situation is more complex, in that the prototypic clinical syndrome of progressive asymmetric rigidity, dystonia, and apraxia can be caused by CBD, PSP, or AD pathology. Currently, the term CBS is used for the clinical syndrome, whereas CBD describes the underlying pathology. For all of the neurodegenerative movement disorders, early-stage clinical diagnosis may be limited in clinical practice in expert and non-expert centres. This is often interpreted as being due to limitations in clinical skills or practice, but this is also likely to relate to the delay in the development of characteristic clinical features, which might not be apparent at the earliest disease stages. The overall accuracy of the diagnosis of PD has been reported to be 76%, but this improves with reassessment by expert clinicians through the disease course.[10,11] In the CAMPAIGN clinical study of incident parkinsonism over 10 years, 9% of patients who entered the study and were diagnosed by expert clinicians in a systematic research assessment as having PD consistent with the Queen Square Brain Bank (QSBB) criteria for PD were later rediagnosed as having alternative conditions.[12] The importance of follow-up is explicit in the QSBB criteria for the diagnosis of PD which include an excellent response to levodopa, persistent asymmetry, emergence of levodopa induced dyskinesia, and disease course of >10 years.[13] Identification of these clinical features is usually not possible at the first clinical assessment, and an adjunctive blood based biomarker which could distinguish PD from MSA, PSP, CBD and ET at the initial assessment would lead to an improvement in clinical practice and trials, particularly in the identification of patients with early stage disease.

3 | BETA-AMYLOID (AB) AND AD PATHOLOGY IN MOVEMENT DISORDERS

Aβ is deposited as amyloid plaques in AD, and there is preferential depletion of the more amyloidogenic Aβ-42 as compared to Aβ-40 in AD pathology. Circulating Aβ-42 levels and the Aβ-42/40 ratio are

reduced in Alzheimer's disease, thought to be due to the preferential accumulation of Abeta42 in amyloid plaques. This can be measured in both CSF and blood, and CSF Aβ-42 and 42/40 ratios have been reliably shown to correlate with the pathological diagnosis of AD and other markers of amyloid pathology such as amyloid-PET.[14] Blood-based assays of Aβ-42 are less reliable than CSF assays, with a reduction of blood Aβ-42 of around 20% in individuals with Alzheimer neuropathology as compared to a reduction of around 50% in CSF Aβ-42.[14] This may relate in part to the peripheral production of Aβ species.

AD pathology is important in neurodegenerative movement disorders in two main areas. Firstly, about one third of cases presenting with CBS have underlying AD neuropathology, with the remainder having 4R-Tau pathology as PSP or CBD.[15] Blood and CSF biomarkers are a promising approach to distinguishing CBS-AD from CBS due to underlying PSP or CBD pathology, and an evaluation of AD biomarkers has been incorporated into the clinical research criteria for CBD.[16] A number of recent studies have confirmed that a substantial proportion of CBS cases have underlying Alzheimer neuropathology as defined by amyloid PET and reduced CSF Aβ-42.[17–20]

AD pathology may also be an important co-pathology and driver of adverse disease outcomes. About 50% of PD patients have developed dementia after 10 years of motor symptoms (PDD), and 50% of patients with PDD have concurrent AD pathology at post-mortem, which is also a common finding in DLB.[21,22] Concurrent AD pathology accelerates the development of dementia in LBD, and there seems to be an interaction between the extent of amyloid, tau, and synuclein pathology.[22] In the analysis of the PPMI de novo PD cohort, a reduction in CSF Aβ-42 and a reduction in the Aβ-42/tau ratio were predictive of cognitive impairment 2 years after study entry.[23]

Previous work has shown that AD CSF biomarkers at baseline are predictive of future dementia and cognitive impairment in PD, suggesting that blood AD biomarkers may also be predictive of PDD and may define a subset of LBD patients with concurrent AD pathology.[23] At a group level, AD biomarkers such as a reduced CSF Aβ-42 or a reduced Aβ-42 / tau ratio are reduced in DLB and PDD compared to controls. A large cross-sectional study of CSF AD biomarkers in LBD showed that 22% of DLB cases had a CSF biomarker profile consistent with AD, as compared with 9% of PDD cases and 3% of PD cases.[24] The group effect differences between PDD/DLB in CSF AD biomarkers has been supported by the work of Chouliaras and colleagues evaluating plasma Aβ-42/40, which showed a mean plasma Aβ-42/40 ratio of 0.060 in LBD as compared with 0.057 in AD pathology cases and 0.065 in controls.[25] Surprisingly, the positive plasma amyloid biomarkers in LBD cases were not reflected in differences in amyloid PET positivity, suggesting that blood AD biomarkers and PET amyloid imaging may be separable in LBD patients.[25] AD biomarkers are unlikely to be useful in distinguishing AD from DLB or PDD but may be helpful in predicting adverse outcomes in patients with PD.

4 | TOTAL TAU (T-TAU) AND PHOSPHO-TAU (P-TAU)

Insoluble tau aggregates are a core pathological feature of AD, PSP, and CBD. Total tau (t-tau) is reliably increased in CSF and blood in AD patients.[26] Increases in tau in blood have been thought to be due to neuronal damage and passive release from neurons, as very high t-tau levels can be measured in some non-AD conditions with neuronal damage such as traumatic brain injury, stroke, and Creutzfeldt-Jakob disease.[27] However, recent studies including studies of the half life of tau in CSF using stable isotope labelling kinetics (SILK) have highlighted the dynamic processes underlying the presence of tau in biofluids, and it is likely that tau measured in CSF likely also relates to active secretion and microglial processing, as well as release from damaged neurons.[27] Despite the widespread deposition of tau in neuronal and glial deposits in PSP, there has been no consistent identification of increased total tau in CSF in PSP patients.[28]

Tau in PSP and AD is hyperphosphorylated at multiple sites, including residues 181 and 217.[29] A great deal of interest has surrounded recent studies measuring p-tau181 and p-tau217 in blood from patients with different neurodegenerative disorders, and it appears that blood p-tau181 and 217 are better measures of AD pathology than plasma Aβ or t-tau. Phospho-tau epitopes can be reliably measured in both plasma and serum. Blood p-tau181 is consistently increased approximately 2–4 fold in patients with Alzheimer's disease syndromes as compared to healthy controls with other clinically diagnosed causes of dementia, including PSP.[30] Furthemore, elevated p-tau 181 correlates with amyloid pathology identified with amyloid-PET and the degree of tau pathology as measured by tau-PET and at post-mortem assessment.[31,32] Recent work has suggested that blood p-tau217 may be an even more sensitive measure of AD pathology.[30,33]

The potential usefulness of plasma p-tau assays in the movement disorder clinic is highlighted by the findings of Thijssen and colleagues. In their work, a substantial proportion of CBS cases had positive amyloid PET scans, indicating underlying AD pathology, and this correlated well with plasma p-tau181 and p-tau217 measures, showing that plasma p-tau181 and p-tau217 can likely distinguish CBS-AD from CBS-PSP and CBS-CBD.[30] Assuming that plasma p-tau assays are adopted in clinical practice, they may well be useful in distinguishing CBS due to AD from CBS due to PSP and CBD.[32,34] Further large scale, multi-centre autopsy confirmed studies will be useful to evaluate the use of plasma p-tau assays for this indication.

A recent study by Rodriguez and colleagues highlights the potential inaccuracy of clinical diagnosis and the role of blood biomarkers in pinpointing the underlying pathology.[35] In a cohort of individuals diagnosed in life as having AD following DSM-4 and NINDS-ADRDA diagnostic criteria, only 75% of patients diagnosed with AD in life had AD pathology at post-mortem. In this study, plasma p-tau181 was highly predictive of AD pathology 8 years before post-mortem examination and was correlated with the degree of tau pathology defined by Braak stages. Highlighting the role of co-pathology,

p-tau181 was elevated in LBD patients with AD copathology, but normal in patients with pure LBD.

This overlap between AD and PD pathology and adverse outcomes may become particularly important if a successful amyloid or tau targeting therapy is developed for AD. Recent studies have addressed this issue. Hall and colleagues divided PDD/DLB patients into patients with and without an abnormal tau PET scan, indicating likely AD co-pathology.[36] Elevated p-tau217 and to a lesser extent p-tau181 separated that LBD group with and without AD co-pathology, similarly to CSF Abeta42 and CSF p-tau-181 measures. In contrast, a similar study defining patients with LBD as having AD pathology with amyloid PET showed no difference in p-tau-181 levels between LBD patients with or without a positive amyloid PET scan.[25]

Despite the hyperphosphorylation of tau in PSP and CBD, multiple studies have been unable to show a difference between p-tau181 and 217 in PSP and CBD patients and controls.[25] Within neurodegenerative diseases, the increases in p-tau seem to be specific to AD, suggesting that this measurable increase relates to the interaction between amyloid plaques and abnormal neurones, rather than being directly related to neuronal release. Differences between PSP-tau and AD-tau suggest that it may be possible to develop specific and sensitive biomarkers for PSP and CBD. Unlike AD, CBD and PSP involve the deposition of phosphorylated four repeat tau, and this tau undergoes specific proteolytic processing generating different tau fragments. Potentially, these differences in the formation and processing of pathologic tau may enable a more precise blood-based biomarker for PSP and CBD, but to date, disappointingly, these biochemical differences have not led to well-replicated blood or CSF biomarkers for PSP and CBD.

Most work in PSP has focussed on CSF assays. An early study identifying a decrease in CSF 33/55 kDa ratio for tau by Western blot in PSP has not been replicated.[37] An immuno-PCR based assay using 4R- and 3R-specific antibodies identified a reduction in four repeat tau in CSF in both PSP and AD, as compared to controls, which is encouraging, although the results suggest there is no distinction between 4R predominant tauopathies and tauopathies involving all six isoforms of tau. A recent study using both a commercial immunoassay and custom ELISA assays reported that there was a decrease in both total and N-terminal tau fragments in PSP CSF.[38] However, most studies have not shown a difference between total tau and tau fragments between PSP patients and controls.[39] A recent study using the ultrasensitive SIMOA assay evaluating N-terminal, mid-terminal, and total tau has shown that these are reliable biomarkers in AD but are normal in PSP.[40] Protein aggregation assays are an alternative approach to the detection of abnormal tau in CSF which appear promising in the definition of abnormal tau species in CSF, as outlined below.

5 | NEUROFILAMENT LIGHT (NF-L)

Nf-L is an axonal structural protein which is highly expressed in large myelinated axons and released from damaged cells, and which shows great promise in improving the diagnosis and prediction of prognosis in neurodegenerative movement disorders. Elevation in CSF and plasma/serum Nf-L can be detected in a range of conditions, including traumatic brain injury, multiple sclerosis relapse, amyotrophic lateral sclerosis, and the Parkinson's plus syndromes PSP, MSA, and CBD. CSF and plasma/serum Nf-L are strongly correlated. There have been inconsistent reports of raised CSF and plasma Nf-L in PD cases as compared to controls, with some authors suggesting that the strong association between Nf-L and age in healthy controls may confound comparisons.[41–45] A meta-analysis of CSF Nf-L showed a slight increase in PD cases, which did not reach statistical significance.[46] If there is an elevation of Nf-L in PD, it is likely to be to a small extent and is unlikely to be helpful as an isolated measure in the early diagnosis of PD.

Serum/plasma Nf-L has been consistently shown to be elevated in the Parkinson's plus syndromes PSP, MSA, and CBS, and elevated Nf-L levels are helpful in distinguishing these conditions from PD.[20,42,44,46] Area under the sensitivity, versus 1-specificity curve (AUC) at varying case/control levels is widely reported as a global measure of the efficiency of a diagnostic test in separating true positives from false positives. In the evaluation of diagnostic tests, an AUC value of >0.80 is considered very good and >0.90 is considered excellent. In established disease, the area under the curve for AUC for plasma Nf-L in distinguishing PD from Parkinson's plus conditions is estimated between 0.85 and 0.91, with a slightly lower AUC in patients with early-stage disease estimated at 0.80.[20,42] Diagnostic separation is most important in early disease stage when clinical diagnosis may be most challenging, when hallmark clinical features may not have emerged. This is particularly relevant in evaluating Nf-L in PSP, as Nf-L may correlate with disease progression over time in PSP. Although these pathfinding studies are promising, further evaluation in naturalistic clinical settings is needed. Specifically, assessment of the added value of blood Nf-L measurement over and above careful application of clinical diagnostic criteria with neuroimaging, and long term follow-up of clinical diagnosis and autopsy evaluation would help cement the clinical role of Nf-L in the differential diagnosis of parkinsonism.

Cross-sectional measures of Nf-L measured in CSF or blood correlate with disease severity, with correlations being seen with the Hoehn and Yahr stage, and more recently, the PSP Rating Scale and the Schwab and England Activities of Daily Living Scale, which survive correction for age and disease duration, implying that this is an independent marker of disease state.[47,48] As well as being associated with clinical severity at baseline, in serial measures, Nf-L progressively increases with time, suggesting that it tracks disease progression.[49] In the davunetide therapeutic trial, there was a progressive increase in CSF Nf-L levels over time with an approximately 15% annual increase in CSF Nf-L.[50] It is unclear how useful increases in Nf-L are in measuring disease progression, although this would be predicted from the cross-sectional and baseline analyses. In the davunetide study, the increase in Nf-L correlated with superior cerebellar volume atrophy and the oculomotor subscale of the PSP-RS, and more longitudinal biomarker data are needed to evaluate the potential usefulness of Nf-L as a disease tracking biomarker.

Blood and CSF Nf-L appears to be a useful prognostic biomarker in PD, i.e., cross-sectional or baseline levels predict the rate of progression and adverse future outcomes. In most but not all PD cohort studies, baseline CSF or blood Nf-L is associated with motor progression measured by the Hoehn and Yahr or MDS-UPDRS scale, functional progression measured with the Schwab and England activities of daily living scale (SEADL) and mortality.[44,45,51,52] Variation between studies may relate to the age at cohort baseline and duration of follow-up, with older study cohorts and longer duration of follow-up providing higher event rates for correlation with prognostic biomarker measurements. Diagnostic accuracy is a possible confounding factor in some of these studies, as inclusion of patients with PSP, MSA, or CBS in PD cohorts with higher baseline Nf-L levels and more rapid progression could bias the results. Gold standard studies in this area will include long-term reevaluation for alternate clinical diagnoses and post-mortem validation.

Baseline Nf-L is also predictive of disease progression in PSP and MSA. Several studies have identified a relationship between the rate of progression as measured by the PSPRS, SEADL, and RBANS or mortality and the level of baseline plasma or serum Nf-L.[28,47,53,54]

6 | ALPHA-SYNUCLEIN

Alpha-synuclein is a natively unfolded 140-amino acid protein which is deposited in fibrils as Lewy bodies and Lewy neurites in PD and DLB and as glial cytoplasmic inclusions in MSA.[4] The normal function of alpha-synuclein probably relates to the modulation of neurotransmitter release, possibly through interaction with SNARE proteins at the synaptic nerve terminal.[4] The importance of alpha-synuclein in neurodegenerative movement disorders is clear from the widespread distribution of alpha-synuclein pathology in PD, DLB, and MSA and the underlying disease genetics.[4] Autosomal dominant coding mutations and copy number variants in alpha-synuclein gene (*SNCA*) cause PD and DLB and common variation at the SNCA locus is associated with PD, DLB and the "prodromal" syndrome REM sleep behaviour disorder.[5] Common variation at the SNCA locus does not appear to be associated with MSA.[55,56] Pathologic alpha-synuclein protein is phosphorylated at Ser-129 and can seed the aggregation and fibrillation of unfolded alpha-synuclein.[4] Alpha-synuclein is a major candidate biomarker for studies of neurodegenerative movement disorders. Total CSF alpha-synuclein has been consistently found to be reduced in patients with PD compared to normal controls.[57,58] However, with respect to blood assays, total alpha-synuclein as measured with ELISA-based techniques has been reported to be increased, decreased, or unchanged in patients with PD compared with controls.[59] More recently, ultrasensitive single-molecule array (SIMOA) assays have been used for alpha-synuclein. These have shown a significant increase in plasma alpha-synuclein in PD cases compared to controls but with a substantial overlap between case and control levels and a receiver operator curve (ROC) of 0.6, meaning that even with the use of ultrasensitive techniques, total blood alpha-synuclein may not be a useful biomarker for PD.[60] Red blood cells are enriched for alpha-synuclein, so blood assays of total alpha-synuclein may easily be confounded by red blood cell contamination.

Potentially, assays targeting abnormal pathological forms of alpha-synuclein may have more specificity in defining PD and. Several studies have reported an increase in plasma alpha-synuclein phosphorylated at Ser-129 as compared to controls, although again there is a substantial overlap between cases and controls.[61] Pathologic alpha-synuclein is thought to form oligomers and then protofibrils before assuming a fibrillar structure. A number of groups have developed oligomer-specific antibodies, implemented in serum or plasma ELISA assays for oligomeric alpha-synuclein with a variety of antibodies and methodological approaches.[62] These assays may be more reliable than blood total alpha-synuclein in distinguishing PD cases from controls. However, a recent study has highlighted the variation in antibodies used in the detection of conformational forms of alpha-synuclein with a lack of specificity in distinguishing between monomers, oligomers, and fibrils, which may currently limit the development and application of these approaches as blood biomarkers.[63,64] A further potential confounding factor in the development of blood oligomer assays is the proposal the alpha-synuclein exists as a stable tetramer in blood, meaning that it may well be difficult to distinguish between pathogenic fibrillar structures and stable "benign" alpha-synuclein tetramers.[65]

7 | MULTIMODAL IMPLEMENTATION

As outlined above, early development of biomarkers is usually carried out with clinically definite disease in which patients meet clinical diagnostic criteria. However, biomarkers are likely to be most useful in "ambiguous" patients where there is clinical uncertainty as to the underlying diagnosis. In this context there may be several lines of evidence that may be used to help define the most likely diagnosis including age, clinical features, and neuroimaging, in addition to blood biomarkers. Most clinicians implicitly or explicitly apply a Bayesian approach in which pre-test and post-test probabilities are considered, and the results of imaging, blood, or CSF biomarkers are evaluated in the light of the most likely underlying diagnosis. This may be particularly important in the evaluation of AD biomarkers where incidental AD co-pathology may be present, particularly with increasing age. In elderly cohorts, "positive" AD biomarkers may not indicate clinical AD, and the results need to be considered with all the available clinical information. In this context, it is worthwhile evaluating the performance of blood biomarkers in very early stage patients where the supporting clinical follow-up information may not be available, in ambiguous patients who may not meet clinical diagnostic criteria for any disease, and in evaluating the added value of blood biomarkers over and above genetic, clinical and imaging data, in a multi-modal approach. The same principle applies to both diagnostic and prognostic biomarkers, and there are well-validated clinical prognostic factors for adverse outcomes in PD. For example, Archer and colleagues recently

showed that plasma Nf-L alone has an AUC for the differentiation of PD from atypical parkinsonism in patients meeting clinical diagnostic criteria of 0.75, whereas adding two MR imaging measures to the plasma Nf-L improved the AUC to 0.93. This type of study needs to be repeated in clinically ambiguous patients. Similarly, we recently showed in an integrated model of the prediction of unfavourable outcomes in PD that age and gender alone had an AUC of 0.71 for the prediction of unfavourable outcomes in PD, whereas an integrated model combining serum Nf-L, age, gender, genetic variables, and clinical variables had an AUC of 0.83 for the prediction of unfavourable outcomes in PD.[44] Future applications are likely to use machine learning-type approaches to aggregate multiple disparate sources of data and are likely to evaluate the performance of biomarkers in real-life population-based studies such as the UK biobank.

8 | PROTEIN AGGREGATION ASSAYS

Recently, there has been a great deal of interest in the development of protein aggregation assays for both alpha-synuclein and tau. These assays (real-time quaking induced conversion—rtQUIC and protein misfolding cyclic amplification—PMCA) assay the kinetics of the formation of abnormal fibrillar protein with a dye such as thioflavin-T when a patient sample is introduced to wild-type unfolded protein. The rtQUIC assay has become widely used as a clinical diagnostic CSF assay in suspected Creutzfeldt-Jakob disease (CJD) where it has a high sensitivity and specificity for sporadic CJD. Recent studies have indicated that CSF PMCA assays for synuclein can distinguish controls, PD, and MSA based on the kinetics of the formation of fibrillar alpha-synuclein.[66] Similarly, rt-QUIC assays have been developed for 4R- and 3R-tau, which appear to be effective in distinguishing PSP, AD, and Pick's disease tau.[67] In addition to the ability to detect abnormal fibrillar protein with aggregation properties, the kinetics of the assays differ between diseases, so, for example, PSP 4R-tau has a different profile to CBD 4R-tau, and this has been validated on both CSF and brain samples.[67] To date, a blood-based rtQUIC assay has not been implemented for CJD or alpha-synuclein, likely related to the low levels of aggregation competent protein in blood, and an effect of haem in red blood cells in quenching the fluorescent response in the thioflavin-based assays.[66,68]

9 | HIGH-THROUGHPUT ASSAYS

Emerging technologies enable the simultaneous measurement of 50-several thousand proteins in blood and CSF, enabling hypothesis-free determination of proteins important for pathogenesis and progression. This technology has the potential to provide new insights into disease pathogenesis as well as the ability to provide information on multi-biomarker profiles or patterns that may distinguish different diseases and disease subtypes. The O-Link platform uses a DNA proximity extension assay (PEA) to amplify low level of protein with DNA-based proximity probe pairs, allowing high levels of multiplexing and target specificity compared to conventional enzyme-based assays.[69] Currently, protein panels are available which assay 384 proteins simultaneously. We have used the multiplex PEA to measure CSF proteins in atypical parkinsonian syndromes and identified reduced levels of FGF-5, FGF-19 and SPOCK1 in MSA cases.

The aptamer-based Somascan platform allows the evaluation of over 4000 proteins in biosamples. A recent analysis of over 4000 proteins in 1599 serum samples from PD patients identified enrichment of proteins in pathways related to axon guidance, complement and coagulation cascades and protein digestion/absorption in PD patients compared to controls.[70] There have been a number of recent studies using high-throughput protein biomarker assays on serum and plasma samples from population-based studies over the last year, and these are likely to expand in scope and number and provide disease- and phenotype-specific profiles.[71,72]

10 | CONCLUSIONS

The development of blood-based biomarkers for AD and the identification of NfL as a prognostic marker and a diagnostic marker for atypical parkinsonism are likely to have an immediate impact on patient selection and stratification of clinical trials and may then be adopted in clinical practice. It is likely that more naturalistic long-term studies will be needed to evaluate the added role of blood biomarkers over and above standard clinical practice and to directly assess the clinical benefit of early diagnosis and more accurate prognosis in everyday practice. It remains the case that the gold standard for most diagnostic studies is clinical diagnosis using standard diagnostic criteria or clinical diagnosis supplemented by PET-protein imaging studies, and in my view, this represents a sound proof of concept for the use of blood biomarkers, rather than a definitive proof of benefit in a routine clinical setting. A multimodal approach where blood biomarkers are combined with demographics, genetics, imaging, and clinical features has the potential to maximally improve diagnostic and predictive accuracy, in addition to enabling the evaluation of the role of biomarkers in their overall clinical context.

Outside of AD-biomarkers and NfL, the development of specific tau and alpha-synuclein-based blood biomarkers for movement disorders has so far been disappointing, but rapid advances in protein aggregation and high-throughput multiplex assays suggest that new biomarker assays, profiles, and panels will continue to be developed, assessed, and integrated into protocols for future clinical trials and clinical practice.

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CONFLICT OF INTEREST

Dr Morris is employed by UCL. In the last 12 months, he reports paid consultancy from Roche and Amylyx; lecture fees/honoraria—BMJ, Kyowa Kirin, Movement Disorders Society. Research Grants from Parkinson's UK, Cure Parkinson's Trust, PSP Association, CBD Solutions, Drake Foundation, Medical Research Council, Michael J Fox Foundation. Dr Morris is a co-applicant on a patent application related to C9ORF72—Method for diagnosing a neurodegenerative disease (PCT/GB2012/052140).

DATA AVAILABILITY STATEMENT

Data sharing not applicable - no new data generated.

PEER REVIEW

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