Tracking and predicting disease progression in Progressive Supranuclear Palsy: CSF and blood biomarkers

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Introduction

Progressive supranuclear palsy (PSP) is a progressive neurodegenerative condition and the most common cause of atypical parkinsonism, with an estimated prevalence of 5-7 per 100,000 **(1)**. The predominant clinical phenotype of PSP is Richardson's syndrome (PSP-RS), which presents with an akinetic-rigid syndrome, falls, a vertical supranuclear gaze palsy and cognitive impairment with executive dysfunction. In recent years, the clinical heterogeneity of PSP has been highlighted by the description of two rarer clinical phenotypes in pathologically confirmed cases of PSP: PSP-Parkinsonism (PSP-P), characterised by asymmetric onset, tremor and a moderate initial therapeutic response to levodopa **(2)**; and pure akinesia with gait freezing (PAGF), characterised by gradual onset of freezing of gait or speech, absent limb rigidity and tremor, no sustained response to levodopa and no dementia or ophthalmoplegia in the first 5 years of disease **(3)**.

Diagnosing PSP, particularly in the early stages of disease, may be challenging as there is a clinical overlap with Parkinson's disease (PD), other atypical parkinsonian conditions such as multiple system atrophy (MSA) and other tauopathies including Alzheimer's disease (AD), frontotemporal dementia (FTD) and corticobasal degeneration (CBD). Furthermore, most patients with PSP present with falls or non-specific visual symptoms, both of which are common in elderly subjects **(4)**. A clinico-pathological study from 2002, which included 20 cases of PSP, found that the positive predictive value of a clinical diagnosis of PSP was 80%, in contrast to the positive predictive value of a clinical diagnosis of PD which was 98.6% **(5)**. However, a recent meta-analysis of 11 clinico-pathological studies showed that the pooled diagnostic accuracy of PD was 80.6% **(6)**, therefore similar to PSP. Furthermore, clinical criteria for the diagnosis of PSP according to the National Institute for Neurological Disorders and Society for PSP **(7)** have low diagnostic sensitivity, such that many patients with pathologically proven PSP will not have been identified according to clinical diagnostic criteria. New diagnostic criteria for PSP are expected to be published by the Movement Disorder Society in due course. For now, pathology at post-mortem remains the gold standard for diagnosing PSP.

Tau in PSP

The pathology of PSP is centred on the structural microtubule associated protein tau, encoded by *MAPT*. In PSP there is neuronal and glial accumulation of hyper-phosphorylated tau aggregates, with neurodegeneration **(8)**. There are several possible links between tau pathology and neurodegeneration including the formation of toxic oligomers, disruption of normal microtubule function **(9)** and cell to cell spread of pathogenic tau **(10)**.

Autosomal dominant mutations in *MAPT*, lead to FTDP-17 **(11)**, and common variation in MAPT predisposes to PSP **(12)**.

Tau pathology in PSP also involves an alteration in tau isoform homeostasis. Tauopathies can be classified according to the predominant isoform of tau that accumulates through alternative splicing of *MAPT*, leading to tau protein with 3 (3R) or 4 (4R) repeats of ~32 amino acids in the carboxy-terminus microtubule binding domain. 4R-tau is the dominant tau isoform in both PSP and CBD, in contrast to mixed 3R/4R-tau in AD **(13)**. Tau hyperphosphorylation and microtubule dysfunction have been targeted in two recent clinical trials **(14)(15)**, although both trials showed no effect on disease modification in PSP.

CSF biomarkers used in the diagnosis of PSP

Many cross sectional CSF studies have assessed whether CSF protein biomarkers can reliably differentiate PSP from healthy controls and patients with relevant differential diagnoses **(see supplementary table for mean biomarker concentrations in PSP and control groups, and p-values for differences between groups)**.

Tau and modified Tau

Based on the pathology, one would expect that increased levels of CSF tau could be detected in PSP. The majority of CSF total or phosphorylated CSF tau (t-tau and p-tau) data reported in the literature is based on commercially available ELISAs, such as the INNOTEST assay (Fujirebio). In these assays, t-tau and p-tau measurements are dependent on anti-tau capture antibodies, such as AT120 and AT270 (pT181), specific for the mid-domain region of the protein, encoded by exons 4-8 **(see figure 1)**.

However, in established PSP disease there has been no observed consistent elevation of CSF t-tau or p-tau compared to healthy controls **(16)(17)**. In addition, although Borroni and colleagues have shown lower levels of the CSF 33kDa/55kDa tau fragment ratio compared to healthy controls and other neurodegenerative conditions including FTD and AD **(18)(19)**, this has subsequently been attributed to assay artefact **(20)**. Regarding tau isoforms, Luk and colleagues showed decreased levels of CSF 4R-tau in PSP and AD groups compared to healthy controls **(21)**, although further replication data is required.

In contrast, raised CSF tau levels form a robust part of the diagnostic criteria for Alzheimer's disease **(22)**. However, it is uncertain why there is a difference between the CSF tau profile in PSP and AD considering that both conditions involve extensive neurofibrillary tangle pathology related to hyperphosphorylated tau protein. A potential explanation for the divergent tau profiles described above is that standard CSF tau ELISA might not detect elevations of specific tau species that are elevated in primary tauopathies such as PSP. In addition, the tau fragment profile is different in AD and PSP, which will determine epitopes and protein levels identified by ELISA. Importantly, most of tau in CSF appears to be in fragments containing N-terminal and/or mid-domain epitopes; virtually no full-length tau containing C-terminal sequences can be detected **(23)**. Whether the concentrations of different fragments differ between different tauopathies is currently unknown. To investigate this possibility, Wagshal and colleagues used novel ELISAs **(24)(see figure 1)** targeting N-terminal and mid-domain epitopes to compare CSF tau concentrations in severity matched AD and PSP patients. This revealed that the PSP group had lower mean CSF N-terminal and mid-domain tau concentrations compared to the AD and control groups. In addition, receiver operating characteristics (ROC) analysis revealed that the N-terminal fragment Tau12-BT2 assay was best at differentiating PSP from AD (area under the curve = 0.948, $p < 0.001$). It is important to note that these novel ELISAs do not distinguish between 3R and 4R-tau isoforms. It is hypothesised that the tau isoform profile may lead to an alteration in tau detectable in CSF in PSP and AD as 4R-tau is released less readily from cells compared to 3R-tau **(25)**.

Non-tau related CSF biomarkers

Non-tau related CSF biomarker candidates have also been studied extensively. The most promising of these have been Neurofilament light and heavy chain (NF-L and NF-H). Similarly to tau, neurofilaments are found in axons of neurons and are important components of the cytoskeleton. However, it appears that neurofilaments are highly expressed in large-calibre myelinated axons in white matter, whilst tau is predominantly expressed in thin unmyelinated axons of the cortex **(26)**. Significantly higher CSF levels of both NF-L **(17)(27)** and NF-H **(28)** have been shown in patients with atypical parkinsonian conditions, including PSP, compared to controls and patients with PD. However, there were no significant differences between the individual atypical parkinsonian conditions. These results are thought to reflect the more extensive and rapid neuronal loss that

occurs in atypical parkinsonian conditions compared to PD. The level of NF-L has also been shown to correlate with disease severity in PD, PSP **(17)**, vascular dementia (VaD) **(29)** and FTD **(30)**.

Other CSF proteins of interest are soluble amyloid precursor protein alpha (sAPPα) and beta (sAPPβ), and YKL-40. sAPP is bound to the mitochondrial outer membrane and therefore may be implicated in mitochondrial dysfunction, which contributes to the development of neurodegenerative conditions. Similarly, YKL-40 has been implicated in neuroinflammation and neurodegeneration and is considered a marker of glial activation. A large cross sectional CSF study by Magdalinou and colleagues showed higher median levels of both NF-L and YKL-40, and lower median levels of both sAPPα and sAPPβ, in PSP compared to PD, AD, FTD and controls **(31)**. The reason for such differences in the levels of sAPP and YKL-40 in PSP is unclear. Finally, there is limited evidence that CSF levels of glial fibrillary acidic protein (GFAP), a protein predominantly expressed in fibrillary astrocytes, are higher in PSP patients compared to controls **(16)**. Replication in larger cohorts is required to further explore the significance of these alternative CSF biomarkers. For now, it should be reiterated that there is a lack of reproducible data for a biomarker that can reliably differentiate PSP from other neurodegenerative conditions.

Blood biomarkers used in the diagnosis of PSP

In contrast to the large number of CSF based diagnostic studies discussed above, we found only four blood based biomarker candidates that have been analysed in diagnostic studies involving PSP patients. Serum uric acid levels have been shown to be lower in PSP patients compared to controls **(32)**, but this was later refuted by a similar cross-sectional study **(33)**. MSA patients showed significantly higher levels of serum insulin-like growth factor-1 compared to controls in a study that also included PD and PSP patients **(34)**. However, one study did reveal significantly elevated levels of both methylmalonate and homocysteine in all patient groups (PD, PSP, ALS) compared to controls **(35)**. The significance of this is unclear but it has been hypothesised that elevated levels of methylmalonate and homocysteine are neurotoxic.

Disease progression in PSP

Longitudinal studies assessing the natural history of PSP reveal that the clinical disease progresses over time. Measuring change in neurodegenerative conditions is central to defining the effects of therapeutic intervention and disease biology. In assessing the PSP rating scale (PSPRS) score, Golbe and colleagues followed up 162 PSP patients over 11 years. They showed that the mean rate of progression was 11.3 points per year and the median actuarially corrected survival was 7.3 years from symptom onset to death. In addition, the PSPRS score was a good independent predictor of survival **(36)**.

However, such scales may be affected by intra- and inter-rater variability. In addition, their use in clinical trials may be hindered by differences in the time interval between pathological disease progression/response to therapeutics and change in clinical state.

Therefore, the need for reliable disease progression biomarkers to complement clinical rating scales is clear. Predicting disease progression provides early prognostic information and may enable better powered clinical trials with more homogenous groups, and give insights into the mediators of disease progression. From a therapeutic trials perspective, measuring non-clinical biomarkers of disease progression may enable early demonstration of target engagement. However, no studies have shown that individual biomarkers are better than clinical scales at tracking disease progression and so, for now, clinical scales remain the only reliable options available. In addition, there are certain limitations encountered with CSF and blood as sources of biomarkers as lumbar puncture is an invasive procedure with potential complications, while protein

concentrations in blood may not reflect the pathological changes in the brain due to variations in blood-brain barrier permeability.

Here we review the reliability of CSF and blood biomarkers in predicting and tracking clinical disease progression in PSP. As well as summarising the literature to date, we also discuss the road ahead.

Methods

We performed a PubMed/Medline search and limited searches to studies reported in English, comparing PSP patients to patients with other neurodegenerative conditions and/or healthy controls **(see figure 2)** up until February 2017. Studies predicting disease progression were defined as longitudinal, prospective studies with baseline blood and/or CSF analysis and clinical assessment followed by at least one subsequent clinical assessment, in which the baseline measures were correlated with subsequent progression. Studies tracking disease progression are defined as longitudinal, prospective studies with baseline blood and/or CSF analysis and clinical assessment followed by at least one subsequent blood and/or CSF analysis and clinical assessment, in which longitudinal measures were assessed for disease progression.

We used searches including the terms 'progressive supranuclear palsy', 'PSP', 'parkinsonism', 'neurodegenerative diseases', 'atypical Parkinsonism', 'blood', 'plasma', 'serum', 'CSF', 'CSF biomarkers', 'biomarker', 'NFL', 'tau', 'tauopathies', 'prospective', 'longitudinal', 'consecutive', 'serial', 'predicting', 'tracking', 'progression' and 'disease progression'. Further references were found manually from identified publications.

All identified studies met a minimum quality score defined by the presence of: at least 5 PSP patients included in the study, with clearly defined diagnostic criteria; a clearly defined primary outcome of the study; a minimum period of 10 months between the first and last clinical assessment, lumbar puncture and/or blood test; clearly defined components of the clinical assessments used in the study; clearly defined CSF sampling protocol and use of assays; appropriate recording of statistical analysis used to interpret results.

Results

We identified five longitudinal studies in total. Four of the identified studies tracked clinical disease progression in PSP patients using CSF and/or blood biomarkers, including one study that was derived from the Davunetide clinical trial **(14)**. One study predicted clinical disease progression using blood biomarkers. We did not identify any studies that used CSF biomarkers to predict progression.

Tracking disease progression in PSP

CSF

Most, but not all of the identified CSF studies **(see table 1 for statistics)** showed that the levels of CSF NF-L increased over time and correlated with clinical disease progression. The one exception was the study by Constantinescu and colleagues who showed that consecutive CSF analysis revealed relatively stable levels of NF-L and GFAP over time in all of the investigated parkinsonian groups **(37)**.

In contrast, Backstrom and colleagues found that CSF NF-L levels was stable in PD patients but rose by almost 30% in PSP patients, over one year when compared to baseline CSF levels **(38)**. As the majority of study participants consisted of PD patients, follow up clinical assessment and analysis of whether CSF biomarkers could predict clinical disease progression was limited to this group and revealed that the baseline triad of high NF-L, low Aβ1-42 and high heart fatty acid-binding protein (HFABP) indicated a high risk of developing Parkinson's disease dementia (PDD). Similarly, a small subgroup of patients, who were part of a larger study by Magdalinou and colleagues, underwent serial CSF analysis and clinical examination to assess disease progression over the course of 1 year. Clinical examination consisted of measuring disease severity using the Hoehn and Yahr (H&Y) staging system and assessing clinical rating scores using the PSPRS and Mattis Dementia Rating Scale (DRS-2). At 1 year they found an increase in H&Y score and PSPRS, and a decrease in DRS-2. These changes were associated with a mean change in levels of NF-L (+540ng/L) **(31)**.

A major source of longitudinal data has come from recent therapeutic trials in PSP. A small subset of patients in the Davunetide trial had serial CSF analysis, measuring levels of CSF Aβ42, t-tau, p-tau and NF-L. Although there were no differences in rates of change between the Davunetide and placebo groups, NF-L was the only CSF biomarker whose concentration showed a statistically significant change over time, with a mean increase in concentration by 755ng/L when Davunetide and placebo patients were grouped together. Although the mean increase in CSF NF-L was lower in the Davunetide group compared to the placebo group (+494ng/L vs +922ng/L), this difference was not statistically significant (p = 0.43). However, the role of CSF NF-L in tracking disease progression was once again highlighted by the fact that the one year change in CSF NF-L levels correlated with an increase in the oculomotor subscale of the PSPRS **(14)**.

CSF biomarker	**Research Group**	**n number of PSP patients**	**Median time between first and last samples (months)**	**Mean interval change in biomarker concentration (median baseline concentration)**	**Associated change in clinical marker (correlation of change in biomarker vs change in clinical marker)**
NF-L	Constantinescu et al **(37)**	14	14.5	**NS** (956.5ng/L)	Not applicable
	Backstrom et al **(38)**	9	12	+1333ng/L (2357ng/L)	Not assessed
	Magdalinou et al **(31)**	9	12	+540ng/L (2219ng/L)	1.2 point increase in mean H&Y score and 14.2 point increase in mean PSPRS score ✝
	Boxer et al **(14)**	22	12	+755ng/L – including active treatment and placebo patients (5185pg/ml)	11.3 point increase in mean PSPRS score. Increase in NF-L concentration correlated with increase in mean oculomotor subscale of PSPRS (Spearman's rho = 0.609; p = 0.003)
GFAP	Constantinescu et al **(37)**	14	14.5	**NS** (760ng/L)	Not applicable

sAPPβ	Magdalinou et al **(31)**	9	12	**NS** (256ng/ml)	Not applicable
Aβ42	Boxer et al **(14)**	22	12	**NS** (384ng/L)	Not applicable
	Backstrom et al **(38)**	9	12	**NS** (486ng/L)	Not applicable
t-tau	Boxer et al **(14)**	22	12	**NS** (59.5ng/L)	Not applicable
	Backstrom et al **(38)**	9	12	**NS** (240ng/L)	Not applicable
p-tau	Boxer et al **(14)**	22	12	**NS** (24.5ng/L)	Not applicable
	Backstrom et al **(38)**	9	12	**NS** (39ng/L)	Not applicable
α-synuclein	Backstrom et al **(38)**	9	12	**NS** (565ng/L)	Not applicable
HFABP	Backstrom et al **(38)**	9	12	**NS** (450ng/L)	Not applicable

† Correlation between change in biomarker and change in clinical marker not calculated

NS No significant change in biomarker concentration over time

Table 1: CSF biomarkers used to track disease progression in PSP

Blood

Longitudinal fluid biomarker analysis in the Davunetide study **(14)** also included plasma NF-H which did not show any significant change in concentration over 1 year, with a median baseline concentration of 760ng/L.

Predicting disease progression in PSP

Blood

We identified a recent longitudinal study of PSP patients by Rojas and colleagues which assessed the ability of baseline plasma NF-L to predict a change in clinical measures using age, gender and baseline MMSE adjusted mixed linear models. Similarly, age and gender controlled Pearson's partial correlations were determined between baseline plasma NF-L levels and changes in regional and whole brain volume. The study showed that high baseline plasma NF-L levels **(see table 2 for statistics)** predict more severe neurologic, cognitive and

functional decline at 1 year follow up, as well as changes in whole brain and SCP volume loss **(39)**. In addition, plasma and CSF NF-L were significantly correlated (r = 0.74, p = 0.002).

Plasma biomarker	Research Group	n number of PSP patients	Mean baseline biomarker concentration (ng/L)	Mean baseline clinical marker score	Statistical analysis of baseline biomarker predicting disease progression
NF-L	Rojas et al **(39)**	147	43.7	High NF-L group PSPRS – 40.0 Low NF-L group PSPRS – 38.1	High baseline plasma NF-L (defined as >36.7pg/ml) group had more severe worsening of PSPRS score over 1 year vs low baseline plasma NF-L (defined as <36.7pg/ml) group, p = 0.02

Table 2: Blood biomarkers used to predict disease progression in PSP

Discussion

There is very limited evidence that CSF and blood biomarkers may have a role in predicting and/or tracking disease progression in PSP. Overall, the studies identified suggest that CSF NF-L tracks clinical disease progression over time, and that this correlates with the PSPRS score. A particularly interesting question that has not been looked at in previous studies is whether or not there are differences in the change in NF-L over time in early vs late stage disease. Other potential markers of tracking disease progression that had favourable results included CSF sAPPβ and GFAP. Of interest, a recent study analysed NF-L changes in plasma, CSF and brain of a variety of mouse models of proteopathic (α-synuclein, tau and β-amyloid) neurodegenerative diseases. Plasma and CSF NF-L levels were strongly correlated, and NF-L increases coincided with the onset and progression of corresponding proteopathic lesions in the brain. In addition, experimental induction of α-synuclein lesions increased plasma and CSF NF-L levels, whilst blocking β-amyloid lesions attenuated the NF-L increase **(40)**. The study therefore concluded that plasma and CSF NF-L may serve as both a marker of disease progression and also as a biomarker for treatment response in proteopathic neurodegenerative diseases. With regards to predicting disease progression, Rojas and colleagues showed that high baseline plasma NF-L is able to predict more severe functional decline **(39)**.

However, when compared to the volume of cross sectional diagnostic studies that have been carried out (a recent review by Magdalinou and colleagues on the diagnostic use of CSF biomarkers in parkinsonian conditions yielded 78 studies **(41)**), there is a clear lack of longitudinal fluid biomarker data on the topic of disease progression in PSP. Of the five studies that were identified, most had small subject numbers, were limited to only 1 year of follow up and exhibited heterogeneity in both the disease and control groups. In contrast, there appears to be a greater depth of studies looking at longitudinal brain changes in PSP patients using a variety of conventional and non-conventional MRI modalities **(42)(43)(44)(45)**. Another major drawback is the lack of combined longitudinal CSF and blood studies - the Davunetide study was the only longitudinal study identified to achieve this.

Other potential future directions on the topic in question include the use of new imaging modalities, including PET imaging with Tau ligands **(46)** and further studies on the role of blood based biomarkers. Of note, future studies may be able to achieve ultra-sensitive measurement of fluid biomarkers by utilising novel technologies such as single molecule array (SIMOA) **(47)**, single molecular counting (SMC) **(48)** and proximity extension assay (PEA) **(49)**. Novel assays, such as the real-time quaking-induced conversion assay (RT-QuIC), can be used to study misfolded protein biomarkers including tau and β-amyloid **(50)**.

This review has highlighted the need for longitudinal studies with large subject numbers that use combined (CSF, blood and imaging) biomarkers. Such studies will first of all aim to replicate the findings of existing longitudinal data on the role of biomarkers in tracking and predicting disease progression. There is also the potential to identify the first reliable PSP-specific biomarker, which may further improve our understanding of the underlying disease mechanisms. We hope that such biomarkers can be used in clinical trials of novel therapeutic targets, where the role of biomarkers as an objective measure of treatment response remains important. We also aim to determine the role of genetics in predicting disease progression and its association with the biomarkers in question. We will carry out these studies using patient data from the PROSPECT study, a UK wide, multi-centre longitudinal study that follows up patients over 5 years.

Figure 1 legend: Modified figure from Wagshal et al **(24)** highlighting the differences in capture antibodies between standard (INNOTEST) and novel tau ELISAs

Figure 2 legend: Flow diagram outlining the selection procedure to identify the 5 studies which were included in this review

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