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A panel of nine cerebrospinal fluid biomarkers may identify patients with atypical parkinsonian syndromes

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Abstract

Background—Patients presenting with parkinsonian syndromes share many clinical features, which can make diagnosis difficult. This is important as atypical parkinsonian syndromes (APSs) such as progressive supranuclear palsy (PSP), multiple system atrophy (MSA) and corticobasal syndrome (CBS) carry a poor prognosis, compared with patients with Parkinson's disease (PD). In addition, there is overlap between APS and dementia diseases, such as Alzheimer's disease (AD) and frontotemporal dementia (FTD).

Objective—To use a panel of cerebrospinal fluid (CSF) biomarkers to differentiate patients with APS from PD and dementia.

Methods—A prospective cohort of 160 patients and 30 control participants were recruited from a single specialist centre. Patients were clinically diagnosed according to current consensus criteria. CSF samples were obtained from patients with clinical diagnoses of PD (n=31), PSP (n=33), CBS (n=14), MSA (n=31), AD (n=26) and FTD (n=16). Healthy, elderly participants (n=30) were

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included as controls. Total τ (t- τ), phosphorylated τ (p- τ), β -amyloid 1–42 (A β 42), neurofilament light chain (NFL), α -synuclein (α -syn), amyloid precursor protein soluble metabolites α and β (soluble amyloid precursor protein (sAPP) α , sAPP β) and two neuroinflammatory markers (monocyte chemoattractant protein-1 and YKL-40) were measured in CSF. A reverse stepwise regression analysis and the false discovery rate procedure were used.

Results—CSF NFL ($p < 0.001$), sAPP α ($p < 0.001$) and α -syn ($p = 0.003$) independently predicted diagnosis of PD versus APS. Together, these nine biomarkers could differentiate patients with PD from APS with an area under the curve of 0.95 and subtypes of APS from one another. There was good discriminatory power between parkinsonian groups, dementia disorders and healthy controls.

Conclusions—A panel of nine CSF biomarkers was able to differentiate APS from patients with PD and dementia. This may have important clinical utility in improving diagnostic accuracy, allowing better prognostication and earlier access to potential disease-modifying therapies.

Introduction

Parkinson's disease (PD) is a progressive neurodegenerative movement disorder characterised by bradykinesia and at least one of three other cardinal signs: resting tremor, rigidity and impaired postural reflexes.¹ There is usually a good and sustained response to levodopa treatment, which improves life expectancy largely by preventing early death from immobility. Atypical parkinsonian syndromes (APSs) are a heterogeneous group of neurodegenerative disorders that are distinct from PD, but share its central characteristic of akinetic rigidity.² The 'atypical' descriptor indicates the presence of features such as early autonomic failure and cerebellar/pyramidal signs in multiple system atrophy (MSA), supranuclear down gaze palsies and dysexecutive syndrome in progressive supranuclear palsy (PSP), dystonia, myoclonus and cortical sensory loss in corticobasal degeneration (CBD) and a more rapid deterioration with earlier postural instability and falls in all three disorders. The duration of disease to death in most cases is less than 10 years. Based on their underlying pathologies, parkinsonian syndromes can be differentiated into synucleinopathies (PD, MSA) and tauopathies (PSP, corticobasal syndrome (CBS)).

Diagnosis rests on clinical acumen supported in some instances by neuroimaging. No diagnostic biomarker currently exists. Misdiagnosis in the early stages is relatively common among parkinsonian syndromes and sometimes occurs with other neurodegenerative conditions such as Alzheimer's disease (AD) and frontotemporal dementia (FTD). In a clinicopathological series, 31% of patients who were clinically diagnosed as CBS had AD underlying pathology³ and clinicopathological overlap between FTD and PSP/CBS has been well established.⁴

When examining cerebrospinal fluid (CSF), it would be ideal to be able to discriminate each different parkinsonian and dementia disorder; but this is not possible yet. A good first step would be able to discriminate PD from other more aggressive forms of parkinsonism, as this has important clinical implications. To investigate this we tested a panel of different CSF markers, including A β 42, total and phosphorylated τ (t- τ and p- τ), which are currently used in the diagnosis of AD. In PD there is evidence that a low A β 42, a marker of plaque pathology, may predict cognitive decline in PD and could be used as a potential prognostic

biomarker of cognitive decline associated with PD. Results have been inconclusive when assessing t- τ and p- τ in the CSF of patients with parkinsonism (for a review see ref. 5). However, they may improve diagnostic accuracy when used in combination with other markers. Neurofilament light chain (NFL), a non-specific marker of axonal degeneration, was also tested. Increased levels of NFL were found in APS and NFL was useful in differentiating APS from PD but not in discriminating between different subtypes of APS. 6,7 Total α -synuclein (α -syn), the most promising biomarker so far in parkinsonian conditions, was included in the panel of markers tested. Results were initially inconclusive but most recent studies have shown a decrease in total α -syn between PD and controls. Some studies have shown reduced α -syn levels in other synucleinopathies, such as MSA, without good discriminatory value between different synucleinopathies (for review see ref. 5 and for a meta-analysis ref. 8). Inflammatory markers included are YKL-40 and monocyte chemoattractant protein (MCP)-1. YKL-40 is a secreted glycoprotein named after its three NH₂-terminal amino acids: tyrosine (Y), lysine (K) and leucine (L), and its molecular weight of 40 kDa.⁹ The protein is highly expressed in astrocytes and microglia.^{10,11} MCP-1 is a small cytokine that is also involved in neuroinflammatory processes associated with neurodegeneration.¹² Soluble amyloid precursor protein α and β (sAPP α and sAPP β) are two soluble metabolites resulting from proteolytic processing of APP, which have been implicated in mitochondrial dysfunction and have shown a potential role in APS diagnosis.⁷

Our aim was to assess the importance of a broad panel of markers representing inflammatory and axonal/synaptic degenerative molecular pathways and their possible diagnostic utility to differentiate PD from APS, as well as to differentiate parkinsonian syndromes from dementia disorders and healthy controls.

Methods

Study participants

This is an observational, prospective, longitudinal study of patients with parkinsonian conditions, dementia disorders and healthy controls. Participants were consecutively enrolled over a 2-year period from 2011 to 2013 from the movement disorders and cognitive clinics at the National Hospital for Neurology and Neurosurgery, Queen Square, London. The diagnoses of probable PD, PSP, CBS, MSA, AD and FTD were based on internationally established operational criteria.^{1,13-17} A group of patients with parkinsonism who did not fit into any of the specific diagnostic categories were included as 'unclassifiable' patients with parkinsonism. We felt it was important to include this study group as such patients pose the greatest diagnostic challenge when seen in clinic.

Patients <40 years old and >85 years old were not included. Study participants were monitored for at least 2 years with a consensus diagnosis by at least two neurologists with experience in movement disorders (AJL, TTW, KB and HRM). Patients with intracranial mass, severe cerebrovascular disease, and infectious, metabolic or systemic diseases affecting the central nervous system were excluded from the study.

Disease duration was recorded and disease severity was evaluated using the Hoehn and Yahr (H&Y) staging system¹⁸ for the patients with parkinsonism. Patients with PSP, PD and CBS

had further assessments, including the Unified Parkinson's Disease Rating Scale (UPDRS)¹⁹ and Mini Mental State Examination (MMSE).²⁰ Patients with PSP had more detailed assessment of disease severity using the PSP rating scale (PSPRS)²¹ and Mattis Dementia Rating Scale (DRS-2).²² The majority of patients with parkinsonism were treated with dopaminergic drugs and all underwent structural brain imaging (MRI/CT).

A subgroup of patients with APS underwent serial CSF examinations over the course of 1 year. Patients were offered participation in the Queen Square Brain Bank brain donation scheme.

Healthy controls were usually spouses or friends of the diseased participants. All healthy controls underwent a thorough clinical and neurological examination, as well as a standardised neuropsychological assessment using the Mattis DRS.²² Individuals with objective or subjective memory problems or parkinsonian symptoms and signs were not included in the control group.

Ethics approval

The study was conducted in accordance with local clinical research regulations and was approved by the London, Queen Square ethical committee. Written informed consent was obtained from all participants, including access to their clinical data and scans.

CSF collection, storage and analysis

A standardised protocol for the collection and storage of CSF was followed (see online supplementary appendix 1). Biomarker levels were measured in the CSF of patients and controls using commercially available immunoassays according to the manufacturers' protocols (see online supplementary appendix 2). All analyses were performed by board-certified laboratory technicians, blinded to clinical data. The analyses were performed using one batch of reagents with intra-assay coefficients of variation below 10%.

Statistical analysis

To compare demographic, clinical characteristics and CSF biomarker data between groups, the normality of data distribution was assessed. Analysis of variances were used for normally distributed data and the Kruskal-Wallis test for skewed data to compare differences among all studied groups. To compare demographic and CSF biomarker data between studied groups, t tests were used for normally distributed data and Wilcoxon rank-sum tests were used for skewed data. Means and 95% CI or medians and IQR are presented accordingly. χ^2 Tests or Fisher's exact tests were used to compare the distribution of categorical variables across the groups. Receiver operating characteristic (ROC) curves were used to investigate the relationship between diagnostic sensitivity and specificity. Selecting biomarkers with an area under the curve (AUC) >0.60, we used a reverse stepwise regression analysis and the false discovery rate (FDR) procedure²³ with control at the 5% level to correct for multiple comparisons. Nuisance covariates (age, gender, disease duration and disease severity) were included in the model.

Based on the ROC analysis the best cut-off values for the CSF markers were determined from the highest Youden's index.²⁴ Correlations between biomarkers were obtained using the Spearman rank correlation (R_s). Statistical analyses were carried out using commercial software (Stata V.12.1). p Values $<5\%$ were considered statistically significant. When adjusted for FDR, we report FDR corrected p values. There were no missing data and outliers were included in the analysis.

Results

Demographic and clinical characteristics

In total, 160 patients and 30 healthy control participants were recruited; PD ($n=31$), PSP ($n=33$), CBS ($n=14$), MSA ($n=31$), AD ($n=26$), FTD ($n=16$) and 'unclassifiable' patients with parkinsonism ($n=9$; table 1). The APS groups had significantly shorter disease duration and significantly greater disease severity (H&Y score) compared with the PD group, but there was no significant difference in gender or age (table 1). Overall, there was a significant difference in age, with controls, patients with AD and FTD being younger, but not among the parkinsonian groups (PD, PSP, CBS, MSA). The disease duration was longer in the PD group, but did not differ among the other disease groups. There was no difference in UPDRS and MMSE within parkinsonian groups.

Associations between demographic/clinical characteristics and CSF biomarkers in patients with parkinsonism

Higher NFL levels correlated with greater disease severity as measured with the H&Y score ($R_s=0.24$) but not with disease duration ($R_s=0.05$). Higher α -syn levels correlated with advancing age and longer disease duration ($R_s=0.34$ and $R_s=0.22$, respectively). There were no other correlations of disease severity or disease duration with any of the other markers. Increased age was associated with higher levels of p - τ and both $sAPP\alpha$ and $sAPP\beta$ ($R_s=0.44$ and $R_s=0.21$, respectively).

Total τ was highly correlated with p - τ and α -syn ($R_s=0.74$ and 0.75 , respectively) and with YKL-40 (0.42). P - τ was highly correlated with α -syn ($R_s=0.77$), which in turn was correlated with $A\beta_{42}$ ($R_s=0.48$), YKL-40 ($R_s=0.44$), and $sAPP\alpha$ and $sAPP\beta$ ($R_s=0.42$ and $R_s=0.47$, respectively). NFL was correlated with YKL-40 ($R_s=0.38$); $sAPP\alpha$ was highly correlated with $sAPP\beta$ ($R_s=0.95$), which in turn was correlated with $A\beta_{42}$ ($R_s=0.41$).

Targeted biomarkers results: unadjusted analysis

For all markers except MCP-1, we found significant differences across the examined groups. MCP-1 levels were significantly higher in MSA versus healthy controls. As expected, $A\beta_{42}$, t - τ and p - τ differed between AD and all other groups, but did not discriminate between parkinsonian syndromes. Healthy controls had the lowest NFL levels (figure 1). APS had higher NFL levels compared with PD, AD and FTD. MSA was the group with the highest NFL levels and the lowest α -syn levels. PD, PSP and healthy controls had similar α -syn results. Similar to NFL, YKL-40 levels were higher in APS compared with PD and FTD. Healthy controls had the lowest YKL-40 levels. $sAPP\alpha$ and $sAPP\beta$ both showed similar

results in that they were both reduced in APS compared with PD. PD, dementia (AD/FTD) and healthy controls had similar levels of sAPP α and sAPP β .

Multivariable analysis

Using ROC curves, these nine biomarkers combined, as well as disease duration and severity (H&Y score) could differentiate patients with PD from APS (PSP, CBS, MSA) with an AUC of 0.95 (95% CI 0.88 to 0.99) and a sensitivity and specificity of 91%. In an FDR corrected reverse stepwise regression analysis, NFL (regression coefficient -0.0001 , $p < 0.001$), sAPP α (regression coefficient 0.0009 , $p < 0.001$) and α -syn (regression coefficient 0.0002 , $p = 0.003$) independently predicted diagnosis of PD versus APS. In addition, disease duration (regression coefficient 0.05 , $p < 0.001$) and disease severity (regression coefficient -0.13 , $p = 0.002$) were also found to be significant. Using ROC curves for these three significant biomarkers and two confounders resulted in an AUC of 0.93 (95% CI 0.87 to 0.99; figure 2).

CSF sAPP α concentrations of 485 ng/mL or higher showed 74% sensitivity and 65% specificity for the diagnosis of PD. CSF NFL concentrations of 1325 ng/L or lower showed 70% sensitivity and 36% specificity for the diagnosis of PD. Using a cut-off point of 1628 ng/L or higher for α -syn gave a sensitivity of 55% and a specificity of 70% differentiating PD from APS.

When we looked into the APS subgroups separately, we found the same panel of nine biomarkers could discriminate between PD and PSP (AUC 0.95, 95% CI 0.87 to 0.99), PD and CBS (AUC 0.98, 95% CI 0.97 to 0.99), and PD and MSA (0.96, 95% CI 0.91 to 0.99). Taking age into account, PSP could be differentiated from MSA with an AUC of 0.84 (95% CI 0.73 to 0.94) with 80% sensitivity and 78% specificity. In an FDR corrected reverse stepwise regression analysis, NFL ($p < 0.01$) and age ($p < 0.001$) independently predicted diagnosis of PSP versus MSA. Using ROC curves for NFL levels and age only resulted in an AUC of 0.81 (95% CI 0.69 to 0.92; figure 3).

There was very good discriminatory power between all parkinsonian groups (PD, PSP, CBS, MSA) and healthy controls with an AUC of 0.98 (95% CI 0.97 to 0.99) using the same panel of nine biomarkers (figure 4A). Parkinsonian groups could be differentiated from dementia disorders with an AUC of 0.90 (95% CI 0.84 to 0.96) (figure 4B). In particular, CBS was discriminated from AD and FTD with an AUC of 0.93 (95% CI 0.85 to 0.99).

Longitudinal data

CSF was collected serially from 10 patients over the course of 1 year; that is, 10–12 months apart. Of those 10 patients, 9 had a clinical PSP diagnosis (2 of them were pathologically confirmed) and 1 had a CBD pathological diagnosis. Disease severity (H&Y score) and clinimetric scales (PSPRS and DRS-2) were also used to assess disease progression.

Within 1 year, there was an increase in H&Y score (mean 1.2, SD 0.82) and in PSPRS (mean 14.2, SD 10.6), and a decrease in DRS-2 (mean -0.2 , SD 6.7). Levels of NFL were increased and levels of sAPP β were decreased over the course of 1 year (mean 540, SD 367; and mean -3 , SD 63, respectively).

Pathologically confirmed/genetically defined group

Since the beginning of the study, 12 patients died and donated their brains for pathological examination. Six patients with PSP, three MSA, two CBD and one AD were diagnosed according to standard pathological criteria. Two of the 11 patients with parkinsonism were misclassified during life (1 patient was clinically diagnosed with PSPRS and had CBD pathology, and 1 was clinically diagnosed with MSA-C and had PSP pathology). In addition, three patients have been genetically defined; one patient with AD (*PSEN1*) and two patients with FTD (*C9ORF72* and *MAPT* 10+16).

Patients with APS with pathologically confirmed diagnoses had significantly higher NFL levels, and significantly lower sAPP α and sAPP β levels compared with those with clinical diagnoses. There was a difference between clinically diagnosed cases and pathologically confirmed cases: -119 (95% CI 231.2 to -6.9) $p=0.038$ and -62.76 (95% CI -125.0 to -0.5) $p=0.048$ in sAPP α and sAPP β , respectively. The log NFL difference was 0.57 (95% CI 0.17 to 0.98) $p=0.006$.

Discussion

Accurate diagnosis is important in neurodegenerative disease to guide clinical management and to provide appropriate advice on prognosis regarding rate of progression and prediction of patients' response to medication. Our panel of nine CSF biomarkers was able to differentiate PD from APS, and the subtypes of APS from one another. In addition, using this panel we were able to differentiate between parkinsonian groups and dementia disorders, as well as healthy controls. Distinguishing PD from more aggressive forms of parkinsonism is a very important step in the diagnosis of parkinsonian syndromes. Of the biomarkers we have evaluated, NFL, sAPP α and α -syn seem to be the most discriminatory.

Neurofilament light chain

NFL levels correlated with greater disease severity, perhaps indicating more widespread or rapid neuronal degeneration, which is characteristic of APS. In our longitudinal group consisting of nine patients with PSP and one CBD (three of whom were pathologically confirmed) we found a progressive increase in NFL and a decrease in sAPP β levels over the course of 1 year. In recent studies, NFL levels were found to be highest in APS and could discriminate APS from PD but could not differentiate between the different APS subtypes.^{6,7,25,26} Contrary to our findings, Constantinescu *et al*²⁵ assessed consecutive analyses of CSF NFL in PD and APS but found no significant changes over 1 year and no correlation with disease severity. However, a recent study showed that higher levels of NFL correlated with greater disease severity in PD, PSP and AD.⁶

sAPP α and sAPP β

We also found that sAPP α levels were lower in APS compared not only with PD, but also with AD. Bech *et al* studied CSF sAPP α and sAPP β levels in 52 patients with parkinsonism (MSA $n=10$, PSP $n=10$, dementia with Lewy bodies (DLB) $n=11$ and PD $n=22$) and found no significant difference between the groups but a trend towards lower levels in MSA and DLB, compared with PSP and PD.¹⁷ The reason for the APP reduction is unclear, but it is

interesting to note that APP is bound to the mitochondrial outer membrane and has been implicated in mitochondrial dysfunction, which may contribute to some neurodegenerative diseases.²⁰ In any case, lower CSF sAPP α levels in APS, including CBS, suggest that APP metabolism is altered in these conditions in a manner which is distinct from that in PD and AD.

Pathologically confirmed group

The fact that we found that pathologically confirmed APS cases had significantly higher NFL levels and significantly lower sAPP α and sAPP β levels than those diagnosed clinically, considerably strengthens our findings and reinforces the importance of pathological diagnosis when interpreting biomarker results, particularly in conditions with overlapping clinical features.

In our pathologically confirmed group, 2 out of 11 patients with APS were misdiagnosed during life, highlighting the need for effective diagnostic markers. In a clinicopathological study of patients with parkinsonism seen by movement disorders specialists, 88% of patients with MSA, 80% with PSP and 30% with CBD were diagnosed correctly during life.²⁷ Older series show less accurate results: approximately 70% accuracy of clinical diagnosis in MSA seen by movement disorders specialists, which can drop to 50% in patients diagnosed by general neurologists.^{28,29} In PSP, correct diagnosis during life can range between 41% and 88%.^{30,31}

α -Synuclein

With regards α -syn our results partly replicate the literature. In our study, α -syn levels were lowest in MSA, but they were not reduced in PD. Several groups have found decreased α -syn levels in MSA compared with controls.^{6,32,33} There are inconsistent results when looking at α -syn levels in PD; most studies have shown decreased levels in PD^{32,34,35} but not all.^{36,37}

YKL-40

YKL-40's exact physiological role is unknown but it has been implicated in neuroinflammation and neurodegeneration and is considered a glial activation marker. In our study, increased levels of YKL-40 were found in APS (in a similar pattern to NFL) and PSP patients had the highest concentration, whereas patients with PD had the lowest. These results are similar to those found in a recent study showing significantly lower CSF YKL-40 levels in PD compared with patients with MSA, PSP, CBD and controls.³⁸

Diagnostic accuracy in dementia and unclassifiable parkinsonian groups

We found very good discriminatory power between all parkinsonian groups and patients with dementia, and healthy controls. CBS could be differentiated from AD and FTD with an AUC of 0.93. Finally, using cut-off points of >485 ng/mL for sAPP α , <1325 ng/L for NFL and >1628 for α -syn, we can speculate that the unclassifiable parkinsonian group is more likely to have an underlying PD rather than APS diagnosis.

Strengths

Our study is a longitudinal, prospective study and a proportion of the patients have now received a definitive pathological or genetic diagnosis. A very strict protocol for the collection and analysis of the samples was carried out in order to control for preanalytical and analytical factors. Our control group only included elderly, healthy controls. This is different to other studies, where 'neurological' controls, including participants with possible neurodegenerative processes, such as patients with mild cognitive impairment or normal pressure hydrocephalus, have been used.^{39,34}

To our knowledge, only one previous study has evaluated the diagnostic accuracy of several biomarkers in dementia and parkinsonism when including a relevant number of patients from PD and APS groups.⁶ Hall and colleagues assessed the accuracy of a panel of five diagnostic CSF biomarkers in patients with dementia and parkinsonism. We found similar results in terms of NFL levels being able to differentiate PD from APS. However, that study did not include YKL-40, MCP-1, sAPP α and sAPP β . In addition, the main emphasis was on dementia disorders (differentiating DLB and PDD from AD) and there was no longitudinal group. Only 1 patient in the APS group had a definitive pathological diagnosis (1%) compared with 11 in our study (14%). In addition, we found that the use of our panel of CSF biomarkers was able to improve diagnostic accuracy from 85% to 90% and showed promise in differentiating between the different atypical parkinsonian groups.

Limitations

APS are uncommon disorders even in specialist tertiary referral centres and, as a consequence, there are relatively small numbers in each group, making multiple comparisons unreliable. Nevertheless, we have identified trends in all our groups (cross-sectional, longitudinal and pathologically confirmed), which can be used to generate further hypotheses. In order to understand fully the value of these biomarkers, a validation cohort and pathological confirmation are needed. Future studies should also address dopaminergic treatment as a potential confounding factor. Our findings were obtained in patients with well-established disease and the majority of the parkinsonian participants were on dopaminergic medication. Finally, some disease groups (such as CBS and FTD) can have different underlying pathologies making their differentiation at a biochemical level even more challenging.

Age may have an impact on CSF biomarker levels. In our study, we found that p- τ , sAPP α and sAPP β levels are positively correlated with increasing age. This needs to be taken into account when interpreting results from participants of different age groups and that is why we included a control group consisting of elderly participants. When differentiating PD from APS, disease duration and disease severity were significant nuisance covariates and were built into the final model. Using nine CSF biomarkers at the same time as a diagnostic tool is not practical or easy to interpret in a clinical setting. We attempted to exclude from the final model biomarkers, such as MCP-1, which contributed very little in terms of increasing diagnostic accuracy in PD versus APS groups. CSF NFL, sAPP α and α -syn levels were the most discriminatory and the combination of these three biomarkers should be included in the final PD versus APS diagnostic model.

Future directions

The role of sAPP α and sAPP β and its mechanism of action should be investigated further in parkinsonian conditions. Microglia activation has been implicated in neurodegenerative conditions and increased CSF YKL-40 in PSP could be a potential specific marker for the disease. YKL-40 should be used in combination with radiological markers, such as midbrain to pons ratio,⁴⁰ to assess whether there is improved diagnostic accuracy. Increasing levels of NFL and decreasing levels of sAPP α and sAPP β in APS could be used as markers of disease progression in clinical trials.

The aim in all neurodegenerative conditions is to find therapies that slow or halt disease progression. These therapies must be offered as early as possible, when the minimum of irretrievable neuronal loss has occurred. Being able to diagnose accurately parkinsonian conditions in the very earliest stages of the disease is an essential first step in order to investigate and apply potential treatments.

Our study suggests diagnostic utility but needs replication in larger cohorts of patients assessed early in the disease course when diagnostic uncertainty is greatest. Those studies should also include pathological confirmation.

Conclusion

A panel of nine CSF biomarkers was able to discriminate PD from APS, and subtypes of APS from one another with levels of NFL, sAPP α and α -syn contributing most to the diagnostic accuracy. This may have important clinical benefit in terms of improving diagnostic accuracy, in turn allowing better prognostication and earlier access to potential disease-modifying therapies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Box plots showing levels of biomarkers in parkinsonian conditions, dementia disorders and healthy controls. Levels of CSF biomarkers in the different diagnostic groups. Box plots of t- τ , p- τ , A β 42, α -syn, NFL, YKL-40, MCP-1, sAPP α and sAPP β . The lower, upper and middle lines of boxes correspond to the 25th centile, 75th centile and median, respectively. The whiskers at the top and bottom extend from the 5th to the 95th centiles, respectively, and the dots represent outliers. CSF, cerebrospinal fluid; PSP, progressive supranuclear palsy; CBS, corticobasal syndrome; MSA, multiple system atrophy; PD, Parkinson's disease; AD, Alzheimer's disease; FTD, frontotemporal dementia; t- τ , total τ ; p- τ , phosphorylated τ ; A β 42, amyloid β 42; α -syn, α -synuclein; NFL, neurofilament light chain; YKL-40, tyrosine (Y), lysine (K) and leucine (L) 40 kDa; MCP-1, monocyte chemoattractant protein-1; sAPP α , soluble amyloid precursor protein α ; sAPP β , soluble amyloid precursor protein β .

Figure 2.

ROC curves showing the three most discriminatory analytes (NFL, sAPP α and α -syn) and two nuisance covariates (disease duration and severity) differentiating PD from APS. (A) Individual ROC curves performed to examine the relationship between diagnostic sensitivity and specificity for the three most discriminatory analytes and two nuisance covariates when differentiating PD from APS. (B) Multivariate discriminant analysis was used to study diagnostic accuracy when the three most discriminatory analytes and two nuisance covariates were studied simultaneously producing a single ROC curve for the diagnostic accuracy of PD versus APS. ROC, receiver operating characteristic; PD, Parkinson's disease; APS, atypical parkinsonian syndrome; α -syn, α -synuclein; NFL, neurofilament light chain; sAPP α , soluble amyloid precursor protein α ; HY score, Hoehn and Yahr score of disease severity.

Figure 3.

ROC curves showing NFL and age, and the combination of the two differentiating PSP from MSA. (A) Individual ROC curves performed to examine the relationship between diagnostic sensitivity and specificity CSF NFL, and age when differentiating PSP from MSA. (B) Multivariate discriminant analysis was used to study diagnostic accuracy when NFL and age were both studied simultaneously producing a single ROC curve for the diagnostic accuracy of PSP versus MSA. PSP, progressive supranuclear palsy; MSA, multiple system atrophy; ROC, receiver operating characteristic; CSF, cerebrospinal fluid; NFL, neurofilament light chain.

Figure 4.

ROC curves showing the combination of all nine analytes differentiating all parkinsonian groups from healthy controls and dementia disorders. (A) ROC curve combining all nine analytes to show the diagnostic accuracy of parkinsonian groups (PD, PSP, CBS, MSA) and healthy controls. (B) ROC curve combining all nine analytes to show the diagnostic accuracy of parkinsonian groups (PD, PSP, CBS, MSA) and dementia disorders (AD and FTD). ROC, receiver operating characteristic; PD, Parkinson's disease; PSP, progressive supranuclear palsy; CBS, corticobasal syndrome; MSA, multiple system atrophy; AD, Alzheimer's disease; FTD, frontotemporal dementia.

Table 1

Characteristics of the study population according to disease group

N	HC 30	PSP 33	CBS 14	MSA 31	PD 31	Unclass 9	AD 26	FTD 16	p Value
Gender n male (%)	15 (50%)	19 (57.6%)	4 (28.6%)	16 (51.6%)	20 (64.5%)	4 (44.4%)	9* (34.6%)	11 (68.8%)	0.17
Age mean (95% CI)	59.8 (56.1 to 63.4)	70.3 [†] (68.2 to 72.4)	69.8 [†] (65.5 to 74.1)	64.3 [†] (62.2 to 66.4)	67.1 [†] (64.0 to 70.2)	72.9 [†] (66.5 to 79.3)	62.8 [†] (57.7 to 68.3)	63.0 [†] (57.7 to 68.3)	<0.0001
DisDur (years) median (IQR)	–	5 (3, 7)	3.5 (2–5)	4 (3–6)	8 [†] (5–15)	3 (2–5)	3 [†] (2–4)	2.5 [†] (2–4.5)	<0.0001
H&Y score mean (95% CI)	–	3.7 (3.4 to 4.1)	3.2 (2.8 to 3.6)	3.2 (2.9 to 3.6)	2.8 [†] (2.4 to 3.1)	2.8 [†] (2.4 to 3.3)	–	–	0.002
t-τ (ng/L) median (IQR)	304 (189, 402)	256 (234, 363)	286 (234, 381)	275 (210, 341)	339 (226, 444)	356 (215, 364)	806 [†] (744 to 868)	242 [†] (219, 362)	<0.0001
p-τ (ng/L) median (IQR)	38 (29, 54)	34 (31, 44)	38 (30, 45)	33 (28, 37)	39 (31, 54)	36 (32, 39)	82 [†] (74 to 90)	35 [†] (30, 45)	<0.0001
Aβ42 (ng/L) mean (95% CI)	960 (847 to 1073)	713 (628 to 798)	715 (553 to 878)	705 (606 to 805)	806 (693 to 919)	691 (528 to 853)	391 [†] (324 to 458)	895 [†] (724 to 1065)	<0.0001
NFL (ng/L) median (IQR)	560 (444, 854)	2219 [†] (1793, 2870)	1937 [†] (1465, 3434)	3024 [†] (1984, 3818)	966 [†] (755, 1841)	1042 [†] (755, 1841)	1231 [†] (1046, 1557)	1227 [†] (925, 2132)	<0.0001
α-syn (ng/L) mean (95% CI)	1782 (1516 to 2048)	1782 [†] (1516 to 2048)	1497 (1183 to 1811)	1347 [†] (1162 to 1531)	1767 [†] (1519 to 2016)	1555 (1245 to 1864)	1947 [†] (1675 to 2218)	1476 [†] (1240 to 1711)	0.002
YKL40 (ng/L × 10 ⁴) mean (95% CI)	12.4 (9.7 to 15.0)	21.5 [†] (18.6 to 24.5)	21.5 [†] (17.3 to 25.8)	19.2 [†] (16.4 to 21.9)	17.3 (12.6 to 22.1)	17.1 (13.8 to 20.4)	18.7 [†] (16.1 to 21.3)	16.2 [†] (13.5 to 18.9)	<0.0001
MCP-1 (ng/L) mean (95% CI)	446 (372 to 520)	527 (472 to 582)	531 (406 to 655)	568 [†] (503 to 633)	460 (321 to 598)	559 (453 to 666)	511 (445 to 576)	447 [†] (382 to 512)	0.11
sAPP _α (ng/mL) mean (95% CI)	576 (500 to 651)	440 [†] (387 to 493)	394 [†] (271 to 516)	410 [†] (347 to 474)	579 [†] (504 to 653)	514 (392 to 636)	557 [†] (479 to 634)	526 (400 to 651)	0.0007
sAPP _β (ng/mL) mean (95% CI)	336 (291 to 382)	256 [†] (224 to 287)	238 [†] (167 to 309)	249 [†] (210 to 289)	318 [†] (281 to 356)	277 (215 to 338)	309 [†] (267 to 352)	283 (217 to 350)	0.009

Only statistically significant results are noted.

α-syn, α-synuclein; AD, Alzheimer's disease; Aβ42, amyloid β 42; CBS, corticobasal syndrome; DisDur, disease duration; FTD, frontotemporal dementia; H&Y score, Hoehn and Yahr score; HC, healthy controls; MCP-1, monocyte chemoattractant protein-1; MSA, multiple system atrophy; NFL, neurofilament light chain; PD, Parkinson's disease; PSP, progressive supranuclear palsy; p-τ, phosphorylated τ; sAPP_α, soluble amyloid precursor protein α; sAPP_β, soluble amyloid precursor protein β; t-τ, total τ; Unclass, unclassifiable patients with parkinsonism; YKL-40, tyrosine (Y), lysine (K) and leucine (L) 40 kDa.

* Compared to PD.

[†] Compared to healthy controls.[‡] Compared to PSP.[§] Compared to CBS.[¶] Compared to MSA.

** Compared to unclassifiable patients with parkinsonism.

^{††} Compared to AD.