α-Synuclein and tau concentrations in cerebrospinal fluid of patients presenting with parkinsonism: a cohort study

Brit Mollenhauer, Joseph J Locascio, Walter Schulz-Schaeffer, Friederike Sixel-Döring, Claudia Trenkwalder, Michael G Schlossmacher

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Paracelsus-Elena-Klinik, Kassel, Germany (B Mollenhauer MD, F Sixel-Döring MD, C Trenkwalder MD); **Department of Neurology and Department of Clinical Neurophysiology, Georg-August University Goettingen, Goettingen, Germany** (B Mollenhauer, C Trenkwalder); **Department of Neurology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA** (J J Locascio PhD); **Department of Neuropathology, Georg-August University Goettingen, Germany** (W Schulz-Schaeffer MD); **Division of Neuroscience, Ottawa Hospital Research Institute, Department of Medicine, and Department of Cellular and Molecular Medicine, University of Ottawa, Ontario, Canada** (M G Schlossmacher MD FRCPC); **and Center for Neurologic Diseases, Department of Neurology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA** (B Mollenhauer, M G Schlossmacher)

Correspondence to: Dr Brit Mollenhauer, Paracelsus-Elena-Klinik, Klinikstrasse 16, D-34128 Kassel, Germany **brit.mollenhauer@pk-mx.de**

or

Dr Michael G Schlossmacher, OHRI-Neuroscience, University of Ottawa, 451 Smyth Road #1462, Ottawa, ON K1H 8M5, Canada **mschlossmacher@ohri.ca**

Summary

Background **Parkinson's disease, dementia with Lewy bodies, and multiple system atrophy are brain disorders characterised by intracellular α-synuclein deposits. We aimed to assess whether reduction of α-synuclein concentrations in CSF was a marker for α-synuclein deposition in the brain, and therefore diagnostic of synucleinopathies.**

Methods **We assessed potential extracellular-fluid markers of α-synuclein deposition in the brain (total α-synuclein and total tau in CSF, and total α-synuclein in serum) in three cohorts: a cross-sectional training cohort of people with Parkinson's disease, multiple system atrophy, dementia with Lewy bodies, Alzheimer's disease, or other neurological disorders; a group of patients with autopsy-confirmed dementia with Lewy bodies, Alzheimer's disease, or other neurological disorders (CSF specimens were drawn ante mortem during clinical investigations); and a validation cohort of patients who between January, 2003, and December, 2006, were referred to a specialised movement disorder hospital for routine inpatient admission under the working diagnosis of parkinsonism. CSF and serum samples were assessed by ELISA, and clinical diagnoses were made according to internationally established criteria. Mean differences in biomarkers between diagnostic groups were assessed with conventional parametric and non-parametric statistics.**

Findings **In our training set (n=273), people with Parkinson's disease, multiple system atrophy, and dementia with Lewy bodies had lower CSF α-synuclein concentrations than patients with Alzheimer's disease and other neurological disorders. CSF α-synuclein and tau values separated participants with synucleinopathies well from those with other disorders (p<0·0001; area under the receiver operating characteristic curve [AUC]=0·908). In the autopsy-confirmed cases (n=41), CSF α-synuclein discriminated between dementia with Lewy bodies and Alzheimer's disease (p=0·0190; AUC=0·687); in the validation cohort (n=407), CSF α-synuclein discriminated Parkinson's disease and dementia with Lewy bodies versus progressive supranuclear palsy, normal-pressure hydrocephalus, and other neurological disorders (p<0·0001; AUC=0·711). Other predictor variables tested in this cohort included CSF tau (p=0·0798), serum α-synuclein (p=0·0502), and age (p=0·0335). CSF α-synuclein concentrations of 1·6 pg/μL or lower showed 70·72% sensitivity (95% CI 65·3–76·1%) and 52·83% specificity (39·4–66·3%) for the diagnosis of Parkinson's disease. At this cutoff, the positive predictive value for any synucleinopathy was 90·7% (95% CI 87·3–94·2%) and the negative predictive value was 20·4% (13·7–27·2%).**

Interpretation **Mean CSF α-synuclein concentrations as measured by ELISA are significantly lower in Parkinson's disease, dementia with Lewy bodies, and multiple system atrophy than in other neurological diseases. Although specificity was low, the high positive predictive value of CSF α-synuclein concentrations in patients presenting with synucleinopathy-type parkinsonism might be useful in stratification of patients in future clinical trials.**

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Introduction

Intracellular accumulation of α-synuclein, a protein that is abundantly expressed in the brain, is a feature of several late-onset synucleinopathies. These disorders predominantly include Parkinson's disease, multiple system atrophy, and dementia with Lewy bodies. Genome-wide association studies of sporadic Parkinson's disease and multiple system atrophy have identified a strong association between disease risk and distinct single-nucleotide polymorphisms (SNPs) in the α-synuclein-encoding gene, *SNCA*. These results add to evidence that α-synuclein misprocessing is a seminal event in synucleinopathies.[1]

The quantification of α-synuclein in CSF with sandwich-type ELISA has been described by several groups in pilot studies.[2–10] Some investigators reported a decrease in the concentration of total CSF α-synuclein in patients with Parkinson's disease and dementia with Lewy bodies when compared with patients with Alzheimer's disease or other neurological disorders. So far, no information is available on CSF α-synuclein concentrations from patients with multiple system atrophy, patients who had autopsy-confirmed parkinsonism, or prospectively studied patients with different forms of parkinsonism.[2–11]

The most common neurodegenerative causes of parkinsonism are Parkinson's disease, multiple system

atrophy, dementia with Lewy bodies, progressive supranuclear palsy, and corticobasal degeneration, with the latter two disorders linked to misprocessing of tau. It is estimated that greater than 70% of clinically diagnosed cases of so-called typical Parkinson's disease feature microscopically detectable α-synuclein-positive inclusions, which accompany the loss of dopamine-producing neurons at autopsy.[12] The remaining proportion encompasses clinical syndromes that present with similar symptoms without evidence of neural synucleinopathy.

Parkinson's disease is diagnosed on the basis of subjective serial examinations to identify its cardinal motor deficits, to show disease progression, to document responsiveness to treatment with levodopa, and to exclude atypical signs. Diagnostic accuracy for Parkinson's disease is about 75% for general neurologists and greater than 90% for movement-disorder specialists.[12] Brain-bank studies in which clinical features have been identified ante mortem,[13] and progress in neuroimaging with radioactively labelled tracers,[14] have improved diagnostic accuracy for typical Parkinson's disease.[12] However, the definitive identification of an α-synuclein-linked variant versus Parkinson's disease with another cause (eg, a Lewy-body inclusion-negative form) cannot be achieved without tissue or DNA analysis. Therefore, a laboratory-based method is needed for the clinical diagnosis of synucleinopathies and for stratification of patients in future cause-directed trials.[15]

Based on our recent pilot study of 100 patients,[3] we speculated that the reduction of CSF α-synuclein concentrations might suggest intraneuronal accumulation (and deposition) of aggregated α-synuclein,[16] whereas its substantial rise probably signals widespread neurodegeneration.[3] We therefore sought to establish whether quantification of α-synuclein—by itself or in combination with other markers—could help in the diagnosis of typical Parkinson's disease in people with parkinsonism and, possibly, of related synucleinopathies (multiple system atrophy and dementia with Lewy bodies) in a larger cohort. Given the evidence of tau misprocessing in progressive supranuclear palsy-type parkinsonism, and because of the recent association of SNPs in the tau-encoding *MAPT* gene with risk of Parkinson's disease,[1] we added CSF tau as a possible laboratory marker for Parkinson's disease. Furthermore, we included the dementia-linked protein variant amyloid β $(Aβ)_{1–42}$ and the total protein concentration to the list of CSF marker candidates to be assessed.

Methods

Participants

According to best practice guidelines for biomarker development,[17] we first quantified CSF values in a training set—a new cross-sectional cohort of patients with a wide range of neurological disorders, including multiple system atrophy, dementia with Lewy bodies, and Parkinson's disease. For this training set, we aimed to recruit a cohort that was larger and diagnostically more diverse than that in our pilot study.[3] The sample size of up to 300 participants was chosen to allow single representation on assay plates for our first-generation ELISA platform (90 wells; 200 µL each) and double representation on assay plates for our second-generation α-synuclein ELISA platform (384 wells; 50 µL). Patients were enrolled at the Departments of Psychiatry and Neurology at the University of Goettingen, Germany, at the Paracelsus-Elena-Klinik, Kassel, Germany (PEKK; a single-site hospital dedicated to the care of patients with movement disorders), and at Brigham and Women's Hospital, Boston, MA, USA. The clinical diagnoses of probable dementia with Lewy bodies, probable multiple system atrophy, probable Alzheimer's disease, and definite Parkinson's disease[3] were made according to internationally standardised criteria.[18–22] Each

	Alzheimer's disease	Neurological controls*	Dementia with Lewy bodies	Parkinson's disease	Multiple system atrophy
Number of patients	62	76	55	51	29
Number of men	22 (36%)	33 (43%)	27 (49%)	32 (63%)	16 (55%)
Age (years)	69 (10); 39–86	54 (21); 15–90	71 (7); 55–85	73 (7); 49–83	67 (7); 52–77
Duration of disease (months)	30 (18); 12–72	..	27 (17); 13–90	146 (77); 24–312	35 (20); 12–84
Dose equivalent of levodopa† (mg)	0	0	107 (220); 0–1065	723 (424); 0–1800	295 (370); 0–1215
CSF total protein (µg/µL)	0·73 (0·19); 0·22–1·20	0·92 (0·46); 0·37–3·07	0·73 (0·27); 0·13–1·76	1·05 (0·39); 0·57–3·20	0·79 (0·15); 0·54–1·07
CSF α-synuclein (pg/µL)	1·85 (1·47); 0·48–7·29	1·73 (1·83); 0·46–7·42	1·42 (1·26); 0·51–7·15	1·19 (0·81); 0·47–4·71	1·24 (0·99); 0·69–6·18
Ratio, CSF α-synuclein to total protein (pg/µg)	2·85 (2·52); 0·62–12·14	1·79 (1·46); 0·48–8·21	2·14 (1·75); 0·30–9·32	1·21 (0·87); 0·43–6·35	1·66 (1·72); 0·79–10·39
CSF tau protein‡ (pg/mL)	726 (314); 178–2064 (n=37)	174 (129); 75–373 (n=6)	361 (270); 103–850 (n=9)	189 (126); 75–626 (n=47)	147 (140); 75–527 (n=10)

Data are mean (SD); range; unless otherwise indicated. When comparing patients with Parkinson's disease without any levodopa treatment and without levodopa ingestion during 12 h before lumbar puncture versus those who had taken the drugs within the past 12 h, we did not find any significant difference in CSF values (CSF α-Syn p=0·39; CSF protein p=0·75). This observation was confirmed with Wilcoxon rank sum testing and in a distribution free raw score permutation test. *Included participants with sudden-onset headache (n=33), chronic inflammatory demyelinating polyradiculitis (n=9), pseudotumour cerebri (n=6), major affective disorder (n=6), focal cerebrovascular disease (n=5), epilepsy (n=4), amyotrophic lateral sclerosis (n=3), multiple sclerosis (n=2), normal-pressure hydrocephalus (n=2), brain tumour (n=2), acute myelitis (n=1), paraneoplastic syndrome (n=1), systemic lupus (n=1), and carcinomatosis meningeae (n=1). †Calculated according to published guidelines.[34,35] ‡Measured only in participants for whom enough CSF was available.

Table 1: **Demographics and CSF values in the training cohort**

participant underwent neuroimaging (CT, MRI, or both) to identify structural causes of illness. Cognition in patients with Alzheimer's disease and dementia with Lewy bodies was assessed by mini-mental state examination scores (Alzheimer's disease mean 18·4 [SD 5·7]; dementia with Lewy bodies 18·7 [4·9]);[23] staging in patients with Parkinson's disease was recorded with Hoehn and Yahr scores during off-periods (mean 3·9 [SD 0·7]). Assigned diagnoses were independently reviewed by a board-certified neurologist with subspecialty training in movement disorders and dementias (BM).

Because clinical diagnosis of dementia with Lewy bodies is consistently less accurate than that of typical Parkinson's disease—in particular in the differentiation of dementia with Lewy bodies from the most common form of dementia, Alzheimer's disease[24]—we assessed CSF concentrations in an independent cohort of patients with a definite diagnosis. CSF specimens were drawn ante mortem during routine clinical investigations; the brains of these individuals were examined at participating sites of the BrainNet autopsy service. These patients were diagnosed with Alzheimer's disease, dementia with Lewy bodies, corticobasal degeneration, cerebrovascular disease, or frontotemporal dementia. Three cases of Huntington's disease were confirmed by genotyping.

For more on the **BrainNet autopsy service** see http://www.brainnet-europe.org/

For validation purposes, between January, 2003, and December, 2006, we prospectively enrolled consecutive inpatients at the PEKK. This cohort comprised patients who were referred by community practitioners for routine hospital admission under the working diagnosis of parkinsonism. The size of this cohort was chosen to ensure sufficient representation of less common causes of parkinsonism (eg, progressive supranuclear palsy); our cutoff size for the creation of a subgroup with parkinsonism was eight, as informed by results from our previously published pilot study (eight patients with prion disease had a detectable difference in their CSF α-synuclein concentrations).[3] The analysis of this cohort was designed specifically to establish whether laboratory values in CSF, serum, or both could assist in the differentiation of synucleinopathy-type parkinsonism from other causes of this clinical syndrome. The inpatient investigation of all people referred for parkinsonism at the PEKK involves brain MRI, polysomnography, smell test, standardised levodopa test, CSF collection, and phlebotomy. Participants with a history of recent stroke or evidence of cerebrovascular changes on MRI according to Fazekas' criteria were excluded.[25]

We separated the participants in the validation cohort into six groups: dementia with Lewy bodies, Parkinson's disease, multiple system atrophy, progressive supranuclear palsy, normal-pressure hydrocephalus, and a heterogeneous group of other parkinsonian cases. Staging of patients with Parkinson's disease was documented by their Hoehn and Yahr score during off periods. Diagnoses were made by board-certified, PEKK-based neurologists with specialty training in movement disorders and independently confirmed by chart review (by BM) on the basis of documented seminal findings with internationally established criteria (including for normal-pressure hydrocephalus and progressive supranuclear palsy).[26,27]

Our study was done in accordance with the Declaration of Helsinki and with informed written consent provided by all patients or by their next of kin in the case of cognitive impairment (mini-mental status examination score[23] of 25 or below). Our study was approved by the

	Neurological controls*	Normal-pressure hydrocephalus	Progressive supranuclear palsy	Dementia with Lewy bodies	Parkinson's disease	Multiple system atrophy
Number of patients	23	22	8	66	273	15
Number of men	17 (74%)	19 (86%)	6 (75%)	47 (71%)	184 (67%)	10 (67%)
Age (years)	73 (8·5); 58–88	77 (6·4); 65–87	72·3 (6·0); 64–82	72 (7·2); 52–85	72 (6·8); 49–89	70 (7·1); 62–83
Duration of disease (months)	62 (48); 16–238	64 (46); 14–228	72 (30); 24–78	46 (47); 13–164	74 (42); 12–296	72 (38); 13–154
Dose equivalent of levodopa (mg)	229 (403); 0–450	308 (363); 0–1305	629 (549); 0–1731	260 (260); 0–1202	773 (434); 0–2743	383 (396); 0–1125
CSF total protein (μg/μL)	1·15 (0·65); 0·55–3·80	1·03 (0·26); 0·63–1·68	1·12 (0·44); 0·62–2·01	1·04 (0·33); 0·4–2·6	1·08 (0·32); 0·37–3·21	0·93 (0·20); 0·50–1·40
CSF α-synuclein (pg/μL)	2·22 (1·31); 0·95–6·07	1·72 (1·12); 0·53–5·87	1·78 (0·91); 0·97–3·81	1·32 (0·62); 0·17–3·09	1·34 (0·81); 0·14–6·62	1·11 (0·45); 0·23–2·00
Ratio, CSF α-synuclein to total protein (pg/μg)	2·17 (1·40); 0·61–6·51	1·71 (1·00); 0·51–4·86	1·66 (0·71); 0·73–2·93	1·36 (0·74); 0·14–4·01	1·35 (1·00); 0·27–10·18	1·31 (0·75); 0·15–3·27
CSF tau protein (pg/mL)	267 (543); 75–2497	157 (163); 75–841	154 (61); 75–226	192 (170); 75–1170	180 (131); 75–832	146 (76); 75–317
CSF amyloid β_{1-42} (pg/mL)	499 (184); 136–914	467 (196); 199–821	529 (176); 300–782	378 (141); 171–754	474 (189); 72–1083	460 (154); 187–752
Serum α-synuclein (pg/μL)	12·62 (7·12); 6·16–36·23	12·40 (7·08); 5·71–34·75	10·42 (1·63); 8·70–13·10	10·29 (4·08); 4·48–23·81	10·74 (4·11); 4·16–24·31	10·47 (5·25); 4·43–18·98

Data are mean (SD); range unless otherwise indicated. *Included patients with parkinsonism linked to the primary diagnosis of Alzheimer's disease (n=3), essential tremor without evidence of Parkinson's disease (also established by a negative nuclear medicine scan of dopaminergic terminals; n=1), drug-induced parkinsonism (n=2), frontotemporal dementia with parkinsonism (n=1), paraneoplastic syndrome (n=2), spinocerebellar ataxia (n=1), or subdural haemorrhage (n=2), or with undetermined cause (n=11).

***Table 2*: Demographics and CSF values in the validation cohort**

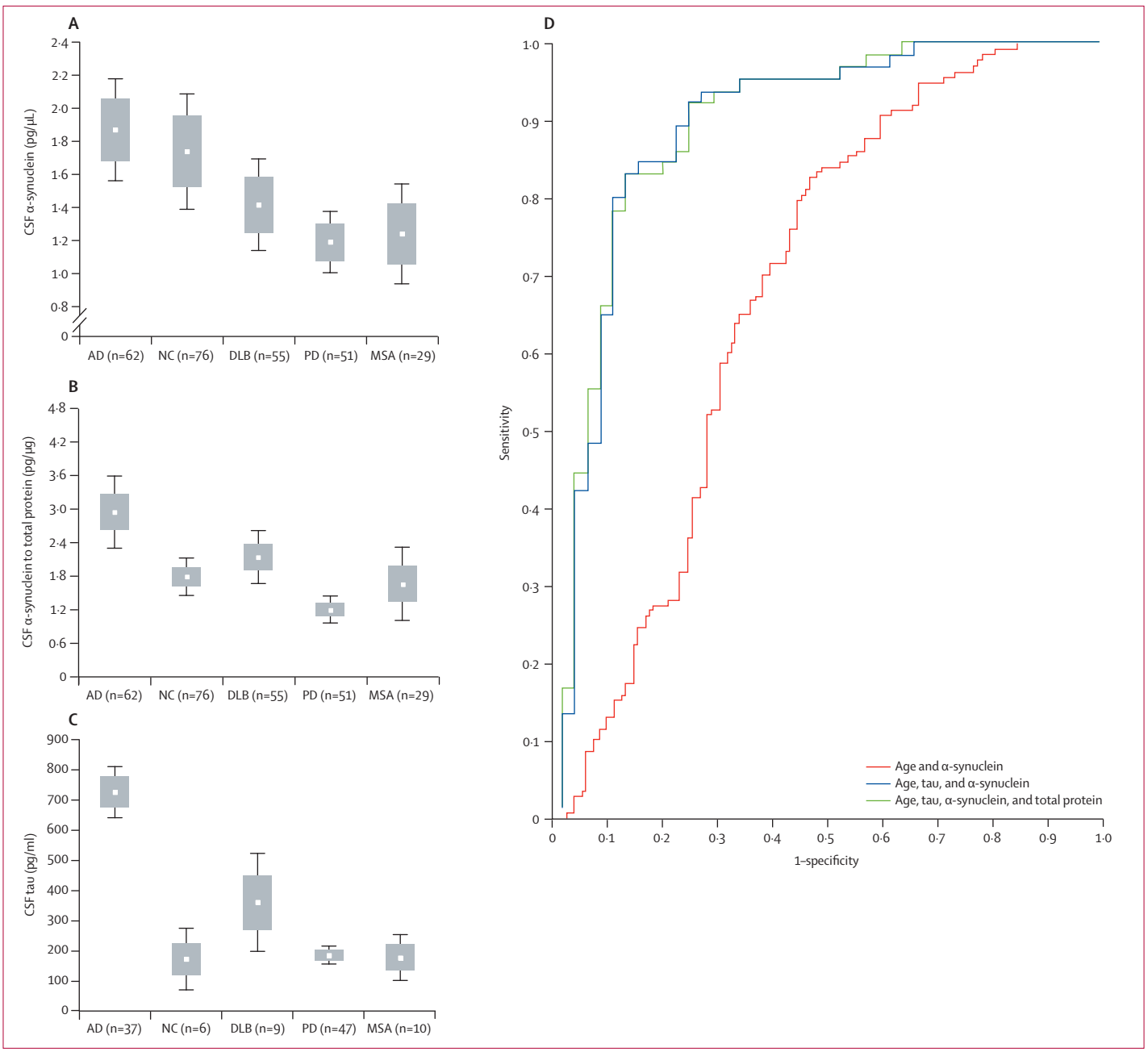

Figure 1: **α-Synuclein and tau in CSF from the training cohort**
ELISA results for total CSF α-synuclein concentration (A; DLB, PD, and MSA differed significantly [p<0·0001] from AD and NC), the ratio of CSF α-synuclein to total CSF protein (B), and total CSF tau concentration (C); white squares represent the mean, grey boxes the standard factor, and bars the 0·89 CI. Eight patients with MSA were diagnosed with the cerebellar variant and 21 with its parkinsonian subtype (no differences were recorded between the two). The webappendix (p 2) gives the values for each participant in A. (D) Logistic-regression analyses for comparison of AUCs for patients with synucleinopathy (ie, PD, MSA, or DLB) versus participants without synucleinopathy (ie, AD or other neurological disorders). The lines depict integrated sensitivity and specificity for two variables (age and CSF α-synuclein; AUC=0·694), three variables (age, CSF tau, and CSF α-synuclein; AUC=0·908), and four variables (age, CSF tau, CSF α-synuclein, and CSF protein; AUC=0·909). Logarithmic transformation of the three CSF variables generated indistinguishable results (AUC=0·713, AUC=0·897, and AUC=0·903, respectively) that were validated by an unbiased, leave-one-out prediction method (AUC=0·697, AUC=0·883, and AUC=0·864, respectively). AD=Alzheimer's disease. DLB=dementia with Lewy bodies. PD=Parkinson's disease. MSA=multiple system atrophy. AUC=area under the receiver operating characteristic curve. NC=neurological controls.

ethics committees at the University of Goettingen, the Board of Trial Registration Hessen (Germany), and Brigham and Women's Hospital. Autopsy consent was provided by the next of kin.

Procedures

CSF was collected according to our previously established, standardised operating procedures by routine lumbar puncture with serial polypropylene tubes. Samples were

	Numbers of patients	Model p value	AUC	Predictors	Corresponding regression coefficients (SE); p values
Training set (n=273)					
PD *vs* AD, NC	53 *vs* 138	<0·0001	0·849	Age CSF α-synuclein CSF total protein	0·0789 (0·0196); <0·0001 −0·7788 (0·2393); 0·0011 2·7421 (0·7647); 0·0003
PD, MSA *vs* AD, NC	82 *vs* 138	<0·0001	0·779	Age CSF α-synuclein CSF total protein	0·0580 (0·0138); <0·0001 −0·6024 (0·1813); 0·0009 1·7489 (0·6017); 0·0037
PD, MSA, DLB *vs* AD, NC	137 *vs* 138	<0·0001	0·712	Age CSF α-synuclein CSF total protein	0·0617 (0·0119); <0·0001 −0·3599 (0·1170); 0·0021 0·6484 (0·4322); 0·1336
PD, MSA, DLB *vs* AD, NC	66 *vs* 43	<0·0001	0·908	Age CSF tau CSF α-synuclein	0·0811 (0·0375); 0·0306 −0·00740 (0·00135); <0·0001 −0·2092 (0·2575); 0·4166
PD, MSA, DLB *vs* AD, NC	65 *vs* 43	<0·0001	0·909	Age CSF tau CSF α-synuclein CSF total protein	0·0865 (0·0387); 0·0255 −0·00731 (0·00140); <0·0001 −0·2091 (0·2595); 0·4204 0·0477 (0·6926); 0·9451
Autopsy cohort (n=41)					
DLB *vs* AD	13 *vs* 21	0·0190	0·687	CSF α-synuclein	−2·8843 (2·2802);..*
DLB *vs* AD	7 *vs* 17	0·2078	0·622	CSF tau	−0·00110 (0·00113);..*
Validation cohort (n=407)					
PD *vs* NC, NPH, PSP	257 *vs* 47	<0·0001	0·706	Age CSF α-synuclein Serum aSyn	−0·0462 (0·0255); 0·0705 −0·5752 (0·1524); 0·0002 −0·00263 (0·00158); 0·0959
PD, DLB *vs* NC, NPH, PSP	314 *vs* 46	<0·0001	0·711	Age CSF tau CSF α-synuclein Serum aSyn	−0·0543 (0·0255); 0·0335 0·00267 (0·00152); 0·0798 −0·6289 (0·1513); <0·0001 −0·00308 (0·00157); 0·0502
PD, DLB, MSA *vs* NC, NPH, PSP	348 *vs* 52	<0·0001	0·702	Age CSF tau CSF α-synuclein	−0·0564 (0·0242); 0·0200 0·000199 (0·000718); 0·7816 −0·6302 (0·1483);<0·0001
PD, DLB, MSA *vs* NC, NPH, PSP	348 *vs* 52	<0·0001	0·729	Age log CSF tau log CSF α-synuclein	−0·0638 (0·0243); 0·0087 0·6878 (0·2711);0·0112 −1·5235 (0·3219); <0·0001

AUC=area under receiver operating characteristic curve. PD=Parkinson's disease. AD=Alzheimer's disease. NC=neurological controls. MSA=multiple system atrophy. DLB=dementia with Lewy bodies. NPH=normal-pressure hydrocephalus. PSP=progressive supranuclear palsy. *With one predictor, p value same as for model.

Table 3: **Summary of statistical analyses of the three cohorts**

processed, as published in detail elsewhere, and assessed if they met CSF quality control criteria.[3,11] A total cell count was established in tube 1 (2 mL). Samples with erythrocyte counts greater than 500 cells per µL CSF in tube 1 were excluded from all analyses. Centrifugation (2000×g; 15 min; 4°C) of cell-free CSF supernatants (tube 2 containing about 10–20 mL) was done within 15 min of collection.[11] Serum was collected by routine phlebotomy before lumbar puncture. All blood and CSF specimens were drawn from fasting patients between 0800 and 0900 h.

Thawed undiluted CSF specimens and diluted serum samples were assessed in duplicate (with the operator blinded to the diagnosis) with a validated sandwich ELISA system (mSA1/Syn1-BB; 384 well-plate format) that measured total α-synuclein (webappendix p 1). α-Synuclein was assessed in all participants. Because tau has an emerging role in the pathogenesis of dementia and parkinsonism,[1] we added the quantification of CSF tau values after the study began in remaining CSF aliquots from the training cohort and in all participants from other cohorts. In view of the established correlation of $A\beta_{1-42}$ with dementia in patients with cognitive impairment attributable to Alzheimer's disease and, possibly Parkinson's disease,[28,29] we also measured CSF $A\beta_{1-42}$ levels in all participants. Because of the high rate of *SNCA* expression during haemopoiesis and the resulting substantially higher α-synuclein concentrations in blood products than in CSF,[11,30] we also measured serum α-synuclein concentrations in the validation cohort. In the validation cohort, we also calculated CSF-to-serum and serum-to-CSF ratios for total α-synuclein, as such indices are important in the diagnosis and causes of several neurological disorders.[31] Total CSF tau, CSF $A\beta_{1-42}$, and CSF protein were measured by commercially available validated ELISA (Innogenetics, Ghent-Zwijnaarde, Belgium) and protein detection kits (Bio-Rad Laboratories, Hercules, CA, USA).[3,32]

Statistical analyses

Logarithmic transformations of some variables were used as appropriate to a particular analysis to meet assumptions of normality of dependent variable residuals or to reduce excessive skewing and outlier influence in predictors for regressions. In addition to conventional parametric and non-parametric statistical tests (eg, multiple regression and ANCOVA with appropriate post-hoc tests [Tukey's]), which assessed mean differences in biomarkers between diagnostic groups, logistic-regression analyses were used. Logistic-regression analyses assessed, in a more clinically useful manner, biological and demographic variables and linear combinations of these, in terms of their predictive value for discriminating between diagnostic groups or sets of such groups (eg, synucleinopathy *vs* non-synucleinopathy groups). In most of these regressions, a backward-elimination method was used whereby an individual predictor or set of predictors were selected out from a superset of predictors by progressively eliminating those with the largest individual p value in the remaining set, one at a time, at each step in the process until only predictors significant at $p \leq 0 \cdot 05$ remained (or sometimes $p < 0 \cdot 1$; models with some non-significant predictors left in are reported for purposes of contrast with other models or to highlight the predictive value of the model as a whole). From these models, we derived odds ratios for predictors, sensitivity and specificity and related statistics, receiver operating characteristic (ROC) curves, and estimates of the area under these curves (AUC; AUC=0·5 indicates no discrimination and a perfect diagnostic test would have AUC=1). In exploring the sensitivity and specificity of various cutoffs for a single predictor (eg, CSF α-synuclein), we used Youden's suggested index[33] to identify optimum cutoffs (ie, the point on a ROC curve that lies farthest above the diagonal line where ordinate equals abscissa, which is thought by

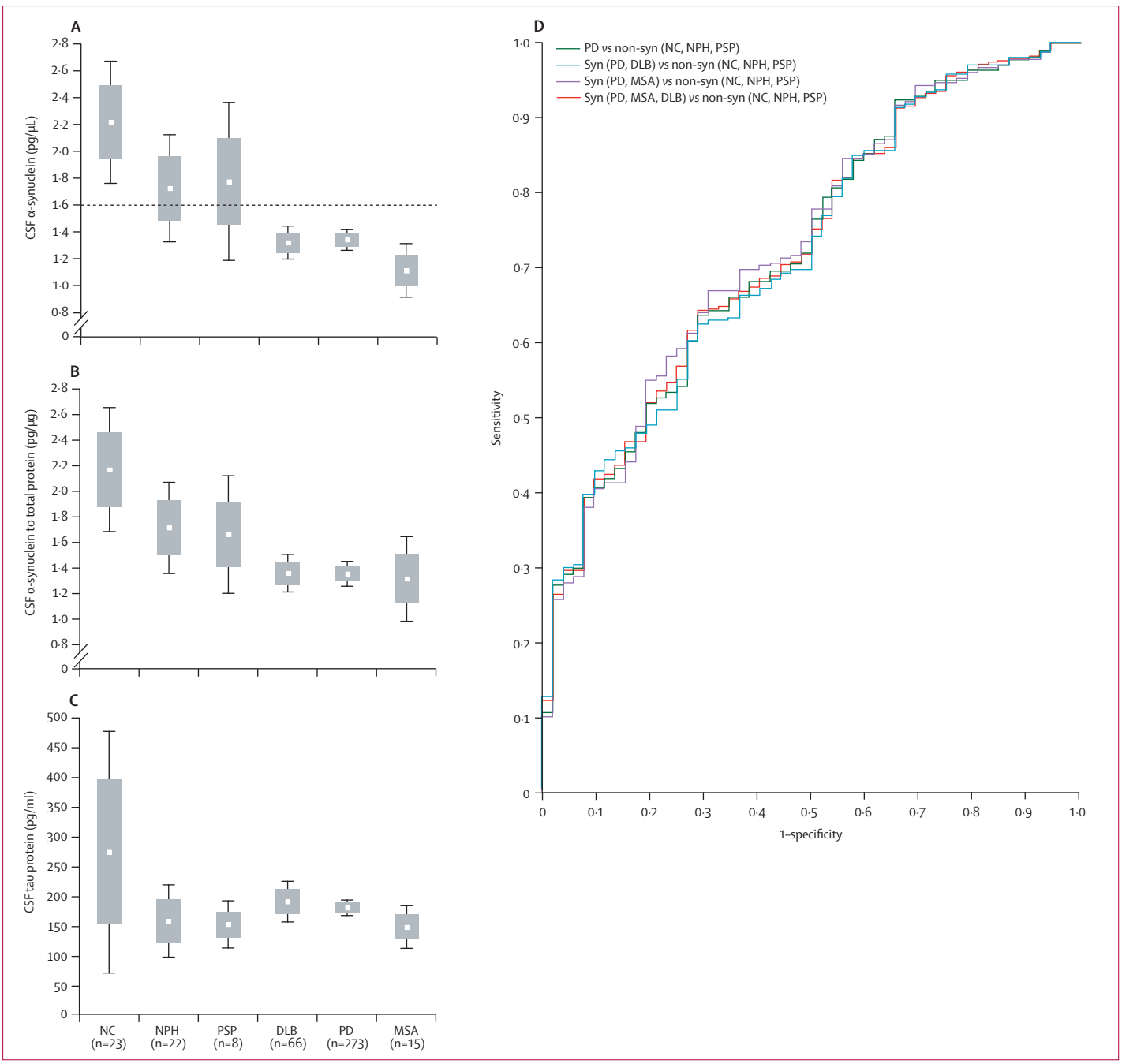

Figure 2: **α-Synuclein and tau in CSF from the validation cohort**
ELISA results for total CSF α-synuclein concentration (A), the ratio of CSF α-synuclein to total CSF protein (B; DLB and PD differed significantly [p<0·05] from the controls), and the total CSF tau concentration (C); white squares represent the mean, grey boxes the standard factor, and bars the 0·89 CI. The dotted line in A shows the best-fit cutoff for CSF α-synuclein to differentiate between participants with DLB, PD, and MSA versus those with NPH, PSP, or other neurological disorders at 1·6 pg/μL. The webappendix (p 2) gives the values for each participant in A. (D) Logistic-regression analyses for comparison of AUC for patients with synucleinopathy (various combinations of participants with PD, MSA, and DLB) versus participants without synucleinopathy (NPH, PSP, or other neurological disorders). The lines depict integrated sensitivity and specificity for the variables CSF α-synuclein, CSF tau, and age. NPH=normal-pressure hydrocephalus. PSP=progressive supranuclear palsy. DLB=dementia with Lewy bodies. PD=Parkinson's disease. MSA=multiple systems atrophy. NC=neurological controls. Syn=synucleinopathy. Non-syn=non-synucleinopathy. AUC=area under the receiver operating characteristic curve.

some to provide the best balance of both good sensitivity and specificity). Because the Youden criterion is not universally accepted, we also computed statistics at slightly different cutoffs to provide a sense of how this would cause results to vary. Statistical analyses were done with SAS version 9.2.

Role of the funding source

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Tables 1 and 2 show the demographics and CSF values of the participants in the training and validation cohorts. In the training cohort, log-transformed values of absolute and relative CSF α-synuclein concentrations showed significant differences in the 273 participants from the five diagnostic groups (table 1), without covariate adjustments (p=0·0156) and after covarying log CSF protein and age (p=0·0006; figure 1). Tukey's post-hoc tests showed that adjusted CSF α-synuclein values differed significantly between the Parkinson's disease and Alzheimer's disease groups (p=0·0002), but not between the dementia with Lewy bodies and Alzheimer's disease groups (p=0·0912).

In logistic-regression analyses, we found that three variables (age, CSF α-synuclein, and CSF protein) separated patients with Parkinson's disease from the combined groups of participants with Alzheimer's disease and other neurological disorders (table 3). When we compared all participants who had suspected synucleinopathy with those who had other diagnoses in this training set, participants with Parkinson's disease, multiple system atrophy, or dementia with Lewy bodies were less well discriminated than were patients with Parkinson's disease or multiple system atrophy (without Lewy body dementia cases), although the discrimination was significant in both cases (table 3). This difference is explained by the intermediate values of CSF α-synuclein in patients diagnosed with dementia with Lewy bodies (figure 1, table 1, and webappendix p 2).

See Online for webappendix

We also measured total tau concentrations in CSF from the 109 of 273 participants in the training cohort from whom sufficient CSF remained available (figure 1).[1] CSF tau levels were significantly different: in participants with Alzheimer's disease compared with the groups of patients with Parkinson's disease, multiple system atrophy, and other neurological disorders, as expected; they also differed in patients with Alzheimer's disease compared with those with dementia with Lewy bodies; and in patients with dementia with Lewy bodies compared with those with Parkinson's disease and multiple system atrophy (table 3). In logistic-regression analyses, the combination of age, CSF tau, and CSF α-synuclein discriminated the group of participants with synucleinopathy (Parkinson's disease, multiple system atrophy, or dementia with Lewy bodies) well from patients with Alzheimer's disease and other neurological disorders (table 3), where total tau was low in the patients with synucleinopathy and high in patients with Alzheimer's disease. When CSF total protein was added as a fourth factor, the AUC was 0·909, but CSF tau and age showed the strongest individual significance in this cohort (table 3).

We assessed CSF values in an independent cohort of 41 patients with dementia, movement disorders, or both, who had confirmation of their diagnosis by autopsy (webappendix p 3). In accordance with our results from the training set, we recorded low CSF α-synuclein levels in all definite cases of dementia with Lewy bodies when compared with Alzheimer's disease and other neurological disorders (table 3, webappendix p 3). We noted no correlation between CSF α-synuclein values in dementia with Lewy bodies and frequency of Lewy bodies in the cortex (data not shown).

We next sought to validate our findings in participants with various forms of parkinsonism. In this validation cohort, the absolute concentration of CSF α-synuclein and its relative amount (CSF α-synuclein/CSF protein) showed significant differences in 407 prospectively assessed participants (p=0·0001 and p=0·0018, respectively) from six diagnostic categories (figure 2, table 2). A backward-elimination multiple-regression model for the variables CSF α-synuclein and CSF tau was fitted with the predictor terms of 14 different biological indices, including diagnosis, age, sex, and drug effect. The model also included quadratic curvilinear effects and interaction terms. The only significant predictor for CSF α-synuclein—after removing non-significant factors—was the clinical diagnosis (p=0·001).

By contrast, the most relevant predictor for CSF tau (final retained model, p<0·0001) was donor age (positive linear), not the clinical diagnosis. We also found a reduction in mean CSF $A\beta_{1-42}$ values in the group of patients with dementia with Lewy bodies; together with their slightly higher mean tau concentrations (figure 2, table 2), this change probably indicates the presence of concomitant Alzheimer's disease pathology.

	Parkinson's disease *vs* non-synucleinopathy				Synucleinopathy *vs* non-synucleinopathy			
	Sensitivity	Specificity	PPV	NPV	Sensitivity	Specificity	PPV	NPV
≤1·4 pg/μL	56·04 (50·2–61·9)	62·26 (49·2–75·3)	88·44 (83·7–93·2)	21·57 (15·1–28·1)	57·06 (51·9–62·2)	62·26 (49·2–75·3)	90·99 (87·2–94·8)	17·84 (12·3–23·4)
≤1·6 pg/μL	70·7 (65·3–76·1)	52·83 (39·4–66·3)	88·53 (84·3–92·8)	25·9 (17·7–34·2)	69·21 (64·4–74·0)	52·83 (39·4–66·3)	90·74 (87·3–94·2)	20·44 (13·7–27·2)
≤1·8 pg/μL	82·78 (78·3–87·3)	45·28 (31·9–58·7)	88·63 (84·7–92·5)	33·8 (22·8–44·8)	81·92 (77·9–85·9)	45·28 (31·9–58·7)	90·91 (87·8–94·1)	27·27 (18·0–36·6)

Data are % (95% CI). PPV=positive predictive value. NPV=negative predictive value.

Table 4: **Sensitivity, specificity, positive predictive values, and negative predictive values, by CSF α-synuclein cutoff value**

A significant (p<0·001) backward-elimination (p=0·1 cutoff) multiple-logistic-regression model suggested three simultaneous predictors for the diagnostic probability of a synucleinopathy versus a non-α-synuclein-related form of parkinsonism (defined, in this cohort, as dementia with Lewy bodies, Parkinson's disease, and multiple system atrophy *vs* normal-pressure hydrocephalus, progressive supranuclear palsy, and other parkinsonism). These predictors were CSF α-synuclein (log p<0·0001), age (p=0·0087), and CSF tau (log p=0·0112). The AUC was 0·729 (figure 2). In our prediction model, the probability of a patient having Parkinson's disease or dementia with Lewy bodies (but not multiple system atrophy) versus progressive supranuclear palsy, normal-pressure hydrocephalus, and other parkinsonism also differed significantly (table 3) as predicted by age, CSF tau, CSF α-synuclein, and serum α-synuclein. The odds ratios were 0·533 for CSF α-synuclein (for a 1·0 pg/μL change; 95% CI 0·391–0·714; p<0·0001), 0·581 for age (for a decade; 0·347–0·947; p=0·0335), 1·305 for CSF tau (100 pg/mL; 1·003–1·816; p=0·0798), and 0·735 for serum α-synuclein (100 units of ELISA signal; 0·539–1·007; p=0·05). Simulation runs verified that the sensitivity and specificity values in this model did not depend on the unequal proportions of participants that represented the six groups (tables 2 and 3, and data not shown).

1·6 pg/μL, calculated by Youden's index, was the best-fit cutoff value for CSF α-synuclein to differentiate between patients with Parkinson's disease, multiple system atrophy, or dementia with Lewy bodies versus patients with normal-pressure hydrocephalus, progressive supranuclear palsy, or other neurological disorders (figure 2, table 4).

When we assessed the absolute or adjusted concentrations of serum α-synuclein in patients with Parkinson's disease, multiple system atrophy, or dementia with Lewy bodies in the validation cohort versus participants without synucleinopathy, we recorded respective differences of p=0·0210 and p=0·0522 in the raw values; however, we did not note any differences for logarithmically transformed values of serum α-synuclein and the ratio of serum α-synuclein to total serum protein concentration (ie, p=0·0715 and p=0·3017, respectively; table 2 and data not shown). We noted a marginal significance (p=0·04) for the log-transformed ratio of CSFα-synuclein to serum α-synuclein in the validation cohort when diagnostic groups were adjusted for sex, age, and drug treatment. However, in Tukey's-adjusted post-hoc tests no significant pair-wise differences between the groups were detected.

Discussion

Our study explored the usefulness of a recently detected marker candidate, CSF α-synuclein, for the diagnosis of patients with Parkinson's disease and related synucleinopathies, alone or together with CSF tau, CSF $A\beta_{1-42}$, CSF protein, and serum α-synuclein. Mean CSF α-synuclein values—but not the concentrations of any other marker candidate assessed—were significantly lower in patients with typical Parkinson's disease, in probable and definite cases of dementia with Lewy bodies, and in probable multiple system atrophy when compared with a wide range of neurological disorders.

So far, CSF α-synuclein has been quantified in participants with neurological disorders in nine cross-sectional studies (panel). Five of these reports[2,3,7,8,10] provide results that are consistent with those from our study; four others do not.[4–6,9] Differences in several variables might have contributed to the differences in results: technological platforms, antibody characteristics, collection protocols, and storage and processing steps (reviewed by Mollenhauer and colleagues[11]). Antibody characteristics in different ELISA protocols and sample collection are probably the most important variables to explain the discrepancy between these reports. Because α-synuclein is abundantly expressed during haemopoiesis,[11,30] sample processing in our cohorts was strictly standardised and donors of CSF specimens with blood contamination were excluded from all further analysis.[36] Moreover, all CSF specimens were from

Panel: Research in context

Systematic review

We searched the PubMed and Medline databases for English language articles published between Jan 1, 1997, and July 31, 2010, with the terms "Parkinson's disease", "Parkinson disease", "dementia with Lewy bodies", "dementia", "multiple-system(s) atrophy", or "synucleinopathy(ies)", and "cerebrospinal fluid", "alpha-synuclein", "SNCA", or "α-synuclein". The results of this search formed the basis of a review article published on Oct 15, 2010;[11] we then did a second search with the same terms for the period of Aug 1, 2010, to Dec 31, 2010. The latter search revealed two additional articles.[9,10] We limited our review and discussion to reports on CSF studies that included patients with parkinsonism, dementia, or both.

Interpretation

Nine cross-sectional studies have quantified CSF α-synuclein as a possible biomarker for parkinsonism, dementia, or both. Our findings are consistent with five of these reports,[2,3,7,8,10] in that CSF α-synuclein concentrations are low in Parkinson's disease or related disorders, but contrast with findings from the other four reports.[4–6,9] This discrepancy needs to be reconciled by comparative, between-centre studies, by further definition of antigen and antibody variabilities, and by standardisation of operating procedures.[11] Our findings that CSF α-synuclein concentrations in dementia with Lewy bodies clustered between those for Alzheimer's disease and Parkinson's disease is consistent with its known neuropathological and biochemical overlap with these disorders. Our results from autopsy-confirmed cases of dementia with Lewy bodies provide insight into CSF α-synuclein concentrations in patients who have a confirmed synucleinopathy diagnosis. Our report also includes the first data for CSF α-synuclein in patients with multiple system atrophy, and shows the biochemical similarities in CSF and serum concentrtations between participants with this disorder and those with Parkinson's disease. In summary, our findings provide further evidence that low α-synuclein concentrations in CSF in patients presenting with parkinsonism have a high positive predictive value but low specificity for an underlying synucleinopathy, and might support use of CSF α-synuclein measurements during stratification of patients in future clinical trials.

inpatients who had fasted for longer than 8 h and had their lumbar punctures done between 0800 and 0900 h, and the specimens were processed, aliquoted, and frozen within 30 min of their collection. We are therefore confident that our biological fluid samples were collected, processed, and analysed according to procedures that had been previously validated and were rigorously implemented.

We found that in dementia with Lewy bodies, a complex disorder that features dementia and parkinsonism, CSF values were often clustered between those for Alzheimer's disease and Parkinson's disease, as shown in our training set (figure 1) but less so in the validation group (figure 2). As we expected, all patients with dementia with Lewy bodies in our autopsy group showed some neuropathological, and thus biochemical, overlap with Alzheimer's disease; the severity of the pathological changes of Alzheimer's disease, as rated by the Consortium to Establish a Registry for Alzheimer's Disease and Braak stages, did not correlate with CSF α-synuclein, but did with CSF tau and CSF $A\beta_{1-42}$ concentrations (data not shown). These findings suggest that all patients with dementia with Lewy bodies in the autopsy cohort were diagnosed correctly while alive, and are consistent with our findings from the training and validation sets. We speculate that low CSF α-synuclein concentrations in some patients with Alzheimer's disease might be concomitant with synucleinopathy (webappendix p 3). Longitudinal studies with additional autopsy data are needed to better differentiate subtypes of dementia with Lewy bodies (such as early versus advanced cases and those with versus without concomitant pathological changes of Alzheimer's disease) and to compare CSF marker values (including for phosphorylated tau, which we did not assess) in patients with dementia with Lewy bodies versus patients with Parkinson's disease, either with or without dementia.[28]

We present the first results for CSF α-synuclein concentrations in patients with multiple system atrophy. Somewhat unexpectedly, we detected a high degree of concordance in CSF α-synuclein values between Parkinson's disease and multiple system atrophy. In its early stages, the parkinsonian variant of multiple system atrophy (MSA-P) is particularly difficult to differentiate from Parkinson's disease.[22] As we show, quantification of total α-synuclein and tau in CSF (and of serum α-synuclein) did not distinguish between these two synucleinopathy disorders, but rather showed their biochemical similarities. A CSF-based distinction between MSA-P and Parkinson's disease would not only be of clinical use but also of pathogenetic relevance in view of the elusive origin of α-synuclein deposits in oligodendroglia of people with multiple system atrophy,[37] where *SNCA* transcripts are reportedly undetectable.[38] Another possible explanation for why total CSF α-synuclein levels are low in Parkinson's disease, dementia with Lewy bodies, and multiple system atrophy is a possible increase in rate of α-synuclein uptake from CSF into neurons and oligodendroglia, thereby potentially allowing intracellular inclusion formation and cell-to-cell propagation of a synucleinopathy. To this end, it will be important to establish whether the recent discovery of elevated oligomeric CSF α-synuclein signals in patients with Parkinson's disease[10] can be extended to specimens from patients with multiple system atrophy and dementia with Lewy bodies. The hypothesis of a trans-synaptic propagation of misfolded α-synuclein species has recently garnered support from results of both cellular and in-vivo experiments.[39]

For validation purposes and to explore potential clinical use, we assessed an additional cohort of patients who were admitted to a single centre under the referral diagnosis of parkinsonism. This hospital is staffed with movement-disorders specialists and uses comprehensive standardised operating procedures in the investigations of all inpatients with parkinsonism, and thus achieves high diagnostic accuracy within a short observation period. In this setting, low mean CSF α-synuclein concentrations were detected in patients with an α-synuclein-related illness compared with a range of other disorders. In this prospectively enrolled cohort, our model revealed three predictors for the diagnostic probability of synucleinopathy: age, CSF tau, and, most significantly, CSF α-synuclein ($p<0·0001$; table 3). However, the corresponding AUC value (0·729), which was confirmed to be independent of the number of patients entered into each group, was lower than the one recorded in our training set (0·908; table 3), which had included many patients without any signs of parkinsonism.

Although CSF α-synuclein was found to be the most predictive variable in our validation group, the lower AUC value in the validation group was due to the substantial overlap of individual CSF α-synuclein concentrations within a small range in the six donor groups presenting with parkinsonism (webappendix p 2); this overlap underlies its relatively low sensitivity and specificity values. Nevertheless, we recorded high positive predictive values with laboratory cutoffs for CSF α-synuclein at 1·6 pg/μL or less or 1·8 pg/μL or less as an indicator of underlying Parkinson's disease (or multiple system atrophy or dementia with Lewy bodies; table 4). Because of the signal overlap (and the overall small range of values), one can argue that the quantification of total CSF α-synuclein in the form of a single laboratory value does not enable neurologists to separate individual patients with α-synuclein-associated Parkinson's disease from all other forms of parkinsonism during routine investigations. Additional markers, such as the joint measurement of oligomeric α-synuclein and as yet undiscovered post-translationally modified α-synuclein species in CSF, might allow a more definitive

laboratory diagnosis of an α-synuclein-associated disorder in the future. Such an algorithm—based on two or more validated biological markers—has recently emerged in the study of Alzheimer's disease.[29,32] Similarly, longitudinal studies with annual CSF collections will establish whether CSF α-synuclein changes can serve as a marker for Parkinson's disease risk or its progression.[2] In our analyses we did not note any correlation between CSF α-synuclein concentrations and the duration of synucleinopathy (eg, correlation coefficient for Parkinson's disease was –0·02 [p=0·8601] in the training set). Furthermore, we did not record any significant effect of dopamine treatment on CSF α-synuclein, CSF tau, and serum α-synuclein concentrations (data not shown). The finding of no significant pair-wise differences between the groups in our post-hoc tests suggest that the low CSF α-synuclein concentrations recorded in participants with synucleinopathy represent changes in the metabolism of α-synuclein in the brain rather than in its filtration rate from blood. To assess a drug-induced effect on marker candidates more definitively, large prospective studies have recently been started (eg, the DeNoPa study[11] and Parkinson's progression markers initiative [PPMI][40]) with long-term follow-up, serial CSF collections, and the enrolment of previously untreated patients with Parkinson's disease as well as healthy controls.

We believe that the most important effect of our results—if confirmed by others—relates to the design of future clinical trials. The differentiation of typical Parkinson's disease versus other parkinsonian disorders can remain challenging, as exemplified in recent trials by a high rate of misdiagnosis at the time of patient screening (≤20%).[41] Cause-directed clinical trials require the identification of the cause of Parkinson's disease in every study participant (stratification).[15] More than 70% of patients with typical Parkinson's disease will be ultimately diagnosed with the Lewy-positive α-synuclein-associated variant at autopsy,[12] thereby providing further rationale to correlate clinical phenotype with objective laboratory evidence to detect α-synuclein misprocessing in living patients. This proportion of synucleinopathy-positive patients—but not the remaining proportion of patients with α-synuclein-negative parkinsonism—and all patients with multiple system atrophy and dementia with Lewy bodies might benefit from α-synuclein-directed pharmacotherapy.

The lowering of the neuronal α-synuclein concentration in vivo to reduce the risk of synucleinopathy, or slow its progression in patients who already have clinical features, has emerged as an attractive therapeutic target for Parkinson's disease researchers. Experimental strategies, which include drugs, RNA-targeting technologies, and antibody-based approaches, are being aggressively pursued (reviewed by Tomlinson and colleagues[42]). We therefore speculate that the high positive predictive value for low CSF α-synuclein levels, which we recorded in our synucleinopathy patients, might aid patient stratification during future trial enrolment. Low CSF α-synuclein concentrations together with other biological markers (eg, oligomeric CSF α-synuclein, hyposmia, or the identification of a bona fide mutation in the *GBA* or *LRRK2* gene) might assist in the selection of those patients who stand to benefit from α-synuclein-directed therapy.

Contributors

BM designed the study and was responsible for sample collection, patient characterisation, and specimen and data processing. JJL oversaw all statistical analyses. WS-S was responsible for collecting autopsy information. FS-D was involved in sample collection and patient characterisation. CT oversaw patient characterisation and assisted in the interpretation of data. MGS supervised sample analysis, and assisted in data interpretation and statistical analyses. BM and MGS wrote the paper. JJL, WS-S, FS-D, and CT co-edited the paper.

Conflicts of interest

BM has received grants from TEVA-Pharma, Desitin, Boehringer Ingelheim and GE Healthcare and honoraria for consultancy from Bayer Schering Pharma AG and for presentations from GlaxoSmithKline and Orion Pharma as well as travel and meeting expenses from Boehringer-Ingelheim and Novartis. MGS has served as an ad hoc, paid consultant for FoldRx, Genzyme, Johnson and Johnson, Amicus Therapeutics, Elan Pharmaceuticals, Novartis, and LINK Medicine, has received payment for a lecture from TEVA Neuroscience, and has a scientific collaboration with Covance, Epitomics, and the Michael J Fox Foundation. CT is employed by Paracelsus, provides expert testimony for Axxonis, serves as a consultant for TEVA Pharma, Boerhinger-Ingelheim, UCB, Solvay, Novartis, Mundipharm, Viofor Pharma, and Cephalon, and has received honoraria for presentations and lectures from TEVA Pharma, Boehringer-Ingelheim, UCB, Viofor Pharma, and Cephalon. WS-S serves as consultant for Bayer Schering Pharma; he has received grants from the VolkswagenStiftung, Deutsche Forschungsgemeinschaft, Alberta Prion Research Institut, Canada, and the European Union, and has received payment for lectures from Bayer Schering Pharma. FS-D has received a grant from the German Parkinson Patient Foundation and honoraria from advisory board meetings from Orion Pharma, as well as payment for lectures by Boehringer-Ingelheim, Orion Pharma, UCB/Schwarz Pharma, and Solvay. JJL declares no conflicts of interest. BM and MGS are listed as co-inventors in a patent application to the US Patent Office related to the quantification of α-synuclein in biological fluids for the purpose of improved diagnosis.

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