Noves aproximacions diagnòstiques a la paràlisi supranuclear progressiva: biomarcadors clínics, en líquid cefaloraquidi i de ressonància magnètica

Cèlia Painous Martí

NOVES APROXIMACIONS DIAGNÒSTIQUES A LA PARÀLISI SUPRANUCLEAR PROGRESSIVA: BIOMARCADORS CLÍNICS, EN LÍQUID CEFALORAQUIDI I DE RESSONÀNCIA MAGNÈTICA

Memòria de tesi doctoral presentada per **Cèlia Painous Martí**
per optar al grau de doctora per la Universitat de Barcelona.

Director i tutor:

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Maig, 2023

INFORME DEL DIRECTOR DE TESI:

El Dr. Yaroslau Compta, doctor en Medicina per la Universitat de Barcelona,

CERTIFICA

Que la memòria titulada "Noves aproximacions diagnòstiques a la paràlisi supranuclear progressiva: biomarcadors clínics, en líquid cefaloraquidi i de ressonància magnètica", presentada per Cèlia Painous Martí, ha estat realitzada sota la meva direcció i considero que reuneix les condicions necessàries per a ser defensada davant el Tribunal corresponent per tal d'optar al Grau de Doctor dins del programa de doctorat de Medicina i Recerca Translacional de la UB.

Barcelona, 10 de maig del 2023

Dr. Yaroslau Compta Hirnyj

Unitat de Parkinson i altres Trastorns del Moviments

Hospital Clínic de Barcelona / IDIBAPS / Universitat de Barcelona

DECLARACIÓ DE L'ORIGINALITAT I BONES PRÀCTIQUES DE LA TESI

El Dr. Yaroslau Compta Hirnyj, Doctor en Medicina per la Universitat de Barcelona

amb DNI 46356704C, i la doctoranda Cèlia Painous Martí amb DNI 43549226Z

DECLAREN QUE

La tesi doctoral, amb títol "Noves aproximacions diagnòstiques a la paràlisi supranuclear progressiva: biomarcadors clínics, en líquid cefaloraquidi i de ressonància magnètica", és original i conté resultats i informació fruit de recerca pròpia i que no s'han plagiat continguts d'altres tesis o publicacions o recerques d'altres autors. Tanmateix, confirmen que s'han seguit els codis ètics i de bones pràctiques per elaborar la tesi.

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Director

Dr. Yaroslau Compta Hirnyj

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Doctoranda

Cèlia Painous Martí

DECLARACIÓ D'AUTORIA DE LA TESI

La doctoranda Sra. Cèlia Painous Martí amb DNI: 43549226Z

DECLARA QUE

És autora de la tesi doctoral titulada "Noves aproximacions diagnòstiques a la paràlisi supranuclear progressiva: biomarcadors clínics, en líquid cefaloraquidi i de ressonància magnètica".

Signat el dia 10 de maig del 2023.

La doctoranda

Cèlia Painous Martí

A la Fina i en Josep,
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ÍNDEX

I. ABREVIATURES I ACRÒNIMS

4R-tau: tau de 4 repeticions

AMS: atròfia multisistèmica

aS: alfa-sinucleïna

DCB: degeneració corticobasal

DCB-SCB: fenotip síndrome corticobasal de la degeneració corticobasal

ELISA: *enzym-linked immunosorbent assay*

FDA: *Food and drug administration*

LCR: líquid cefaloraquidi

MAPT: *microtubule-associated protein tau*

MP: malaltia de Parkinson

NFL: neurofilament de cadena lleugera

NINDS-SPSP: *National Institute of Neurological Disorders and Stroke and Society for Progressive Supranuclear Palsy*

PCR: *polymerase chain reaction*

PET: tomografia d'emissió de positrons

PSP: paràlisi supranuclear progressiva

PSP-CBS: PSP síndrome corticobasal

PSP-F: PSP frontal

PSP-P: PSP parkinsonisme

PSP-PGF: PSP amb congelació progressiva de la marxa

PSP-PI: PSP inestabilitat postural

PSP-SL: PSP variant de la parla i el llenguatge

RM: ressonància magnètica

RT-QuIC: *real time quaking-induced conversion*

SC: subjectes control sense malaltia neurològica

SIMOA: *single molecule array*

SR: Síndrome Richardsoniana

II. ENUMERACIÓ DELS ARTICLES QUE COMPONEN LA TESI

Aquesta tesi doctoral es presenta en forma de compendi d'articles que corresponen a la mateixa unitat temàtica: l'estudi de biomarcadors diagnòstics (clínics, proteics i radiològics) de la paràlisi supranuclear progressiva.

Els objectius d'aquesta tesi són avaluar els símptomes que ens poden ajudar a realitzar un diagnòstic precoç de la malaltia i els possibles biomarcadors proteics i radiològics que ens permetin diferenciar-la d'altres parkinsonismes neurodegeneratius. Aquests objectius es desenvolupen en els següents tres articles científics originals.

- Article 1: **Painous C**, Martí MJ, Simonet C, Garrido A, Valldeoriola F, Muñoz E, Cámara A, Compta Y. Prediagnostic motor and non-motor symptoms in progressive supranuclear palsy: The step-back PSP study. Parkinsonism Relat Disord. 2020;74:67-73. doi: 10.1016/j.parkreldis.2020.03.003. (JCR classificació a la categoria de "Clinical Neurology": 51/208. IF 4.89; Q1)
- Article 2: Compta Y*, **Painous C***, Soto M*, Pulido-Salgado M, Fernández M, Camara A, Sánchez V, Bargalló N, Caballol N, Pont-Sunyer C, Buongiorno M, Martin N, Basora M, Tio M, Giraldo DM, Pérez-Soriano A, Zaro I, Muñoz E, Martí MJ, Valldeoriola F. Combined CSF α-SYN RT-QuIC, CSF NFL and midbrain-pons planimetry in degenerative parkinsonisms: From bedside to bench, and back again. Parkinsonism Relat Disord. 2022;99:33-41. doi: 10.1016/j.parkreldis.2022.05.006. (JCR classificació a la categoria de "Clinical Neurology": 51/208. IF 4.89; Q1).

  Aquest article en el seu vessant tècnic de laboratori es va incloure a la tesi doctoral de Marta Soto Gimeno titulada «Uncovering novel

biomarkers for Parkinson's disease and its prodromal stages» dirigida pels Drs. Mario Ezquerra i Rubén Fernández-Santiago i presentada el passat 23 de febrer de 2023 dins del programa de doctorat de Biomedicina de la UB. En la present tesi es presenten els aspectes d'interpretació clínica dels resultats de biomarcadors. Això es fa d'acord amb la normativa vigent en ser tant Marta Soto com Cèlia Painous coautores primeres amb igual contribució.

- Article 3: **Painous C***, Pascual-Diaz S*, Muñoz-Moreno E, Sánchez V, Pariente JC, Prats A, Soto M, Fernández M, Pérez-Soriano A, Camara A, Muñoz E, Valldeoriola F, Caballol N, Pont-Sunyer C, Martin N, Basora M, Tio M, Rios J, Martí MJ, Bargalló N, Compta Y. Midbrain and pons MRI shape analysis and its clinical and CSF correlates in degenerative parkinsonisms: a pilot study. Eur Radiol. 2023. doi: 10.1007/s00330-023-09435-0. Epub ahead of print. (JCR classificació a la categoria de "Radiology, Nuclear Medicine and Imaging", 22/321. IF 7.034; Q1)

III. RESUM

La paràlisi supranuclear progressiva (PSP) és un parkinsonisme neurodegeneratiu caracteritzat patològicament pel dipòsit neuronal i glial de la proteïna tau de 4 repeticions (4R-tau) a diferència de la malaltia de Parkinson (MP) on la proteïna de dipòsit es l'alfa-sinucleïna (aS). Degut a que comparteix signes i símptomes amb altres parkinsonismes neurodegeneratius, el diagnòstic diferencial pot ésser difícil sobretot en les fases inicials de la malaltia. A hores d'ara no disposem de biomarcadors fiables i el diagnòstic definitiu de la PSP és patològic. *In vivo* només es pot arribar a un diagnòstic de PSP suggestiva (certesa baixa), possible (certesa intermèdia) o probable (certesa alta però incompleta) mitjançant criteris clínics. Per tant, l'estudi de biomarcadors que ajudin a realitzar un diagnòstic més precís i en una fase precoç de la malaltia és essencial.

Així doncs, aquesta tesi vol aprofundir en el coneixement dels biomarcadors diagnòstics a la PSP. Hem formulat les següents hipòtesis: 1) existeixen símptomes prediagnòstics a la PSP que difereixen de la fase prediagnòstica de la MP; 2) la combinació de biomarcadors permet diferenciar la PSP d'altres parkinsonismes; 3) la morfologia o forma ("shape") per ressonància magnètica (RM) del tronc de l'encèfal també difereix entre PSP i altres parkinsonismes i es correlaciona amb indicadors clínics i bioquímics de gravetat de la malaltia. En conseqüència hem plantejat els següents objectius: 1) avaluar els símptomes que precedeixen el diagnòstic per a definir-ne la fase prediagnòstica i comparar-los amb la MP; 2) avaluar el rendiment de la combinació de diferents biomarcadors proteics en líquid cefaloraquidi (LCR) i radiològics per RM en comparació a d'altres parkinsonismes neurodegeneratius incloent la nova tècnica d'agregació RT-QuIC; 3) avaluar les diferències entre PSP i altres parkinsonismes amb la nova aproximació de RM anomenada *shape analysis* i els seus correlats clínics i en LCR.

El primer objectiu es tracta en el primer article d'aquesta tesi on s'analitzen de forma restrospectiva els símptomes que poden presentar els pacients amb PSP anys abans de rebre el diagnòstic (prediagnòstics) i es comparen les troballes amb dos grups controls (un format per pacients amb MP i l'altre per subjectes control sense malaltia neurològica (SC)). En la nostra cohort, els pacients amb PSP presenten una major prevalença d'alguns símptomes motors i no motors que poden estar presents fins a 10 anys abans del diagnòstic. La presència d'aquests símptomes combinada amb l'absència d'altres símptomes prediagnòstics més propis de la MP proporciona una alta capacitat predictiva per a diferenciar PSP de MP.

El segon objectiu es recull al segon estudi, centrat en la capacitat diagnòstica de la combinació de diferents biomarcadors en LCR i radiològics. En la nostra cohort, la combinació d'una àrea mesencefàlica reduïda a la RM, nivells elevats de neurofilament de cadena lleugera (NFL) i la negativitat d'agregació d'aS per RT-QuIC, mostra una excel·lent capacitat discriminant entre taupaties (PSP i degeneració corticobasal (DCB)) i altres tipus de parkinsonismes.

Per últim, al tercer article s'explora el tercer objectiu: valorar si els diferents parkinsonismes presenten patrons de *shape analysis* (estudi de forma més que de superfície o voulm) diferencials a nivell del tronc encefàlic. Tot i que aquesta tècnica mostra resultats menys significatius a la PSP que a d'altres parkinsonismes neurodegeneratius, sí que mostra correlacions significatives amb indicadors clínics de gravetat i amb el biomarcador d'agressivitat de la neurodegeneració (nivells elevats de NFL en LCR).

En resum, aquesta tesi aporta nou coneixement sobre biomarcadors diagnòstics en una malaltia de difícil i complex diagnòstic. Els símptomes prediagnòstics poden ser d'utilitat per a realitzar un diagnòstic precoç de la PSP, i la combinació de biomarcadors ens pot ajudar en el diagnòstic diferencial amb d'altres parkinsonismes. Les troballes d'aquesta investigació són doncs de potencial rellevància clínica, i obren noves i interessants línies d'investigació.

1. INTRODUCCIÓ

1. INTRODUCCIÓ

1.1. DEFINICIÓ DE PARÀLISI SUPRANUCLEAR PROGRESSIVA

La paràlisi supranuclear progressiva és una malaltia minoritària, un dels parkinsonismes neurodegeneratius més discapacitants i un dels diagnòstics diferencials més freqüents i difícils amb la MP. Anatomopatològicament, es caracteritza per l'agregació i dipòsit anòmal de la proteïna 4R-tau, motiu pel qual s'engloba dins de les 4R-taupaties. La presentació clàssica, ara coneguda com a síndrome Richardsoniana (PSP-SR), es va descriure l'any 1964. Es caracteritza per una síndrome rígida-acinètica simètrica associada a inestabilitat postural amb caigudes cap enrere des de l'inici, alteracions oculomotores de predomini en el pla vertical i alteracions pseudobulbars, cognitives i conductuals (1). Durant les dècades següents, emperò, s'han descrit un ampli espectre de nous fenotips clínics que poden presentar-se de forma similar a altres malalties neurodegeneratives i que s'han incorporat als actuals criteris diagnòstics (2). A més, s'ha optimitzat l'estimació de la incidència i prevalença de la malaltia, s'han descrit detalladament les característiques clíniques i perfilat la història natural, i des d'una vessant neuropatològica, s'ha objectivat l'important paper que juga la proteïna tau en la seva fisiopatologia (3).

1.1.1. Epidemiologia

Al llarg dels anys els valors de les estimacions de la prevalença de la PSP han anat augmentant. Inicialment, s'estimava que la prevalença era d'aproximadament 1,4/100.000 habitants (4), mentre que estudis més recents descriuen una prevalença de 5 a 8,3 casos per cada 100.000 habitants (5,6). La incorporació de nous fenotips clínics diferents del PSP-SR i un major coneixement i reconeixement de la malaltia poden haver contribuït a aquest increment. Alguns estudis anatomopatològics han reportat una prevalença

d'aproximadament 18 casos/100.000 (7,8), suggerint que si es té en compte tot l'espectre fenotípic de la PSP probablement la prevalença real de la malaltia és més elevada.

1.1.2. Neuropatologia

La PSP s'engloba dins de les taupaties, malalties caracteritzades patològicament per pèrdua neuronal, gliosis i acumulació i dipòsit de proteïna tau en diverses àrees cerebrals. La proteïna tau està codificada pel gen MAPT (*microtubule-associated protein tau* en anglès) localitzat en el cromosoma 17, i s'encarrega de l'estabilització dels microtúbuls axonals. Aquesta proteïna presenta sis isoformes com a resultat d'un *splicing* alternatiu en el gen MAPT. Segons si contenen tres o quatre repeticions del domini d'unió al microtúbul en la part carboxi-terminal de la molècula a l'exó 10, aquestes isoformes es classifiquen com a tau de tres (3R-tau) o quatre repeticions (4R-tau). Segons el tipus de taupatia predomina una o altra isoforma (9-12). En el cas de la PSP, la 4R-tau és la isoforma predominant que es troba en estat d'hiperfosforilació, entre d'altres modificacions post-traduccionals. Aquests canvis faciliten la formació d'agregats neuronals de tau, anomenats cabdells neurofibrilars globosos que s'associen a mort neuronal i gliosi. La proteïna tau també es diposita a cèl·lules glials, fonamentalment en forma d'astròcits en plomall i de cossos espirals oligodendroglials. Aquests canvis neuropatològics es localitzen predominantment al mesencèfal, nucli subtalàmic, ganglis basals i, en menor grau, en àrees corticals (predominantment a escorça frontoparietal prerolàndica) i nucli dentat (13). Aquesta distribució pot ésser variable segons el fenotip clínic (**Figura 1**). Així doncs, els fenotips de predomini motor presenten el gruix de patologia a nivell subcortical, i fenotips amb clínica de predomini cortical, presenten més patologia tau cortical (11, 14).

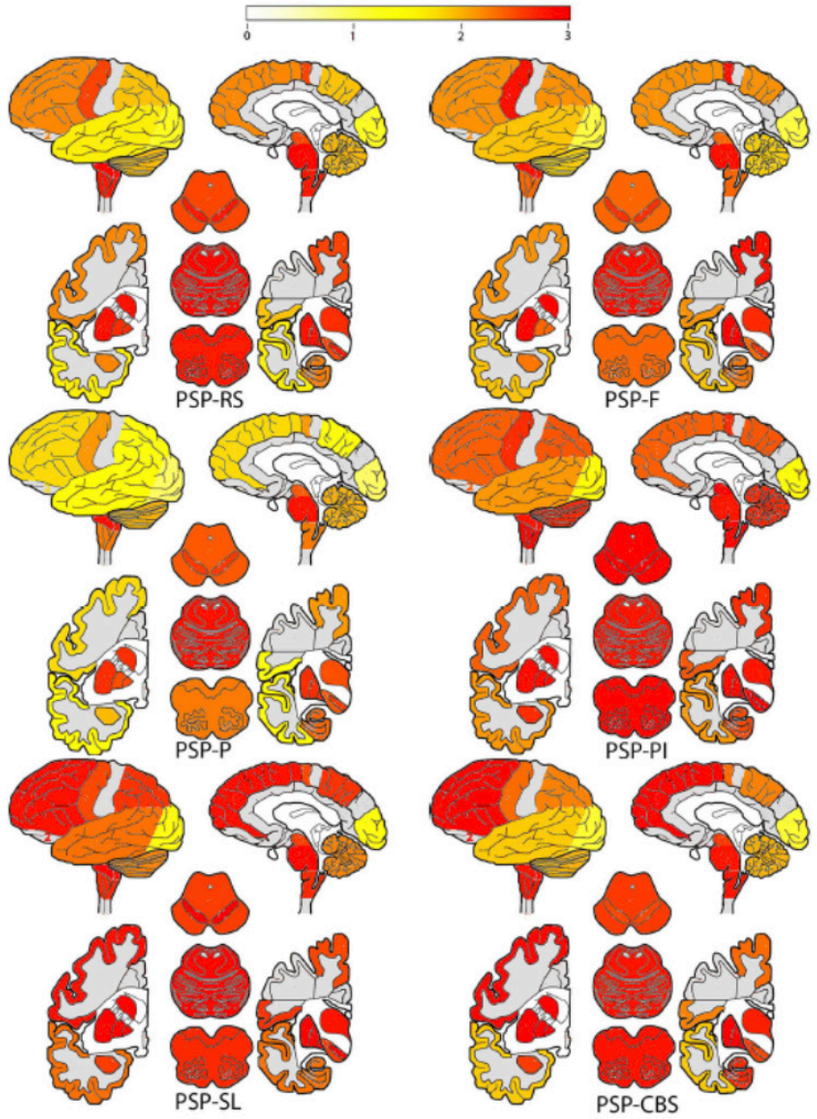


Figura 1. Extreta de «Distribution patterns of tau pathology in progressive supranuclear palsy». Acta Neuropathol. 2020;140(2):99-119.

Mapa de calor de les puntuacions totals de tau en la PSP-SR, la variant PSP frontal (PSP-F), PSP-parkinsonisme (PSP-P), PSP-inestabilitat postural (PSP-PI), PSP-variant de la parla i el llenguatge (PSP-SL) i síndrome PSP-corticobasal (PSP-CBS). La gravetat de la patologia tau va del blanc (absent), passant pel groc i el taronja, fins al vermell (greu). Les regions corticals de color gris indiquen que la regió no ha estat avaluada.

1.1.3. Etiopatogènia

Dilucidar els mecanismes fisiopatogènics subjacents és bàsic per a la identificació de biomarcadors diagnòstics i dianes terapèutiques. La recerca prèvia s'ha centrat majoritàriament en factors ambientals i genètics, alteracions moleculars i, més recentment, en el mecanisme de propagació de les taupaties.

Factors ambientals: A l'estudi ENGENE-PSP s'observà que un menor nivell educatiu i l'exposició a l'aigua de pou i a residus industrials rics en metalls pesants, eren factors relacionats amb un major risc de desenvolupar PSP (15,16). Un estudi independent també va documentar que l'exposició laboral a metalls pesants estava associada amb el risc de desenvolupar PSP (17). En estudis efectuats a l'illa caribenya de Guadalupe el consum d'alts nivells d'annonacina, un inhibidor del complex mitocondrial 1 que es troba a la família de fruita tropical Annonaceae, es va associar amb el desenvolupament de PSP o a un altre parkinsonisme atípic (18,19). També s'ha suggerit que en dones, l'augment d'exposició als estròsgens pot estar associada amb una menor probabilitat de desenvolupar-la (20).

Genètica: La base genètica de la PSP ha guanyat una atenció creixent, i molts estudis han suggerit que la genètica juga un paper en la susceptibilitat per a presentar-la i que mutacions de determinats gens condueixen directament a PSP. La PSP és generalment una malaltia esporàdica, però rarament s'han reconegut formes familiars (21,22) i en una sèrie, fins a un 7% dels pacients amb PSP-SR complien criteris per a un mode d'herència de transmissió autosòmica dominant (21) tot i que aquests resultats no es van replicar en un estudi independent (15). Mutacions en el gen MAPT poden produir PSP, així com demència frontotemporal familiar, parkinsonisme lligat al cromosoma 17 (FTLD-17), afàsia progressiva primària i degeneració corticobasal (DCB) (23-26).

Una troballa important va ser la de l'associació de l'haplotip H1 amb no només la PSP sinó també la MP si bé apuntant que és un factor

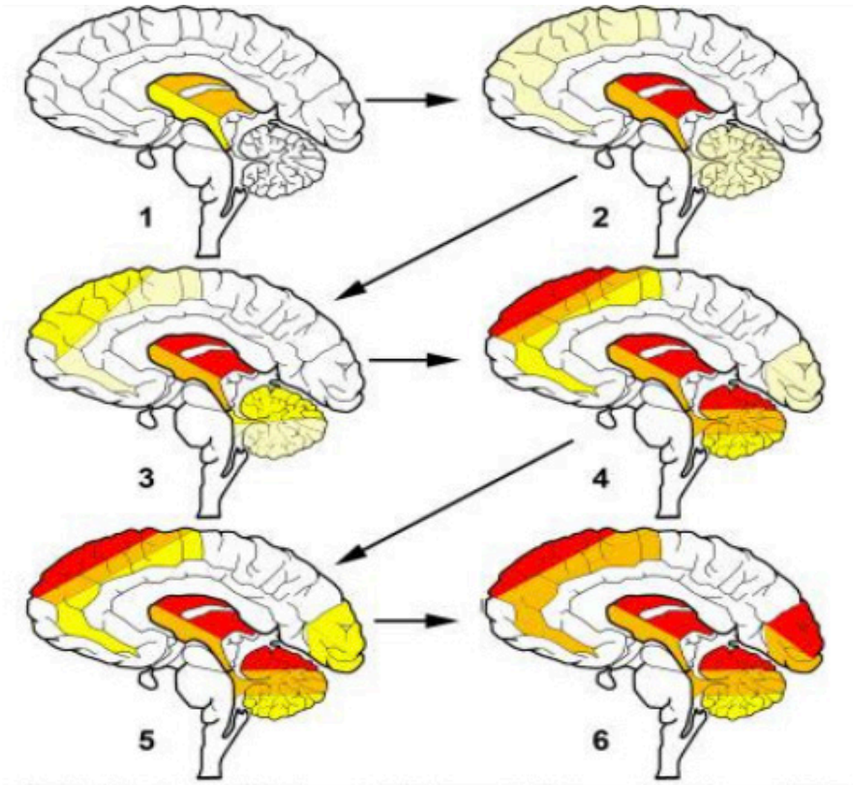
potencialment implicat però insuficient, ja que és present en entorn d'un 50% de la població general (27,28).

L'haplotip H1, a més, és més comú en PSP-SR que en PSP-P (29) i eleva 5,6 vegades el risc de desenvolupar la malaltia (27). Un estudi d'associació de tot el genoma (GWAS) validat patològicament va confirmar l'associació a variants MAPT i a l'haplotip H1, i va identificar altres loci genètics, incloent MOBP (proteïna oligodendroglial bàsica associada a la mielina), STX6 (implicada en la fusió de vesícules a la xarxa de Golgi i estructures endosòmiques) i EIF2AK3 (implicat en la síntesi de proteïnes davant l'excés d'estrès del reticle endoplasmàtic) (30-33). Aquestes variants s'han validat en un segon estudi GWAS que també identificà SLCO1A2, implicat en el transport de ions, i DUSP10, que està implicat en el transport de tau (34). Jabbari i col. van dur a terme un estudi estudi GWAS mitjançant la comparació de casos PSP-RS amb casos PSP-noRS suggerint que la variant intrònica rs564309 del gen de la proteïna 11 (TRIM11), pot ser un modificador genètic del fenotip clínic (35). Aquest estudi obre noves direccions per dilucidar el paper que juga la genètica en l'heterogeneïtat clínica de la PSP. Jabbari i col. també han mostrat l'associació del gen LRRK2, causa monogènica de penetrància variable de la MP, amb la supervivència a la PSP (36).

Estrès oxidatiu, disfunció mitocondrial i inflamació: a la PSP, com en altres malalties neurodegeneratives, s'ha demostrat la disfunció mitocondrial i l'estrès oxidatiu in vitro i en teixits humans (37-40). L'activitat enzimàtica mitocondrial es redueix i la peroxidació lipídica augmenta, la qual cosa condueix a un estrès oxidatiu excessiu (39-42). Pel que fa a la inflamació s'han trobat nivells més elevats de citocina IL1β a les regions cerebrals afectades per PSP correlacionant-se amb els nivells d'activació microglial (43). Altres estudis han observat que l'activació microglial es correlaciona amb el dipòsit de tau (44) i nivells més alts de citocines proinflamatòries en el LCR dels pacients amb PSP en comparació amb els pacients amb MP (45). Aquestes observacions han donat lloc a assaigs amb agents antiinflamatoris o antioxidants. La teràpia amb CoQ10 va mostrar millores en el metabolisme

cerebral, així com en les puntuacions totals de l'escala de severitat de la PSP i d'avaluació frontal en un petit assaig de sis setmanes (46). En canvi, un estudi de seguiment durant un any de CoQ10 no va mostrar diferències significatives en les escales avaluades entre el grup de PSP i el placebo i va tenir una alta taxa d'abandonament de pacients amb malaltia més greu, dificultant la interpretació dels resultats (47). Els assaigs amb riluzol i rasagilina no han demostrat un benefici significatiu (48,49).

Propagació de Tau semblant al prió: En la PSP, la patologia tau es limita inicialment al sistema pàl·lido-luyso-nigral i després afecta els ganglis basals, els nuclis pontins i el nucli dentat, i finalment, els lòbuls frontal i parietal, augmentant amb el temps no només l'extensió sinó també la gravetat de la malaltia. (50,51) (**Figura 2**). La hipòtesi de la propagació semblant als prions ha ofert una explicació per a aquesta via jeràrquica de neurodegeneració (52). Diverses dades suggereixen que la tau fibril·lada de manera anòmala és capaç d'actuar com a plantilla per induir el plegament incorrecte de la tau monomèrica normal conduint a la propagació de la malaltia d'una manera similar a com ho fan les malalties priòniques o altres proteïnes com l'aS en la malaltia de Parkinson (53). Estudis *in vivo* en models animals que utilitzen fibril·les preformades (54,55), homogeneïtzats de cervells de malalts (56) i altres tècniques (57,58) han demostrat la propagació distal de la patologia tau mitjançant propagació transinàptica (59,60). Es creu hi ha «soques» específiques de tau capaces de sembrar patologies diferents (57,61,62).



REGION	GP		STN		STR		FR		DE/CB		OC
Cell Stage	N/O		N		A		A		N/O		A
1	+/++	AND/OR	+/++	AND/OR	+/++	AND	-	AND	-	AND	-
2	++/+++	AND	++/+++	AND	++/+++	AND	-/+	AND/OR	-/+	AND	-
3	++/+++	AND	++/+++	AND	++/+++	AND	-/+ + +	AND	+ + -/+	AND	-
4	++/+++	AND	++/+++	AND	++/+++	AND	+ ++/+++ ++/+++	AND	++/+++ ++/+++ +	AND	- -/+
5	++/+++	AND	++/+++	AND	++/+++	AND	+ ++/+++ ++/+++	AND	++/+++ ++/+++ +	AND	+
6	++/+++	AND	++/+++	AND	++/+++	AND	++/+++	AND	++/+++ ++/+++ +	AND	++/+++

Figura 2. Extreta de «Distribution patterns of tau pathology in progressive supranuclear palsy». Acta Neuropathol. 2020;140(2):99-119.

En aquesta figura es representa l'extensió de la patologia tau i es proposa un estadiatge neuropatològic, basat en la localització i intensitat de patologia tau.

1.1.4. Complexitat clínica

La PSP és una malaltia esporàdica, de curs progressiu i sol iniciar-se entre els 60 – 65 anys. Com s'ha comentat prèviament, el fenotip clàssic de la malaltia és la SR però al llarg dels anys s'han anat descrivint altres fenotips.

PSP-Síndrome Richardsoniana

Els símptomes inicials més freqüents són les caigudes de repetició enrere, la inestabilitat de la marxa, malaptesa, disàrtria (molt característica una veu lenta i arrossegada), canvis de la personalitat (apatia, desinhibició, irritabilitat), bradifrènia, problemes executius (dificultats en la planificació, *multitasking*) i alteracions oculomotores caracteritzades a l'inici per alentiment i disminució de l'amplitud dels moviments sacàdics verticals i posteriorment paràlisi supranuclear de la mirada de predomini vertical que si bé poden ser només troballa exploratòria, poden causar molèsties als pacients, com ara visió borrosa o diplòpia (3).

PSP-Parkinsonisme (PSP-P)

Presenta un curs clínic més benigne en comparació amb la SR. La clínica és similar a la MP, essent característic a l'inici un parkinsonisme asimètric amb o sense tremolor de repòs i amb una resposta lleu-moderada a la levodopa, però de vegades molt marcada. Amb la progressió de la malaltia, molts dels pacients acaben evolucionant a un fenotip Richardsonià (29).

PSP amb congelació progressiva de la marxa (en anglès *progressive gait freezing*, PGF)

Durant anys els pacients presenten com a únic símptoma un trastorn de la marxa. Aquest primerament es caracteritza per bloqueigs a l'inici de la marxa i/o en els girs, i posteriorment va evolucionant a *freezing* complet de la marxa. A més, els pacients poden presentar bloqueigs de la parla o en les tasques manuals (per exemple l'escriptura). La PSP-PGF és altament predictiva de patologia PSP subjacent (3,63).

PSP - Síndrome corticobasal (PSP-SCB)

Existeix una considerable superposició genètica, neuropatològica i clínica entre la PSP i la degeneració corticobasal (DCB). Així doncs, els criteris actuals de la DCB reconeixen la síndrome PSP-SR causada per patologia DCB i els criteris de la PSP el fenotip PSP-SCB causat per patologia PSP. El fenotip PSP-SCB es caracteritza per la combinació variable de rigidesa, distonia i bradicinèsia asimètrica en extremitats, juntament amb clínica cortical i a vegades síndrome de l'extremitat estranya (aliena), amb poca o nul·la resposta a la levodopa. És una presentació infreqüent de PSP i la seva diferenciació amb el SCB causat per DCB és impossible de realitzar sense examen neuropatològic (64,65). Tanmateix, la PSP és una de les causes més freqüents de SCB, forma de presentació més característica de la DCB (64).

PSP variant de la parla i el llenguatge (PSP-SL)

Els nou criteris (2) reconeixen un nou fenotip que inicia amb alteració del llenguatge en forma d'afàsia no fluent i/o apràxia del llenguatge sense altres símptomes motors acompanyants inicialment.

PSP amb presentació frontal (PSP-F)

És un fenotip infreqüent caracteritzat per la presència de trets clínics similars a la variant conductual de la degeneració frontotemporal anys abans que aparegui la clínica motora. Es caracteritza per un precoç i progressiu deteriorament de la personalitat, del comportament social, la conducta i la cognició (66).

PSP amb clínica predominantment cerebel·losa (PSP-C)

És un fenotip molt infreqüent que es presenta amb atàxia cerebel·losa com a símptoma inicial i predominant, podent evolucionar posteriorment cap a PSP-SR. En un estudi neuropatològic es van detectar 5 PSP-C d'un total de 1085 PSP definitives. 4 d'aquests casos presentaven clínica suggestiva d'atròfia multisistèmica (AMS) però sense una marcada disautonomia (67).

Degut a la seva infreqüència i perquè una síndrome atàxica és molt més freqüent en altres malalties neurodegeneratives, aquest fenotip no s'inclou en els últims criteris diagnòstics de la PSP (2).

Taupaties 4R

De forma creixent fins i tot en l'àmbit clínic molts autors parlen de taupaties 4R. Dins d'aquest grup s'engloben la PSP i la DCB que són taupaties primàries caracteritzades principalment pel dipòsit de 4R-tau. La correlació entre la presentació clínica fenotípica i l'entitat neurpatològica subjacent és un repte diagnòstic en les taupaties 4R. Una determinada entitat neuropatològica pot tenir presentacions clíniques heterogènies i, sovint, és difícil predir la patologia subjacent en funció de la síndrome clínica. És per això que als actuals criteris diagnòstics de la MDS (2) introdueixen la categoria «4R-taupatia probable» com a paraigües per a malalties difícils de diferenciar clínicament i que comparteixen en part un mecanisme patològic comú.

1.1.5. Actuals criteris diagnòstics de la International Parkinson and Movement Disorders Society

Actualment, el diagnòstic de certesa d'aquesta malaltia es realitza amb criteris anatomopatològics i, en la fase pre-mortem, únicament es pot arribar a un diagnòstic de PSP possible o probable utilitzant criteris diagnòstics clínics. Els criteris clínics del *National Institute of Neurological Disorders and Stroke and Society for Progressive Supranuclear Palsy* (NINDS-SPSP) han sigut els més utilitzats des de la seva creació en el 1996 fins al 2017 (1). Són criteris amb una elevada especifictat per al diagnòstic de PSP, però la seva sensibilitat és baixa perquè no considera variants diferents a la SR (68). La detecció d'aquestes dificultats junt amb una millora del reconeixement d'aquestes formes no Richardsonianes van motivar la creació d'uns nous criteris diagnòstics (2). Aquests nous criteris, publicats per la *International Parkinson and Movement Disorders Society* en el 2017 (2), expandeixen l'espectre clínic de la PSP incorporant nous fenotips, no considerats en els

criteris previs (1), i introduint una nova categoria anomenada «suggestiva de PSP». Aquesta nova categoria inclou signes clínics subtils o lleus que són suggestius que hi pugui haver una anatomia patològica de PSP subjacent, però que no són suficients per a complir criteris de possible o probable PSP. L'expansió dels fenotips clínics i la inclusió d'aquesta nova categoria permetrien realitzar un diagnòstic en fases més precoces (69,70).

1.1.6. Període prediagnòstic

Encara que no existeixen definicions establertes, segons la presència i tipus de símptomes podríem dividir el període prediagnòstic en la PSP en fase presimptomàtica, prodròmica, suggestiva de PSP i aquella fase en què els pacients ja compleixen criteris de PSP però el diagnòstic no s'ha fet (retard en el diagnòstic).

La fase presimptomàtica es produeix en individus asimptomàtics però que presenten canvis neuropatològics de PSP. Avui en dia, aquesta fase només pot identificar-se post-mortem mitjançant l'observació de canvis histològics en individus sense símptomes clínics (71-73).

La fase prodròmica es pot definir com a aquella on les alteracions anatomopatològiques ja són prou rellevants per a ser simptomàtiques, però encara no hi ha un quadre clínic prou definit per pensar en l'existència de la malaltia. En aquest sentit, diversos estudis comunitaris amb resultats neuropatològics han demostrat troballes histopatològiques congruents amb PSP en individus que no havien rebut el diagnòstic d'aquesta malaltia, però que presentaven símptomes motors i/o neurocognitius lleus (8,71,73). Això està indicant l'existència d'una fase prodròmica de durada desconeguda, on molt probablement el procés neurodegeneratiu no es troba tan establert i, per tant, l'aplicació de potencials teràpies modificadores de la malaltia seria de gran interès.

La fase suggestiva de PSP representaria una fase més tardana que l'anterior, on ja hi ha un conjunt de símptomes que poden fer pensar en l'existència d'una PSP, però encara no hi ha prou evidència clínica com per a

establir un diagnòstic fiable. Aquesta fase s'ha intentat capturar en els nous criteris diagnòstics (2) afegint la categoria de «PSP suggestiva». Inherent a la definició de «PSP suggestiva» hi ha un cert grau d'incertesa sobre si l'individu progressarà a PSP possible o probable. En el futur, biomarcadors diagnòstics específics per a PSP poden ajudar a mitigar o reduir aquesta incertesa.

La importància de la identificació precoç dels pacients rau en oferir la possibilitat d'incloure els pacients en fases inicials en assaigs clínics de fàrmacs amb potencial capacitat modificadora de la malaltia, per al desenvolupament de noves eines diagnòstiques, però també per a poder donar una informació del pronòstic a pacients i família. Amb l'objectiu de poder identificar els pacients de forma més precoç i aprofundir en el coneixement de la fase prediagnòstica, vam realitzar el primer treball que conforma aquesta tesi («Prediagnostic motor and non-motor symptoms in progressive supranuclear palsy: The step-back PSP study»).

1.2. BIOMARCADORS

En la recerca de la PSP, hi ha hagut interès per a trobar marcadors que ens ajudin a predir o excloure la patologia d'aquesta malaltia.

1.2.1. Biomarcadors clínics

A nivell clínic, Respondek i col·laboradors (3) van realitzar un estudi retrospectiu investigant la presència de símptomes i signes en una cohort neuropatològica multicèntrica de persones amb PSP i van comparar els resultats amb altres malalties que poden presentar-se de forma similar (MP, DCB, AMS parkinsoniana, demència frontotemporal). Van trobar que la PSP només es diagnosticava correctament en vida en un 25% i 63% en les primeres i últimes visites, respectivament. Una de les raons era la variabilitat fenotípica de la PSP que no quedava recollida en els antics criteris diagnòstics (1), els quals sí que eren molt específics per a la PSP-SR. Com a característiques altament específiques (> 80%) de PSP en les etapes inicials de la malaltia (< 3 anys des de l'inici dels símptomes) van trobar: paràlisi

supranuclear de la mirada, alteració dels moviments sacàdics, inestabilitat postural, caigudes i *freezing* de la marxa. Hi havia altres característiques que presentaven una bona sensibilitat en el transcurs de la malaltia, però a expenses d'una baixa especifictat, entre aquests: disfunció frontal, disfàgia, disàrtria i parkinsonisme amb tremolor de repòs, +/- asimètric i amb +/- resposta a la levodopa. Per últim, esmenten que no van ser capaços de definir cap fase prodròmica característica de la malaltia. Els resultats d'aquest estudi, entre d'altres, es van utilitzar per a la creació dels nous criteris diagnòstics (2), basats principalment en trets clínics.

Atès que les alteracions oculomotores són un tret característic de la PSP, Pagonabarraga i col·laboradors han elaborat una escala per a l'avaluació estandarditzada d'aquestes anomalies, aconseguint diferenciar pacients amb PSP en un estadi inicial de pacients amb MP (74).

1.2.2. Biomarcadors neuroradiològics

En el camp de la neuroimatge i la PSP s'han fet importants avenços en les últimes dues dècades, però, així i tot, encara no disposem de cap marcador radiològic diagnòstic o pronòstic validat (2,75). Cal la realització de més estudis per a validar mesures estandarditzades que puguin ser utilitzades en la pràctica clínica i a nivell individual. A més, la majoria d'estudis s'han realitzat en pacients en fases avançades i que presenten un fenotip SR. Per tant, disposem de poca informació sobre marcadors en fases més primerenques de la malaltia i en pacients amb fenotip no SR (76).

1.2.2.1. *Ressonància magnètica estructural*

L'atròfia mesencefàlica és el signe objectivat amb més consistència i permet diferenciar la PSP d'altres parkinsonismes. A nivell visual, de forma qualitativa, s'han descrit el signe del colibrí (aplanament o concavitat del tegmèntum mesencefàlic) en el pla mig sagital i, en el pla axial, els signes de «Mickey Mouse» (els peduncles cerebrals mesencefàlics es veuen arrodonits en comptes de rectangulars) i de «morning glory» (concavitat dels marges laterals del tegmentum mesencefàlic) (75) (**Figura 3**). Aquests

marcadors presenten una elevada especificitat però una sensibilitat subòptima, no essent presents en una proporció important de pacients, sobretot en fases inicials de la malaltia (77-83). Quantitativament, existeixen algunes mesures lineals simples com el diàmetre antero-posterior del mesencèfal, que han presentat resultats variables en la diferenciació de la PSP amb altres parkinsonismes i manquen de validació externa i, per tant, de generalització. Més enllà del mesencèfal, hi ha altres estructures cerebrals que poden ajudar-nos en el diagnòstic de la PSP. Diversos estudis ecogràfics (84,85) i de ressonància magnètica (86,87), han demostrat un engrandiment del tercer ventricle i que aquest es va dilatant al llarg del temps en la PSP. L'amplitud del tercer ventricle normalitzat pel diàmetre intern del crani s'ha proposat recentment com a un marcador senzill que pot ajudar en el diagnòstic diferencial entre PSP i MP, presentant una bona precisió diagnòstica (corba ROC >0,90) (86). Altres biomarcadors que han resultat ser útils per a diferenciar PSP-SR de MP i AMS-P són les àrees del mesencèfal i protuberància i en especial la ràtio entre elles (àrea mesencefàlica / àrea protuberància) que presenta una sensibilitat i especificitat majors a 85% en la majoria d'estudis (80,87-90). Aquestes mesures encara no tenen un punt de tall ben establert per diferenciar resultats patològics dels que no ho són i poder-ho aplicar a nivell individual i en la pràctica clínica.

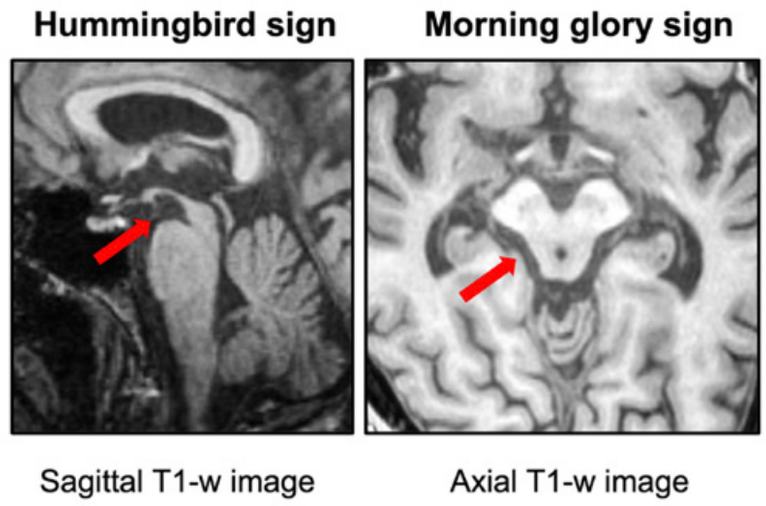


Figura 3. Extreta de «Magnetic Resonance Planimetry in the Differential Diagnosis between Parkinson's Disease and Progressive Supranuclear Palsy». Brain Sci. 2022;12(7):949.

Aquesta imatge mostra a l'esquerra un tall de RM en T1 en el pla mig sagital on s'observa el signe del «colibrí», causat per atròfia mesencefàlica dorsal i amb significativa preservació de la protuberància en un pacient amb PSP. A la dreta, veiem un tall en el pla axial amb el signe de «glory morning» caracteritzat per una concavitat dels marges laterals del tegmentum mesencefàlic en un pacient amb PSP.

A més, com que els peduncles cerebel·losos superiors també es troben atròfics en la PSP, s'ha afegit la seva mesura desenvolupant-se així l'índex MRPI («magnetic ressonance parkinsonism index») (**Figura 4**). Aquest índex està compost pel quocient entre l'àrea mesencefàlica (M_A) i l'àrea de la protuberància (P_A) així com el diàmetre del peduncle cerebel·lós mig (PCM) i el diàmetre del peduncle cerebel·lós superior (PCS). Presenta una elevada sensibilitat i especificitat per a la diferenciació de PSP amb MP, AMS i parkinsonisme vascular. Tot i així, la seva complexitat rau en que la mesura detallada d'aquestes diferents estructures, sobretot dels PCS, pot ser difícil de realitzar de forma reglada en els diferents centres i per tant difícil de generalitzar. Per últim, s'ha creat una segona versió de l'índex MRPI (MRPI 2.0) que afegeix la mesura del diàmetre del 3r ventricle millorant la diferenciació

de la PSP-P amb la MP (91). Per tal de millorar la seva reproductibilitat la mesura dels índex MRPI i MRPI 2.0 s'han automatitzat (86,92). Tot i així, continuen essent eines poc utilitzades en la pràctica clínica i això podria ser degut a que els resultats són depenents de les cohorts utilitzades i no hi ha punts de tall ben establerts.

Figura 4. Extreta de «Magnetic Resonance Planimetry in the Differential Diagnosis between Parkinson's Disease and Progressive Supranuclear Palsy». Brain Sci. 2022;12(7):949.

En aquesta figura es mostra com es mesuren (a) les àrees en el pla mig sagital del mesencèfal i protuberància, així com (b,c) els diàmetres dels peduncles cerebel·losos mitjos i inferiors per a poder calcular l'índex MRPI. Si a més s'afegeixen (d) els diàmetres a nivell anterior, mig i posterior del tercer ventricle i de (e) les banyes frontals dels ventricles laterals, es pot calcular el MRPI 2.0.

1.2.2.2. *Shape analysis*

La *shape analysis* permet detectar l'estretament local, és a dir atròfia, en regions específiques d'estructures complexes. Aquesta tècnica ha anat adquirint més rellevància en l'última dècada per a l'estudi de malalties neurodegeneratives com la malaltia d'Alzheimer o la MP (93-98). En la MP

mitjançant *shape analysis* s'han trobat diferències significatives amb SC en diferents estructures subcorticals, inclosos el nucli subtalàmic (95), el globus pàl·lid (96) i l'estriat (97,98). Pel que fa a l'aplicació d'aquesta tècnica en parkinsonismes atípics hi ha molt pocs estudis. Dos grups han comparat les diferències en la forma a nivell de ganglis basals entre pacients amb PSP i SC trobant diferències significatives en tàlem i caudat uns (99), i en estriat els altres (100). Només un estudi ha explorat les diferències resultants de la comparació de diferents parkinsonismes neurodegeneratius. Aquest estudi, compost per una mostra petita (5 PSP, 6 DCB, 9 MP, 12 CS), també s'ha centrat en l'anàlisi d'estructures subcorticals supratentorials trobant atròfia local bilateral en el tàlem ventral anterior i lateral en el grup PSP+DCB en comparació amb els altres grups (101). Prèviament al tercer estudi d'aquesta tesi, no hi havia cap article publicat sobre el paper de la *shape analysis* en l'estudi del tronc encefàlic en parkinsonismes degeneratius.

1.2.2.3. *Tomografia per emissió de positrons*

La imatge molecular de dipòsits de tau per tomografia d'emissió de positrons (PET) s'ha centrat sobretot en la malaltia d'Alzheimer, la taupatia més freqüent. S'han desenvolupat i estudiat diversos traçadors de proteïna tau coneguts com de primera generació: 18F-5105, 18F-FDDNP, 18F-THK523 i 11C-PBB3 (102).

El 18F-Flortaucipir (abans AV-1451 i T807) és el traçador de tau més investigat fins a l'actualitat i ha estat aprovat el 2020 per la *Food and drug administration* (FDA). Flortaucipir s'uneix a filaments helicoidals aparellats en taupaties 3R/4R (103) i presenta patrons de retenció correctes tant en malaltia d'Alzheimer amnèsica (104,105) com en les variants no amnèsiques (106,107). Tanmateix, la retenció sembla ser menys robusta en les taupaties 4R (103,108,109). Tot i que es poden demostrar diferències a nivell de grup entre PSP i controls sans, en gran part degut a l'augment de la retenció als ganglis basals i les regions nigrals (104,110-112), les diferències a nivell de pacients individuals no són clares, amb superposició significativa de la captació, unió del traçador fora de l'objectiu (*off-target binding*, havent-

se postulat unió inespecífica a la MAO-B i la neuromelanina) i augment de captació en sinucleïnopaties incloent formes esporàdiques (AMS) i genètiques (casos associats a mutació de la dinactina [DCTN-1 o síndrome de Perry]) (110,113,114).

Això ha fet que es desenvolupi una segona generació de traçadors PET-tau que estan mostrant una captació *off-target* més baixa i amb potencial per a les taupaties no Alzheimer. Així, en un estudi multicèntric transversal efectuat amb 60 pacients amb PSP s'ha demostrat la capacitat del traçador 18F-PI-2620 per unir-se a la isoforma 4R tau a múltiples regions diana de PSP subcorticals, diferenciant els pacients amb PSP dels controls a nivell individual (115) amb una moderada a elevada precisió. En canvi, un segon estudi amb una mostra molt menor (n PSP = 3) i utilitzant un temps d'adquisició més tardà (60-90 minuts), només va trobar augment de la captació en globus pàl·lid i no en altres estructures afectades habitualment en la PSP (116). A l'espera de nous estudis de mida mostral suficient, aquest nou traçador sembla prometedor.

Els traçadors PET dirigits a la micròglia activada (11C-(R) PK11195) poden ser útils per avaluar la inflamació associada a la neurodegeneració en PSP i altres malalties relacionades com la DCB (117,118).

Tot i que l'ús de PET està molt estès i accessible en hospitals acadèmics del nostre entorn, aquesta indicació encara no està del tot establerta i disponible al nostre entorn i roman experimental, a més de costosa, si bé menys invasiva que altres potencials biomarcadors, com els de LCR (vegi's més endavant).

1.2.3. Biomarcadors de PSP en biofluïds

Ara per ara no disposem de marcadors biològics específics per a PSP. Tradicionalment, s'han emprat tècniques d'immunodetecció tipus ELISA (enzym-linked immunosorbent assay), on la proteïna d'interès és capturada per un anticòs unit al fons dels pouets de la placa d'ELISA, per després ser detectada per un segon anticòs unit a un cromòfor, fet que permet mesurar

l'absorbància del color que en quantificar-se permet calcular la concentració (habitualment en pg/mL). Els nivells de la proteïna tau, incloses les mesures mitjançant ELISA de la tau total (t-tau) i la tau fosforilada (p-tau) en LCR, al contrari que en la malaltia d'Alzheimer, no solen estar elevades de manera diferencial a la PSP (119-121). Borroni i col·laboradors (122) varen observar que la ràtio tau 33 kDa/55 kDa es trobava significativament reduïda en LCR de pacients amb PSP en comparació amb els controls sans de similar edat i en pacients amb diverses malalties neurodegeneratives. Aquestes troballes no es van replicar en un estudi posterior (123). Un altre estudi multicèntric liderat pel Reta Lila Weston Institute de Queen's Square, Londres, i amb participació del nostre equip, va emprar immuno-PCR en comptes de les tècniques usuals d'immunodetecció. En la immuno-PCR no es mesura l'absorbància d'un anticòs de detecció marcat amb un cromòfor que s'uneix al complex anticòs de captura / proteïna, sinó l'amplificació mitjançant PCR d'un fragment de DNA unit a l'anticòs de detecció amb l'objectiu d'optimitzar la sensibilitat de la detecció. En aquest estudi es van analitzar mitjançant aquesta tècnica d'immunoPCR els nivells de les isoformes 4R de tau en LCR de controls i pacients amb MP, PSP i DCB així com Alzheimer. Si bé els nivells eren reduïts significativament en PSP envers controls, també hi hagué reduccions amb gran superposició de valors als grups MP i Alzheimer, limitant el valor d'aquesta tècnica (124).

El NFL, proteïna altament expressada en axons mielinitzats de gran calibre, és abocada al LCR en context de mort neuronal, constituint doncs un biomarcador de neurodegeneració. Ha demostrat en estudis mitjançant ELISA en LCR ser un marcador sensible però inespecífic, de manera que el seu valor no és diagnòstic sinó pronòstic. Així doncs, el NFL es troba elevat a LCR de pacients amb PSP i altres síndromes parkinsonianes atípiques i permet diferenciar-les de la malaltia de Parkinson, però no entre elles (121,125-127). Gràcies a la tecnologia SIMOA (*single molecule array*) que permet la detecció de concentracions molt menors (ng/L), s'han pogut determinar també els NFL en sang. Estudis primerencs varen trobar correlació lineal significativa entre NFL sèric amb les concentracions de NFL en LCR, si bé en general

amb coeficients de correlació més aviat modests (125,128-130). Nivells de NFL sèric basals més elevats s'han associat amb pitjors resultats clínics i radiològics longitudinals en la PSP (131). Així, el NFL sèric és un potencial biomarcdor subrogat o substitut de resposta clínica en assaigs terapèutics i de fet ja s'està emprant com a variable secundària o exploratòria de resposta en alguns assaigs actualment en marxa (per exemple l'estudi de UCB per testar la seguretat i tol·lerabilitat del fàrmac UCB0107 en pacients amb PSP).

Tornant a la tau com a element clau de la neuropatologia subjacent a la PSP, una altra potencial manera de mesurar-la després dels fracassos de les tècniques d'immunodetecció com l'ELISA, és el concepte de proteïnes que s'autopropaguen com a característica causal de les malalties neurodegeneratives. Això ha donat lloc al desenvolupament de sistemes d'amplificació de proteïnes plegades anormalment in vitro, com ara l'amplificació cíclica de mal plegament de proteïnes (PMCA) i la conversió induïda per agitació en temps real (RT-QuIC o *Real-Time Quaking-Induced Conversion*). La tècnica RT-QuIC es va desenvolupar per a detectar la proteïna priònica (PrPSc) i es basa en l'agregació de substrat de proteïna recombinant afegida a una mostra biològica induïda per llavors («seeds») de prions presents en aquella mostra biològica. La reacció s'amplifica per cicles alternats de sacsejada i repòs, i es detecta perquè els agregats que es formen es tenyeixen amb tioflavina, resultant en una fluorescència detectable amb un lector de plaques de fluorescència. El mètode inicialment limitat al camp de les prionopaties s'ha estès recentment al diagnòstic de les sinucleïnopaties (MP idiopàtica i genètica i demència amb cossos de Lewy, amb resultats més inconsistents en l'AMS) (132-137). Una revisió sistemàtica i metaanàlisis realitzades per Yoo i col·laboradors, demostraven una sensibilitat i especificitat agrupades de les tècniques d'amplificació de proteïnes plegades anormalment in vitro de 0,91 (0,87-0,94) i 0,96 (0,93-0,98) per a les malalties amb cossos de Lewy i de 0,63 (0,24-0,90) i 0,97(0,93-0,99) per a l'AMS (138). També s'ha demostrat que el RT-QuIC dona positivitat i proporciona una bona capacitat predictiva pel

desenvolupament de MP o DCL en pacients amb TCSREM idiopàtic, un símptoma prodròmic de sinucleïnopatia (139).

Aquestes tècniques també s'estan expandint a les taupaties. Els assaigs de tau RT-QuIC per a la detecció selectiva i la distinció dels agregats tau 3R, 4R i 3R/4R suggereixen que aquestes conformacions estructurals es poden amplificar de manera específica i diferencial, donant suport al diagnòstic específic de la taupatia i discriminant les taupaties d'altres malalties amb plegament incorrecte de proteïnes. L'assaig original tau RT-QuIC es va desenvolupar per a la tau 3R de la malaltia de Pick (140). Utilitzant fragments de 3R-tau com a substrat (K19CFh), el 3R tau RT-QuIC mostrà una alta selectivitat per a les llavors tau de Pick en el teixit cerebral i les mostres de LCR post-mortem. A continuació es va publicar el primer assaig 3R/4R tau RT-QuIC per a la detecció de filaments tau en la malaltia d'Alzheimer en teixit cerebral, conegut com a AD RT-QuIC (141). Posteriorment, es va millorar la fiabilitat de l'assaig incloent un únic substrat de 3R-tau, conegut com a assaig K12 RT-QuIC (142), capaç de detectar llavors 3R-tau d'homogenats de cervell provinents de persones amb malaltia de Pick (3R-tau), malaltia d'Alzheimer (3R/4R-tau) i encefalopatia crònica traumàtica (3R/4R-tau).

Finalment, s'han desenvolupat assaigs addicionals per a la detecció d'agregats 4R tau (143). El 4R RT-QuIC demostra una selectivitat preferencial per a les taupaties 4R incloent PSP i DCB, permetent una elevada detecció de llavors 4R-tau en teixit cerebral i LCR post-mortem, però amb molta menys sensibilitat per al LCR de pacients pre-mortem (143). Encara no disposem de cap altre treball que hagi pogut replicar els resultats.

2. HIPÒTESIS

2. HIPÒTESIS

1. Els canvis neuropatològics en la PSP s'inicien anys abans del diagnòstic traduint-se en la presència de símptomes motors i no motors diferencials;
2. La combinació de biomarcadors en LCR i de mesures planimètriques per RM de mesencèfal permet diferenciar significativament la PSP d'altres parkinsonismes;
3. La morfologia (*shaping*) per RM del tronc de l'encèfal en la PSP presenta patrons diferencials respecte a altres parkinsonismes neurodegeneratius i es correlaciona amb indicadors clínics i bioquímics de gravetat de la malaltia.

3. OBJECTIUS

3. OBJECTIUS

1. Analitzar la presència de símptomes motors i no motors abans del diagnòstic de PSP, estimar el risc de PSP en relació a aquests símptomes i el seu temps d'aparició per a definir la fase prediagnòstica de la PSP, i comparar-los amb els símptomes prediagnòstics de la MP.
2. Comparar la combinació de biomarcadors de neurodegeneració i agregació proteica en LCR i de planimetria de mesencèfal per RM entre la PSP i els altres parkinsonismes degeneratius.
3. Comparar patrons de *shaping* per RM a nivell del tronc de l'encèfal en la PSP i altres parkinsonismes degeneratius i analitzar-ne les correlacions amb variables clíniques i biomarcadors de neurodegeneració en LCR.

4. MATERIAL, MÈTODES I RESULTATS

4. MATERIAL, MÈTODES I RESULTATS

4.1. ARTICLE 1

Prediagnostic motor and non-motor symptoms in progressive supranuclear palsy: The step-back PSP study.

Painous C, Martí MJ, Simonet C, Garrido A, Valldeoriola F, Muñoz E, Cámara A, Compta Y. Parkinsonism Relat Disord. 2020;74:67-73. doi: 10.1016/j.parkreldis.2020.03.003.

Resum article 1:

Símptomes motors i no motors en la fase prediagnòstica de la paràlisi supranuclear progressiva: The Step-Back Study.

Objectius: Analitzar els símptomes que precedeixen el diagnòstic de la PSP, estimar el risc de PSP en relació a aquests i comparar-los amb els símptomes prediagnòstics de la MP.

Metodologia: Estudi retrospectiu de casos i controls mitjançant revisió d'històries clíniques i entrevista estructurada amb pacients i els seus cuidadors. Vam analitzar un total de 35 símptomes dividits en vuit dominis: visual, mareig, motor, estat anímic/apatia, cognitiu, conducta, son, gastrointestinal/urinari i miscel·lània. Per a comparar variables entre grups vam utilitzar tests no paramètrics i per a analitzar quins símptomes predeien el diagnòstic de PSP vs. MP vam aplicar models de regressió logística binària ajustats per edat i sexe. El nivell de significació es va establir en $p < 0,05$ corregit per *false discovery rate* per a comparacions múltiples.

Resultats: Vam incloure 150 subjectes: 50 amb PSP (38% dones, edat 75,8) i un grup control, emparellat per edat i sexe, compost per 50 amb MP i 50 SC. Els subjectes amb PSP van presentar una freqüència més elevada de símptomes en els dominis motor, estat anímic/apatia, cognitiu, conducta i mareig en comparació amb els subjectes amb MP. Més d'un 50% de les caigudes, depressió, ansietat i apatia prediagnòstiques, i més d'un 30% dels problemes de la marxa prediagnòstics, van aparèixer entre 3 i 10 anys abans del diagnòstic. La combinació de símptomes prediagnòstics amb l'absència d'altres símptomes prediagnòstics més propis de la MP van permetre discriminar PSP de MP de forma fiable i amb àrees sota la corba majors a 0,70.

Conclusions: Els pacients amb PSP presentaven una major prevalença d'alguns símptomes motors i no motors podent estar presents fins a 10 anys abans del diagnòstic. La combinació d'aquests símptomes prediagnòstics proporcionava una alta capacitat predictiva per a diferenciar PSP de MP. La definició d'una fase prediagnòstica en la PSP pot permetre la identificació de pacients en estadis inicials de la malaltia.

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Prediagnostic motor and non-motor symptoms in progressive supranuclear palsy: The step-back PSP study

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ABSTRACT

Background: Improved knowledge of the prediagnostic phase of progressive supranuclear palsy (PSP) might provide information on when and how the disease starts, along with the opportunity to test therapies in disease stages with lesser neurodegeneration.
Objective: To explore the symptoms in years preceding the PSP diagnosis.
Methods: This is a single-center retrospective case-control study based on clinical charts review and a structured interview to PSP patients and their caregivers. Prediagnostic symptoms were defined as those present more than one year before the diagnosis. We explored 35 symptoms in the following domains: visual, dizziness, motor, mood/apathy, cognitive, behavioral, sleep, gastrointestinal/urinary and miscellaneous. Non-parametric statistics were applied, with significance set at <0.05 (FDR-corrected).
Results: We included 150 subjects: 50 PSP patients (38% females, age 75.8) and an age- and sex-matched control group of 50 Parkinson's disease (PD) and 50 subjects (CS) without neurodegenerative disease. The frequencies of visual, motor, cognitive, behaviour and dizziness domains were significantly higher in PSP vs. PD, and so were the motor, mood/apathy, cognitive, behaviour and dizziness ones in PSP vs. CS. Over 50% of prediagnostic falls, apathy and anxiety, depression and memory-attention-executive symptoms, and over 30% of gait disturbances started more than three and up to ten years before the diagnosis. PSP patients had more consultations to ENT and ophthalmologists than PD patients.
Conclusion: PSP patients present a broad variety of motor and non-motor symptoms several years before the diagnosis. The definition of a prediagnostic PSP phase might be helpful to identify patients in early disease stages.

1. Introduction

Progressive supranuclear palsy (PSP) is an atypical parkinsonism presenting with several different motor and non-motor phenotypes, and with an underlying 4-repeat tauopathy [1]. Its definite diagnosis requires neuropathological examination [2]. In 2017 the Movement Disorders Society published the new PSP diagnostic criteria [3], which incorporate a new category called "suggestive of PSP". This new category includes subtle or mild clinical signs that are suggestive of underlying PSP pathology, but do not meet criteria for possible or probable PSP and could provide an opportunity for earlier diagnosis. An early and correct diagnosis is crucial for the outcome of ongoing and future clinical trials, at present mostly focused on tau-directed agents as potential disease-modifying treatments [4,5].

Several neuropathological community-derived studies have shown PSP pathology in elders not having been diagnosed with PSP [6–8]. Some of these cases had mild motor and neurocognitive features indicating that a PSP prediagnostic phase of unknown duration could exist [7,8].

In spite of this preliminary neuropathological evidence, no clinical studies have specifically looked at symptoms of the prediagnostic phase of PSP. We hypothesized that the prediagnostic phase in PSP consists in subtle or mild motor and non-motor symptoms. The study of this prediagnostic PSP phase might provide information on when and how PSP starts, as well as the opportunity to test experimental therapies in disease stages with theoretically less advanced neurodegeneration.

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Accordingly, we aimed to describe the motor and non-motor symptoms of the prediagnostic phase of PSP.

2. Methods

2.1. Design

This is a single-center retrospective longitudinal case-control study conducted at the Parkinson's Disease & Movement Disorders Unit of Hospital Clinic in Barcelona.

2.2. Participants

We have studied a cohort of 150 participants: 50 patients with PSP, 50 with Parkinson's disease (PD) but no other central neurological disease, and 50 control subjects (CS), most of them with hemifacial spasm (46/50), but all without known neurodegenerative disease or major psychiatric disorder. PSP patients have been visited at our Unit and received the diagnosis between 2012 and 2018. All met the MDS PSP diagnostic criteria [3] of probable or possible PSP in their last visit. Individuals with dementia, vascular parkinsonism, neurodegenerative parkinsonism other than PSP, or drug-induced parkinsonism were excluded. The study received approval from the ethics committee of our institution. All patients signed written informed consent.

2.3. Procedures and variables

General demographic data including age, sex, age of onset and disease duration were collected by trained neurologists through a retrospective structured review of the clinical charts and a standardized face-to-face questionnaire (see below) to patients and their caregivers. Charts were searched manually by following a protocolized algorithm that was applied equally to all the groups. Clinical charts were from Hospital Clinic Barcelona and we reviewed consultations to neurologist, as well as to other specialists, including the general practitioners and the emergency department. In case of discordant results between clinical charts and interview, clinical chart data prevailed as we considered these at lower risk of temporal bias. In case of discordant results in the interview among PSP patients and their caregivers, taking into account the lack of insight of many patients with PSP have, the report of the caregiver prevailed.

Age of onset was considered as the age when a first symptom was attributable to either parkinsonism as recorded by a general neurologist or to PSP as recorded by a movement disorders specialist according to the medical records. To define both the time of diagnosis and subsequently the age at diagnosis we considered two settings: the time to specific clinical diagnosis of PSP and the time to diagnosis of parkinsonism in cases in whom parkinsonism preceded the PSP diagnosis. We did so because parkinsonism is a syndrome strongly linked with PSP but not properly a symptom, and the goal of the study was assessing prediagnostic symptoms of PSP. Parkinsonism was defined by the treating neurologist, as the presence of bradykinesia in combination with either rest tremor, rigidity, or both.

PSP phenotypes were defined according to the first clinical presentation as this was deemed more likely to be related to prediagnostic symptoms than the phenotype of established disease appearing later on.

We considered as prediagnostic symptoms those that occurred at least one year before the diagnosis to attenuate the possibility of bias due to diagnostic delay (see Suppl. Fig. 1). We also assessed the percentage of patients having the prediagnostic symptoms three or more years before the diagnosis. We gave relevance to these one- and three-year windows as they have been used in the former and current PSP diagnostic criteria, respectively [3,9]. Prediagnostic symptoms were considered as present when they had been present at least for 3 months and in case of falls had to be occurred at least twice in a year.

We applied a modified version of the published "ONSET PD study" questionnaire [10], to fit with this PSP-focused study, covering 35 symptoms in nine domains: "visual", "dizziness", "motor", "mood/apathy", "cognitive", "behavioral", "sleep", "gastrointestinal/urinary" and "miscellaneous". Under the cognitive domain we included speech and memory-attention-executive complaints by clinical judgment. "Mood/apathy" included sadness, anxiety, irritability and apathy, as recorded in the clinical chart or in the questionnaire. The domain "behavioral" incorporated aggressiveness and behavioral change, which included symptoms of disinhibition, hyperorality and hygiene changes. "Visual" domain included blurred vision, photophobia, unspecific visual symptoms, diplopia and burning eyes. Finally, the "motor" domain included falls, gait disturbances, tremor and clumsiness. We recorded if prediagnostic falls and gait disorder, which were the most common motor symptoms (see results), presented or not in association with other motor features. See Supplementary Table 1 for other domains.

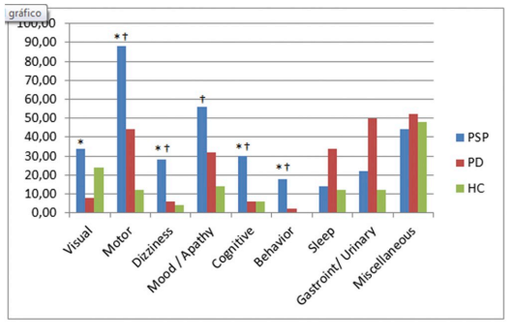


Fig. 1. Comparison of the frequency of prediagnostic symptoms grouped in domains among PSP vs. PD vs. CS.
* Significant ($p < 0.05$) comparison between PSP vs. PD.
† Significant ($p < 0.05$) comparison between PSP vs. CS.

The time when symptoms were first noticed was also recorded (<1, 1–3, 3–5, 5–7, 7–10 and > 10 years before the diagnosis in PSP and PD and before the interview in CS). Finally, we also collected the visits to other specialists before the diagnosis.

2.4. Statistical analysis

No formal sample size and statistical power calculations were done due to the rarity of the disease and after the sample size of previous single-center PSP studies. In terms of matching, participants were age- and sex-matched at the group level, with PD and PSP participants being also matched for disease duration. To minimize the difference in recall bias between PSP and CSs, we also matched CSs according to PSP time of diagnosis. As an example, if a given CS was matched with a PSP patient diagnosed 2 years ago, all the symptoms reported by the CS the previous 3 years (2 for matching with the PSP subject + 1 as defined in methods) were excluded.

We calculated the median time from the first estimated prediagnostic symptom to diagnosis. To control for potential spurious association in the case of symptoms very distant from diagnosis, we calculated the frequency of prediagnostic symptoms both including and excluding those having started more than 10 years ago.

Qualitative variables are presented as absolute and relative frequencies and were compared by means of Fisher's exact test. Quantitative data are presented as median and interquartile range (IQR) and were compared using Kruskal-Wallis test or Mann Whitney's *U* test, as appropriate. We applied binary logistic regression models adjusted for potential modifiers such as age at inclusion and sex to test the most frequent (>0.20) and significant prediagnostic features as predictors (independent variables) of PSP (outcome as compared to either PD or CS or PD + CS [no-PSP]). The absence of non-motor features that were significantly more frequent in PD compared to PSP was also used as independent variable. These symptoms were grouped under the category of "lack of PD prodromal features". The number of covariates in each model was limited according to the size of the set or subset of cases included in the model in order to avoid overfitting. These models resulted in their respective Odds ratios (ORs) and their 95% confidence intervals (95% CIs). Finally, we run ROC curves with their respective areas under the curve (AUCs), 95% CIs and sensibilities (Se) and specificities (Sp). Additionally we looked at the association between the prediagnostic symptoms and the PSP phenotypes by means of Fisher's exact test.

All statistical tests were two-tailed, with p-value set at <0.05, with adjust for multiple comparisons by means of Benjamini-Hochberg false discovery rate (FDR) [11]. No imputation was made for missing data. Subjects missing values in a particular field were not included in the analysis for that particular outcome. Data analysis was carried out using Stata 14.0 (Stata Corp, College Station, TX) for Windows and IBM SPSS statistics software version 24.0 (Armonk, NY:IBM Corp).

3. Results

3.1. Study population

PSP participants had a median age of 75.8 [IQR: 71.9–81.9], a median disease duration of 2.8 [IQR: 1.7–4.6] years and 38.0% were women. PSP participants did not significantly differ from both control groups in age and sex, nor disease duration (in the case of PD) (Table 1).

PSP-RS was the most frequent phenotype (54.0%), followed by PSP-P (20.0%) and PSP-PGF (18.0%) (Table 1). Patients with PSP were classified as "probable PSP" in a 96.0% and as "possible PSP" in a 4.0% at last visit. From the PSP group, 7 patients died and 2 of them were brain bank donors and confirmed to have PSP.

3.2. Prediagnostic symptoms in PSP: frequency and timing to diagnosis

The median time from the first estimated prediagnostic symptom to diagnosis was 81.7 months [IQR: 49.2–123.4]. When excluding symptoms having started more than 10 years ago, the median timespan was 75.6 months [IQR 46.2–94.5]. For the most frequent PSP subtypes, the median time was 71.8 months [IQR 46.2–96.4] in PSP-RS, 112.1 months [IQR 91.6–122.8] in PSP-P and 123.5 months [IQR 93.6–130.5] in PSP-PGF (Kruskall-Wallis p-value: 0.01; U Mann Whitney p-value: 0.03 in PSP-RS vs. PSP-P; 0.03 in PSP-RS vs. PSP-PGF; 0.39 in PSP-P vs. PSP-PGF; all pair-wise p-values FDR corrected).

The most frequent prediagnostic symptoms in PSP were falls (54.0%), gait disturbances (52.0%), depression (42.0%), pain (36.0%), visual symptoms (34.0%), clumsiness (30.0%), dizziness (28.0%), anxiety (26.0%) and apathy (20.0%). Proportion of prediagnostic symptoms did not differ between PSP phenotypes.

Prediagnostic falls and gait disturbances were present, without other motor symptoms, in 42.4% and 24% of PSP participants for more than one year. Regarding the time of onset of prediagnostic symptoms (Table 2), among the most frequent ones (>20%), over 50% of prediagnostic falls, apathy, anxiety, depression and memory-attention-executive symptoms, and over 30% of gait disturbances started more than 3 and up to 10 years before the diagnosis of PSP or parkinsonism. By contrast, those reported more frequently within 3 years of diagnosis were visual symptoms (82.3%) and clumsiness (87.0%).

3.3. Prediagnostic domains in PSP vs. PD and PSP vs. CS (Fig. 1)

When compared to PD, PSP patients presented significantly higher frequencies of "visual" (34.0 vs. 8.0%, p = 0.012), "motor" (88.0 vs. 44.0%, p < 0.001), "cognitive" (30.0 vs. 6.0%, p = 0.012), "behaviour" (18.0 vs. 2.0%, p = 0.039) and "dizziness" (28.0 vs. 6.0%, p = 0.019) domains. Likewise, the frequencies of "motor" (88.0 vs. 12.0%, p < 0.001), "mood/apathy" (56.0 vs. 14.0%, p < 0.001), "cognitive" (30.0 vs. 6.0%, p = 0.011), "behaviour" (18.0 vs. 0.0%, p = 0.011) and "dizziness" (28.0 vs. 4.0%, p = 0.01) domains were significantly higher in PSP vs. CSs.

Prediagnostic symptoms in PSP vs. PD patients (Supplementary Table 2).

With regard to the motor domain, PSP patients presented more prediagnostic falls (54.0 vs. 8.0%, p < 0.001) and gait problems (52.0 vs. 10.0%, p < 0.001), whereas prediagnostic tremor was more frequent in PD (10.0 vs. 32.0%, p = 0.035). Regarding cognitive complaints, speech (18.0 vs. 0.0%, p = 0.011) but not memory-attention-executive (22.0 vs. 6.0%, p = 0.084) complaints were significantly more frequent in PSP. In terms of psychiatric symptoms, anxiety (26.0 vs. 12.0%, p = 0.190) and depression (42.0 vs 24.0%, p = 0.154) symptoms did not differ statistically, but PSP patients were more apathetic (20.0 vs. 2.0%, p = 0.023).

Hyposmia (16.0 vs. 60.0%, p < 0.001), constipation (12.0 vs. 44.0%, p = 0.005), and sleep problems such as frequent nightmares (2.0 vs. 26.0%, p = 0.005) and dream enacting behaviour (2.0 vs. 24.0%, p = 0.009), were more frequent in PD, with no differences in insomnia or daytime sleepiness.

Prediagnostic symptoms in PSP patients vs. CS subjects (Supplementary Table 3).

Statistically significant differences between groups were observed in the majority of motor symptoms (falls p < 0.001, gait disturbances p < 0.001, clumsiness p < 0.001), depression (42.0 vs. 8.0%, p < 0.001), apathy (20.0 vs. 2.0%; p = 0.023), irritability (14.0 vs. 0%, p = 0.032), speech problems (18.0 vs. 0.0%, p = 0.011) and dizziness (28.0 vs. 4.00%, p = 0.010).

Table 1
Descriptive data of PSP, PD and CS participants.

Characteristic	PSP patients (n = 50)	PD patients (n = 50)	CS (n = 50)	P value
Female, n (%)	19, (38.0%)	17, (34.0%)	20, (40.0%)	0.870*
Age, median [IQI]	75.8 [71.9–81.9]	74.0 [70.1–78.7]	72.3 [68.0–79.2]	0.161**
Requested recall time period, median [IQI]	2.8 [1.7–4.6]	3.2 [2.5–4.9]	2.8 [1.7–4.3]	0.365**
PSP phenotype, n (%)		–	–	–
RS	27, (54.0%)			
P	10, (20.0%)			
PGF	9, (18.0%)			
SL	2, (4.0%)			
F	1, (2.0%)			
CBS	1, (2.0%)			

*By means of Chi2 exact test.
**By means of Kruskall-Wallis.

Table 2
Relationship of time of onset of prediagnostic symptoms and time of diagnosis.

		Timespan before the diagnosis of PSP or parkinsonism							Timespan before the diagnosis of PD or parkinsonism						
		Prediagnostic symptoms							Prediagnostic symptoms						
	Onset before diagnosis; n (% of all PSP patients)	S ≤ 1 year; N (%)	1< S ≤ 3 y; N (%)	3< S ≤ 5 y N (%)	5 < S ≤ 7 y N (%)	7 < S ≤ 10 y N (%)	S > 10 y N (%)		Onset before diagnosis; n (% of all PD patients)	S ≤ 1 year; N (%)	1< S ≤ 3 y; N (%)	3< S ≤ 5 y N (%)	5 < S ≤ 7 y N (%)	7 < S ≤ 10 y N (%)	S > 10 y N (%)
Diplopia	5 (10.0)	1 (20.0)	3 (60.0)		1 (20.0)			Diplopia	3 (6.0)	2 (66.7)		1 (33.3)			
Blurred	4 (8.0)		1 (25.0)	3 (75.0)				Blurred	–						
Photophobia	1 (2.0)				1 (100)			Photophobia	–						
Burning	5 (10.0)			3 (60.0)	1 (20.0)	1 (20.0)		Burning	–						
Uncomfortable	2 (4.0)	1 (50.0)		1 (50.0)				Uncomfortable	1 (2.0)			1 (100)			
Nonspecific visual symptoms	7 (14.0)	1 (14.3)	1 (14.3)	5 (71.4)				Nonspecific visual symptoms	2 (4.0)			1 (50.0)	1 (50.0)		
Visual	17 (34.0)	3 (17.6)	11 (64.7)	2 (11.8)	1 (5.9)			Visual	6 (12.0)	2 (33.3)	3 (50.0)	1 (16.7)			
Hyposmia	8 (16.0)			1 (12.5)			7 (87.5)	Hyposmia[2m]	30 (60.0)		1 (3.3)	2 (6.7)	1 (3.3)	4 (13.3)	20 (66.6)
Falls	33 (66.0)	6 (18.2)	8 (24.2)	15 (45.5)	3 (9.1)	1 (3.0)		Falls	8 (16.0)	4 (50.0)	1 (12.5)	3 (37.5)			
Gait disturbance	29 (58.0)	3 (10.3)	17 (58.6)	7 (24.1)	2 (6.9)			Gait disturbance	13 (26.0)	8 (61.5)	3 (23.1)	2 (15.3)			
Tremor	9 (18.0)	4 (44.4)	5 (55.6)					Tremor	30 (60.0)	14 (46.7)	9 (30.0)	3 (10.0)	1 (3.3)		3 (10.0)
Clumsiness	23 (46.0)	8 (34.8)	12 (52.2)	2 (8.7)	1 (4.3)			Clumsiness	19 (38.0)	13 (68.4)	5 (26.3)	1 (5.3)			
Voice	15 (30.0)	9 (60.0)	4 (26.7)	2 (13.3)				Voice	5 (10.0)	4 (80.0)		1 (20.0)			
Dysphagia	8 (16.0)	7 (87.5)					1 (12.5)	Dysphagia	1 (2.0)	1 (100)					
Frequent nightmares	1 (2.0)		1 (100)					Frequent nightmares	14 (28.0)	1 (7.1)	3 (21.4)	4 (28.6)	1 (7.1)	2 (14.3)	3 (21.4)
Dream enacting behaviour	2 (4.0)	1 (50.0)	1 (50.0)					Dream enacting behaviour	12 (24.0)		3 (25.0)	2 (17.0)	1 (8.0)	2 (17.0)	4 (33.0)
Insomnia	8 (16.0)	2 (25.0)	4 (50.0)		1 (12.5)	1 (12.5)		Insomnia	2 (4.0)		2 (100)				
Day Sleep	4 (8.0)	2 (50.0)	1 (25.0)	1 (25.0)				Day Sleep	3 (6.0)	2 (66.7)				1 (33.3)	
Depression	24 (48.0)	3 (12.5)	3 (12.5)	9 (37.5)	6 (25.0)	1 (4.2)	2 (8.3)	Depression	15 (30.0)	3 (20.0)	4 (26.7)	4 (26.7)	2 (13.3)	1 (6.7)	1 (6.7)
Anxiety	13 (26.0)		4 (30.8)	6 (46.2)	3 (23.1)			Anxiety	6 (12.0)		2 (33.3)	2 (33.3)		1 (16.7)	1 (16.7)
Apathy	11 (22.0)	1 (9.1)	3 (27.3)	4 (36.4)	2 (18.2)	1 (9.1)		Apathy	3 (6.0)	2 (66.7)	1 (33.3)				
Irritability	10 (20.0)	3 (30.0)	1 (10.0)	5 (50.0)	1 (10.0)			Irritability	1 (2.0)		1 (100)				
Aggressiveness	3 (6.0)		1 (33.3)	2 (66.7)				Aggressiveness	1 (2.0)					1 (100)	
Change behaviour	5 (10.0)	1 (20.0)	3 (60.0)		1 (20.0)			Change behaviour	–						
Memory/Attention/ executive problems	15 (30.0)	4 (26.7)	2 (13.3)	7 (46.7)	2 (13.3)			Memory/ attention/ executive problems	5 (10.0)	2 (40.0)	1 (20.0)	1 (20.0)	1 (20.0)		
Speech	9 (18.0)		4 (44.4)	3 (33.3)	2 (22.2)			Speech	–						
Pain	19 (36.0)	1 (5.3)	5 (26.3)	6 (31.6)	4 (21.1)	3 (15.8)		Pain	20 (40.0)	1 (5.0)	5 (25.0)	9 (45.0)	4 (20.0)	1 (5.0)	
Fatigue	5 (10.0)		1 (20.0)	3 (60.0)	1 (20.0)			Fatigue	5 (10.0)	2 (40.0)	2 (40.0)	1 (20.0)			
Dizziness	18 (36.0)	4 (22.2)	8 (44.4)	6 (33.3)				Dizziness	5 (10.0)	2 (40.0)	3 (60.0)				
Faint	4 (8.0)	1 (25.0)		2 (50.0)		1 (25.0)		Faint	–						
Constipation	6 (12.0)		1 (16.7)	1 (16.7)	1 (16.7)		3 (50.0)	Constipation [1m]	27 (54.0)	5 (18.5)	2 (7.4)	3 (11.1)	1 (3.7)	5 (18.5)	10 (37.0)
Urinary urgency	7 (14.0)	2 (28.6)	2 (28.6)	3 (42.9)				Urinary urgency	4 (8.0)	1 (25.0)	2 (50.0)			1 (25.0)	
Urinary incontinence	4 (8.0)	3 (75.0)	1 (25.0)					Urinary incontinence	3 (6.0)		1 (33.3)	1 (33.3)		1 (33.3)	
Urinary frequency [1m]	3 (6.0)		1 (33.3)			1 (33.3)		Urinary frequency	4 (8.0)	1 (25.0)		2 (50.0)	1 (25.0)		
Sexual dysfunction	3 (6.0)		1 (33.3)	1 (33.3)	1 (33.3)			Sexual dysfunction	9 (18.0)	1 (11.1)	2 (22.2)	1 (11.1)	5 (55.6)		

Xm X symptom/s was/were reported as prediagnostic (>1 year from diagnosis of PD/parkinsonism), but the patient neither the family remembered when exactly the specific symptom/s had started.

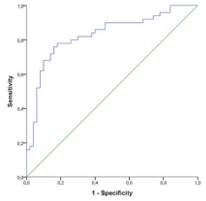
Table 3
Prediagnostic symptoms ability to differentiate PSP from PD.

		95% confidence interval			
PSP vs PD	Odds ratio	Lower margin	Upper margin	p-value	
Model 1					
Age	1.05	0.99	1.12	0.119	
Sex	0.82	0.33	2.03	0.676	
Cognitive	4.94	1.25	19.54	0.023	
Apathy	11.08	1.20	102.38	0.034	
Se: 50					
Sp: 80					
AUC: 0.70	0.60		0.81		
Model 2					
Age	1.03	0.96	1.11	0.390	
Sex	1.30	0.47	3.59	0.616	
Cognitive	11.32	1.03	18.67	0.045	
Falls	4.39	3.37	37.9	0.000	
Se: 60					
Sp: 90					
AUC: 0.79	0.77		0.88		
Model 3					
Age	1.01	0.94	1.09	0.766	
Sex	1.45	0.50	4.21	0.496	
Visual	12.11	3.49	42.06	0.000	
Falls	5.07	1.33	19.38	0.018	
Dizziness	5.16	1.13	23.47	0.034	
Se: 78					
Sp: 82					
AUC: 0.83	0.75		0.91		
Model 4					
Age	1.07	0.99	1.15	0.068	
Sex	0.79	0.27	2.29	0.658	
Cognitive	8.91	1.88	42.30	0.006	
Visual	8.27	2.11	32.49	0.002	
Lack of PD prodromal features*	9.03	3.08	26.47	0.000	
Se: 78					
Sp: 74					
AUC: 0.85	0.77		0.93		
Model 5					
Age	1.05	0.97	1.14	0.206	
Sex	1.16	0.37	3.56	0.801	
Visual	6.41	1.49	27.57	0.012	
Falls	11.99	3.07	46.91	0.000	
Lack of PD prodromal features*	7.81	2.55	23.89	0.000	
Se: 78					
Sp: 82					
AUC: 0.88	0.82		0.95		
Model 6					
Age	1.07	0.99	1.16	0.109	
Sex	0.94	0.31	2.86	0.911	
Apathy	12.11	1.12	130.32	0.040	
Falls	10.32	2.73	39.03	0.001	
Lack of PD prodromal features*	7.42	2.49	22.12	0.000	
Se: 78					
Sp: 78					
AUC: 0.87	0.80		0.94		

(continued on next page)

Table 3 (*continued*)

Model 7				
Age	1.06	0.99	1.13	0.110
Sex	0.91	0.35	2.38	0.848
Visual	6.01	1.71	21.11	0.005
Cognitive	5.31	1.28	22.13	0.022
Apathy	9.82	0.99	97.08	0.051
Se: 62				
Sp: 82				
AUC: 0.76		0.66	0.85	



Significant binary logistic regression models with different combinations of demographic and prediagnostic symptoms as predictors (independent variables) and PSP as outcome (dependent variable).
*PD prodromal features include: hyposmia, constipation, dream enacting behaviour and frequent nightmares.

3.4. Visits to other specialists in the prediagnostic phase

PSP patients had more consultations to ENT (p = 0.028) and ophthalmologist (p = 0.016) before the diagnosis compared to PD patients, but not to CSs.

3.5. Prediagnostic symptoms ability to differentiate PSP from PD or CS

The age- and sex-adjusted model that included visual problems, falls and lack of PD prodromal features as independent predictors was the model with better ability to differentiate PSP from PD. Other predictive models age- and sex-adjusted, with AUCs equal or greater than 0.85, included the combination of lack of prodromal PD features plus falls and apathy or visual and cognitive problems as independent variables. The other predictive models with their ROC analyses and curves can be seen in Table 3.

In the age- and sex-adjusted binary logistic regression models, comparing PSP and CSs either depression, cognitive problems or apathy + falls were predictive of PSP with AUCs equal or higher than 0.80 (Supplementary Table 4). The best predictive model in this group was the one that included gait problems, depression and dizziness. The age- and sex-adjusted model comparing PSP to non-PSP (PD + CS) is presented in Supplementary Table 4.

4. Discussion

To the best of our knowledge, this is the first study exploring the symptoms of the prediagnostic phase of clinically diagnosed PSP. In our cohort patients with PSP presented prediagnostic motor symptoms, too subtle or mild to allow the PSP diagnosis. PSP patients also featured prediagnostic non-motor features (mostly affective, cognitive and behavioral). Remarkably, part of the cases presented these prediagnostic symptoms between 3 and up to 10 years before the diagnosis.

Both, motor and non-motors features have been recognized since PSP description and included in different diagnostic criteria over the decades up until the most recent ones [3]. However, as usual with clinical diagnostic criteria in neurodegenerative diseases, the combinations of symptoms allowing for a greater specificity usually imply a diagnostic delay. Accordingly, the last PSP diagnostic criteria have included the "suggestive of" category, as a means to identify early cases. Still, there is scanty evidence of prediagnostic PSP. Candidate prediagnostic symptoms might include both motor symptoms (as unsteadiness, falls and eye movements abnormalities not meeting the requirements of the diagnostic criteria) and non-motor symptoms (typically the ones resulting from cognitive and neuropsychiatric dysfunction) [12].

In our cohort motor problems as falls and gait disturbances were common prediagnostic symptoms in keeping with previous clinicopathological studies [13], although those could be overrepresented due to the predominance of motor PSP phenotypes. Hence, falls appeared not associated with other motor symptoms in part of the cases and in a remarkable proportion of participants more than three years before the diagnosis. This allows us to interpret falls in these cases as a true prediagnostic feature rather than the result of diagnostic delay (see further on). To a smaller extent, gait disturbances were also the sole motor symptom during more than a year. Of note, both falls and gait disturbances often associated with other prediagnostic non-motor symptoms such as mood/apathy, cognitive or visual features were predictive of PSP in our logistic binary regression models.

In terms of prediagnostic cognitive symptoms, cognitive dysfunction in PSP has a prevalence over 50% [14] chiefly characterized by subcortical and frontal dysfunction [15] as reduced verbal fluency and impaired executive functions [15, 16]. In addition, PSP patients can also present speech and language disturbances [17]. Under the domain "cognitive" we included attention-memory-executive and speech problems. The frequency of prediagnostic cognitive symptoms in our PSP cohort was lower than the prevalence found in established PSP patients, but still significantly higher compared to PD and CS groups. When analyzed independently, speech but not attention problems remained significantly higher in PSP. This could be due to speech problems being more specific and earlier feature in PSP compared to PD, where attention problems are common in early disease.

Neuropsychiatric symptoms like apathy and depression have been widely described in PSP patients [15,18], and are important determinants of quality of life and potentially treatable [19]. The frequency of depression was high (over 40%), even more than PD (24%), and the frequency of apathy was of 20%. Several studies have shown depressive symptoms as the most frequent mood disturbance in PSP [18,20] being along with apathy more severe than in PD and multiple system atrophy (MSA) [15]. By contrast, prediagnostic anxiety was non-significantly more frequent in PSP (26%) than in PD (12%) or CSs (12%) in keeping with previous research in established PSP compared to MSA and PD [21] or CSs [18]. Finally, in our cohort PSP had more prediagnostic irritability when compared with CS (14 vs. 0%) and they presented more behavioral problems overall than PD or CS, in line with what has been observed after the diagnosis [20].

Prediagnostic visual symptoms also were more frequent in PSP than PD (34% vs. 8%, p = 0.012). PSP patients also had more prediagnostic visits to ophtalmologists compared to the PD group, but not CSs. This is very likely due to the fact that CSs were mostly patients with hemifacial spasm, frequently referred to ophtalmologists. Most importantly, while previously visual complaints have been found to occur earlier than other symptoms [13], in our case visual symptoms appeared mostly within three years from diagnosis, and may be preceded by other prediagnostic symptoms like the neuropsychiatric ones.

Conversely, in terms of timing to diagnosis, over 50% of prediagnostic falls, apathy, anxiety, depression and memory-attention-executive symptoms, and over 30% of gait disturbances started more than 3 and up to 10 years before the diagnosis of PSP or parkinsonism. Thus, as stated above, we consider these symptoms unlikely to be due to diagnostic delay, which has decreased from 43.76 months in the 1990s to 29.15 months in the 2010s [22].

Finally, our results have replicated the observation that PD patients more frequently presented some features such as hyposmia, dream enactment, nightmares and constipation. The absence of these symptoms also was predictive of PSP in our logistic binary regression models. Thus, the absence of these prodromal PD symptoms in the presence of other "pro-PSP" features might enhance the ability to identify very early PSP.

Our study has limitations. As already mentioned, it is a retrospective single-center study and our cohort might be biased to motor PSP phenotypes as patients were recruited from a Movement Disorder's Unit. Potential recall errors may have biased data collection of the presence and onset of prediagnostic symptoms. For example, cognitive and neuropsychiatric symptoms could be harder to remember or more affected by lack of insight relative to motor symptoms as falls. We also recorded the cognitive symptoms and domain as per clinical chart review and the questionnaire, but without a specific neuropsychological study. In studies as ours, discrepancies between patients and their caregivers or between clinical charts and interviews must also be taken into account. Another limitation is that PSP patients have been compared to PD patients, but not to other parkinsonisms (vascular, Lewy Body) with which it shares early symptoms (falls, gait, cognition). This implies that specificity of the aforementioned predictive models must be interpreted with caution. Finally, we do not have neuropathological diagnosis confirmation for the majority of the cases. However, in the last follow-up visit the majority of PSP patients met criteria for probable PSP and two of them have been neuropathologically confirmed. Further prospective longitudinal studies with larger sample size will help confirming or not our findings, which might help to better define the different profiles of participants in such studies.

In summary, our findings suggest that PSP patients may present with a broad variety of not only motor, but also non-motor clinical features several years before the diagnosis. Hence, falls, cognitive and mood disturbances might be considered as key features of the prediagnostic phase of PSP and useful for selection of enriched populations in prospective studies to further define this prediagnostic phase and its implications in future research of novel therapies.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parkreldis.2020.03.003.

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4.2. ARTICLE 2

Combined CSF α-SYN RT-QuIC, CSF NFL and midbrain-pons planimetry in degenerative parkinsonisms: From bedside to bench, and back again.

Compta Y*, Painous C*, Soto M*, Pulido-Salgado M, Fernández M, Camara A, Sánchez V, Bargalló N, Caballol N, Pont-Sunyer C, Buongiorno M, Martin N, Basora M, Tio M, Giraldo DM, Pérez-Soriano A, Zaro I, Muñoz E, Martí MJ, Valldeoriola F. Parkinsonism Relat Disord. 2022;99:33-41. doi: 10.1016/j.parkreldis.2022.05.006.

(*Autors amb contribució igual al treball)

Resum article 2:

Combinació de RT-QuIC d'aS en LCR, NFL en LCR i planimetria de mesencèfal i protuberància en parkinsonismes degeneratius.

Objectius: Analitzar la capacitat diagnòstica resultant de la combinació de diferents marcadors diagnòstics (biològics i radiològics) en els parkinsonismes degeneratius, inclosa la PSP.

Metodologia: vam recollir dades demogràfiques i clíniques i vam posar en funcionament el RT-QuIC d'aS al nostre laboratori en una cohort transversal de 112 participants: 19 SC, 20 amb MP, 37 amb AMS, 23 amb PSP i 13 amb DCB. També vam determinar els NFL en LCR per ELISA i en un subgrup de 74 participants (10 SC, 9 amb MP, 26 amb AMS, 19 amb PSP, 10 DCB) vam obtenir mesures planimètriques en RM de mesencèfal i protuberància de forma automatitzada.

Resultats: En la MP el RT-QuIC d'aS presentava una sensibilitat del 75% i a l'AMS de l'11%. En els SC i PSP+DCB la tècnica va resultar en especificitats del 100 (19/19) i 89% (32/36), respectivament. Pel que fa als NFL i les mesures planimètriques en RM vam replicar troballes descrites prèviament en la literatura. Els resultats de l'anàlisi de corbes ROC resultants dels models de regressió logística binària combinant diferents biomarcadors es resumeixen en: MP vs. no-MP: AUC=0,961 (95%CI=0,897–1,000), p<0,001, sensibilitat/especificitat = 78,0/94,0; AMS vs. no-AMS: AUC=0,936 (95%CI=0,881–0,991), p<0,001, sensibilitat/especificitat = 84,0/83,0; taupaties vs. no- taupaties: AUC=0,933 (95%CI=0,875–0,990), p<0,001, sensibilitat/especificitat = 86,0/82,0.

Conclusions: la combinació de diferents biomarcadors pot ser d'utilitat en el diagnòstic diferencial de la PSP amb diferents parkinsonismes neurodegeneratius i pot incrementar el rendiment diagnòstic en comparació amb la utilització dels biomarcadors de forma aïllada.

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Combined CSF α-SYN RT-QuIC, CSF NFL and midbrain-pons planimetry in degenerative parkinsonisms: From bedside to bench, and back again

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ABSTRACT

Introduction: Differential diagnosis between Parkinson's disease (PD) and atypical parkinsonisms (APs: multiple system atrophy[MSA], progressive supranuclear palsy[PSP], corticobasal degeneration[CBD]) remains challenging. Lately, cerebrospinal fluid (CSF) studies of neurofilament light-chain (NFL) and RT-QuIC of alpha-synuclein (α-SYN) have shown promise, but data on their combination with MRI measures is lacking.
Objective: (1) to assess the combined diagnostic ability of CSF RT-QuIC α-SYN, CSF NFL and midbrain/pons MRI planimetry in degenerative parkinsonisms; (2) to evaluate if biomarker-signatures relate to clinical diagnoses and whether or not unexpected findings can guide diagnostic revision.
Methods: We collected demographic and clinical data and set up α-SYN RT-QuIC at our lab in a cross-sectional cohort of 112 participants: 19 control subjects (CSs), 20PD, 37MSA, 23PSP, and 13CBD cases. We also determined CSF NFL by ELISA and, in 74 participants (10CSs, 9PD, 26MSA, 19PSP, 10CBD), automatized planimetric midbrain/pons areas from 3T-MRI.
Results: Sensitivity of α-SYN RT-QuIC for PD was 75% increasing to 81% after revisiting clinical diagnoses with aid of biomarkers. Sensitivity for MSA was 12% but decreased to 9% with diagnostic revision. Specificities were 100% against CSs, and 89% against tauopathies raising to 91% with diagnostic revision. CSF NFL was significantly higher in APs. The combination of biomarkers yielded high diagnostic accuracy (PD vs. non-PD AUC = 0.983; MSA vs. non-MSA AUC = 0.933; tauopathies vs. non-tauopathies AUC = 0.924). Biomarkers-signatures fitted in most cases with clinical classification.
Conclusions: The combination of CSF NFL, CSF RT-QuIC α-SYN and midbrain/pons MRI measures showed high discriminant ability across all groups. Results opposite to expected can assist diagnostic reclassification.

Abbreviations: MRI, magnetic ressonance imaging; RT-QuIC, real time - quaking induced conversion; CSF, cerebrospinal fluid; NFL, neurofilament light-chain; PD, Parkinson's disease; PSP, progressive supranuclear palsy; MSA, multiple system atrophy; CBD, corticobasal degeneration; AP, atypical parkinsonism.

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1. Introduction

Neurodegenerative parkinsonisms are classified into synucleinopathies (Parkinson's disease(PD); multiple system atrophy(MSA)), or tauopathies (progressive supranuclear palsy(PSP); corticobasal degeneration(CBD)), depending on the underlying proteinopathy [1]. Despite the use of consensus criteria, differentiating PD from atypical parkinsonisms (APs: PSP, CBD and MSA) remains challenging, even in expert sites [2] as reliable biomarkers are lacking.

Increased cerebrospinal fluid (CSF) levels of neurofilament light chain (NFL) discriminate PD from APs, but are unspecific among APs [3]. Lately, real-time quaking-induced conversion (RT-QuIC) of alpha-synuclein (α-SYN) is being consistently found to be positive in PD and negative in tauopathies, albeit with low sensitivity in MSA [4,5], except for a study with 75% sensitivity, but longer time to positivity and lower amplification than PD [6]. A single published study has reported improved differentiation between PD and APs by combining NFL and α-SYN RT-QuiC, but without other clinical or imaging biomarkers [7]. MRI mid-sagittal planimetry has shown reduced midbrain and pons areas in PSP and MSA, respectively [8,9].

To the best of our knowledge, the combination of CSF NFL (as a biomarker of neuronal/axonal damage and proxy of aggressiveness of neurodegeneration), CSF α-SYN RT-QuiC (as a proxy of underlying abnormal and disease-causing aggregation of α-synuclein) and MRI brainstem planimetry (as indicators of differential predominance of midbrain [PSP] vs. pons [MSA] atrophy) remains unexplored in neurodegenerative parkinsonisms.

Accordingly, we hypothesized that: a) biomarkers-combination yields high diagnostic accuracy allowing for separating parkinsonisms on α-synuclein, aggressiveness, and regional brainstem atrophy axes; b) "biomarker-signatures" relate to clinical diagnoses consistent with different combinations of the aforementioned biomarkers; c) unexpected biomarker findings can, in part of cases at least, relate to phenotypic differences, or guide diagnosis revision. The objectives were thus to explore the combined biomarkers discriminant ability across degenerative parkinsonisms, to assess the association of "biomarker-signatures" with each diagnostic group, and to revisit the clinical features and the diagnosis of cases with unexpected biomarker results.

2. Methods

Design: Multicentre cross-sectional study centralized at the Parkinson's Disease & Movement Disorders Unit of Hospital Clinic Universitari de Barcelona.

Participants: The cohort recruited between 2015 and 2021 consisted of 112 participants: 20PD, 37MSA, 23PSP, 13CBD and 19 control subjects (CSs). Automatized MRI midbrain/pons areas planimetry was available for 74 participants (9PD, 26MSA, 19PSP, 10CBD, 10CSs). All CSF samples were used to set up α-SYN RT-QuIC at our lab. All diseased-participants fulfilled the "probable" (or "clinically established" in PD) category of their respective diagnostic criteria [10–13] upon recruitment, with at least two-year follow-up. CSs were aged>55, with CSF obtained before the injection of spinal anaesthesia for knee surgery, and no neuro-psychiatric condition by clinical history and exam. Secondary parkinsonism and large vascular MRI abnormalities were exclusion criteria, and so was Alzheimer's disease CSF profile [14] in CBD patients. The Ethics Committee approved the study and all participants signed their informed consent.

Clinical assessment: Demographic and clinical variables were sex, age, disease duration at inclusion, the Unified Parkinson's Disease Rating Scale part III (UPDRS-III) [15] and the Hoehn and Yahr (HY) rating scale [16]. Cognition was evaluated with the Montreal Cognitive Assessment (MoCa) [17] in all participants except in MSA patients, whom were assessed by the Mini Mental test (MMSE) [18]. We also administered the PSP rating scale (PSPRS) [19] to PSP, CBD and CSs and the Unified Multiple System Atrophy Rating Scale (UMSARS) [20] in MSA patients.

CSF protocol: All CSF samples were obtained using the protocol published elsewhere [21], via lumbar puncture at the L3-L4 level with a 22-gauge needle, between 8 and 10 a.m. The first 2 mL were used for routine studies. CSF was processed within 30min of obtention, centrifuged 10 min at 2000 rcf and 4 °C, and stored at −80 °C. CSF NFL levels were measured with a commercial ELISA NF-light kit (Umandiagnostics, Sweden) (NF-light datasheet). All samples were run in duplicate together with blank (sample diluent), calibrator solutions, and an internal control sample, evenly distributing all groups among runs. All standard curves fulfilled the quality control criteria established by the manufacturers and R2 values range between 0.999 and 1. For all analysis we used a single concentration value per case in pg/mL (the mean of duplicates; all duplicates had a variation coefficient <20%).

CSF α-SYN RT-QuIC was performed in black 96-well optical bottom plates (Thermo Scientific Nunc MicroWell) measuring all samples in triplicate. Samples from all groups were evenly distributed among runs and an RT-QuIC positive PD sample was added to each plate as positive control. Each well was preloaded with eight 0.5 mm zirconia/silica beads (BioSpec Products) and 85 μl of RT-QuIC reaction mix containing 100 mmol/L phosphate buffer (Ph 8.2), 10 μmol/L Thioflavin T (ThT) and 0.1 mg/ml recombinant human α-synuclein (Sigma-Aldrich). Reactions were seeded with 15 μl of CSF. Plates were then sealed with a sealing tape (Thermo Scientific Nunc) and incubated in a FLUOstar Omega (BMG Labtech) for 120h at 42 °C setting cycles of 90 s shaking at 600 rpm and 14 min resting. Fluorescence was measured at the end of each cycle at an emission wavelength of 482 nm after an excitation at 448 nm. Positivity was defined as ≥2 of 3 replicates over 2SDs above the mean of relative fluorescence units (RFUs) in CSs at 60h and 120h similar to previous studies [4].

MRI planimetry: Brainstem was parcelled using the Brainstem Bayesian FreeSurfer module [22]. First, for each subject, the T1-weighted image was aligned with a template in the MRI to identify the midsagittal plane. To account for variability in the alignment, 10 slices around the central sagittal slice were evaluated to identify the midsagittal slice, which was defined as the one containing the smaller midbrain area. To automatically assess midsagittal midbrain (M_A) and pontine (P_A) areas, the number of voxels segmented as midbrain and pons in the midsagittal slice were counted and multiplied by voxel size. Values are reported in cm^2. M_A and P_A were also manually assessed as previously described [23]. Two independent raters (VS and CP) performed the measurements blinded to the clinical information. To assess the intrarater reliability, a second blinded evaluation was made 1 month after the first evaluation by one of the raters (CP). Intraclass correlation coefficient (ICC) was calculated and considered as poor if ICC <0.40, fair if ICC between 0.40 and 0.59, good if ICC between 0.60 and 0.74, and excellent if ICC between 0.75 and 1.00 [24]. Intra-manual and manual-automatic agreements were excellent and good to excellent, respectively.

Statistical analyses: Statistical power calculation considering prior literature [4–6] resulted in a sample size of at least 80 individuals (20 PD, 20 MSA, 16 PSP, 8 CBD, 16 CSs) to obtain an ≥80% accuracy in the estimation of a proportion by means of a two-tailed 95% confidence interval (95%CI) under a dichotomic assumption (α-SYN RT-QuIC + vs. -, p = 50%). Qualitative variables are presented as absolute and relative frequencies; quantitative data as median and interquartile range (IQR). Non-parametric tests (Fisher's exact test for qualitative variables and Mann Whitney's U or Kruskal-Wallis test for continuous ones) were applied. Non-parametric analysis of covariance with age as covariate was used to compare NFL, M_A and P_A variables across groups. Spearman's correlations (rho), linear regressions and the respective scatter plots were used to evaluate linear correlation across quantitative clinical and biomarker variables.

Univariate binary logistic regression models and receiver operating characteristic (ROC) curves statistics with their area under the curves (AUCs), 95%CIs and sensitivities (Se)/specificities (Sp) were calculated for discrimination of PD vs. non-PD (CSs + MSA + PSP + CBD), MSA vs. non-MSA (CSs + PD + PSP + CBD) and tauopathies (PSP + CBD) vs. non-tauopathies groups (CSs + PD + MSA). NFL, α-SYN RT-QuiC, M_A or P_A were used as independent variables. Then, multivariate binary logistic regression models combining CSF NFL, α-SYN RT-QuiC, M_A and P_A were calculated, but limiting the number of covariates to minimize the risk of overfitting. The NFL variable was treated as a continuous and binary variable, using as cut-off 1315.40 pg/mL after the ROC curve analysis of CSF NFL levels in APs vs. non-APs (see details in Results).

As a post-hoc exploratory analysis we defined the following biomarkers signatures: "CS signature" = none biomarker altered or no clinical symptoms ± a single altered biomarker; "PD signature" = positive (+) α-SYN RT-QuIC & negative (−) NFL levels and normal MRI values; "tauopathy signature" = + NFL & midbrain atrophy; "MSA signature" = + NFL & pons atrophy. We analysed the proportion of each signature in each diagnostic group.

Finally, and as a "real life" approach to explore the hypothetic post-hoc ability of the studied biomarkers to confirm or challenge the initial diagnosis we revisited all participants' diagnoses according to α-SYN RT-QuIC (positive or negative) and NFL levels (< or >1315.40 pg/mL) results. In Results we summarize cases with unexpected findings and which of them could be reclassified considering both the biomarkers and the clinical follow-up after CSF collection, as in the rest of cases clinical diagnosis and biomarker findings were consistent and did not raise diagnostic doubts. We then accordingly recalculated sensitivities and specificities.

Statistical tests were two-tailed, with significance set at ≤0.05, corrected for multiple comparisons by false-discovery-rate(FDR) [25]. Missing values were not included in the analysis for its outcome. Data analysis was performed with IBM SPSS version 24.0 (Armonk, NY; IBM).

3. Results

Demographic and clinical data: No demographic differences were found except for younger age in MSA. Likewise, UPDRS, PSPRS, MoCa and HY did not significantly differ across parkinsonisms, but were worse in these groups vs. CSs (Table 1).

α-SYN RT-QuIC: In PD patients the proportion of positive seeding was significantly higher than in all other groups (Table 1), with 15/20 of them being positive (sensitivity = 75%). Sensitivity was much lower in MSA (4/33 = 12%). Conversely, none of the CSs and only 4 of 36 tauopathy cases were false positives (Table 1; Fig. 1A–C), resulting in specificities of 100% in PD vs. CSs, and 89% in PD vs. tauopathies. Univariate logistic regression followed by ROC curve analyses demonstrated the following accuracy for the discrimination of: PD vs. non-PD: AUC = 0.832 (95%CI = 0.748–0.895), p < 0.001, Se/Sp: 75.0/91.3; MSA vs. non-MSA: AUC = 0.573 (95%CI = 0.474–0.655), p = 0.06. In the case of PD, since disease duration was significantly longer than in other conditions, we run a binary logistic regression model for PD vs. no-PD with CSF α-SYN RT-QuIC as predictor covaried by disease duration along with the ROC curve resulting from the propensity scores of the regression model, showing that CSF α-SYN RT-QuIC remains an independent significant predictor of PD when adjusting for disease duration (Suppl. Fig 2).

CSF NFL: APs had significantly higher CSF NFL levels than PD and CSs (Table 1). ROC curve analysis of APs vs. non-APs yielded AUC = 0.953 (95%CI = 0.885–0.983), p < 0.0001, with an optimal cut-off of 1315.40 pg/mL (Se = 88%; Sp = 97%). Albeit levels were significantly higher in MSA vs. tauopathies, the difference was marginal with large overlap (AUC = 0.631; 95%CI = 0.504–0.757; p = 0.046; Se/Sp = 25.0/80.4). PD had significant higher NFL vs. CSs (Table 1; AUC = 0.743 (95%CI = 0.577–0.890), p = 0.017, Se/Sp = 58.8/68.8.

MRI planimetry: M_A was significantly smaller in PSP vs. all other

Table 1
Demographic, CSF and MRI measures across the different diagnostic groups.

	CS (n = 19)	PD (n = 20)	MSA (n = 37; 22MSAp [60%], 15MSAc [40%])	PSP (n = 23; Richardson's 15[65%], non-Richardson's 8 [35%])	CBD (n = 13)	p-value *
Age (in years)	72.2 [66.9–74.8]	70.5 [64.9–74.7]	62.5 [55.9–66.8]	74.5 [71.3–79.5]	70.3 [68.3–74.5]	**All groups:** 0.001; **PSP/MSA**: <0.001; **MSA/CS**: 0.006; **MSA/PD**: 0.010; **MSA/CBD**: 0.002;
Female sex (%)	11 (57.9)	11 (55.0)	17 (45.9)	9 (39.1)	10 (76.9)	
Disease duration (in years)	NA	7.8 [4.5–15.6]	4.4 (3.1–6.8)	4.4 (3.1–6.8)	5.1 (3.7–6.8)	
UPDRS	0 [0–2.0]	32 [23–50]	44.5 [42–70]	36 [25.5–45]	48 [42–65]	**PSP/CS**: <0.001; **MSA/CS**: <0.001; **CBD/CS**: <0.001; CBD/PD: 0.254; **PD/CS**: <0.001
PSPRS	0 [0–0]	NA	NA	35 [22–47]	36 [30–43]	**PSP/CS**: <0.001; **CBD/CS**: <0.001
UMSARS	NA	NA	44 [32–69]	NA	NA	
MoCa	26 [26–28]	20.5 [10–27]	NA	22 [16–25]	19 [9.5–26.5]	**PSP/CS**: 0.006
MMSE	NA	NA	28 [28–30]	NA	NA	
HY > 2 (%)	0 (0)	8 (53.3)	25 (69.4)	17 (81.0)	8 (61.5)	**PSP/CS**: <0.001; **MSA/CS**: <0.001; **CBD/CS**: 0.001; **PD/CS**: <0.001
α-SYN RT-QuIC N (+)/N (−)	0/19	15/5	4/33	3/20	1/12	**All groups:** <0.001; **PSP/PD:** <0.001; **MSA/PD:** <0.001; **CBD/PD:** <0.001; **PD/CS:** <0.001
CSF NFL (pg/mL)	656.2 [504.2–867.0]	953.7 [697.4–1237.5]	2879.2 [1939.4–3840.9]	1897.5 [1554.3–2441.2]	2161.5 [1701.6–2330.0]	**All groups:** < 0.001; **AP/PD + CS:** <0.001; **PSP/PD:** < 0.001; **PSP/CS:** <0.001; **MSA/CS:** <0.001; **MSA/PD:** <0.001; **CBD/CS:** < 0.001; **CBD/PD:** <0.001; **PD/CS:** <0.001
M_A (cm^2)	1.1 [1.1–1.2]	1.3 [1.2–1.4]	1.3 [1.2–1.4]	0.9 [0.8–1.0]	1.0 [0.9–1.1]	**All groups:** <0.001; **PSP/MSA:** <0.001; **PSP/PD:** <0.001; **PSP/CS:** 0.013; **MSA/CBD:** 0.004; **CBD/PD:** < 0.001;
P_A (cm^2)	4.6 [4.4–5.6]	4.9 [4.5–5.1]	3.9 [3.4–4.4]	4.7 [4.4–5.2]	4.5 [4.1–4.9]	**All groups:** 0.002; **PSP/MSA:** 0.003; **MSA/CS:** 0.017; **MSA/PD:** 0.008;

Quantitative variables are presented as median [IQR] and compared between groups using Kruskal-Wallis or U Mann Whitney tests as appropriate.
Categorical variables are presented as absolute frequency (proportion) and compared between groups using Fisher's exact test.
*p-values results are presented FDR-corrected. Only significant results are listed.

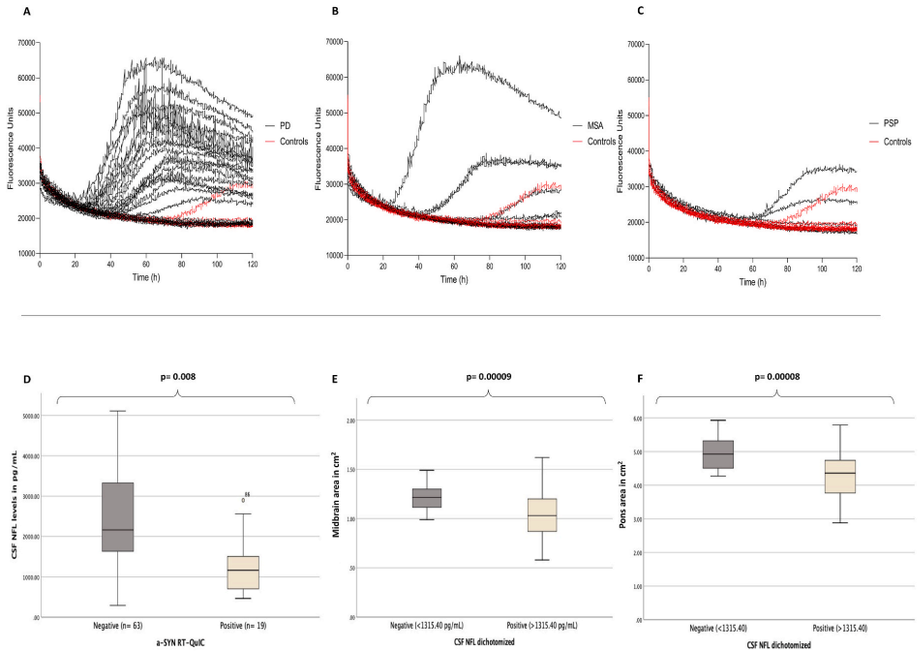
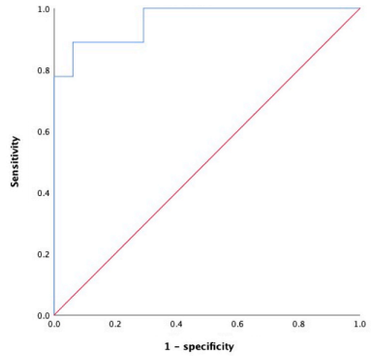


Fig. 1. Representative examples of CSF α-SYN RT-QuIC using FLUOSTAR OMEGA. Examples are from patients studied in the same experiment. Figure legend: A = 7 out of 9 patients PD cases were positive in contrast to none of the 4 CSs; B = 2 of 4 MSA patients were positive compared to the 4 negative CSs; C = 3 of 3 PSP samples did not reach the threshold of 2 of 3 replicates required for positivity as happened with the 4 CSs; D = Significantly Higher CSF NFL levels in participants with negative compared to those with positive α-SYN RT-QuIC; E = significantly smaller midbrain area in participants with positive vs. negative CSF NFL (according to the cut-off with best sensitivity and specificity for differentiating atypical parkinsonisms from PD); F = significantly smaller pons area in participants with positive vs. negative CSF NFl; G = significant negative correlation between pons area and CSF NFL levels driven by MSA cases (the higher the CSF NFL levels, the smaller the pons area); H = significant positive correlation between PSP scores and CSF NFL levels driven by tauopathy-cases (PSP or CBD) (the higher the CSF NFL levels, the higher, thus worse, the PSP scores); I = significant negative correlation between PSP scores and midbrain area driven by tauopathy cases (the smaller the midbrain area, the higher, thus worse, the PSP scores).

groups except CBD, which had smaller M_A than PD and MSA. MSA presented smaller P_A vs. all other groups except CBD (Table 1). ROC curve analyses for M_A were as follows: PD vs. non-PD: AUC = 0.836 (95%CI = 0.734–0.913), p-value<0.001, Se/Sp = 66.7/81.5; tauopathies vs. non-tauopathies: AUC = 0.846 (95%CI = 0.750–0.923), p < 0.001, Se/Sp = 75.9/82.2. For P_A the results were: PD vs. non-PD: AUC = 0.683 (95%CI = 0.571–0.792), p = 0.110; MSA vs. non-MSA: AUC = 0.797 (95%CI = 0.688–0.882), p < 0.001, Se/Sp = 73.1/75.0.

Gender perspective: No significant gender differences were found for positive CSF α-SYN RT-QuIC (22.4% women; 18.5% men; p = 0.65), high CSF NFL (59.3% women; 67.2% men; p = 0.44), midbrain atrophy (48.6% women; 39% men, p = 0.49) and pons atrophy (40% women; 39% men, p = 1.00). ROC analyses yielded significant accuracies for CSF α-SYN in PD, CSF NFL in APs, M_A in tauopathies and P_A in MSA in both

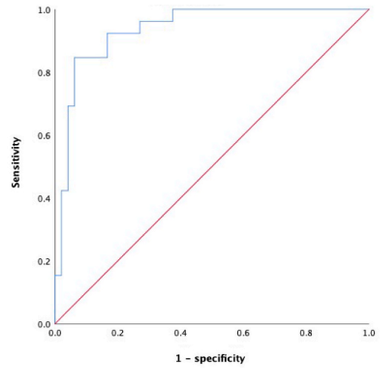
A



n= 74 (9 PD vs. 65 no-PD)
AUC= 0.961
95%CI= 0.897-1.000
P= 0.000008

ROC curve of PD vs. non-PD groups (propensity scores from binary logistic regression model combining α-SYN RT-QuIC, CSF NFL levels and MRI midbrain area)

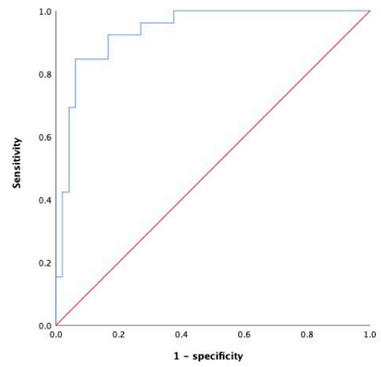
B



n= 74 (26 MSA vs. 48 no-MSA)
AUC= 0.936
95%CI= 0.881-0.991
P= 0.0000004

ROC curve of MSA vs. non-MSA groups (propensity scores from binary logistic regression model combining α-SYN RT-QuIC, CSF NFL levels, MRI pons area and age)

C



n= 74 (29 tauopathies vs. 45 non-tau participants)
AUC= 0.933
95%CI= 0.875-0.990
P= 0.00000000004

ROC curve of tauopathies vs. non-tau groups (propensity scores from binary logistic regression model combining α-SYN RT-QuIC, CSF NFL & MRI midbrain & pons areas)

Fig. 2. ROC curves of propensity scores from different binary logistic regression models combining biomarkers: [A] model covaried for RT-QuIC, NFL and midbrain area in PD vs. non-PD: AUC = 0.961 (95%CI = 0.897-1.000), p < 0.0001, Se/Sp = 78.0/94.0; [B] model covaried for RT-QuIC, NFL, pons area and age in MSA vs. non-MSA: AUC = 0.936 (95%CI = 0.881-0.991), p < 0.0001, Se/Sp = 84.0/83.0; [C] model covaried for RT-QuIC, NFL, midbrain and pons areas in tauopathies vs. non-tauopathies: AUC = 0.933 (95%CI = 0.875-0.990), p < 0.0001, Se/Sp = 86.0/82.0.

women and men (Suppl. Fig. 1).

Biomarker-biomarker and clinical-biomarker associations: CSF NFL levels were significantly higher in participants with negative α-SYN RT-QuIC (driven by APs) and midbrain and pons areas were significantly smaller in participants with high CSF NFL levels (driven by PSP and MSA cases, respectively) (Fig. 1D–F). Significant negative correlations were found between pons area and CSF NFL levels driven by MSA cases (the higher the CSF NFL levels, the smaller the pons area) and between PSPRS and midbrain area (the smaller the midbrain area, the higher the PSP scores) (Fig. 1G and I). Significant positive correlation was found between PSPRS and CSF NFL levels (the higher the CSF NFL levels, the higher the PSP scores) (Fig. 1H). The proportion of + α-SYN RT-QuIC, + NFL and midbrain/pons atrophy did not significantly differ either between MSA-p and MSA-c, or between Richardson's and non-Richardson's-PSP except for significantly greater midbrain atrophy in Richardson's vs. non-Richardson's-PSP.

Combined biomarkers diagnostic accuracy: The results of ROC curve analyses using propensity scores from binary logistic regression analyses combining biomarkers are shown in Fig. 2A–C and summarized as follows: PD vs. non-PD: AUC = 0.961 (95%CI = 0.897–1.000), p < 0.0001, Se/Sp = 78.0/94.0; MSA vs. non-MSA: AUC = 0.936 (95%CI = 0.881–0.991), p < 0.0001, Se/Sp = 84.0/83.0; tauopathies vs. non-tauopathies: AUC = 0.933 (95%CI = 0.875–0.990), p < 0.0001, Se/Sp = 86.0/82.0.

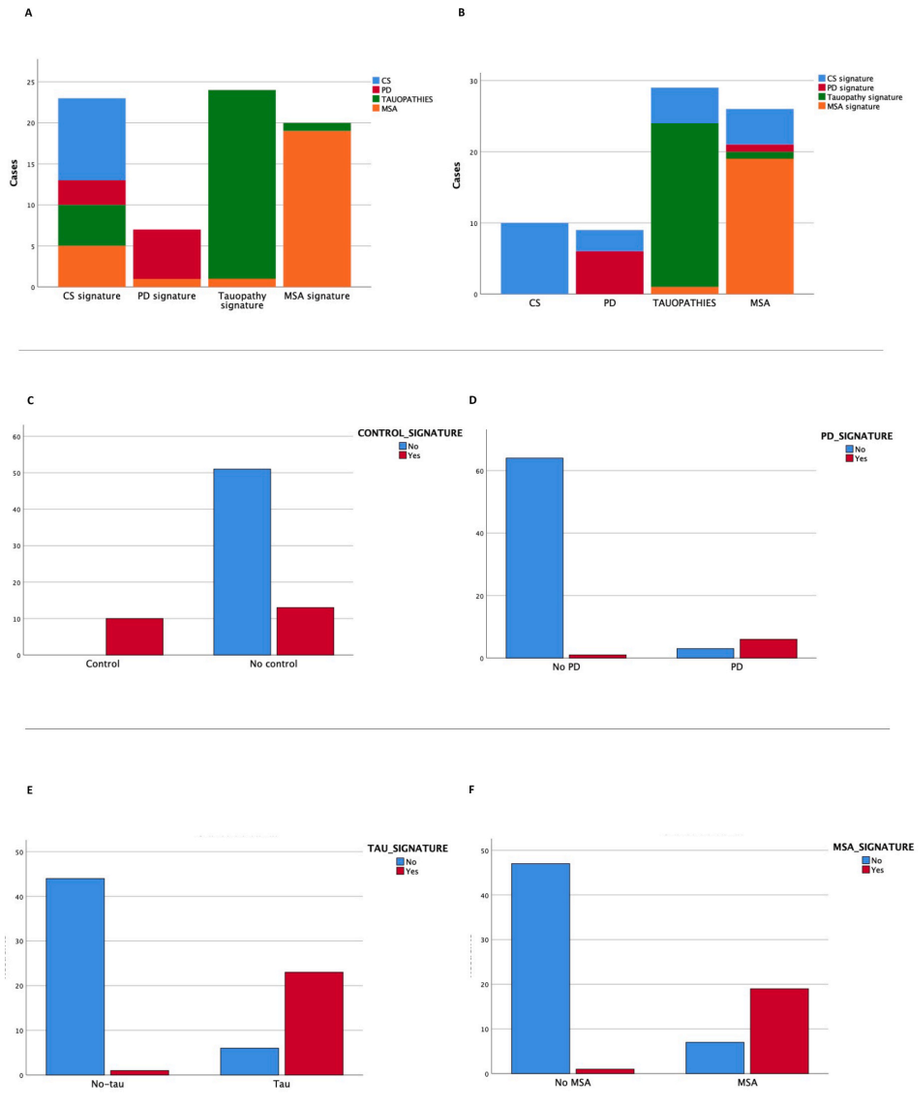


Fig. 3. Biomarkers-signatures association with clinical classification: A = predominance of cases with fitting clinical classification in each biomarkers-signature; B = predominance of cases with fitting biomarkers-signature in each clinical classification; C = predominance of lack of "control-signature" among non-control participants; D = predominance of lack of "PD-signature" among non-PD participants; E = predominance of "tau-signature" among tauopathies (PSP + CBD); F = predominance of "MSA-signature" in MSA.

Biomarkers-signatures: All CSs (10/10) had "CS signature". Among PD, 77.7% (7/9) had "PD signature" (the remaining 23.3% (2/9) "CS signature"). In clinically diagnosed tauopathies, 79.8% (23/29) had "tauopathy signature", 17.2% (5/29) "CS signature" and 1 subject "MSA signature". Patients with tauopathy and "CS signature" presented lower PSPRS (17 [15–17] vs. 35.5 [30–47], p = 0.0064) and UPDRS (22 [22–27] vs. 43 [28.5–50] p = 0.0302) scores compared to patients with tauopathy and "non-CS signature. Among MSA 73.1% (19/26) had "MSA signature", 19.2% (5/26) "CS signature", one "tauopathy signature" and another one "PD signature" (Fig. 3A–F).

3.1. Unexpected biomarkers findings: post-hoc diagnostic and Se/Sp revision

α-SYN RT-QuIC: Of the 5 PD cases with negative α-SYN RT-QuIC, one had presented with asymmetrical rigid-akinetic parkinsonism and modest response to levodopa along with vascular risk factors (which are not an exclusion criterion for PD) and mild microvascular damage in brain MRI, thus being classified as PD at the time of obtention of CSF. Thereafter, though, the patient added more vascular lesions on MRI and did not improve with further increase of levodopa, but subsequently stabilized, not progressively worsening in the setting of strict control of vascular risk factors. Of the 4 MSA cases with positive α-SYN RT-QuIC, one had normal CSF NFL values and normal MRI, as well as, eventually, response to high doses of levodopa with typical rest tremor, allowing for reclassifying him as PD. Exclusion of the former case and inclusion of the latter one would increase sensitivity from 75% to 80% (16/20). The other 3 MSA cases with positive α-SYN RT-QuIC had high CSF NFL levels and a clinical picture strongly suggestive and meeting the accepted MSA diagnostic criteria. This along with the fact that the remaining MSA cases with negative α-SYN RT-QuIC also fulfilled the accepted MSA diagnostic criteria, would leave post-hoc sensitivity of α-SYN RT-QuIC for MSA as low as 9% (3/32).

In terms of "false" + for α-SYN RT-QuIC among tauopathies, all 3 PSP participants with positive α-SYN RT-QuIC meet the MDS PSP diagnostic criteria (two with high CSF NFL levels; two with PSP-RS phenotype, one with the PSP-PGF variant). Conversely, the only CBD case with positive α-SYN RT-QuIC presented with asymmetrical parkinsonism with apraxia elements, but at follow-up has a mixed clinical picture including hyposmia, RBD and hallucinations besides parkinsonism which make of PD of the Lewy body dementia variant [16] the most likely diagnosis at last follow-up. This would increase the above recalculated post-hoc sensitivity of α-SYN RT-QuIC for PD from 80% to 81% (17/21), and specificity in PD vs. tauopathies to 91% (32/35).

CSF NFL: Two MSA cases had normal CSF NFL levels, one already has been commented above and has been clinically and paraclinically reclassified as PD; the other one has a diagnosis of MSA-cerebellar type with 11 years of history and mild dysautonomia and still relatively preserved on motor grounds, with negative studies for CAG trinucleotide repeat expansion SCAs. The two PSP cases with low instead of high CSF NFL levels had PSP-P and PSP-PGF phenotypes with long and benign evolution. Likewise, of the two CBD cases with low CSF NFL levels one has 7 years of history with typical corticobasal features, wheelchair-bound and with important left-side apraxia, still cognitively spared; the other one also has parkinsonism and corticobasal features contralateral to the most affected side on presynaptic dopaminergic imaging as the other case, but in this one the follow-up is even longer (9 years) and milder (still able to walk).

4. Discussion

We set up CSF α-SYN RT-QuIC at our site in a cohort of 112 participants ranging from controls to the spectrum of PD and APs (MSA, PSP and CBD). We also report for the first time that the combination of CSF RT-QuIC α-SYN, CSF NFL and midbrain/pons MRI measures in degenerative parkinsonisms yields high accuracy. "Biomarkers signatures" fitted in most cases with clinical classification at recruitment, while part of cases with either false positive or false negative results could be clinically reclassified or their unexpected biomarker findings could be explained by their phenotype and disease progression.

To date several studies have shown high sensitivity and specificity of CSF α-SYN RT-QuIC, particularly for PD, and more inconsistently for MSA. Our results are in keeping with this literature [3–6,26]. Our finding of low sensitivity of α-SYN seeding in MSA deserves further comment. Attempts to detect α-synuclein in MSA have been challenging with most/several studies using CSF RTQuIC reporting low sensitivities, ranging from <10% [5,7] to 35% [26], except in the study of Poggioni et al. who detected a 75% sensitivity. By contrast, two studies using protein misfolding cyclic amplification (PMCA), a seeding assay similar to RTQuIC, have shown promising results. Shahnawaz and colleagues reported that patients with MSA presented significantly lower fluorescence signal compared to PD, but with 65 out of 75 CSF MSA samples containing α-SYN aggregates. In addition, they demonstrated distinct conformational strains between MSA and PD [27]. The second study [28] also found high sensitivity in two different cohorts (100% and 94% sensitivity). This discrepancy between sensitivity results could be explained by differences in the reaction buffers or recombinant α-SYN used in the different studies suggesting that inherent heterogeneity in α-synuclein seeding occurs in MSA [29].

In terms of combining biomarkers, the combination of CSF RT-QuIC α-SYN and CSF NFL has already been shown to result in improved discriminant ability, with positive CSF RT-QuIC α-SYN among PD and high CSF NFL in APs driving the results [6]. Likewise our MRI findings are in line with previous literature [8,9].

The "biomarkers signatures" we have defined fitted largely with the initial clinical classification. Adding also to the clinical significance of these biomarkers on an individual basis we have seen that in part of patients with unexpected biomarkers findings such results could be attributed to phenotypic peculiarities. Two PSP patients with normal (low) CSF NFL levels have PSP-parkinsonism and PSP-freezing variants, associated with lesser aggressiveness and longer survival [30], which fit with normal (low) CSF NFL levels. Likewise, cases with MSA and CBD with low CSF NFL levels also had longstanding and rather benign disease course. Alternatively, unexpected results in RT-QuIC can be a clue for clinicians to reconsider the diagnosis: one patient initially labelled as PD was reclassified as vascular parkinsonism; another with MSA and positive CSF α-SYN RT-QuIC but normal CSF NFL and MRI was relabelled as PD. Another case with asymmetric apraxia eventually disclosed RBD and presented visual hallucinations and cognitive fluctuations fitting with PD of the dementia with Lewy bodies variant [13]. This post-hoc revision of clinical diagnoses based on biomarkers findings is to be taken with caution, as alternative explanations for false negative or positive results might be failure of the technique itself in some cases, or the presence of co-pathology in false positive cases, as in the case of positive CSF α-SYN RT-QuIC in PSP (13%), which is similar to previously reported postmortem Lewy co-pathology in PSP of 8% [31].

This study is not without limitations. The diagnosis is clinical, not neuropathologically confirmed, limiting the interpretability of the current findings. However, the participants included fulfilled the category of "probable" for their respective diagnosis criteria which provides high diagnostic accuracy and, patients have been systematically examined by neurologists with expertise in parkinsonism. The study findings do not allow for differentiating MSA vs. tauopathies or between tauopathies (PSP vs. CBD) solely relying on CSF α-SYN RT-QuIC or CSF NFl levels. However, and this was one of the goals of the paper, when combining CSF and MRI biomarkers both statistically and under the "profiles" approach there is a hint that these combinations can be helpful, while waiting for more specific CSF α-SYN RT-QuIC and for validated CSF RT-

QuIC for 4r-tau and/or reliable α-synuclein and tau PET tracers. As additional limitations we have not a validation cohort, nor early disease or unclassifiable cases. The sample size is relatively small, particularly in the case of the subgroup with MRI measures available, yet in the range of previous studies [3–6,8,9], and above our sample size calculation. All this along with the fact that we have replicated prior published results gives robustness to our data. However, further studies with larger samples are needed. Our post-hoc recalculation of sensitivities and specificities can be criticized too, but we have done this under the "real clinical practice perspective", in a similar way to when a dopamine imaging scan comes back negative leading to reconsider clinical diagnosis. While obviously this comparison is perhaps premature now, we believe that the section of clinically revisited diagnosis and sensitivities/specificities is of clinical interest. Finally, as mentioned above, we have not assessed RT-QuIC for 4R-tau, expected to mirror in tauopathies the accuracy of RT-QuIC for α-SYN in synucleinopathies, but still needing further validation [32]. Future studies combining CSF α-SYN RT-QUIC with 4R-tau RT-QuIC or PET imaging of 4R-tau [33] might improve diagnosis of tauopathies and even track the effect of potential disease modifying strategies.

In conclusion, the combination of CSF NFL, CSF α-SYN RT-QuIC and midbrain/pons MRI areas yielded high diagnostic accuracy in degenerative parkinsonisms and in most cases the "biomarkers signatures" fitted with the clinical classification. Unexpected results related to phenotypic peculiarities (low CSF NFL in benign phenotypes) or assisted diagnostic reclassification (false + and - CSF α-SYN RT-QuIC). Longitudinal studies of larger cohorts with improved molecular and imaging biomarkers are warranted.

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Declaration of competing interest

The authors declare that they have no conflict of interest relevant to the current study.

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YC wants to dedicate this work to, and express his solidarity with, his motherland Ukraine: only construction, never destruction, is the way for humanity to reach progress and prosperity. Слава Україні!

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parkreldis.2022.05.006.

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4.3. ARTICLE 3

Midbrain and pons MRI shape analysis and its clinical and CSF correlates in degenerative parkinsonisms: a pilot study.

Painous C*, Pascual-Diaz S*, Muñoz-Moreno E, Sánchez V, Pariente JC, Prats-Galino A, Soto M, Fernández M, Pérez-Soriano A, Camara A, Muñoz E, Valldeoriola F, Caballol N, Pont-Sunyer C, Martin N, Basora M, Tio M, Rios J, Martí MJ, Bargalló N, Compta Y. Eur Radiol. 2023. doi: 10.1007/s00330-023-09435-0.

(*Autors amb contribució igual al treball)

Resum article 3:

Shape analysis de mesencèfal i protuberància en parkinsonismes neurodegeneratius i anàlisis de les seves correlacions clíniques i en LCR: un estudi pilot.

Objectius: mitjançant RM realitzar una anàlisi de la forma (shape analysis) del tronc de l'encèfal en la PSP i altres parkinsonismes neurodegeneratius, així com analitzar la seva associació amb variables clíniques i de LCR.

Metodologia: recollida de variables clíniques i demogràfiques, determinació de mesures planimètriques i de shape analysis en RM i quantificació de NFL en LCR de 84 participants: 21 PSP, 14 DCB, 12 MP, 26 AMS i 11 SC.

Resultats: la PSP presenta estretament focal (equival a atròfia) en àrees petites del mesencèfal posterior i superior, així com de la protuberància rostral en comparació amb l'AMS. L'AMS presenta significativament una major atròfia del tronc de l'encèfal, principalment en la protuberància. La DCB presenta atròfia focal en diverses àrees petites de la protuberància i mesencèfal en comparació amb la MP i els SC. Pel que fa a les mesures planimètriques i NFL, repliquem resultats publicats anteriorment a la literatura. L'atròfia objectivada mitjançant shape analysis del tronc de l'encèfal es relaciona amb un pitjor estat motor en tots els parkinsonismes i amb nivells més alts de NFL en l'AMS, la PSP i la MP.

Conclusions: els parkinsonismes atípics presenten patrons diferents de shape analysis del tronc de l'encèfal. La shape analysis té un valor discriminant limitat en la PSP, però mostra correlacions significatives amb indicadors de gravetat clínica i d'agressivitat de la neurodegeneració (nivells elevats de NFL en LCR).

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NEURO

Midbrain and pons MRI shape analysis and its clinical and CSF correlates in degenerative parkinsonisms: a pilot study

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Abstract

Objectives To conduct brainstem MRI shape analysis across neurodegenerative parkinsonisms and control subjects (CS), along with its association with clinical and cerebrospinal fluid (CSF) correlates.

Methodology We collected demographic and clinical variables, performed planimetric and shape MRI analyses, and determined CSF neurofilament-light chain (NfL) levels in 84 participants: 11 CS, 12 with Parkinson's disease (PD), 26 with multiple system atrophy (MSA), 21 with progressive supranuclear palsy (PSP), and 14 with corticobasal degeneration (CBD).

Results MSA featured the most extensive and significant brainstem shape narrowing (that is, atrophy), mostly in the pons. CBD presented local atrophy in several small areas in the pons and midbrain compared to PD and CS. PSP presented local atrophy in small areas in the posterior and upper midbrain as well as the rostral pons compared to MSA. Our findings of planimetric MRI measurements and CSF NfL levels replicated those from previous literature. Brainstem shape atrophy correlated with worse motor state in all parkinsonisms and with higher NfL levels in MSA, PSP, and PD.

Conclusion Atypical parkinsonisms present different brainstem shape patterns which correlate with clinical severity and neuronal degeneration. In MSA, shape analysis could be further explored as a potential diagnostic biomarker. By contrast, shape analysis appears to have a rather limited discriminant value in PSP.

Key Points

- *Atypical parkinsonisms present different brainstem shape patterns.*
- *Shape patterns correlate with clinical severity and neuronal degeneration.*
- *In MSA, shape analysis could be further explored as a potential diagnostic biomarker.*

Keywords Multiple system atrophy · Progressive supranuclear palsy · Shape analysis · Neurofilament protein · Parkinsonian disorders

Abbreviations

AP	Atypical parkinsonism
CBD	Corticobasal degeneration
CS	Control subjects
CSF	Cerebrospinal fluid
M_A	Midbrain area
MFSDA	Multivariate functional shape data analysis
MRI	Magnetic resonance imaging
MSA	Multiple system atrophy
MSA-C	MSA-cerebellar
MSA-P	MSA-parkinsonian
NfL	Neurofilament-light chain
P_A	Midsagittal pontine area
PD	Parkinson's disease
PM	Pons to midbrain ratio
PSP	Progressive supranuclear palsy
PSP-P	PSP parkinsonism
PSP-PGF	PSP-progressive gait freezing
PSP-RS	PSP-Richardson's syndrome

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Introduction

Structural MRI has been widely studied in the differential diagnosis of neurodegenerative parkinsonisms: Parkinson's disease (PD), multiple system atrophy (MSA), progressive supranuclear palsy (PSP), and corticobasal degeneration (CBD). Many studies have focused on the brainstem due to its frequent involvement in atypical parkinsonisms (AP: PSP, MSA, CBD). Atrophy of the midbrain in PSP [1–3] or the pons in MSA [4, 5] is a well-known example. Structural MRI brainstem measures include morphological markers ("hummingbird" sign in PSP[6]; "hot cross bun" sign in MSA [7]), quantitative measures (midbrain anterior–posterior diameter and brainstem midsagittal areas or volumes) [8], and specific ratios [9–11]. Several of these measurements can discriminate between PD, PSP, and MSA in isolation [10–12] or combined with other biomarkers [13]. However, results have been variable for morphological [14] and the antero-posterior diameter of the midbrain [8, 9].

An alternative approach is shape analysis, which detects local narrowing in specific regions of complex structures, as opposed to conventional volumetric analysis only reporting changes in the overall volume [15]. This technique has been used in neurodegenerative parkinsonisms to study local atrophy patterns in the basal ganglia, the thalamus, and the hipoccampus [16–21], but not the brainstem, nor its CSF biomarkers correlates either, to the best of our knowledge.

With the hypothesis that brainstem MRI shape analysis might differentiate neurodegenerative parkinsonisms, we aimed at characterizing and comparing brainstem shape changes across these conditions, as well as analyzing the clinical and biological correlates of shape changes. As secondary goals, we intended to replicate prior findings of cerebrospinal fluid (CSF) levels of neurofilament-light chain (NfL) and automatic measures of the pons to midbrain ratio (PM) ratio.

Methods

Design

This is a cross-sectional study of patients recruited between 2015 and 2020 at the Parkinson's Disease & Movement Disorders Unit of the Hospital Clinic in Barcelona as part of different research projects implying the availability of both high-field MRI and CSF samples for almost each participant.

Participants

There was not a formal sample size calculation, but rather a post hoc analysis of the aforementioned projects considering that sample size was in the range of prior published studies on this topic, along with the uniqueness of our cohort due to having available both MRI and CSF data in almost all patients (unlike previous published literature where MRI shape analyses were not correlated with CSF findings) [16–21]. Hence, we included 84 subjects from two prospective biomarkers studies (Supplementary Fig. 1) conducted at our unit with 32 participants previously described in two reports on CSF cytokine levels and longitudinal clinical progression in MSA, respectively, thus not overlapping with the current study [22, 23]. All diseased-participants fulfilled the "probable" (or "clinically established" in PD) category of their respective diagnostic criteria [24–27]. CS were individuals over > 55 years, undergoing intradural anesthesia for knee surgery who, as per thorough clinical history and examination (including a Montreal Cognitive Assessment [28] (MoCa) score ≥ 26), did not have any neurological or psychiatric condition. Vascular or drug-induced parkinsonism, large vascular MRI abnormalities, and Alzheimer's disease CSF biochemical profile in patients with corticobasal syndrome [29] were exclusion criteria. The study received approval by the Ethics Committee. All participants signed informed consent.

Clinical procedures

Movement disorders specialized neurologists (Y.C., C.P., A.P.) collected the following demographic and clinical variables of all participants: age at disease onset, sex and age and disease duration at the time of the study procedures. Cognition was assessed by means of the MoCa [24] (except Mini Mental test (MMSE) [30] in MSA as part of an independent protocol [23]). Motor assessments were based on the Unified Multiple System Atrophy Rating Scale (UMSARS) [31] in MSA patients, the PSP Rating Scale (PSPRS) [32] in PSP, and the subscale of the Unified Parkinson's Disease Rating Scale (UPDRS part III) [33] in all subjects except in MSA subjects. Hoehn and Yahr classification (HY) [34] was obtained for all the participants. Disability assessment was based on the Schwab and England Activities of Daily Living (SEADL) scale [35].

CSF collection, storage, and analyses

CSF samples were obtained via lumbar puncture at the L3–L4 level with a 22-gauge needle, between 8 and 10 a.m. The first 2 mL was used for routine studies. CSF was processed within 30 min of collection, centrifuged at 2000 rcf and 4 °C for 10 min, and stored at − 80 °C [36]. CSF NfL levels were measured with a commercial ELISA kit (Umandiagnostics, Sweden). The samples were run together with blank (sample diluent), the (prepared) calibrator solutions, and the appropriate control always in duplicate (a single concentration value in pg/mL was calculated as the mean of the duplicates; all with a variation coefficient < 20%).

MRI acquisition

MRI was performed within 2 months of lumbar puncture. MRI was acquired with a 3-T Prisma Siemens scanner, including sagittal T1-weighted volumes acquired with 3-dimensional magnetization prepared rapid gradient echo (3D-MPRAGE) sequences with TR = 2.4 s, TE = 2.22 ms, FlipAngle = 8; and isometric voxel size of $0.8 \times 0.8 \times 0.8 mm^3$. Regarding the participants coming from previous studies (see above), 23 subjects were acquired with TE = 2.98 ms, TR = 2.98 s, and voxel size $1 \times 1 \times 1$ mm^3. The T1-weighted MRI acquisition parameters for the other 10 subjects ranged from TE = 2.17 to 6.30 ms, TR = 14.40 ms to 2.4 s, and voxel sizes between $0.94 \times 0.94 \times 0.94$ and $1.2 \times 1.05 \times 1.05$ mm^3. Details on the number of acquisitions with each protocol per group can be found in Supplementary table 1. Supplementary Fig. 2 shows the compatibility between acquisition protocols in planimetry metrics and shape analyses.

Morphometric MRI measurements

Brainstem automatic measures (Supplementary Fig. 3)

Brainstem structures were parcelled using the Brainstem Bayesian FreeSurfer module [37]. First, for each subject, the T1-weighted image was aligned with a template in the MRI to identify the midsagittal plane. To account for variability in the alignment, 10 slices around the central sagittal slice were evaluated to identify the midsagittal slice, which was defined as the one containing the smaller midbrain area. To automatically assess midsagittal midbrain area (M_A) and midsagittal pontine area (P_A), the number of voxels segmented as midbrain and pons in the midsagittal slice were counted and multiplied by voxel size and PM was calculated. To validate the results of the automated measurements, the M_A and P_A measurements were replicated manually (Supplementary Fig. 4), as previously described [38], by two independent anatomical experts ("Manual 1" and "Manual 2") blinded to clinical information. After the validation of the automatic method, the remaining stages were automatically performed.

Shape analysis

Pons and midbrain regions obtained from the FreeSurfer Brainstem Bayesian parcellation module were merged and modelled using Spherical Harmonics Point Distribution Models (SPHARM-PDM) obtained from the SlicerSALT software (http://salt.slicer.org/) [15, 39]. For each subject, a brainstem surface containing 1002 vertices was generated and centered in a common space. Morphological vertex-level group differences were analyzed using a multivariate functional shape data analysis (MFSDA) [40], including age as a covariate, due to significantly younger age in MSA vs. the other subgroups (see results), in keeping with known age at onset of these conditions [41]. Multiple comparisons were controlled by family-wise error (FWE) (corrected significance threshold ≤ 0.05). Finally, the distance between each vertex in the subject mesh and the corresponding vertex in the control average mesh was correlated with CSF NfL, UPDRS, PSPRS, UMSARS, and SEADL by Spearman partial correlation covaried for age. We interpreted narrowing as atrophy and enlargement as lack of atrophy or compensatory enlargement in a region near an atrophic area.

Statistics

Sample size was defined on a pragmatic basis considering previous studies and the rarity of atypical parkinsonisms. Qualitative variables are presented as frequencies and were compared by means of Fisher's exact test. Quantitative data are presented as median/interquartile range (IQR) and were compared using Kruskal–Wallis test or Mann Whitney's *U*-test, as appropriate. CSF and morphometric quantitative MRI biomarkers were compared between diagnostic groups using non-parametric analysis of covariance with age as covariate. To study the influence of CSF and MRI biomarkers (independent variables) on clinical variables (dependent variables) in parkinsonian disorders, we first transformed into ranges the independent variables and then applied multiple linear regression limiting covariables to age to minimize the risk of overfitting. For statistical purposes, HY was converted to a binary variable as HYbin: I-II vs. III-V.

To verify intra-rater and automatic-to-manual agreement, intraclass correlation coefficients (ICC) of agreement and consistency were calculated. These were considered as poor (ICC < 0.40), fair (0.40–0.59), good (0.60–0.74), and excellent (0.75–1.00) [42].

Statistical tests were two-tailed, with significance set at ≤ 0.05, corrected for multiple comparisons by false-discovery rate (FDR) [43] (except for shape analysis, FWE-corrected; see above). Subject missing values in a particular field were not included in the analysis for that particular outcome. Data analysis was carried out using Stata 16.0 (Stata Corp) for Windows and IBM SPSS statistics software version 24.0 (Armonk, NY:IBM Corp).

Results

Demographic and clinical data

We included 21 patients with PSP (14 PSP-Richardson's syndrome (PSP-RS), 6 PSP parkinsonism (PSP-P), and 1

PSP-progressive gait freezing (PSP-PGF)), 14 CBD, 26 MSA (18 MSA-parkinsonian (MSA-P) and 8 MSA-cerebellar (MSA-C)), 11 PD, and 12 control subjects. The clinical diagnosis was neuropathologically confirmed in three participants (PD, CBD, and MSA; 1 each). Demographic and clinical data are shown in Table 1. There were no significant differences in disease duration, sex, and age between groups, except for MSA patients who were younger than PSP, PD, and CBD. There were no significant differences in UPDRS, HY, MoCa, and SEADL across the parkinsonian disorders.

Table 1 Comparison of demographic and clinical data across the different study groups

	PSP ($n=21$)	CBD ($n=14$)	MSA ($n=26$)	PD ($n=11$)	CS ($n=12$)	p value*
Age†	74.5 [70.0–77.4]	69.8 [67.7–74.7]	62.3 [55.1–66.8]	74.7 [64.9–77.1]	71.8 [62.7–73.7]	All groups: 0.001; PSP/CBD: 0.315; PSP/MSA: < 0.001; PSP/PD: 0.768; PSP/CS: 0.162; CBD/MSA: 0.001; CBD/PD: 0.759; CBD/CS: 0.872; MSA/PD: 0.030; MSA/CS: 0.058; PD/CS: 0.457
Gender (females)	7 (33.3)	11 (78.6)	11 (42.3)	6 (54.5)	5 (41.7)	All groups: 0.218; PSP/CBD: 0.072; PSP/MSA: 0.758; PSP/PD: 0.469; PSP/CS: 0.818; CBD/MSA: 0.140; CBD/PD: 0.594; CBD/CS: 0.218; MSA/PD: 0.818; MSA/CS: 1; PD/CS: 0.810
Disease duration†	5.4 [3.1–7.3]	5.1 [3.7–6.8]	3.8 [2.5–6.4]	7.8 [1.2–13.5]	-	All groups (except CS): 0.759; PSP/CBD: 1; PSP/MSA: 0.457; PSP/PD: 0.832; CBD/MSA: 0.558; CBD/PD: 0.768; MSA/PD: 0.540;
UPDRS	37 [26–44]	48 [42–65]	-	29 [23–43]	-	PSP/CBD/PD: 0.191; PSP/CBD: 0.118; PSP/PD: 0.697; CBD/PD: 0.218
UMSARS	-	-	44 [32.5–67.5]	-	-	
PSPRS	35 [30–43]	-	-	-	-	
HY_{bin} ($HY < 3$)	4 (19.05)	5 (35.71)	8 (30.77)	6 (54.55)		All groups (except CS): 0.440; PSP/CBD: 0.631; PSP/MSA: 0.697; PSP/PD: 0.155; CBD/MSA: 1; CBD/PD: 0.631; MSA/PD: 0.457
MoCa	19 [16–25]	19 [9.5–26.5]	-	20.5 [10–27]	26 [26–28.5]	PSP/CBD/PD: 0.997; PSP/CBD: 0.972; PSP/PD: 0.923; **PSP/CS:** 0.020; CBD/PD: 0.818; CBD/CS: 0.118; PD/CS: 0.789
MMSE	-	-	28 [26.5–30]	-	-	
Schwab and England	60 [45 – 75]	45 [40–60]	50 [40 – 75]	85 [50 – 95]	100 [97.5 – 100]	All groups (except CS): 0.138; PSP/CBD: 0.144; PSP/MSA: 0.859; PSP/PD: 0.439; PSP/CS: 0.001; CBD/MSA: 0.118; CBD/PD: 0.111; CBD/CS: 0.001; MSA/PD: 0.218; MSA/CS: 0.001; PD/CS: 0.051

Quantitative variables are presented as median [IQR] and compared between groups using Kruskal–Wallis or Mann–Whitney U tests as appropriate

Categorical variables are presented as absolute frequency (proportion) and compared between groups using Fisher's exact test

†At the time of the lumbar puncture and MRI (expressed in years)

*p values results are presented FDR-corrected

Statistical significant differences between groups are marked in bold

MSA patients presented a median of 28 points in the MMSE (normal value ≥ 24).

CSF biomarkers

These are summarized in Supplementary table 2. CSF NfL levels were significantly higher in atypical parkinsonisms vs. PD and CS.

MRI quantitative measures

Automatic and manual measures are summarized in Supplementary table 2 and Supplementary table 3, respectively. For inter-rater agreement and consistency coefficients, see Supplementary table 4. MRI quantitative automatic measures at group level demonstrated that in PSP the M_A was significantly reduced, except when comparing with CBD, and the PM ratio was significantly increased when comparing with all the other groups. CBD patients presented significantly lower M_A compared to PD and CS, and the PM ratio was significantly lower when compared to PSP and higher when compared to MSA and PD. Finally, MSA patients presented significantly lower P_A than PSP, PD, and CS, and lower PM ratio than PSP and CBD. Significant associations of CSF and automatic MRI measures with clinical variables are summarized in Supplementary table 5.

Shape analysis

Comparison of brainstem shape across parkinsonian disorders and control subjects

Areas with statistically significant shape differences are shown in Fig. 1. MSA was the group with the most extensive significant atrophy of the pons and midbrain when compared to PD and CS and mainly in the lateral inferior pons (including the middle cerebellar peduncle) when compared to PSP. CBD presented significant atrophy in several small areas in the pons and midbrain when compared to PD and CS. PSP presented significant atrophy in the upper posterior midbrain and small areas in the rostral pons when compared to MSA. For descriptive purposes, distance maps between average group shapes can be found in Supplementary Fig. 5 and information regarding the parcellation of the brainstem for the corresponding shape analysis in Supplementary Fig. 6.

Association of brainstem shape and clinical variables

We found significant positive correlations between higher motor scales' scores (UMSARS in MSA, PSPRS in PSP, and UPDRS in CBD and PD) and greater brainstem shape atrophy across the different parkinsonisms (Fig. 2). Specifically, in MSA, these positive correlations were present mostly in 2 small areas in the rostral upper midbrain and in a greater area in the dorsal and lateral pons. In CBD, scattered areas of positive correlations were seen in the pons. In PSP, these were present in the central posterior midbrain, the dorsal pons, the lateral midbrain, and the lateral and inferior rostral pons. In PD, positive correlations were seen in a few small areas in the rostral and dorsal pons.

We also found significant predominantly negative correlations between SEADL scores and brainstem shape alterations (that is, the more atrophy the lower SEADL scores) in CBD, PSP, and PD. Specifically, negative correlations predominated in the midbrain in CBD and

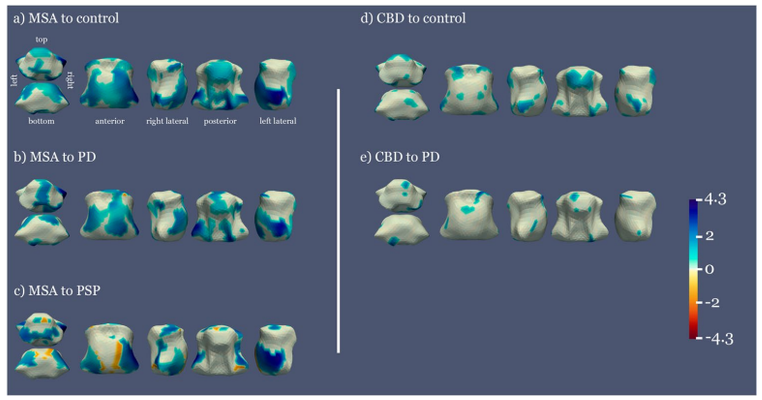

Fig. 1 Mean distance between the average shape of each pair of groups, significant results. Color areas represent the distance between average shapes in the regions where significant differences between groups are observed ($p < 0.05$, FWE corrected). Warm to cold colormap showing areas in blue where the first group is significantly narrower than the second group and red for the opposite case. The vertical barcode represents the distance between groups in millimeters, 4.3 mm being the maximum distance found between the two groups

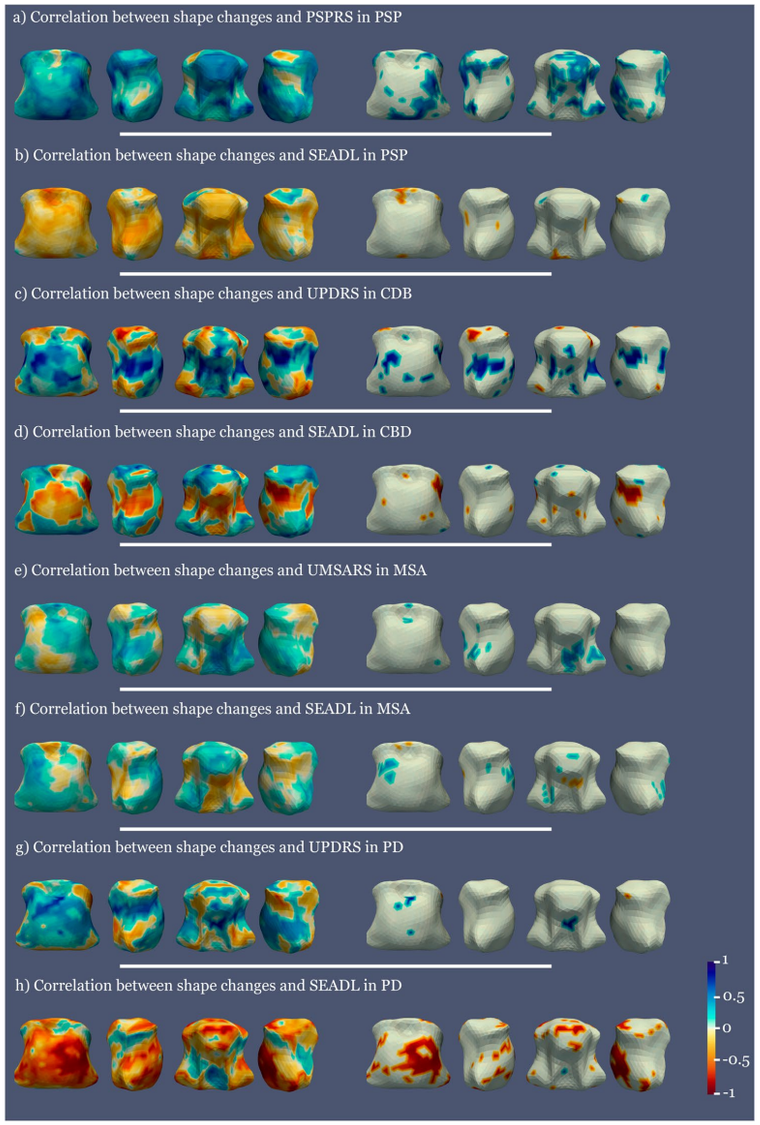

Fig. 2 Correlation maps between clinical variables and shape atrophy in PSP, CBD, MSA, and PD compared to controls. The left column shows all the correlation values and the right column only the significant ones. Blue represents positive correlations and red negative correlations. All analyses covaried for age at inclusion

PSP, and were scattered through the midbrain and the pons in PD. In MSA, there was a more mixed pattern of both negative and positive correlations (that is, atrophy or enlargement of brainstem shape was related to lower SEADL scores depending on the area) with the negative correlation surfacing in the middle posterior pons.

Association of brainstem shape and CSF NfL

A positive correlation between CSF NfL levels and shape (the higher CSF NfL levels, the more atrophy in these areas) in PSP, MSA, and PD was seen in several small midbrain and pons areas. In CBD, significant negative correlations

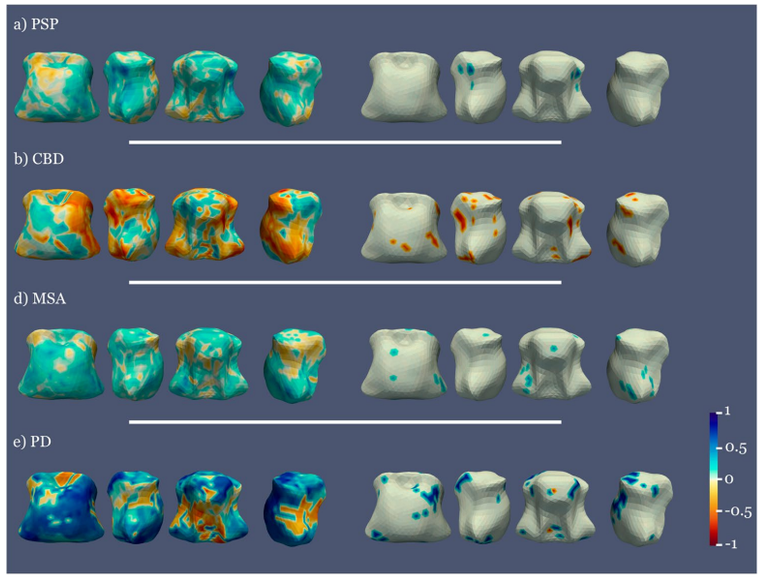

Fig. 3 Correlation between NfL and shape atrophy in the diseased groups with respect to controls. The left column shows all the correlation values and the right column only the significant ones. Blue represents positive correlations and red negative correlations. All analyses covaried for age at inclusion

(enlargement of these areas related to increased NfL) were seen (Fig. 3).

Discussion

We herein report for the first time: (1) different brainstem atrophy patterns across neurodegenerative parkinsonisms with remarkable differences in MSA; (2) local atrophy association with clinical and CSF variables across the spectrum of degenerative parkinsonisms. In addition, we have replicated previous findings of PM ratio [44, 45] and CSF NfL levels [46] in degenerative parkinsonisms.

The novelty from our study comes from brainstem shape analysis. Shape analysis has become of increasing interest in neurodegenerative diseases, as Alzheimer and Parkinson's disease. Significant shape differences have been found between PD and control subjects in different subcortical structures, including the subthalamic nucleus [16], the globus pallidus [17], and the striatum [18, 19]. To our knowledge, studies comparing shape differences between atypical parkinsonism and healthy controls have only been performed in PSP [20, 21]. Moreover, only one study has focused on shape analysis differences between neurodegenerative parkinsonian disorders, focusing on subcortical supratentorial structures instead of the brainstem with a small sample size (5 PSP, 6 CBD, 9 PD, 12 healthy controls), and reporting significant local bilateral atrophy in the ventral anterior and ventral lateral thalamus in PSP + CBD vs. the other groups [47].

To interpret narrowing as atrophy and enlargement as inflammation or regional elastic compensation, first we compared diseased vs. control groups, then compared diseased groups, shifting from an atrophy vs. non-atrophy paradigm to an atrophy-differences one. In this vein, our results show a gradation ranging from a more extensive affectation in MSA, followed by CBD to a more limited one in PSP (Fig. 1). Shape analysis results are interpreted based on the brainstem architecture. When a global atrophy is present in the brainstem nuclei, we found significant atrophy in a larger part of the brainstem surface due to collapse of the brainstem scaffold. On the other hand, when atrophy is present in superficial structures, such as the motor tracts contained in the crus cerebri or the midbrain tectum, we obtain more localized significant atrophy surfaces. Hence, comparing MSA vs. controls, we found global surface atrophy of the pons due to atrophy of the middle cerebellar peduncles (lateral region) and an anterior–posterior collapse possibly driven by changes in pontine nuclei and transverse fibers. Midbrain surface anterior and posterior alterations (colliculi and tectum), with no significant results in the lateral regions, may also indicate an anterior–posterior collapse. Involvement of corticospinal tract in MSA [26] or of a wider region of the crus cerebri or part of the substantia nigra was also found, since atrophy of the central nuclei such as the raphe would not lead to a collapse in a structure so distal

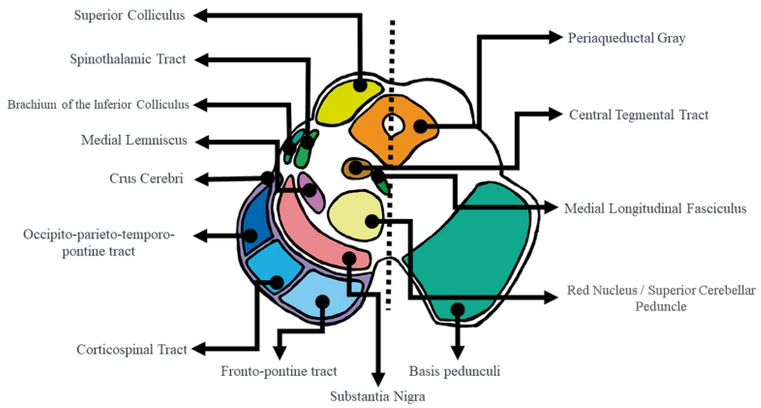

Fig. 4 Diagram of the midbrain anatomy at the level of the superior colliculi. The diagram highlights the important contribution of the cerebral peduncles to the midbrain shape, which might turn these structures, rather spared in PSP, into "shape-preserving structures" in contrast to more large shape collapse of the pons in MSA due to more extensive pontine involvement in this condition

thanks to the robust architecture of the brainstem. In MSA vs. PD, local atrophy was similar but more restricted, whereas in MSA vs. PSP higher atrophy in the middle cerebellar peduncles was consistent with previous imaging and pathological knowledge [10, 48]. In the case of CBD, focal atrophy in the tectum and several small areas in the pons was observed [49].

The similar atrophy gradation of MSA and CBD vs. CS (more marked) and vs. PD (more limited) is clinically and radiologically plausible since PD is the diseased group with lesser brainstem affection [2], hence lying between APs and CS.

Greater atrophy in PSP in the superior colliculus (upper midbrain) indicates greater dento-rubro-thalamic tract and red nucleus involvement (Fig. 4) in keeping with vertical sight limitation in PSP patients, to which the superior colliculus is critical [50]. We interpret the rather restricted narrowing in PSP as follows: while in PSP atrophy is important in the whole midbrain as captured by other measures (MRPI or PM ratio), its predominance in the tectum could turn the rather spared cerebral peduncles into "shape-preserving structures" that might account for lesser ability of MRI shape analysis to detect midbrain atrophy. Alternatively, the inclusion of different PSP phenotypes, some of them with lesser burden of brainstem pathology [2, 51], might have increased the variability and reduced the significance of shape results in this group.

Correlations with clinical and CSF variables

Greater brainstem shape atrophy was associated with worse motor state in all parkinsonisms and worse daily living function scores in CBD, PSP, and PD, while in MSA patients the correlations with SEADL were mixed: the negative correlation (the more narrowing, the lower SEADL) arose in the posterior middle pons, where tegmental pontine nucleus involvement has been correlated with severe MSA-related orthostatic hypotension, one of the most disabling MSA symptoms [52]. The positive correlations (enlargement related to lower SEADL) might be due either to a ceiling effect or to relative enlargement of certain areas in the setting of atrophy of diseased regions.

The association of greater atrophy with higher NfL levels in PSP, MSA, and PD is in keeping with the notion that CSF NfL levels indicate neuroaxonal damage. The finding of higher NfL associated with lesser atrophy in CBD is difficult to interpret. A stochastic association is unlikely due to stringent FWE correction and multivariate analyses. An alternative explanation is that it could reflect ongoing neuronal injury in areas still undergoing inflammation before atrophy [53]. In CBD, post-mortem [54] and in vivo studies [55] have demonstrated microglial activation in areas associated with tau pathology including the brainstem.

APs presented higher CSF NfL levels compared to PD and CS, but no significant differences among themselves, with increasing NfL levels significantly associated with disease severity, in agreement with previous literature [56, 57].

Shape analysis provides novel and complementary information with respect to other traditional atrophy metrics and can contribute to a better understanding of the pathological processes. First, compared to traditional indices based on planimetric measurements, shape analysis provides 3D sensitivity, that is, it is able to detect changes in all the structure, not only in the MR slice of interest. In this sense, clear differences between MSA and PSP were found by shape analysis out of the midsagittal plane. With regard to other methods quantifying atrophy as a decrease in the total volume of the anatomical region, since they provide one only measure for the whole region, they might not be sensitive to scenarios where the difference is not only the global decrease or volume but rather

the specific location of the volume decrease (as shown in the comparison between MSA and PSP).

Our study is not without limitations. The different subgroups size is relatively small, yet in the range of previous published studies on MRI shape in parkinsonisms [17, 20, 21] and remarkable considering the rareness of the atypical parkinsonisms, as well as the fact that it is challenging having both MRI and CSF from the same subjects, which is unique to our study relative to prior literature [16–21]. Moreover, we have applied statistical correction for multiple comparisons and limited covariables in regression analyses to age at inclusion, thus respectively minimizing the risk of stochastic results and of overfitting. On the other hand, the fact that the sample size is small increases the risk of statistical error type II, that is, falsely rejecting the alternative hypotheses. As our study is positive with several significant findings, the potential limitation of our sample size would be that of underestimating, rather than overestimating, our findings. Neuropathological diagnosis confirmation is lacking in most cases. However, those having come to autopsy were confirmed, and for the rest strict diagnostic criteria were applied and cases with corticobasal syndrome with CSF Alzheimer-profile were excluded. The sample size was small, yet in the range of previous studies [2, 13, 58]. We considered MSA patients as a sole group but did not assess separately MSA-C and MSA-P. However, MSA-C and MSA-P share involvement of the same brain structures including the brainstem and accordingly in the diagnostic criteria the radiological findings of one variant are accepted to assist the diagnosis of the other one [26, 59]. Age differed among groups as expected since MSA usually has younger age at onset [41], but we covaried analyses for age and moreover it is unlikely that differences in age drove the results when most significant differences were obtained in the MSA (that is, the younger) group. Another limitation is the difference in T1-weighted MRI parameters as participants came from different projects. Although de novo acquisitions were acquired with the same acquisition protocol, the images from previous projects had been acquired with different parameters. The automatic methods for brainstem segmentation have shown to be robust against differences in acquisition parameters [60]. On the other hand, recent studies have shown that shape features are more robust to acquisition parameters than features related to volume or intensity [61], pointing to reliability of shape analysis even in case of differences in acquisition.

In conclusion, we have found different patterns of local brainstem atrophy across atypical parkinsonisms by means of MRI shape analyses in association with clinical and CSF indicators of disease severity. More specifically, shape analysis might be further explored as a potential MSA diagnostic biomarker. In contrast, and despite its significant clinical and CSF correlations, in PSP shape analysis appears to be of rather limited discriminant value. Our results remain preliminary and additional prospective studies of larger cohorts will help confirm or not our findings and should further assess the combination of MRI shape analysis and CSF biomarkers.

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Data Availability Data will be made available upon reasonable and justified request.

Declarations

Guarantor The scientific guarantors of this publication are the corresponding authors on behalf of their institutions: Hospital Clínic de Barcelona, IDIBAPS and University of Barcelona.

Conflict of interest None of the authors has any conflict of interest relevant to the topic of this manuscript.

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Informed consent Written informed consent was obtained from all participants in this study.

Ethical approval Institutional Review Board approval was obtained.

Study subjects or cohorts overlap Out of the 84 participants, 32 were previously reported in two published studies with no overlap with the current one:

- Compta Y, Dias SP, Giraldo DM, et al. (2019) Cerebrospinal fluid cytokines in multiple system atrophy: A cross-sectional Catalan MSA registry study. Parkinsonism Relat Disord 65:3–12.
- Pérez-Soriano A, Giraldo DM, Ríos J, Muñoz E, Compta Y, Martí MJ; Catalán MSA Registry (CMSAR) (2021) Progression of Motor and Non-Motor Symptoms in Multiple System Atrophy: A Prospective Study from the Catalan-MSA Registry. J Parkinsons Dis 11(2):685–694.

Methodology
- prospective
- cross-sectional
- observational
- multicenter study

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5. DISCUSSIÓ

5. DISCUSSIÓ

A la PSP un diagnòstic de certesa requereix l'examen neuropatològic post-mortem i, per tant, en vida només podem arribar a un diagnòstic de probable PSP aplicant els criteris clínics diagnòstics. Amb els treballs que configuren aquesta tesi hem volgut aprofundir en la recerca de marcadors que ens ajudin al diagnòstic d'aquesta malaltia.

Al primer treball hem pretès abordar el concepte de malaltia prediagnòstica que està sent treballat de forma molt intensiva i extensiva des de fa dècades ja a la malaltia d'Alzheimer i a la MP, però roman inexplorat als parkisonismes atípics, en general, i a la PSP, en particular. Fer un diagnòstic precoç de la malaltia és important per en un futur poder aplicar potencials teràpies modificadores del curs de la malaltia, abans que el procés neurodegeneratiu s'hagi establert de manera irreversible. Sabem per estudis neuropatològics comunitaris que poden existir canvis neuropatològics de la malaltia en persones que no presenten símptomes o que presenten alguns signes motors o neurocognitius subtils i aïllats (8,72,73). Això està indicant l'existència d'una fase prediagnòstica de duració indeterminada i poc coneguda. Amb el nostre estudi vam voler analitzar des d'una perspectiva clínica aquesta fase i en ser el primer treball d'aquest tipus ha representat un avenç en aquesta àrea, motiu pel qual se li va dedicar un editorial que s'adjunta com a annex.

En la nostra cohort vam trobar que les persones amb PSP i MP presentaven símptomes prediagnòstics diferencials amb corbes ROC amb una àrea sota la corba major a 0,70. Aquests símptomes prediagnòstics en la PSP comprenien símptomes motors, massa subtils o lleus per permetre el diagnòstic de PSP, i no motors (majoritàriament afectius, cognitius, conductuals, però també mareig). Entre els símptomes motors prediagnòstics, les caigudes i el trastorn de la marxa eren freqüents i en

un 50 i 30%, respectivament, podien estar presents més de 3 anys abans del diagnòstic. A més, en un 42 i 24% respectivament, aquests símptomes es presentaven aïlladament, no s'associaven a altres símptomes motors, suggerint que eren símptomes prediagnòstics reals i no deguts a un retard en el diagnòstic. Dins del domini "cognitiu" vam incloure problemes d'atenció-memòria-executius i de la parla. Els problemes de la parla, però no els altres, van ésser més freqüents en la fase prediagnòstica de la PSP en comparació amb els pacients amb MP. Això podria ser degut al fet que els problemes de la parla són una característica més específica i precoç en la PSP en comparació amb la MP on els problemes d'atenció poden ser freqüents i aparèixer ja en fases inicials (144,145). Pel que fa als símptomes neuropsiquiàtrics, tot i que la depressió s'ha associat clàssicament a la MP, no vam trobar diferències significatives en quant a símptomes depressius prediagnòstics entre els dos parkinsonismes, resultat d'acord amb literatura prèvia on es mostra que la depressió és freqüent en la PSP (146,147). Els pacients amb PSP sí que presentaven més apatia i irritabilitat prediagnòstiques. Finalment, els pacients amb MP també presentaven major freqüència de certs símptomes com tremolor, hipòsmia, clínica suggestiva de trastorn de conducta del son REM i restrenyiment, molts dels quals s'han proposat com a indicadors de la presència d'acúmul anòmal d'alfa-sinucleïna de tipus Lewy subjacent (148-150).

Una part significativa dels casos presentava aquests símptomes prediagnòstics entre 3 i fins a 10 anys abans del diagnòstic i, per tant, com que el temps de retard en el diagnòstic ha anat disminuint de forma considerable les darreres dècades (44 mesos el 1990; 29 mesos el 2010) considerem poc probable que siguin exclusivament resultat d'un diagnòstic tardà, sobretot aquells més allunyats en el temps del diagnòstic clínic (151).

Aquest és el primer estudi clínic específicament dissenyat per a descriure els símptomes prediagnòstics i el seu moment d'aparició en la PSP. Posteriorment, altres estudis clínics han explorat i analitzat aquesta fase utilitzant diferents metodologies. Kwasny i col. (152) van fer un estudi de casos i controls amb informació provinent d'una base de dades electrònica

d'atenció primària per a identificar característiques clíniques presents en els pacients que posteriorment rebrien el diagnòstic de PSP. Van incloure 152 PSP i 3122 subjectes controls. Utilitzant tècniques de *machine learning*, sense una hipòtesi a priori, van trobar un total de 9 variables associades significativament a la fase prediagnòstica de la PSP. Aquestes es van dividir en variables associades a la malaltia (ús de fàrmacs anticolinèrgics, problemes motors, cognitius, del tracte urinari/ restrenyiment i depressió), possibles factors de risc modificables (diabetis i malaltia cerebrovascular) i errors diagnòstics comuns (problemes vestibulars i altres malalties neurodegeneratives). La presència d'aquest conjunt de variables en un pacient conferia un elevat risc de rebre un subseqüent diagnòstic de PSP. La principal limitació d'aquest estudi és que els pacients no havien estat valorats per neuròlegs i, per tant, algunes de les variables identificades com a prediagnòstiques podrien ser conseqüència d'un retard en el diagnòstic.

En un altre treball, Street i col. (153) a partir de la cohort prospectiva "Baseline UK Biobank" en la que es realitza una visita basal amb tests psicomotrius a tots els participants, identifiquen 176, 2526 i 5404 subjectes que han rebut posteriorment el diagnòstic de PSP, MP o cap dels dos, respectivament. En aquesta visita basal, realitzada una mitjana de quasi 8 anys abans de rebre el diagnòstic tant de PSP com de MP, ja s'observaven diferències motores i cognitives en els grups de PSP i MP respecte al grup control però no entre els grups de PSP i MP. La presència d'aquestes troballes suggeriria l'existència d'una fase prediagnòstica de llarga evolució amb canvis subtils tant en la funció motora i cognitiva encara que no específics d'una o altra malaltia.

Entre les principals limitacions del primer estudi de la present tesi caldria destacar que es tracta d'un estudi retrospectiu i, per tant, hi ha la possibilitat d'un biaix de memòria dels participants, si bé aquest es va intentar minimitzar a través de la valoració conjunta amb la informació registrada a la història clínica prèvia. La presència de símptomes cognitius o psiquiàtrics no s'han avaluat mitjançant escales reglades sinó a través de la història clínica i un qüestionari estandarditzat. No tenim resultats patològics

de la major part dels pacients, si bé 3 pacients amb PSP van ser donants de cervell i es confirmà el diagnòstic (dos amb resultats coneguts en el moment de la publicació del treball i un en el que s'ha arribat al diagnòstic de PSP definitiva posteriorment), i la majoria complien criteris de PSP probable (que té un percentatge d'encert diagnòstic elevat) en l'última visita.

Al segon treball de la present tesi hem seguit la línia del grup d'estudiar biomarcadors en l'àmbit dels parkinsonismes degeneratius, establerta els darrers 15 anys, amb estudis sobre biomarcadors de demència a la MP i de diferents vies patogèniques (coenzima Q10, citoquines) en l'atròfia multisitèmica (154-157). En aquest estudi a més hem dut a terme el treball de durant 2 anys posar en marxa al nostre laboratori la tècnica RT-QuIC en LCR per a l'aS a més de començar a determinar els NFL en LCR mitjançant ELISA.

A la PSP, igual que en la resta de malalties neurodegeneratives, la realització d'un diagnòstic el més precís possible durant la vida és de gran importància en la recerca per així poder classificar els pacients en fenotips més concrets, crear cohorts més homogènies i, per tant, reduir l'heterogeneïtat dels resultats. L'última dècada s'han fet grans avenços en la cerca de marcadors diagnòstics en la PSP i altres parkinsonismes neurodegeneratius, però encara no en disposem de cap que presenti una fiabilitat excel·lent. A més, els biomarcadors actuals poden servir per a diferenciar entre certes patologies però no aportar informació per al diagnòstic diferencial amb d'altres. Per exemple, l'agregació aS RT-QuIC o els nivells de NFL poden ajudar en el diagnòstic diferencial de la MP vs. els parkinsonismes atípics però no entre aquests últims. És per això, que la idea de combinar diferents biomarcadors és cada cop més atractiva pels investigadors.

En aquest segon article de la present tesi, hem demostrat que la combinació de diferents marcadors biològics i radiològics pot ser d'utilitat en el diagnòstic diferencial de la PSP amb diferents parkinsonismes neurodegeneratius i pot incrementar el rendiment diagnòstic en comparació amb la utilització dels

biomarcadors de forma aïllada. En l'estudi de Quadalti i col. (158) ja havien demostrat una millor habilitat diagnòstica entre MP i parkinsonismes atípics amb la combinació d'aS RT-QuIC i els nivells de NFL respecte a la utilització d'aquests de forma aïllada. Nosaltres, combinant nivells de NFL, resultats d'aS RT-QuiC i mesures planimètriques en ressonància magnètica hem obtingut una bona fiabilitat diagnòstica, amb excel·lents àrees sota la corba i majors sensibilitat i especificitat. En concret per a la PSP, englobada dins del grup de taupaties amb la DCB-SCB, respecte als altres grups diagnòstics la combinació de biomarcadors conferia una corba ROC amb AUC de 0,93 (95%CI = 0,875-0,990, p < 0,0001) i unes sensibilitat i especificitat del 86,0 i 82,0%, respectivament.

També hem demostrat que la revisió post-hoc del diagnòstic de pacients amb resultats inesperats en els NFL i RT-QuIC d'aS permetia la reclassificació diagnòstica d'alguns pacients i, en altres, ajudava a entendre peculiaritats fenotípiques. Per exemple, els pacients amb PSP i NFL normals (n=2) presentaven fenotips (PSP-P i PSP-PGF) que s'han associat a un curs menys agressiu i a una supervivència més llarga. De forma semblant, els pacients amb AMS i DCB-SCB amb resultats normals en els nivells de NFL, també havien presentat un curs més benigne a l'esperat. Alternativament, els resultats inesperats en RT-QuIC poden ser un senyal per a reconsiderar el diagnòstic: un pacient inicialment etiquetat com a MP amb resultat negatiu de RT-QuIC va ser reclassificat com a parkinsonisme vascular; dos altres casos amb resultats positius primer classificats com AMS i DCB-SCB van ser reclassificats com a MP. Aquesta revisió *post-hoc* s'ha d'interpretar amb cautela, ja que hi podrien haver hipòtesis alternatives que expliquessin els resultats «falsament» positius o negatius com, per exemple, un error de la pròpia tècnica. En el cas dels falsos positius, també podrien ser deguts a la presència de copatologia. Per exemple, en el nostre estudi un 13% dels pacients amb PSP presentaven resultats positius d'aS RT-QuIC, percentatge que estaria en el rang de la copatologia Lewy post-mortem reportada anteriorment en PSP (de 8 a 22%) (159-162).

Les principals limitacions d'aquest article són la falta de confirmació neuropatològica en la majoria dels casos i la relativa petita mostra de participants, sobretot controls i MP, perquè la resta de subgrups són relativament grans considerant la raresa de les malalties i la mida mostral de bibliografia de la mateixa temàtica.

Per últim, destacar com s'ha dit prèviament que aquest treball ha permès la posada en marxa de la tècnica RT-QuIC per aS no només al nostre laboratori experimental sinó al centre de diagnòstic biomèdic del nostre hospital on des de fa més d'un any que es pot sol·licitar com a prestació diagnòstica assistencial (s'adjunta certificat de la direcció de l'hospital reconeixent el nostre paper en la implantació d'aquest nou test diagnòstic).

Finalment, al tercer treball aportem la novetat de la *shape analysis* del tronc cerebral mitjançant RM cerebral. Aquesta tècnica ha anat adquirint un interès creixent en les malalties neurodegeneratives com l'Alzheimer i la MP (95-98) però encara no s'havia utilitzat mai per a l'anàlisi del tronc encefàlic en el diagnòstic diferencial de la PSP amb altres parkinsonismes neurodegeneratius.

Els nostres resultats mostren una gradació que va des d'una afectació més extensa en l'AMS, seguida per la DCB-SCB fins a una més limitada en la PSP. Hem centrat la interpretació dels resultats de la shape analysis que presentem en base a l'arquitectura del tronc de l'encèfal. Quan hi ha una atròfia generalitzada dels nuclis del tronc encefàlic, trobem una atròfia en una part més extensa de la superfície del tronc encefàlic a causa del col·lapse d'aquesta estructura. En canvi, quan l'atròfia es troba present en estructures més superficials, com per exemple en els tractes motors que passen pel crus cerebri o pel tèctum mesencefàlic, obtenim una atròfia més localitzada.

La *shape analysis* en la PSP va detectar una major atròfia del colliculus superior (part superior del mesencèfal) que seria indicativa de la implicació del tracte dentorubrotalàmic i del nucli vermell. Aquesta afectació del colliculus superior es tradueix amb la característica afectació dels moviments oculars en el pla vertical (163). Tot i així, la PSP presentava globalment una

afectació de la *shape analysis* molt més restringida, en comparació amb l'AMS i la DCB- SCB, que hem interpretat de la següent manera: tot i que l'atròfia del mesencèfal és freqüent i significativa en la PSP i pot evidenciar-se amb altres mesures (l'índex MRPI o el ratio PM), el fet de que afecti predominantment el tèctum mesencefàlic amb preservació important dels peduncles cerebrals, podria convertir aquests últims en estructures que «conserven la forma» provocant una menor capacitat de la *shape analysis* per a detectar canvis. Una explicació alternativa podria ser que la inclusió de diferents fenotips de PSP, alguns d'ells amb menor càrrega de patologia a nivell mesencefàlic (51,89), podria haver augmentat la variabilitat del grup reduint, per tant, la possibilitat d'obtenir resultats significatius.

En la comparació de la *shape analysis* entre AMS i controls, vam trobar una atròfia global de la superfície de la protuberància secundària a l'atròfia dels peduncles cerebel·losos mitjans (situats en la regió lateral) i al col·lapse antero-posterior, possiblement provocat per canvis en els nuclis pontins i les fibres transversals. Les alteracions en la superfície anterior i posterior del mesencèfal (collicul i tèctum), sense una atròfia significativa en les regions laterals, també sembla indicar un col·lapse antero-posterior. També es va objectivar la implicació del tracte corticoespinal en l'AMS (164) o d'una regió més àmplia del crus cerebri o de part de la substància negra, ja que l'atròfia de nuclis centrals del rafe no donarien lloc a un col·lapse de l'estructura tan distal gràcies a la robusta arquitectura del mesencèfal. En la comparació AMS vs. MP aquesta atròfia localitzada era similar però més restringida, mentre que en la comparació AMS vs. PSP objectivàvem una major atròfia dels peduncles cerebel·losos mitjos consistent amb troballes radiològiques i anatomopatològiques descrites prèviament (88,165). En el cas de la DCB-SCB, es va observar atròfia local del tèctum i de diverses petites àrees en la protuberància (166).

El fet que tant l'AMS com la DCB-SCB presentessin una atròfia més marcada quan es comparaven respectivament amb els SC i un patró similar però més limitat quan es comparaven respectivament amb la MP, és plausible tan clínica com radiològicament ja que la MP seria el grup dels

parkinsonismes amb menys afectació del tronc encefàlic (89), a mig camí entre els parkinsonismes atípics i els SC.

Pel que fa específicament a la PSP, les troballes positives d'aquest tercer treball radicarien en les correlacions amb variables clíniques i de LCR: una major atròfia detectada per shape analysis s'associava amb un pitjor estat motor i una pitjor funcionalitat, donant plausibilitat clínica a les troballes de la *shape analysis* a la PSP, malgrat ser aquestes més restringides que als altres parkinsonismes. A la DCB-SCB i la MP les correlacions entre *shape* i variables clíniques va anar paral·lela a la PSP. Per la seva banda, als pacients amb AMS les correlacions amb l'escala SEADL eren mixtes: observàvem una correlació negativa (és a dir, a major atròfia, pitjor puntuació en l'escala SEADL) en la part mitja posterior de la protuberància, on l'afectació dels nuclis pontins dels tegmèntum s'ha vist correlacionada amb una major gravetat de la hipotensió ortostàtica en l'AMS, un dels símptomes més discapacitats d'aquesta malaltia (167). Les correlacions positives (augment de la forma associat a una pitjor puntuació en l'escala SEADL) podrien ser degudes a un efecte sostre o a l'ampliació relativa (no real) de determinades àrees en el context d'atròfia de les regions afectades per la patologia.

L'associació entre una major atròfia determinada per *shaping* i nivells més elevats de neurofilament en la PSP, l'AMS i la MP és congruent amb el fet que els NFL indiquen degeneració neuroaxonal. En canvi, l'associació de nivells alts de NFL amb una menor atròfia en la DCB-SCB és més difícil d'explicar, essent poc probable que sigui deguda a l'atzar perquè hem utilitzat mètodes de correcció estrictes en les anàlisis multivariants. Una hipòtesi alternativa seria que estem veient una lesió neuronal en curs, zones que presenten inflamació com a pas previ de presentar atròfia (44). Recolzant aquesta possibilitat, en la DCB, estudis in vivo (168) i post-mortem (118) han demostrat activació de la micròglia en àrees associades a patologia tau incloent el tronc de l'encèfal.

D'acord amb la literatura prèvia, els parkinsonismes atípics presentaven nivells més elevats de NFL en comparació amb la MP i els SC, però no entre

ells, i uns nivells de NFL més elevats s'associaven a una major gravetat de la malaltia (127,169).

La *shape analysis* aporta informació novedosa i complementària en comparació amb altres tècniques tradicionals i pot ajudar a millorar el coneixement del procés patològic subjacent. Primer, en comparació amb mesures planimètriques, la *shape analysis* mostra informació tridimensional, per tant és capaç de detectar canvis a nivell de tota l'estructura i no només en un tall específic. En aquest sentit, s'han trobat clares diferències entre la PSP i l'AMS fora del pla mig sagital. En comparació amb mètodes que quantifiquen l'atròfia com una disminució del volum de tota la regió anatòmica, al proporcionar una única mesura per tota la regió pot no ser sensible a canvis en regions específiques i no indiquen la localització de les regions on hi ha atròfia. La correlació ja esmentada entre atròfia determinada per *shaping*, sobretot a la part superior del tronc de l'encèfal en àrees típiques afectades en la PSP, i pitjors puntuacions a escales clíniques i de funcionalisme i nivells més elevats de NFL en LCR dona consistència interna als resultats i alhora és coherent amb literatura prèvia emprant altres aproximacions (127,169).

Direccions futures en la recerca a partir del que hem après amb aquesta tesi inclouen el registre de PSP multicèntric de la província de Barcelona amb biorepositori (148/U/2020, IP Dr. Compta), finançat per la Marató de TV3. Aquest registre on ja s'han inclòs més de 120 casos amb diferents categories de certesa diagnòstica ens permetrà 1) fer un seguiment a una mostra més àmplia i ben caracteritzada de pacients i tenir l'opció a llarg termini de tenir la confirmació neuropatològica al menys de part d'aquests casos, 2) estudiar si la combinació dels biomarcadors utilitzats en el segon article de la present tesi ofereix una bona capacitat diagnòstica en fases inicials de la malaltia amb la possibilitat d'afegir biomarcador RT-QuIC específic de 4R-tau o explorar altres aspectes clínics dels diferents fenotips de la PSP com l'afectació del son o oculomotora quantificada o fer estudis d'epigenètica en fibroblasts i 3) analitzar amb més profunditat si els resultats dels biomarcadors poden ser variables en funció del fenotip

i la durada o estadi de la malaltia. A més, una de les mancances en la determinació d'alguns paràmetres que hem estudiat, com per exemple són la determinació d'agregació d'aS, dels NFL o les mesures planimètriques en RM, és la falta d'homogenització de les tècniques entre laboratoris/centres i l'absència de punts de tall ben definits entre lo patològic i la normalitat. El biorepositori esmentat ens permetrà col·laborar amb altres laboratoris i centres i realitzar estudis comparatius per veure si els resultats presenten concordança i està més a prop de la generalització d'aquestes tècniques, a més de fer factible l'establiment de punts de tall uniformes. Per últim, hi ha la necessitat de trobar un biomarcador de taupatia. En aquest sentit, el registre de PSP també inclou la col·laboració amb l'equip del Dr. Byron Caughey de l'NIH de les Muntanyes Rocalloses (Hamilton, Montana, EUA) per a validar el RT-QuIC de 4R-tau (Annex 3 i 4) i el nostre equip ha rebut un beca interCIBER (CIBERBBN i CIBERNED) per a posar en marxa l'adquisició de PET de tau amb el traçador PI2620 (LMI) i la seva quantificació en pacients amb PSP.

6. CONCLUSIONS

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1. Els subjectes amb PSP poden presentar una àmplia varietat de símptomes motors i no motors fins a 10 anys abans del diagnòstic per tant no atribuïbles al retard diagnòstic i que a més són diferents als que presenten en la MP.
2. Símptomes com les caigudes, problemes cognitius i de l'estat anímic són elements destacats de la fase prediagnòstica de la PSP i poden ser útils per a la selecció de poblacions amb majors probabilitats de desenvolupar PSP en un futur.
3. La combinació de nivells elevats de NFL en LCR, negativitat de el RT-QuIC d'aS en LCR i reducció planimètrica del mesencèfal a la RM aporta una major precisió en el diagnòstic diferencial de la PSP en comparació amb la utilització de les diferents anàlisis de forma aïllada.
4. Els resultats inesperats dels biomarcadors poden ajudar a reorientar el diagnòstic o a entendre peculiaritats de la malaltia a nivell fenotípic o de diferències evolutives.
5. Els diferents parkinsonismes degeneratius presenten patrons diferencials de *shape analysis* del tronc encefàlic, essent aquests més extensos a l'AMS i més limitats en la PSP.
6. No obstant, les alteracions de *shaping* a la PSP es correlacionen significativament amb una major gravetat clínica i amb nivells elevats de NFL en LCR, biomarcador d'agressivitat de la neurodegeneració, reforçant el potencial rol dels NFL com a eina pronòstica i possible biomarcador de resposta a futures teràpies.

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8. ANNEXOS

8. ANNEXOS

8.1. EDITORIAL

Meissner WG, Höglinger GU. Looking into the prediagnostic phase of progressive supranuclear palsy. Parkinsonism Relat Disord. 2020 May;74:74-75. doi:10.1016/j.parkreldis.2020.04.006.

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Looking into the prediagnostic phase of progressive supranuclear palsy

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The early diagnosis of progressive supranuclear palsy (PSP) remains challenging. Early identification is not only important to inform patients and caregivers about the prognosis, but also for the enrollment of patients in early disease stages into disease-modification trials where treatments are expected to be the most effective, and for the development of new objective diagnostic tools (e.g. imaging, fluid biomarker).

The MDS-sponsored PSP Study Group has recently revised the diagnosis criteria for PSP [1,2]. One main objective of this revision was to improve the sensitivity in early disease stages, which was 24% at the first visit for former criteria [1,3]. With the addition of akinesia and cognitive dysfunction as functional domains, the revised PSP criteria include a broader repertoire of clinical symptoms and allow the diagnosis of several distinct PSP predominance types that have emerged in recent years [4]. They also include a category for cases with a diagnosis that is "suggestive" of PSP. These patients show early signs of PSP that do not reach the threshold for "possible" or "probable" PSP. It is hoped that cohorts of patients with "suggestive" PSP will inform about the progression in early disease stages, similar to at risk populations that were recently identified for other neurodegenerative disorders (e.g. pure autonomic failure and rapid eye movement sleep behavioral disorder for synucleinopathies).

Neurofilament light chain is emerging as an interesting prognosis fluid biomarker and there has been substantial progress over the past two decades in brain imaging for the diagnosis and prognosis of PSP [5]. Imaging studies have mostly focused on PSP with Richardson's syndrome (PSP-RS) and have rarely included other PSP predominance types. Based on the results of imaging studies, atrophy or hypometabolism in midbrain relative to pons on MRI or [18F]fluorodeoxyglucose PET have been included as supportive features to revised diagnosis criteria. However, brain MRI can be normal in early disease stages and midbrain atrophy can be missing or only appear in advanced disease stages in non PSP-RS predominance types. Hence, the refinement of clinical PSP predominance types and a better characterization of the earliest disease stages remain critical steps.

In the current issue, Painous and colleagues report the results of a retrospective longitudinal case-control study where they compared prediagnostic motor and non-motor symptoms in 50 PSP patients with 50 age- and sex-matched PD patients and 50 controls (mostly neurological controls without neurodegenerative disorder) [6]. PSP-RS was the predominance type in around 50%, while 20% showed either PSP with predominant parkinsonism (PSP-P) or PSP with progressive gait freezing (PSP-PGF). At the last clinical visit, 96% had a diagnosis of "probable" PSP according to MDS PSP criteria and the diagnosis was confirmed post-mortem in two patients.

Symptoms were collected through a questionnaire that was previously used in the ONSET PD study to describe the presence and perceived onset of non-motor symptoms in PD [7]. This questionnaire covers 35 symptoms in nine domains. Frequent prediagnostic symptoms in PSP were falls (54%), gait disturbances (52%), depression (42%), pain (36%), visual symptoms (34%), cognitive dysfunction (30%), voice impairment (30%), clumsiness (30%), dizziness (28%), anxiety (26%) and apathy (20%). They were more frequent in PSP compared to PD, whereas a significant proportion of PD patients also reported prediagnostic depression. Noteworthy, more than 50% of prediagnostic falls, apathy and anxiety, and more than 30% of gait disturbances and depression appeared more than three years before the diagnosis. The proportion of prediagnostic symptoms did not differ between PSP phenotypes, but were perceived by PSP-P and PSP-PGF patients for a longer period prior to diagnosis, probably because of the slower progression of these predominance types compared to PSP-RS. As expected, hyposmia (16%), constipation (20%) and dream enacting behavior (4%) were less prevalent in PSP and more frequent in PD.

Noteworthy, PSP patients had more consultations to ENT and ophthalmologists than PD patients in the prediagnostic phase illustrating the importance to create awareness among these specialties for their contribution to an earlier PSP diagnosis.

Based on their findings, Painous and colleagues tested the diagnostic accuracy of variable combinations of prediagnostic symptoms for the distinction between PSP and PD. The model including visual problems, falls and lack of PD prodromal features as independent predictors showed the best ability to differentiate PSP from PD with a sensitivity of 78% and a specificity of 82%.

The study has some limitations, in particular the retrospective design with potential recall bias and autopsy confirmation in only two PSP patients. In addition, the comparison did not include other parkinsonian disorders that share with PSP early symptoms such as falls, gait disturbances or cognitive impairment (e.g. vascular parkinsonism, Lewy body dementia and multiple system atrophy). Nevertheless, it

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provides important information about the prediagnostic phase of PSP and suggests that core clinical features of current diagnosis criteria and additional symptoms appear several years before the diagnosis in a large number of PSP patients. The results will also be helpful for further shaping the new category of cases with a diagnosis that is "suggestive" of PSP. As next steps, it would be important to continue to look into the prediagnostic phase of PSP by assessing well-characterized post-mortem confirmed series and, ultimately, large prospective cohorts including confirmatory objective biomarkers such as Tau PET.

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8.2. CERTIFICAT DE LA IMPLANTACIÓ DE LA TÈCNICA RT-QUIC D'ALFA-SINUCLEÏNA

Certificat de la Direcció de l'Hospital Clínic conforme la contribució del nostre equip de recerca a la implantació de la tècnica RT-QuIC d'alfa-sinucleïna en LCR com a prestació diagnòstica assistencial.

CLÍNIC BARCELONA
Hospital Universitari

A quien corresponda,

El investigador Yaroslau Compta Hirnyj y su equipo a través del proyecto financiado por la AES del ISCIII con número PI1700096 y titulado **"Amplificación de autoagregación de alfa-sinucleína y 4R-tau en tejido cerebral y líquido cerebrospinal mediante RT-QuIC como biomarcador diferencial de parkinsonismos degenerativos"** han contribuido con los experimentos de este proyecto a la implantación asistencial de la técnica RT-QuIC para alfa-sinucleína en líquido cefalorraquídeo como biomarcador de enfermedades con depósito de esta proteína (en especial, la enfermedad de Parkinson) en el Hospital Clínic de Barcelona. Gracias a ello, esta prueba ahora está disponible como test diagnóstico rutinario y se une a los ya existentes de ELISA de tau y amiloide-beta en líquido cefalorraquídeo y de RT-QuIC de proteína priónica, también en líquido cefalorraquídeo. Ello ofrece la posibilidad, única en nuestro entorno, de realizar estudios moleculares multimodales *in vivo* de las principales enfermedades neurodegenerativas por su frecuencia (Alzheimer y Parkinson) o sus implicaciones en salud pública y seguridad biológica (Creutzfeldt-Jakob).

Atentamente,

Dr. Antoni Castells
Director Médico
Hospital Clínic

Dr. Manel Juan
Jefe de Servicio de Inmunología

Barcelona, 9 de marzo de 2022

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8.3. CARTA DE COL·LABORACIÓ

Carta de col·laboració amb Byron Caughey en estudis de RT-QuIC de 4R-tau en CR.

National Institute of Allergy and Infectious Diseases

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Jan 30, 2020

Dr. Yarkoslau Compta
Hospital Clinic de Barcelona
Barcelona, Spain

Dear Dr. Compta,

This letter is to express my enthusiastic willingness to collaborate on your grant proposal to use RT-QuIC assays as part a larger clinical cohort and biomarker study of progressive supranuclear palsy (PSP) cases associated with the Barcelona PSP Registry. As you know, my lab has developed an ultrasensitive tau RT-QuIC assays for pathologic tau aggregates in brain and cerebrospinal fluid of PSP cases (Saijo et al., *Acta Neuropath* 2020 139(1):63-77). More extensive evaluation of this tau seed amplification assay, and its diagnostic utility, is still much needed. I think that the project that you propose will be a significant step in this direction, and I am delighted to offer my support for your application. We will perform RT-QuIC experiments on your samples and help you to get the assay running in your laboratory.

I will be able to contribute approximately 5% of my effort to this work, subject to availability of time and resources. This collaboration is part of my official duties as a federal employee at the National Institute of Health (NIH), National Institute of Allergy and Infectious Diseases (NIAID), and no funds from the grant will be used in intramural research, neither will I accept any form of remuneration, whether in the form of salary, honoraria, or travel expenses. Further, in keeping with the mission of NIAID to promote and facilitate biomedical research and the dissemination of new knowledge, we would supply requested research materials and technical expertise not only to you, but also to other interested and qualified parties for research purposes. Approval for this collaboration has been granted by Steven M. Holland, M.D., Director of the Division of Intramural Research (DIR) at NIAID.

Sincerely,

Byron Caughey, Ph.D.
Senior Investigator,
National Institute of Allergy and Infectious Diseases, NIH

Approved: _____
Steven M. Holland, M.D., Director

8.4. CORBES DE RT-QUIC DE 4R-TAU EN LCR

Exemples de corbes de RT-QuIC de 4R-tau en LCR en un cas amb MP (negatiu) i un amb PSP (positiu) del nostre biorepositori analitzades al laboratori de Byron Caughey.