ΕΛΛΗΝΙΚΗ ΝΕΥΡΟΛΟΓΙΚΗ ΕΤΑΙΡΕΙΑ
ΑΛΚΜΑΝΟΣ 10, 115 28, ΑΘΗΝΑ
ΕΝΤΥΠΟ ΚΛΕΙΣΤΟ ΑΡ. ΑΔΕΙΑΣ 815/94 ΚΕΜΠ ΚΡ.
ΠΛΗΡΩΜΕΝΟ ΤΕΛΟΣ
ΚΩΔΙΚΟΣ ΕΝΤΥΠΟΥ: 017159

ISSN: 2732-9119

ΕΛΛΗΝΙΚΗ ΝΕΥΡΟΛΟΓΙΚΗ ΕΤΑΙΡΕΙΑ
HELLENIC NEUROLOGICAL SOCIETY
www.enee.gr

Archives of Clinical Neurology

www.jneurology.gr

OFFICIAL JOURNAL OF THE HELLENIC NEUROLOGICAL SOCIETY

ΕΙΔΙΚΟ ΤΕΥΧΟΣ/SPECIAL ISSUE
BIOMARKERS IN PARKINSON'S DISEASE AND ATYPICAL PARKINSONISM / ΒΙΟΔΕΙΚΤΕΣ ΣΤΗΝ ΝΟΣΟ ΤΟΥ ΠΑΡΚΙΝΣΟΝ ΚΑΙ ΤΑ ΑΤΥΠΑ ΠΑΡΚΙΝΣΟΝΙΚΑ ΣΥΝΔΡΟΜΑ

Τόμος 33 - Τεύχος 2

Vol. 33 - Issue 2

ΑΡΘΡΑ / ARTICLES

Μάρτιος - Απρίλιος 2024 / March - April 2024

Διμηνιαία έκδοση της
Ελληνικής Νευρολογικής Εταιρείας
Αλκμάνος 10, Αθήνα 115 28
Τηλ.: 210 72.47.056 - Fax: 210 72.47.556
www.enee.gr info@jneurology.gr

Κωδικός Διεύθυνσης Εποπτείας ΜΜΕ: 7159
ISSN 1106 - 3106

Αρχεία Κλινικής Νευρολογίας

www.jneurology.gr

Τόμος 33, Τεύχος 2, Μάρτιος - Απρίλιος 2024

Περιεχόμενα

Official Journal of the

Hellenic Neurological Society

10, Alkmanos str., Athens
Tel.: 210 72.47.056 - Fax: 210 72.47.556
www.enee.gr info@jneurology.gr
e-submission: submission@jneurology.gr

OWNER

HELLENIC NEUROLOGICAL SOCIETY
10 Alkmanos str, Athens
115 28 - Greece

PRINTED EDITION AND PDFs

Lychnia S.A.
7 Andravidas str., Athens
136 71, Hamomilo Aharnon
Tel.: 210 34.10.436 - 1, Fax: 210.34.25.967
www.lyhnia.com

SUBSCRIPTION FEES

HNS Members Free

Archives of Clinical Neurology

www.jneurology.gr

Volume 33:2 March - April 2024

Contents

Βιοδείκτες στην νόσο του Πάρκινσον και τα Άτυπα Παρκινσονικά Σύνδρομα.

Αγαπητοί συνάδελφοι,

Η Ιδιοπαθής Νόσος ή Σύνδρομο Πάρκινσον (ΝΠ) είναι μια ετερογενής διαταραχή με πολλαπλά φαινοτυπικά χαρακτηριστικά[1]. Ο ελληνικός όρος «ετερογενής» αναφέρεται στην περίπτωση αυτή σε «…πολλές αιτιολογίες ή κάτι το οποίο αποτελείται από ανόμοια ή διαφορετικά συστατικά ή μέρη…»

Πράγματι, η κρατούσα σήμερα άποψη είναι ότι η νευροεκφυλιστική διαδικασία της ΝΠ εξαρτάται από περισσότερο του ενός παθογενετικούς μηχανισμούς. Αυτή η αντίληψη συνέχεται με την αυξανόμενη ανάγκη για βιοδείκτες για την ανίχνευση και την πιο αντικειμενική αποτύπωση της βιολογίας της νόσου.[2]

Έτσι, οι βιοδείκτες ως «ένα καθορισμένο χαρακτηριστικό που μετριέται ως δείκτης φυσιολογικών βιολογικών διεργασιών, παθογόνων διεργασιών ή αντιδράσεων σε έκθεση ή παρέμβαση» [3] πιθανότατα θα βελτιώσουν την ακρίβεια της έγκαιρης διάγνωσης, θα αποσαφηνίσουν τους υπότυπους ή παραλλαγές της ΝΠ, θα εξατομικεύσουν τη θεραπεία και θα βελτιώσουν τις κλινικές μελέτες. [4]

Για παράδειγμα, οι δύο κλινικές μελέτες τροποποίησης της νόσου που στόχευαν τη συσσωματωμένη α-συνουκλεΐνη, και που απέτυχαν στους στόχους τους (PASADENA[5], SPARK[6]), θα μπορούσαν να είχαν στεφθεί με επιτυχία εάν είχαν χρησιμοποιήσει πιο ειδικούς βιοδείκτες για την επιλογή ασθενών, την παρακολούθηση και τα αποτελέσματα.

Αυτή η άποψη αποκτά σπουδαιότητα υπό το φως των πρόσφατων εξελίξεων στην ανάπτυξη βιοδεικτών. Το τελευταίο χρονικό διάστημα έχουν προκύψει αρκετοί αξιόπιστοι βιοδείκτες, με την βοήθεια νέων τεχνικών όπως η alpha-synuclein seed amplification assay (αSyn-SAA).[7]

Επιπλέον, η ύπαρξη γενετικών μορφών PD έχει δυνητικό αντίκτυπο στη θεραπευτική. Τα αιτιολογικά γονίδια ή οι γενετικοί παράγοντες κινδύνου (γονιδιωματικοί βιοδείκτες) αντιπροσωπεύουν έναν πιθανό στόχο για θεραπείες τροποποίησης της νόσου στην ΝΠ, αλλά επίσης, σε συνδυασμό με πολλά άλλα στοιχεία εξατομικευμένης ιατρικής και διεπιστημονικών δεδομένων, συμβάλλουν στην ακριβή κλινική διαχείριση αυτής.[8]

Η έκδοση λοιπόν ενός θεματικού τεύχους (στα πλαίσια των Αρχείων Κλινικής Νευρολογίας) με αντικείμενο τους βιοδείκτες στην ΝΠ αλλά και τις λοιπές κινητικές διαταραχές, θα ήταν κάτι επίκαιρο και χρήσιμο.

Εκ μέρους του Κλάδου Κινητικών Διαταραχών της ΕΝΕ
Παντελής Στάθης, MD PhD

Biomarkers in Parkinson Disease and Atypical Parkinsonism

Dear colleagues,
Idiopathic Parkinson's Disease or Syndrome (iPD) is a heterogenous disorder with multiple phenotypic characteristics[1]. The Greek term "heterogenous" refers at this point to "...several etiologies or consisting of dissimilar or diverse ingredients or constituents..."
Indeed, the present notion is that PD's neurodegenerative process depends on more than one pathogenic mechanism. This notion stresses the increasing need for biomarkers to detect and more objectively quantify disease biology[2].
So, biomarkers as "A defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes or responses to an exposure or intervention" [3] would probably improve the accuracy of early diagnosis, clarify subtypes, customize therapy, and accelerate clinical trials.[4]
For example, the two clinical disease modification trials targeting aggregated α-synuclein, that failed to reach their goals (PASADENA[5], SPARK[6]) could have been crowned with success if they had used more specific biomarkers for patient selection, monitor and outcomes.
This view acquires considerable value in the light of recent biomarker developments. Several reliable biomarkers have recently emerged, from newly developed assays such as the alpha-synuclein seed amplification assay (αSyn-SAA).[7]
In addition, the existence of genetic forms of PD has a potential impact on therapeutics. Causative genes or genetic risk factors (genomic biomarkers), represent a potential target for disease-modifying therapies in PD but also, be aggregated with several other data of personalized medicine and multidisciplinary input, contribute to precision clinical management of PD.[8]
Therefore, the publication of a thematic issue (within the Archives of Clinical Neurology) on the topic of biomarkers in PD and other atypical Parkinsonian syndromes, would be something timely and useful.

On behalf of the Movement Disorders Branch of the Greek Neurological Society
Pantelis Stathis MD, PhD

References-Βιβλιογραφικές Αναφορές

[1] Kempster PA, Hurwitz B, Lees AJ. James Parkinson's Chimera: syndrome or disease? J R Coll Physicians Edinb 2017; 47: 190–5 | doi: 10.4997/JRCPE.2017.220
[2] Chahine LM, Merchant K, Siderowf A, Sherer T, Tanner C, Marek K, Simuni T. Proposal for a biological staging system for Parkinson's disease. Journal of Parkinson's Disease 2023;13: 297–309 DOI 10.3233/JPD-225111
[3] FDA-NIH Biomarker Working Group. BEST (Biomarkers, Endpoints, and other Tools) Resource. Silver Spring (MD): Food and Drug Administration (US); Bethesda (MD): National Institutes of Health (US), www.ncbi.nlm.nih.gov/books/NBK326791/ (2016, accessed 22 September 2017)
[4] Parnetti L, Gaetani L, Eusebi P, et al. CSF and blood biomarkers for Parkinson's disease. Lancet Neurol 2019; 18: 573–86.
[5] Pagano G, Taylor KI, Anzures-Cabrera J, et al. Trial of prasinezumab in early-stage Parkinson's disease. N Engl J Med 2022; 387: 421–32.
[6] Lang AE, Siderowf AD, Macklin EA, et al. Trial of cinpanemab in early Parkinson's disease. N Engl J Med 2022; 387: 408–20.
[7] Siderowf A, Concha-Marambio L, Lafontant DE, et al Assessment of heterogeneity among participants in the Parkinson's Progression Markers Initiative cohort using α-synuclein seed amplification: a cross-sectional study. Lancet Neurol. 2023 May;22(5):407-417. doi: 10.1016/S1474-4422(23)00109-6. PMID: 37059509.
[8] Marras C, Chaudhuri KR, Titova N, Mestre TA. Therapy of Parkinson's Disease Subtypes. Neurotherapeutics 2020;17:1366–1377

Συντακτική Ομάδα (Editorial Board)

Συντακτική Ομάδα (Editorial Board)

25. Χ. Κρόγιας (Ruhr University of Bochum, Bochum, Germany)
26. Β. Λιούτας (Harvard University, Boston, USA)
27. Π. Μήτσιας (Πανεπιστήμιο Κρήτης, Ηράκλειο & Wayne State University, Detroit, USA)
28. Γ. Ρούντολφ (Γενικό Νοσοκομείο Παπαγεωργίου, Θεσσαλονίκη)
29. Α. Σαφούρης (Νοσοκομείο Metropolitan, Πειραιά)
30. Κ. Σπέγγος (Νοσοκομείο Υγεία, Αθήνα)
31. Γ. Τσιβγούλης (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών & University of Tennessee Health Sciences Center, Memphis, USA)

Παιδονευρολογία
1. Β. Δάρας (Harvard University, Boston, USA)
2. Α. Ευαγγελίου (Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, Θεσσαλονίκη)
3. Δ. Ζαφειρίου (Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, Θεσσαλονίκη)
4. Α. Παπαβασιλείου (Ιασώ Παίδων, Αθήνα)

Κλινική Νευροφυσιολογία
1. Ε. Αναγνώστου (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)
2. Π. Ζης (Πανεπιστήμιο Κύπρου, Λευκωσία, Κύπρος)
3. Ι. Καράκης (Emory University, Atlanta, USA)
4. Β. Κιμισκίδης (Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, Θεσσαλονίκη)
5. Π. Κοκότης (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)
6. Α. Κωδούνης (251 Γενικό Νοσοκομείο Αεροπορίας, Αθήνα)
7. Α. Μπονάκης (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)
8. Μ. Παπαδοπούλου (Πανεπιστήμιο Δυτικής Αττικής)
9. Χ. Πιπερίδου (Δημοκρίτειο Πανεπιστήμιο Θράκης, Αλεξανδρούπολη)
10. Ε. Σταμπουλής (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)
11. Δ. Τσίπτσιος (Δημοκρίτειο Πανεπιστήμιο Θράκης, Αλεξανδρούπολη)
12. Ε. Χρόνη (Πανεπιστήμιο Πάτρας, Πάτρα)

Άνοια
1. Π.Ιωαννίδης (Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, Θεσσαλονίκη)
2. Ε.Καπάκη (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)
3. Χ.Μπούρας (University of Geneva, Geneva, Switzerland)
4. Γ.Παρασκευάς (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)
5. Ν.Σκαρμέας (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)
6. Μ.Τσολάκη (Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, Θεσσαλονίκη)

Επιληψία
1. M. Reuber (University of Sheffield, UK)
2. Α. Αγαθονίκου (Γενικό Νοσοκομείο Αθηνών ΚΑΤ, Αθήνα)
3. Α. Αρζιμάνογλου (University Hospital of Lyon, Lyon, France)
4. Ι. Καράκης (Emory University, Atlanta, USA)
5. Β. Κιμισκίδης (Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, Θεσσαλονίκη)
6. Μ. Κουτρουμανίδης (Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom)
7. Χ. Πιπερίδου (Δημοκρίτειο Πανεπιστήμιο Θράκης, Αλεξανδρούπολη)
8. Π. Πολυχρονόπουλος (Πανεπιστήμιο Πάτρας, Πάτρα)
9. Αικ.Τερζούδη (Δημοκρίτειο Πανεπιστήμιο Θράκης, Αλεξανδρούπολη)

Συντακτική Ομάδα (Editorial Board)

Κεφαλαλγία
1. Χ.Αρβανίτη (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)
2. Θ. Αβραμίδης (Γενικό Νοσοκομείο Αθηνών Ερυθρός Σταυρός, Αθήνα)
3. Μ. Βικελής (Αθήνα)
4. Κ. Γυμνόπουλος (Γενικό Νοσοκομείο Θεσσαλονίκης Άγιος Λουκάς, Θεσσαλονίκη)
5. Π. Μήτσιας (Πανεπιστήμιο Κρήτης, Ηράκλειο & Henry Ford Hospital - Wayne State University, Detroit, USA)
6. Δ. Μητσικώστας (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)
7. Γ. Ρούντολφ (Γενικό Νοσοκομείο Παπαγεωργίου, Θεσσαλονίκη)

Ιστορία της Νευρολογίας
1. Α. Καράβατος (Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, Θεσσαλονίκη)
2. Λ. Τριάρχου (Πανεπιστήμιο Μακεδονίας, Θεσσαλονίκη)

Επεμβατική Νευρολογία
1. N. Goyal (University of Tennessee Health Sciences Center, Memphis, USA)
2. A. Sarraj (The University of Texas McGovern Medical School, Houston, USA)
3. Α. Σαφούρης (Νοσοκομείο Metropolitan, Πειραιά)

Κινητικές Διαταραχές
1. Μ. Αρναούτογλου (Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, Θεσσαλονίκη)
2. Ζ.Μ. Κεφαλοπούλου (Πανεπιστήμιο Πάτρας, Πάτρα)
3. Σ. Κονιτσιώτης (Πανεπιστήμιο Ιωαννίνων, Ιωάννινα)
4. Σ. Μποσταντζοπούλου (Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, Θεσσαλονίκη)
5. Μ. Πολίτης (University of Exeter, UK)
6. Μ. Σταμέλου (University of Marburg, Germany)
7. Λ. Στεφανής (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)

Νευρογενετική
1. K. Kleopa (Cyprus Institute of Neurology and Genetics, Cyprus)
2. Ε. Δαρδιώτης (Πανεπιστήμιο Θεσσαλίας, Λάρισα)
3. Γ. Κούτσης (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)
4. Δ. Μόνος (University of Pennsylvania, Philadelphia, USA)
5. Γ. Ξηρομερίσιου (Πανεπιστήμιο Θεσσαλίας, Λάρισα)
6. Γ. Χατζηγεωργίου (Πανεπιστήμιο Κύπρου, Λευκωσία, Κύπρος)

Νευροανοσολογία
1. R. Gold (Ruhr University of Bochum, Bochum, Germany)
2. M. Hadjivassiliou (University of Sheffield, UK)
3. Κ. Βουμβουράκης (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)
4. Ν. Γρηγοριάδης (Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, Θεσσαλονίκη)
5. Ε. Δαρδιώτης (Πανεπιστήμιο Θεσσαλίας, Λάρισα)
6. Γ. Δερετζή (Γενικό Νοσοκομείο Παπαγεωργίου, Θεσσαλονίκη)
7. Ε.Μ. Ευαγγελοπούλου (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)
8. Ι. Ηλιόπουλος (Δημοκρίτειο Πανεπιστήμιο Θράκης, Αλεξανδρούπολη)
9. Λ. Κάππος (University of Basel, Basel, Switzerland)
10. Κ. Κυλιντηρέας (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)
11. Δ. Μόνος (University of Pennsylvania, Philadelphia, USA)
12. Μ. Μποζίκη (Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, Θεσσαλονίκη)
13. Τ. Ντόσκας (Ναυτικό Νοσοκομείο Αθηνών, Αθήνα)
14. Π. Παπαθανασόπουλος (Πανεπιστήμιο Πάτρας, Πάτρα)

15. Γ. Τζάρτος (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)
16. Γ. Χατζηγεωργίου (Πανεπιστήμιο Κύπρου, Λευκωσία, Κύπρος)

Νευροεντατική

1. J. Chang (MedStar Washington Hospital Center)
2. T. Steiner (University of Heidelberg, Heidelberg, Germany)
3. Π. Βαρελάς (Albany Medical College, Albany, USA)
4. Κ. Δημητριάδης (Ludwig-Maximillians University Munich, Germany)
5. Δ. Κάζης (Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, Θεσσαλονίκη)
6. Χ. Κρόγιας (Ruhr University of Bochum, Bochum, Germany)
7. Γ. Ρούντολφ (Γενικό Νοσοκομείο Παπαγεωργίου, Θεσσαλονίκη)

Εκπαίδευση στη Νευρολογία

1. Θ. Αβραμίδης (Γενικό Νοσοκομείο Αθηνών Ερυθρός Σταυρός, Αθήνα)
2. Κ. Βαδικόλιας (Δημοκρίτειο Πανεπιστήμιο Θράκης, Αλεξανδρούπολη)
3. Π. Βαρελάς (Albany Medical College, Albany, USA)
4. Κ. Βουμβουράκης ((Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)
5. Ν. Γρηγοριάδης (Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, Θεσσαλονίκη)
6. Ε. Δαρδιώτης (Πανεπιστήμιο Θεσσαλίας, Λάρισα)
7. Γ. Δερετζή (Γενικό Νοσοκομείο Παπαγεωργίου, Θεσσαλονίκη)
8. Π. Ζης (Πανεπιστήμιο Κύπρου, Λευκωσία, Κύπρος)
9. Κ. Κυλιντηρέας (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)
10. Π. Μήτσιας (Πανεπιστήμιο Κρήτης, Ηράκλειο & Henry Ford Hospital - Wayne State University, Detroit, USA)
11. Ι. Μυλωνάς (Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, Θεσσαλονίκη)
12. Γ. Ρούντολφ (Γενικό Νοσοκομείο Παπαγεωργίου, Θεσσαλονίκη)
13. Λ. Στεφανής (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)
14. Γ. Τσιβγούλης (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών & University of Tennessee Health Sciences Center, Memphis, USA)
15. Γ. Χατζηγεωργίου (Πανεπιστήμιο Κύπρου, Λευκωσία, Κύπρος)

Νευρομυϊκές διαταραχές

1. C. McDermott (University of Sheffield, UK)
2. Θ. Αβραμίδης (Γενικό Νοσοκομείο Αθηνών Ερυθρός Σταυρός, Αθήνα)
3. Π. Δαβάκη (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)
4. Θ. Ζαμπέλης (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)
5. Β. Ζούβελου (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)
6. Π. Ζης (Πανεπιστήμιο Κύπρου, Λευκωσία, Κύπρος)
7. Ι. Μαυρομάτης (Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, Θεσσαλονίκη)
8. Γ. Παπαδήμας (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)
9. Α. Παπαδημητρίου (Πανεπιστήμιο Θεσσαλίας, Λάρισα)
10. Δ. Παρίσης (Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, Θεσσαλονίκη)
11. Ε. Σταμπουλής (Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα)
12. Ν. Τάσκος (Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, Θεσσαλονίκη)

Νευρο-ογκολογία

1. Α. Κυρίτσης (Πανεπιστήμιο Ιωαννίνων, Ιωάννινα)

Συντακτική Ομάδα (Editorial Board)

Editorial Board

Editorial Board

Editorial Board

15. Tzartos J (National & Kapodistrian University of Athens, Athens, Greece)
16. Voumvourakis K (National & Kapodistrian University of Athens, Athens, Greece)

Neurointensive care

1. Dimitriadis K (Ludwig-Maximillians University Munich, Germany)
2. Chang J (MedStar Washington Hospital Center)
3. Kazis D (Aristotle University of Thessaloniki, Thessaloniki, Greece)
4. Krogias C (Ruhr University of Bochum, Bochum, Germany)
5. Rudolf J (Papageorgiou Hospital, Thessaloniki, Greece)
6. Steiner T (University of Heidelberg, Heidelberg, Germany)
7. Varelas P (Albany Medical College, Albany, USA)

Neurology Education

1. Avramidis T (Red Cross Hospital, Athens, Greece)
2. Dardiotis E (University of Thessaly, Larissa, Greece)
3. Deretzi G (Papageorgiou Hospital, Thessaloniki, Greece)
4. Grigoriadis N (Aristotle University of Thessaloniki, Thessaloniki, Greece)
5. Hadjigeorgiou GM (University of Cyprus, Nicosia, Cyprus)
6. Kilidireas K (National & Kapodistrian University of Athens, Athens, Greece)
7. Milonas I (Aristotle University of Thessaloniki, Thessaloniki, Greece)
8. Mitsias P (University of Crete, Heraklion, Greece & Wayne State University, Detroit, USA)
9. Rudolf J (Papageorgiou Hospital, Thessaloniki, Greece)
10. Stefanis L (National & Kapodistrian University of Athens, Greece)
11. Tsivgoulis G (National & Kapodistrian University of Athens, Athens, Greece & University of Tennessee Health Sciences Center, Memphis, USA)
12. Vadikolias K (Democritus University of Thrace, Alexandroupolis, Greece)
13. Varelas P (Albany Medical College, Albany, USA)
14. Voumvourakis K (National & Kapodistrian University of Athens, Athens, Greece)
15. Zis P (University of Cyprus, Nicosia, Cyprus)

Neuromuscular disorders

1. Avramidis T (Red Cross Hospital, Athens, Greece)
2. Chroni E (University of Patras, Patras, Greece)
3. Davaki P (National & Kapodistrian University of Athens, Greece)
4. McDermott C. (University of Sheffield, UK)
5. Mavromatis I (Aristotle University of Thessaloniki, Greece)
6. Papadimas G (National & Kapodistrian University of Athens, Athens, Greece)
7. Papadimitriou A (University of Thessaly, Larissa, Greece)
8. Parissis D (Aristotle University of Thessaloniki, Greece)
9. Stamboulis E (National & Kapodistrian University of Athens, Athens, Greece)
10. Taskos N (Aristotle University of Thessaloniki, Greece)
11. Zouvelou V (National & Kapodistrian University of Athens, Athens, Greece)
12. Zis P (University of Cyprus, Nicosia, Cyprus)

Neurooncology

1. Kyritsis A (University of Ioannina, Ioannina, Greece)

Neuro-opthalmology

1. Anagnostou E (National & Kapodistrian University of Athens, Athens Greece)
2. Evdokimidis I (National & Kapodistrian University of Athens, Athens, Greece)
3. Iliopoulos I (Democritus University of Thrace, Alexandroupolis, Greece)

Άρθρα...

FLUID BIOMARKERS IN PARKINSON'S DISEASE

Anastasia Bougea[1], Nikolas Papagiannakis[1], Charikleia-Ilianna Botonaki[2], Leonidas Stefanis[1]

[1] *1st Department of Neurology and Movement Disorders, Medical School, Eginition Hospital, National and Kapodistrian University of Athens*
[2] *Medical School, Aristotle University of Thessaloniki, Thessaloniki, Greece*

Abstract
Parkinson's disease (PD) is a chronic, debilitating neurodegenerative disorder characterized clinically by a variety of progressive motor and non-motor symptoms. Currently, there is a dearth of diagnostic tools available to predict, diagnose or assessdisease risk or progression, leading to a challenging dilemma within the healthcare management system. The search for a reliable biomarker for PD that reflects underlying pathology is a high priority in PD research. With the advent of the recent alpha-synuclein Seeding Amplification Assays (SAA), mainly applied in the Cerebrospinal Fluid (CSF), a new era in PD biomarkers has commenced. However, such assays, despite their high sensitivity and specificity for PD or its prodromal forms, are at this point used only as a research tool, and they are not quantitative or reflective of disease severity. Currently, there are no reliable biomarkers predictive of progressionof motor and non- motor symptoms. A combination of multiple biomarkers might facilitate earlier diagnosis and more accurate prognosis in PD. In this review, we focus on the recent developments of fluid biomarkersin different biological liquids (CSF, blood, saliva) for PD.

Keywords: Parkinson's disease (PD), fluid biomarkers, non-motor symptoms, cerebrospinal fluid, blood, saliva, alpha-synuclein

ΥΓΡΟΒΙΟΔΕΙΚΤΕΣ ΣΤΗ ΝΟΣΟ ΠΑΡΚΙΝΣΟΝ

Αναστασία Μπουγέα[1], Νικόλας Παπαγιαννάκης[1], Χαρίκλεια Ηλιάνα Μποτονάκη[2], Λεωνίδας Στεφανής[1]
[1] *Α Νευρολογική Κλινική και Κινητικών Διαταραχών, Ιατρική Σχολή, Αιγινήτειο Νοσοκομείο Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών*
[2] *Ιατρική Σχολή, Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, Θεσσαλονίκη*

Περίληψη
Η νόσος του Πάρκινσον (ΝΠ) είναι μια χρόνια, εξουθενωτική νευροεκφυλιστική διαταραχή που χαρακτηρίζεται κλινικά από ένα ευρύ φάσμα κινητικών και μη συμπτωμάτων. Επί του παρόντος, υπάρχει έλλειψη διαθέσιμων διαγνωστικών εργαλείων για την πρόβλεψη, τη διάγνωση ή την εκτίμησητου κινδύνου ή της εξέλιξης της νόσου, οδηγώντας σε δυσεπίλυταδιλήμματα στο σύστημα διαχείρισης της υγειονομικής περίθαλψης. Η αναζήτηση ενός αξιόπιστου βιοδείκτη για την ΝΠ που αντικατοπτρίζει την υποκείμενη παθολογία αποτελεί υψηλή προτεραιότητα στην έρευνα για την ΝΠ. Η πρόσφατη εφαρμογή των SeedingAmplificationAssays (SAAs) της α-συνουκλείνης, ιδιαίτερα στο Εγκεφαλονωτιαίο Υγρό (ΕΝΥ), αποτελεί την αρχή μιας νέας εποχής στους βιοδείκτες της νόσου. Οι βιοδείκτες όμως αυτοί, παρά την υψηλή ευαισθησία και ειδικότητα για τις πρόδρομες μορφές και την εγκατεστημένη ΝΠ, χρησιμοποιούνται επί του παρόντος μόνο ερευνητικά, δεν είναι ποσοτικοί,και δεν αντικατοπτρίζουν την βαρύτητα της νόσου. Επί του παρόντος, δεν υπάρχει κανένας αξιόπιστος βιοδείκτης που να μπορεί να προβλέψει την εξέλιξη των κινητικών και μη κινητικών συμπτωμάτων. Ένας συνδυασμός πολλαπλών βιοδεικτών μπορεί να διευκολύνει την πρώιμη διάγνωση και την ακριβέστερη πρόγνωση στην ΝΠ. Σε αυτήν την ανασκόπηση, εστιάζουμε στις πρόσφατες εξελίξεις των βιοδεικτώνγια τη ΝΠ σε διαφορετικά βιολογικά υγρά (σε ΕΝΥ, αίμα, σίελο).

Λέξεις- κλειδιά: νόσος του Πάρκινσον (ΝΠ), υγροβιοδείκτες, μη κινητικά συμπτώματα,ΕΝΥ, αίμα, σίελος, α-συνουκλεΐνη

1. INTRODUCTION

Neurodegenerative diseases present a major problem for public health compromising the quality of life in today's aging population. Parkinson's disease (PD) affects 4.5 million worldwide, and it is predicted that this number will triple by 2030with enormous personal and societal consequences[1]. PD is an heterogeneous disease, with a wide array of motor (tremor, rigidity, and bradykinesia) and non-motor (sleep disorder, hyposmia, constipation, depression/ anxiety) symptoms resulting from pathology in both the central and peripheral nervous systems[2]. Clinical diagnosis of PD is not always easy, and is only feasible when 50-60% of substantia nigra dopaminergic neurons are lost[3]. Importantly, the misdiagnosis rate can be as high as 25% in early stages of PD[4]. Moreover, currently available therapies are limited to stabilizing or ameliorating symptoms or slowing symptomatic progression, but without having a clear effect on the progression of neurodegenerative mechanisms. These facts highlight the need for the development of biological indicators to enable timely and accurate diagnosis, both in terms of daily practice and as regards the appropriate choice of patients for therapeutic protocols of drugs under development.

A biomarker is defined as: ''A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention''[5]. From a methodological point of view, biomarkers can be categorized as clinical, imaging, biochemical, and genetic. Given the proximity of CSF to the central nervous system (CNS), this biofluid is the ideal source for diagnostic markers of ongoing pathological processes, although it is not a good matrix for monitoring drug effects or other variables over time, because of the need for repeated lumbar punctures. In this context, blood and saliva samples provide less invasive biomarkers on which OMICS, such as proteomics, metabolomics and lipidomics,could be applied, to capture collectively complex biological processes that could be defining of particular disease states. However, the use of OMICS in such biofluids is generally challenging, due to the overarching influence of comorbities in characterizing these matrices, as well as the fact that these biofluids are dissociated from the brain.It should be kept in mind that the usefulness of biomarkers islinked to the possibility of making an early diagnosis, and of enrollment of patients in conceptually novel clinical trials to test experimental disease modifying drugs[6].

So far, there have been several systematic review articles addressing the utility of diagnostic and prognostic biomarkers in PD[7]. The present review is a critical overview of fluid biomarkers (CSF, blood, saliva) in PD, discussing their strengths and limitations, as well as providing suggestions for future research.

2. PROTEIN BIOMARKERS FOR PARKINSON'S DISEASE

2.1 Abnormal Protein Accumulation and Aggregation Related Biomarkers

2.1.1 Alpha-synuclein in biological fluids

Alpha-synuclein (α-Syn) is the most important molecule in the pathogenesis of PD[8]. Variations in the *SNCA* gene encoding α-Syn were the first described genetic mutations identified as a causative agent for PD[9].α-Syn is a small cytoplasmic protein consisting of 140 amino acids. It is expressed in the central nervous system (CNS), particularly at the presynaptic neuronal terminals, but its physiological role is not well understood[10].

The protein itself can misfold into pathogenic species, whose aggregation forms the Lewy bodies, a pathological hallmark of PD [11]. α-Syn oligomers which are precursors to LBs are also toxic to cells[12]. Transgenic mouse models based on α-Syn overexpression can result in a PD-like phenotype, the effects of which include nigral degeneration, motor symptoms and response to levodopa therapy[13].This protein has attracted research attention as a potential biomarker for PD. Taking into account that abnormal α-Syn accumulation in the brain is likely the main cause of PD, itis highly probable that its accumulation in bodily fluids may reflect the abnormalities in the brain of PD patients[14].

Table 1 summarizes studies of fluid biomarkers in PD.The most obvious fluid to search for α-Syn is the CSF, as it is the fluid that has the greater proximity and it is influenced directly from brain processes[15]In three meta-analysis, CSF total α-Syn is lower in patients with PD compared with that of healthy controls[16-18].This phenomenon is largely attributed to a decrease of soluble brain α-Syn, as a result of its deposition in aggregates, akin to what is thought to happen with beta-amyloid deposition in Alzheimer's Disease (AD). The lowering of CSF totalα-Syn in PD is of a small magnitude, 10-15%. The number of longitudinal studies is limited with contradictory results. One study found that higher initial levels predicted worse progression and cognitive decline[19],while three other studies found no such effect[20-22].Additionally, one of them did not find any meaningful changes in CSF α-Syn levels in a 4-year span[21].

However, CSF α-Syn is not considered useful as a diagnostic biomarker, due to low accuracy. A meta-analysis found that it has a pooled sensi-

Table 1 Summary of selected studies of fluid biomarkers in Parkinson disease

References	Sample	Biofluid /Biomarker	Main outcomes
Hall et al.,2015	42PD+ 69C (BioFinder Study) at 2ys	CSF/ A-Syn CSF/Aβ42,t-tau,p-tau CSF/NfLs	Higher α-Syn in PD vs C Lower Aβ42 levels were associated with worsening of performance on delayed memory recall. high levels of p- tau were associated with worsening in motor symptoms
Steward et al.,2014	>300 unmedicated PD pts (DATATOP study) with 9ys follow-up	CSF/ α-Syn	Lower α-Syn predict cognitive decline but not motor progression
Mollenhauer et al., 2019	376 drug naïve PD + 173 HC (PPMI) with 24,36 mths	CSF/ total α-Syn	Lower CSF α-Syn in PD at 24, 36 mths. CSF α-Syn did not correlate with longitudinal MDS-UPDRS motor scores or DAT.
Forland et al., 2018	27PD pts + 18C with 2, 4 ys follow-up	CSF/ total α-Syn	total α-Syn did not predict motor or cognitive decline
Majbour et al.,2016	121PD pts (DATATOP study) with 2ys follow-up	CSF/ total, oligomeric, p-α-Syn	increase in total and oligomeric α-Syn levels and a decrease in p- α-Syn. oligomeric-α-Syn/total-α-Syn ratio was associated with postural and gait instability
Foulds et al., 2013	189 PD pts+ 91HC with 4-6 mths	Plasma/ total,p-α-Syn	Higher,p-α-Syn but not total α-Syn in PD vs HC
Mullin et al., 2019	82 GBA carriers +35C over 5ys follow-up	Serum, Saliva/ total α-Syn	High serum α- Syn in one GBA carrier who develop PD
Mollenhauer et al., 2017	173 PD + 112 HC (PPMI) at 6, 12mthsfollow-up	CSF/ A-Syn CSF/Aβ42,t-tau,p-tau	CSF biomarkers remained stable over 6 and 12 months and did not correlate with changes in UPDRS or DAT
Hansson et al., 2017	254PD pts (BioFinder Study)	CSF/NfLs	Blood NfL levels discriminate between PD and APD.
Terrelonge et al., 2016	104 PD, 11 MSA, 13 PSP with 5 to 9 ys follow-up	CSF/ α-Syn CSF/Aβ42,t-tau,p-tau CSF/NfL,FA	In PD, high NfL, low Aβ1-42, and high FA at baseline were related to future PDD
Liu et al., 2015	713PD (DATATOP study)	CSF, Serum/ urate	High CSF, Serum urate at baseline were associated with slower rates of clinical decline.
LeWitt et al., 2017	PD collected twice with an interval of up to 2 years	Plasma/medium-long chain FA, phenylalanine (aspartylphenylalanine, benzoate), serine metabolism (serine), Purine metabolism (inosine)	Increased FA, phenylalanine and serine metabolism Decreased Purine metabolism
Pellecchia et al., 2017	42PD with a4-y follow-up	Serum/ uric acid	lower levels of serum uric acid in the early disease stages are associated to the later occurrence of MCI

Brockmann et al. 2015	30sPD, 12 PD-GBA+,5PD-LRRK2+ over 3 ys follow-up	CSF/Aβ42,t-tau,p-tau	All three PD cohorts showed lower levels of Aβ42 sPD, GBA-PD but not PD-LRRK2+ with lower levels of t-tau and p-tau Higher baseline p-tau with more accelerated cognitive deterioration over time in LRRK2-PD and GBA-PD, but not in sPD. PD-GBA+ more rapid disease progression of motor and cognitive decline compared with nonGBA-PD
Ahmadi Rastegar et al., 2019	160sPD+LRRK2-PD (PPMI) over 2ys	Serum/27 cytokines	PDGF is elevated in LRRK2-PD compared to sPD GCSF, IL8, IL17A, IL10 associated with motor severity scale IL-6 and IL-4 associated with depression scale

Aβ42:amyloid-beta 42; APOE: CSF: cerebrospinal fluid; DJ-1: deglycase-1; FA: fatty acid; GBA: glucocerebrosidase; Halogenation markers*: AOPP, 3-chlorotyrosine, Mieloperoxidase , Hydrogen peroxide; HC: healthy controls; Hcy: Homocysteine; LRRK2: Leucine-rich repeat kinase 2; MCI: mild cognitive impairment; MDS-UPDRS: Movement Disorder Society- Unified Parkinson's Disease Rating Scale; MSA: Multiple System Atrophy;mths: months; NfL : Neurophilaments; mths: months; p-tau: phosphorylated tau; PD: Parkinson's Disease; PDD:Parkinson's Disease Dementia; PSP: Progressive Supranuclear Palsy; pts: patients; s: sporadic

tivity between 78% (62%–88%) and 88% (95% CI 84%–91%), and a specificity between 40% (35%–45%) and 57% (36%–76%)[17].On the other hand, there is someevidence that oligomeric α-syn is increased in the CSF taken from PD patients compared to healthy controls[23].The ratio of CSF oligomeric α-Syn to total α-Syn improved the diagnostic performance of oligomeric α-Syn alone, with an area under the curve (AUC) of up to 0.78 (sensitivity 82%, specificity 64%)[24].

There is the possibility that α-Syn levels reflect neuronal damage, since values increase in other non-synuclein related neurodegenerative disorders, like AD and Creutzfeldt-Jakob disease[25]. Interestingly, the different mutual interactions among α-Syn species and the different role of each protein in the pathogenetic mechanisms could explain the differences in terms of clinical phenotype[26].The association between CSF α-Syn and memory and language in AD suggests either that reduced CSF α-Syn also partly reflects global impaired neuronal/synaptic function, or that non-specific overall cognitive deterioration is accelerated in the presence of synuclein related pathology[27].This fact could help explain the variable association of CSF α-Syn levels with PD across studies. As with total α-syn levels, the use of CSF α-Syn species is not recommended in clinical practice. There is high heterogeneity across studies, a result that could be attributed to the co-existence of several unstandardized methods for the measurements.

In addition to methodological differences in the quantification of α-Syn, blood contamination of CSF during lumbar puncture is an important limitation of CSF α-Syn measurement. Blood α-Syn levels are much higher than those in CSF, because red blood cells are a major source of α-syn. Haemolysisin the course of sample collection and processing should be considered as a confounding factor for quantification of α-Syn level in CSF and blood. Other factors such as level fluctuations over time and drug treatment may have less effect on the level of α-Syn in CSF, however, most of the studies failed to address these issues. Further validation studies are needed before CSF totalα-Syn is included in routine clinical practice.

Compared to CSF, blood is a less costly and relatively non-invasive, easy-to-access biomarker for PD.In a recent meta-analysis of ten studies, total plasma α-Syn was found to be higher in patients with PD compared with controls[28]. Overall, Foulds et al. [29]conclude that the plasma level of p-α-Synhas potential value as a diagnostic tool, whereas the level of total α-Syn could act as a surrogate marker for the progression of PD. On the other hand, α-Syn oligomers or phosphorylated

forms gave inconclusive outcomes[30]. Nevertheless, in a longitudinal survey of glucocerebrosidase (GBA) mutation carriers, the one subject who developed PD had the highest levels of α-Syn in the entire cohort, while the severity of GBA mutations appeared to correlate with the concentration of serum α-Syn[31].A particular biosample that may be of interest is that of erythrocytes, as, as mentioned, they are a rich source of α-Syn. Idiopathic PD and GBA-PD patients appear to have increased levels of oligomeric α-Syn in erythrocyte membranes compared to age- and sex-matched controls [32], and similar findings have also been reported by others.

Of particular interest in the context of biomarker research is the packaging of α-Syn into exosomes and its subsequent release into the circulation. Exosomes are formed within the endosomal system of cells and are released into the extracellular space upon fusion of multivesicular bodies with the plasma membrane[33]. The sorting of α-Syn into exosomes is thought to involve interactions with lipid membranes, as well as specific protein-protein interactions with components of the endosomal sorting complex required for transport (ESCRT) machinery[33]. Lower levels of CSF exosome-associated α-Syn were observed in PD patients[34]. These findings suggest that exosomal α-Syn in the CSF holds promise as a diagnostic biomarker for PD, although further validation in larger cohorts and longitudinal studies is warranted. Recently, Yan et al. found that both plasma exosomal α-Syn and plasma neural-derived exosomal α-Syn were elevated in PD patients compared to healthy controls, whereas only plasma neural-derived exosomal α-Syn were elevated in the RBD group [35]. Niu et al. [36]showed that plasma neuronal exosomal a-Syn had a greater power for diagnosing early-stage PD compared with other studies [37,38]. Several factors including plasma storage condition, disease staging and sample preparation, might explain the different results. Jiang et al. [39]found that the levels of serum-neuronal exosome α-Syn were elevated in early stage PD, even in patients with REM sleep behavior disorder (RBD), but not sufficiently sensitive and specific to be used as a diagnostic marker. These increased levels of α-Syn in serum-neuronal exosomes remained elevated with disease progression, suggesting them as a potential pharmacodynamic biomarker for α-Syn targeting therapies in PD. Furthermore, neural-derived exosomal α-Syn in the serum may help to identify different motor types in PD(non-tremor-dominant vs tremor dominant group)[40].

Although the use of saliva to measure α-Syn is also an attractive possibility for biomarker assessment, as its collection is easy, non-invasive and lacks possible blood contamination, there is conflicting evidence about the total α-Syn levels in saliva of PD patients compared to healthy controls[41-43].The discrepancy among these studies can be attributed to several factors, including the small number of samples, heterogeneous study groups, and analytical issues of salivary α-Syn quantification. The majority of the included studies failed to describe such procedures in detail, and furthermore they lacked homogeneity since protocols varied. Consequently, diagnostic performance of total or oligo salivary α-Syn assays is not yet at the level needed to justify their introduction into clinical practice[44].

Seeding Amplification Assay

The Seeding Amplification Assay is the newest and most promising technique of detecting abnormal aggregate-proneα-Syn species, primarily in the CSF.

The "Protein Misfolding Cyclic Amplification(PMCA)" and the "Real-Time Quaking-Induced Conversion(RT-QuIC)" are two ultrasensitive protein amplification methods for the identification of pathological protein aggregates, that were initially created for the field of prion disorders to detect PrPsc. PMCA was developed by Soto et al. in 2001, followed by the development of RT-QuIC by Atarashi et al. in 2011[45]. Because of the efficacy of RT-QuIC technique for the detection of prion diseases and since α-Syn seems to follow similar mechanisms of aggregation to the prion protein, similar misfolded-protein amplification techniques have been applied in brain homogenates and CSF samples from patients diagnosed with synucleinopathies for the identification of misfolded α-Syn [45]. These assays include RT-QuIC, and a newly developed "aSyn-PMCA" assay, similar methodologically to RT-QuIC, and they have recently been reported under the consensus term, seed amplification assays(SAAs)[46].These techniques mimic in vitro the in vivo protein misfolding and aggregation process seen in CJD[47]. The fundamental idea of these techniques is comparable to that of a polymerase chain reaction (PCR): at the cost of the substrate (protein monomer), a template (protein aggregate) is growing in a cyclic reaction, leading to a rise in template units[45]. If PrPSc is present in the test sample, the normally solubleprion protein (substrate) gets converted from a highly alpha-helical structure into an amyloid fibril, rich in beta-sheet. Samples are incubated in a buffer solution, at a defined temperature, which contains the substrate (protein monomer). Preformed aggregates of the sample serve as templates, which polymerize at their extremities at the cost of the substrate. The grown aggregates are fractured into smaller pieces and additional polymerization sites are generated during the shaking/sonication step. In order to induce an exponential amplification of the pathological ag-

Table 2 Studies on aSyn-SAAs using CSF and BH samples

Author	Assay	Sample	Autopsy	Disease	Number of samples(cases/ controls)	Sensi-tivity	Specific-ity
Fairfoul et al., 2016	RT-QuIC	CSF	YES	PD iLBD/AD other parkinso-nian disorders	2/20 13/20 29/20	100% 15% 75,9%	100% 100% 100%
			NO	PD other parkinso-nian disorders	20/15 3/15	95% 100%	100% 100%
Shahnawaz et al., 2017	PMCA	CSF	NO	PD other parkinso-nian disorders	76/97 20/97	88% 90%	94% 94%
Groveman et al, 2018	RT-QuIC	CSF	NO	PD other parkinso-nian disorders	12/31 17/31	92% 94%	100% 100%
Kang et al., 2019	RT-QuIC PMCA	CSF CSF	NO NO	PD PD	105/79 105/79	96,2% 95,2%	82,3% 89,9%
Manne et al, 2019a	RT-QuIC	CSF BH	NO YES	PD PD other parkinso-nian disorders	15/11 11/19 5/19	100% 90,9% 100%	100% 100% 100%
Garrido et al., 2019	RT-QuIC	CSF	NO	LRRK2-PD iPD NMCs of LRRK2	15/10 10/10 16/10	40% 90% 18,8%	80% 80% 80%
Van Rumund et al., 2019	RT-QuIC	CSF	NO*Å	PD other parkinso-nian disorders	53/52 29/52	84% 55,2%	98% 98%
Bongianni et al., 2019	RT-QuIC	CSF	YES	LBD/AD LBD/PART LBD/CJD other parkinso-nian disorders	15/49 2/49 3/49 8/49	93,3% 100% 66,6% 100%	95,9% 95,9% 95,9% 95,9%
Rossi et al., 2020	RT-QuIC	CSF	YES	Mixed LBD*Ç other parkinso-nian disorders	7/81 16/81	85,7% 87,5%	98,8% 98,8%
			NO	PD other parkinso-nian disorders	71/62 111/62	100% 71,2%	98,4% 98,4%
Shahnawaz et al., 2020	PMCA	CSF	NO	PD other parkinso-nian disorders	94/56 75/56	93,6% 84,6%	100% 100%
Orrúet al., 2020	RT-QuIC	CSF	NO	PD	108/85	97%	87%
Singer et al., 2020	PMCA	CSF	NO	PD other parkinso-nian disorders	16/29 75/29	100% 94,7%	100% 100%
Concha-Marambio et al., 2021	PMCA	CSF	NO	PD SWEDD	30/30 20/30	96,2% 20%	96,7% 96,7%
Rossi et al., 2021	RT-QuIC	CSF	NO	MCI-LB	81/58	95,1%	96,6%

Author	Assay	Sample	Autopsy	Disease	Number of samples(cases/ controls)	Sensi-tivity	Specific-ity
Bargar et al., 2021b	RT-QuIC	CSF	YES	PD other parkinso-nian disorders	88/68 58/68	98% 98%	100% 100%
Brockmann et al., 2021	RT-QuIC	CSF	NO	sporadic PD PD GBA PD LRRK2 PD recessive*Ñ NMCs*⁴ other parkinso-nian disorders	107/26 99/26 9/26 20/26 14/26 49/26	91% 86,8% 78% 50% 14% 85,7%	92% 92% 92% 92% 92% 92%
Poggiolini et al., 2021	RT-QuIC	CSF	NO*⁴	PD other parkinso-nian disorders	74/55 69/55	89% 68,1%	96% 96%
Compta et al., 2022	RT-QuIC	CSF	NO	PD other parkinso-nian disorders	20/19 37/19	75% 12%	100% 100%
Hall et al., 2022	RT-QuIC	CSF	NO	PD other parkinso-nian disorders Controls convert-ed to LBD	50/47 29/47 2/47	94% 65,5% 100%	83% 83% 83%
			YES	standard LBD*⁴ non-standard LBD*⁴	25/53 23/53	100% 57%	94% 94%
Garrido et al., 2022	RT-QuIC	BH SN	YES	LRRK2-PD LTP+ LTP+ controls	3/7*⁴ 7/7	100% 100%	100% 100%
		BH AC	YES	LRRK2-PD LTP+ LTP+ controls	3/8 7/8	100% 100%	50% 50%
		CSF	YES	LRRK2-PD LTP+ LTP+ controls	2/6 6/6	100% 83%	100% 100%
Siderowf et al., 2023	RT-QuIC	CSF	NO	PD SWEDD NMCs of GBA NMCs of LRRK2 other parkinso-nian disorders	545/163 54/163 151/163 159/163 51/163	87,7% 9,3% 7,3% 8,8% 86,2%	96,3% 96,3% 96,3% 96,3% 96,3%
Concha-Marambio et al., 2023	PMCA	CSF	NO	PD other parkinso-nian disorders	95/64 36/64	95,7% 86,1%	96,9% 96,9%

***1 Only** 2% ofthe cases are autopsy-confirmed.*2 Mixed LBD includes CJD with DLB (n = 2), CJD with brain-stem LBD (n = 3), and other primary diagnoses with limbic LBD (n = 1) or brainstem LBD (n = 1).*3Recessive PD includes patients with mutations in parkin, PINK-1 or DJ-1. *4 Non manifesting carriers include Carriers of GBA(n=10),LRRK2(n=3) or recessive(n=1) *5 32 out of 55 controls were autopsy samples. *6standard LBD includ-ed cases with PD, PD with AD and DLB. *7 non-standard LBD includes AD with Lewy Bodies not meeting criteria for DLB or PD, and incidental LBD *8 Controls include LRRK2-PD without LTP and LTP- controls. Abbreviations: AC= anterior cingulate gyrus, SN= substantia nigra, LTP= Lewy Type pathology, NMCs=non-manifesting carriers of mutations in genes related to LBD.

Table 3 Studies on aSyn-SAAs using peripheral tissue samples

Author	Assay	Sample	Autopsy	Disease	Number of samples(cases/ controls)	Sensitivity	Specificity
De Luca et al., 2019	OM	RT-QuIC	NO	PD other parkinsonian disorders	18/18 11/18	56% 82%	83% 83%
Stefani et al., 2021	OM	RT-QuIC	NO	PD other parkinsonian disorders	41/59 63/59	46,3% 44,4%	89,8% 89,8%
Bargar et al., 2021a	OM	RT-QuIC	NO	PD other parkinsonian disorders	13/11 30/11	69% 63%*Å	91%*Å 91%
Manne et al., 2020	Frozen SKIN	RT-QuIC	YES	PD	25/25	96%	96%
	FFPE SKIN	RT-QuIC	YES	PD	12/12	75%	83%
Wang et al., 2021	Abdominal SKIN	RT-QuIC	YES	PD LBD other parkinsonian disorders	47/43 7/43 3/43	94% 100% 67%	98% 98% 98%
		PMCA	YES	Synucleino-pathies*Ç	32/8	82%	96%
	Scalp SKIN	RT-QuIC	YES	PD	20/10	100%	100%
	Biopsy SKIN*Ñ	RT-QuIC	NO	PD	20/21	95%	100%
		PMCA	NO	PD	10/10	80%	90%
Donadio et al., 2021	SKIN*⁴	RT-QuIC	NO	PD LBD other parkinsonian disorders	6/18 4/18 8/18	100% 75% 62,5%	83% 83% 83%
	CSF	RT-QuIC	NO	PD LBD other parkinsonian disorders	2/13 2/13 4/13	100% 100% 50%	100% 100% 100%
Mammana et al., 2021	SKIN cervical	RT-QuIC	YES	PD iLBD other parkinsonian disorders	1/40 7/40 1/40	100% 85,7% 100%	97,5% 97,5% 97,5%
	SKIN thigh	RT-QuIC	YES	PD iLBD other parkinsonian disorders	1/39 6/49 1/39	100% 66,7% 100%	100% 100% 100%
	CSF	RT-QuIC	YES	iLBD other parkinsonian disorders	4/30 1/30	75% 100%	100% 100%
	SKIN cervical	RT-QuIC	NO	PD other parkinsonian disorders	4/15 4/15	100% 100%	93,3% 93,3%
	SKIN thigh	RT-QuIC	NO	PD other parkinsonian disorders	4/11 7/11	50% 100%	90,9% 90,9%

Author	Assay	Sample	Autopsy	Disease	Number of samples(cases/ controls)	Sensitivity	Specificity
	SKIN leg	RT-QuIC	NO	PD other parkinsonian disorders	5/15 4/15	80% 100%	100% 100%
	CSF	RT-QuIC	NO	PD other parkinsonian disorders	7/27 11/27	100% 100%	100% 100%
Kuzkina et al., 2021	SKIN*4	RT-QuIC	NO	PD	34/30	82%	85%
Kuzkina et al., 2023	SKIN*4	RT-QuIC	NO	PD other parkinsonian disorders	39/23 38/23	87,2% 97,4%	87% 87%
Fenyi et al., 2019	GI rectum	PMCA	NO	PD	4/4	25%	75%
	GI sigmoid	PMCA	NO	PD	12/7	58,3%	100%
	GI antrum	PMCA	NO	PD	2/-	100%	—
Manne et al., 2019b	SMG	RT-QuIC	YES	PD iLBD	13/16 3/16	100% 100%	94% 94%
Chahine et al., 2023	SMG	RT-QuIC??	NO	PD	41/14	73,2%	78,6%
	CSF	PMCA?	NO	PD	54/21	92,6%	90,5%
Luan et al., 2022	SALIVA	RT-QuIC	NO	PD other parkinsonian disorders	75/36 18/36	76% 61,1%	94,4% 94,4%
Okuzumi et al., 2023	SERUM	IP/RT-QuIC	NO	PD Parkin-PD other parkinsonian disorders	221/128 17/128 58/128	95% 0% 65,5%	91,4% 91,4% 91,4%

***1The group** of other parkinsonian disorders includes 20 MSA-P patients and 10 MSA-C patients.Each sample was analyzed by two different labs. The results for PD and MSA-P subjects showed an interrupter agreement of 100% between the two labs. Among the MSA-C patients, one was positive at USA-lab(10% sensitivity) and none was positive at ITA-lab(0% sensitivity) and among healthy controls, specificities of 91% and 100% were reached at USA-lab and ITA-lab, respectively. *2 Synucleinopathies include PD cadavers (n = 24), LBD cadavers (n = 5) and MSA cadavers (n = 3).*3 Biopsy skin samples were obtained from the leg or the posterior cervical region. *4 Biopsy skin samples were obtained from C7, thigh and leg. *5 Biopsy skin samples were obtained from C7, Th10, Thigh and lower leg. *6 Biopsy skin samples were obtained from the leg, C7 or Th10. Abbreviations: FFPE Formalin-fixed paraffin-embedded,IP/RT-QuICimmunoprecipitation-based real-time quaking-induced conversion,

gregates, incubation and fragmentation steps are repeated in a cyclic process several times[45].

Up to date, these seeding aggregation assays have been tested with multiple studies in CSF and Brain Homogenate (BH) samples for the detection of synucleinopathies as presented in table 2. Although the initial protocols were tested in BH and CSF samples, these assayshave been now also applied to a variety of biospecimens, such as olfactory mucosa (OM), gastrointestinal tract, skin, serum, submandibular gland and saliva, as presented in table 2, with promising results.

So far, many studies have tested α-Syn-seeding activity in synucleinopathy cases, via RT-QuIC and PMCA assays, with the use of CSF samples[48]. A number of studies resulted, with the use of aSyn-PMCA assay, in 88-100% sensitivity rates and 89.9% - 100% specificity rates for discriminating between PD patients and healthy controls[49-51].Studies testing RT-QuIC assay in autopsy-derived CSF samples have demonstrated 98-100% sensitivity and 100% specificity for differentiating between PD cases and

controls.Many studies, that have used CSF samples from living patients with PD and non-synucleinopathy controls, showed sensitivity and specificity rates of 75-100% and 80-100%, respectively[46,52,53].

In 2022, Wang et al.[54] conducted the first meta-analysis for the diagnostic accuracy of α-Syn -RT-QuIC in synucleinopathies. They reached a sensitivity of 91% (95% CI: 0.85-0.94) and a specificity of 95% (95% CI: 0.90-0.97) for distinguishing between Lewy Body disease patients and controls. The Lewy Body Disease group included PD, DLB, PAF, iRBD and mixed cases of LBD, while the control group consisted of MSA patients, patients with other neurological diseases and healthy subjects.

A systematic review and metaanalysis was conducted in 2023 by Grossauer et al.[48], with the aim to evaluate the diagnostic accuracy ofCSFα-Syn-SAAs in differentiating synucleinopathies from controls. The results showed a sensitivity of 88% (95% CI, 0.87-0.95) and a specificity of 95% (95% CI, 0.92–0.97) in differentiating synucleinopathies from non-synucleinopathies.

Given its potential application as a biomarker for alpha-synucleinopathies, the detection of α-Syn-seeding activities inother biological fluids or peripheral tissuesbeyond CSF and BH is of primary interest. Recently, a number of studies have applied the α-Syn-SAAs in various peripheral tissue samples and biological fluids (Table 3).

Although the use of α-Syn -SAAs in olfactory mucosa samples represents an appealing method, due to the low invasiveness of nasal swabbing in comparison with lumbar puncture or biopsies, the studies conducted so far in these samples showed high specificities but relatively moderate sensitivities in discriminating PD patients from controls[55-57].α-Syn-SAAs have also been applied in Submandibular gland biopsy samples of PD patients with very promising results [58,59]. Nevertheless, the invasiveness of this biopsy procedure makes these samples less appealing for clinical application[60]. Saliva represents a very attractive method to detect α-Syn seeding activity, as it is non-invasive. However, so far the results from one study with the use of salivary RT-QuICdid not result in high sensitivity for PD[61]. Another study used a modified RT-QuIC assay, namely IP (immunoprecipitation)/RT-QuIC, in serum. The results are very promising, showing high sensitivity and specificity for discriminating PD patients from controls, but further verification studies are needed.

A number of studies have tested the aSyn-SAAs in skin biopsy samples from both living patients and cadavers with PD, with most of them resulting in high diagnostic accuracies for PD. Manne et al.[58] showed that the RT-QuIC assay in frozen skin biopsiesfrom PD cadavers and controls resulted in higher sensitivity and specificity than FFPE (formalin-fixed paraffin-embedded) skin biopsies. Wang et al. [62] compared the diagnostic accuracy of skin-RT-QuIC and skin-aSyn-PMCA, showing that among living PD patients and controls, RT-QuIC assay resulted in higher sensitivity and specificity than α-Syn-PMCA.The diagnosticaccuracy also varied in somestudies, depending on thebiopsysite, although a specific pattern has not yet been identified. Interestingly, among skin biopsies from cervical region, thigh and leg, Mammana et al.[63] showed that skin biopsies from thigh had the lowest sensitivity and specificity for PD diagnosis in living patients, although these results were not reproduced in autopsy-derived samples.

A recent meta-analysis compared the diagnostic accuracy of various biospecimens with the use of α-Syn-SAAs. α-Syn-SAAs could discriminate PD patientsfromhealthycontrolsor non-neurodegenerative neurologicalcontrols in CSF sampleswith 91% sensitivity(95% CI 0.89-0.92) and 95% specificity (95% CI 0.94-0.96); in OM samples with 51%sensitivity (95% CI 0.39-0.62)and 91% specificity (95% CI 0.84-0.96); in skin samples with 91% sensitivity (95% CI 0.86-0.94) and 92% specificity (95% CI 0.87-0.95); in saliva samples with 79% sensitivity (95% CI 0.70-0.86) and 88%specificity (95% CI 0.77-0.95); in submandibular gland samples with 80% sensitivity (95% CI 0.66-0.89) and a specificity of 87% (95% CI 0.69-0.96); in gastrointestinal (GI) tract samples with 44%sensitivity (95% CI 0.30-0.59) and 92% specificity (95% CI 0.79-0.98)[64].

Overall, the α-Syn SAAs, given their high sensitivity and specificity, have changed the landscape of biomarkers in PD, although at this point in time they remain a research tool, as they have not been fully validated clinically. There are issues with the need for specialized equipment, the difficulties in implementing the assay reliably in some laboratories, and the lack of standardized procedures, as each laboratory uses slightly different protocols. An issue in point is the large discrepancy across laboratories in the differential diagnosis of PD from MSA. Furthermore, these assays at themomentare not quantitative, and cannot reliably assess disease progression, and therefore represent more state rather than trait markers.

2.1.2Classic AD type biomarkers (amyloid, tau, phospho tau)

In PD, apart from the core pathological hallmark of LBs, up to 20-30% of patients show coexistent Alzheimer disease (AD) pathology in the form of, more commonly, extracellular beta-amyloid (diffuse Aβ and neuritic) plaques and, more rarely, intracellular aggregates of the hyperphosphorylated tau

protein (total t-tau) in neurofibrillary tangles (NFTs) and neuropil threads (NTs). These neuritic plaques include a dense core of amyloid beta peptides mainly β-amyloid1-42 (Aβ42), while NFTs consists of tau phosphorylated at threonine 181 (Tp-181).

Because t-tau is considered a marker of neurodegeneration, its levels are purported to change later during the progression of the disease correlating with clinical symptom severity. This is in contrast to Aβ42 values which become abnormal before alterations in other AD biomarkers and cognitive symptoms are detected[65]. Lower CSF Aβ42 has been shown to predict the subsequent development of cognitive decline in non demented PD[66-68]. Importantly, as CSF t-tau reflects the intensity of acute neuronal damage and chronic neuronal degeneration, elevated t-tau levels in PD were correlated with cognitive decline over time in one study [69], however this was not the case in other studies [66-68]. Mollenhauer et al.[70] showed that p-tau was increased marginally over a short period of time (6-12 months) in PD compared to HC, however again this was not the case in most other studies. A study done by Majbour et al.[23] revealed no significant change in levels of t-tau, p-tau, Aβ40, and Aβ42in PD patients over a two-year period, which may be too short. Overall, it appears that t-tau or p-tau are not strong candidates for diagnostic markers or predictors of cognitive decline in PD. Low CSF Aβ42, on the other hand, is an established predictor of cognitive decline in PD, as it has been borne out in numerous studies with longitudinal observations.

2.1.3 **Neurofilaments**

Neurofilaments (NFs) are prominent components of large myelinated axons; forthis reason, an increase in their CSF and blood concentration is considered a sensitive marker of white matter axonal degeneration[71]. This process is not typical in early stages of PD which may explain the lack of a significant difference in CSF and blood NfL in PD patients compared with controls[72]. Conversely, our recent meta-analysis showed that CSF NFLs may be used as a biomarker in discriminating atypical parkinsonian disorders (progressive supranuclear palsy, multiple system atrophy, and corticobasal syndrome) from PD with high diagnostic accuracy at an early stage of disease[73](Table 1). Since NfL levels in blood show a strong correlation with those in CSF, serum NfL may represent a non-invasive, cost-efficient and widely accessible biomarker that could be easily implemented in clinical practice and allow monitoring disease progression[74,75]. A recent longitudinal study showed that both serum and CSF NfL are associated with worse progression of depression and anxiety. Serum NfL showed stronger associations with non-motor symptoms, suggesting it could potentially be used as a non-invasive marker of non-motor progression for PD [76]. However, NFs failed to have prognostic value in terms of motor progression over 2 years in patients with PD[19].

3. METABOLITE BIOMARKERS FOR PARKINSON'S DISEASE

Before the discovery of genetic forms of PD and the development of sensitive assays to detect proteins associated with PD pathology in body fluids, biomarker studies for PD focused on changes in small molecules/metabolites (mainly in CSF), such as catecholamines, serotonin, aminoacids (including neurotransmitters like GABA, glycine, glutamate or precursors of the monoamine neurotransmitters including phenylalanine, tyrosine, tryptophan and related compounds), using HPLC with electrochemical, fluorescent or UV detection[77].Correlations with the progression of PD were found for changes in phenylalanine, purine and FA metabolism, serine, polyamines and tryptophan metabolism via the kynurenine pathway in CSF, plasma and urine. However, there are profound metabolic effects of dopaminergic treatment on aromatic amino acid metabolism (tyramine, tryptophan) of PD patients. Of note, in a prospective study of unmedicated PD patients, LeWitt et al.[78]showed that CSF homovanillic acid is a poor predictor of PD progression, but that several purines (compounds with xanthine structure) and some medium- or long-chain FA correlated strongly with worsening of UPDRS scores.

Other small molecules of interest are glutathione and purine metabolites, including uric acid (UA), because of their role as antioxidants. Serum UA levels are higher in prodromal PD subjects with ongoing dopaminergic degeneration compared to those with manifest PD[79].Lower levels of serum UA in the early disease stages are associated to the later occurrence of mild cognitive impairment (MCI) in an early PD cohort [80]. These findings suggest that the serum UA levels might be a potential biomarker to indicate the risk and progression of PD. However, confounding factors of the included studies such as genetic ,clinico- demographics (age, disease duration and stage, diagnosis criteria and treatment status) and lifestyle (diet, diuretics and alcohol consumption)factors which could affect UA levels should be taken in account in interpreting the above results. Metabolite profiling of body fluids of PD is a powerful tool to identify novel biomarkers for early diagnosis, prognosis and monitoring of disease progression(Table 1).Further validation in larger longitudinal studies, as well as in PD patients with specific gene mutations, will be of great interest.

4. LYSOSOMAL-RELATED BIOMARKERS FOR PARKINSON'S DISEASE

The process leading to accumulation of aggregated α-Syn has been associated with the impairment of the autophagy-lysosomal pathway, which represents one of the main routes for the intracellular degradation of α-Syn.GBA1 mutation carrier status is the most common genetic risk factor for α-Syn aggregation leading to PD. In the prospective BioFIND cohort, there was a significant reduction of CSF β-glucocerebrosidase (GCase) (–28% in PD vs controls) and cathepsin D (–21% in PD vs controls) activity in patients with PD; a similar trend was also observed for β-hexosaminidase activity (–9% in PD vs controls)[81]. In this cohort, 13% of patients with PD and 5% of healthy controls were carriers of mutations in the GCase coding gene (GBA). Although GCase activity was lower in carriers versus non-carriers (–27%), the overall decrease was present independent of GBA mutation carrier status (–25% in non-carrier patients with PD vs non-carrier controls). Diagnostic accuracy was suboptimal for GCase (sensitivity 67%, specificity 77%) and cathepsin D (sensitivity 61%, specificity 77%). The diagnostic performance improved when combining the panel of all of the measured lysosomal enzymes activities (sensitivity 71%, specificity 85%) and further increased when amyloid, tau, and α-Syn pathology markers were added to the model. It should be noted however that other studies have failed to find a decrease of peripheral GCase activity in iPD vs. controls, whereas a decrease of such activity in heterozygote GBA mutation carriers is consistently observed [82]. In contrast, other indices of peripheral lysosomal function, such as Hsc70, reflective of the process of Chaperone-Mediated Autophagy (CMA) may be decreased in iPD [82].

5. NEUROINFLAMMATORY REACTION RELATED BIOMARKERS

Evidence has shown an interplay between neuroinflammation and other proposed pathogenic mechanisms of PD, such as mitochondrial dysfunction and oxidative stress, while there is also involvement of parkinsonian genes, such as α-Syn, Parkin and DJ-1 in innate immune responses. Pro-inflammatory cytokines produced by microglia activation further promote the production of immune markers, nitric oxide, and reactive oxygen species. Post-mortem and biofluid (blood, CSF) studies reported that increased inflammatory profiles are associated with clinical subtypes of PD, promoting an accelerated motor and non-motor phenotype[83-85]. Elevated CSF ICAM-1, Interleukin-8, MCP-1, MIP-1 beta, SCF and VEGFlevels are prospectively related with a raised risk of cognitive impairment in PD patients[86]. Serum levels of MCP1 IL-8, IL-10, and GCSF were also positively correlated with serial changes in UPDRS III score, suggesting that higher levels of these biomarkers are associated with faster motor progression[87]. Therefore, a number of pro-inflammatory cytokines could be potential biomarkers for evaluating the severity of motor and cognitive impairment in PD patients(Table 1). Importantly, medications targeting the inflammatory mediators may provide an effective treatment strategy for PD.

6. MIRNAS AND CIRCRNAS AS BIOFLUID BIOMARKERS FOR PD

miRNAs are small (22 nt) double-stranded RNA molecules that regulate gene expression via binding to the 3′ UTR of mRNA targets. The expression of different miRNAs (appx. 2000 miRs characterized in humans) is strongly dependent on physiological and pathological stimuli and reflects the functional state of a cell, making the miRNA signature an interesting biomarker candidate in various diseases.

CSF and its unique proximity to the brain makes it a promising biofluid source for miRNAs capable of reflecting neurodegenerative changes in the brain. A recent meta-analysis identified several CSF-based studies which demonstrated an interesting trend of inversely mirroring changes in the CNS. [88].For example, upregulated levels of CSF miR-205-5p were reported by Marques et al.[89], but such levelswere downregulated in both the SN and the striatum. This trend was also reflected in the upregulated levels of miR-7-5p and miR-218-5p in the CSF and corresponding downregulation in the SN and prefrontal cortex[90,91].

Compared to CSF, blood-based miRNA biomarkers offer the advantage of being minimally invasive and have the potential to facilitate large-scale screening and longitudinal monitoring of PD patients. Several studies have reported altered expression levels of specific miRNAs in the blood of PD patients[92-95]. For example, miR-124-3p, miR-132-3p and miR-433-3p were found to be upregulated in plasma but downregulated in the prefrontal cortex[95-97]. Additionally, downregulation of miR-15b-5p, miR-29a-3p and miR-221-3p was reported in plasma with upregulation reported in the putamen, anterior cingulate gyrus and prefrontal cortex[95,98-100]Interestingly, the downregulation of miR-19b in serum samples of patients with RBD might predict the conversion into PD within a 4-year period of follow-up after RBD diagnosis[101]. Unfortunately, further comparable longitudinal studies validating these results are still missing.

Another emerging area of interest in the field of miRNA biomarkers for PD is the potential use of

salivary miRNAs for disease diagnosis and monitoring. Few studies have reported altered expression patterns of specific miRNAs in the saliva of PD patients[102,103]. For instance, a study by Cressatti et al. identified significantly dysregulated salivary miRNAs, including miR-153, miR-223, and miR-1, in PD patients compared to healthy controls[104]. These findings suggest that salivary miRNAs hold promise as non-invasive biomarkers for PD and merit further research to elucidate their clinical potential.

Circular RNAs (circRNAs) are an emerging class of endogenous RNAs abundantly expressed in eukaryotics[105]. Amongst peripheral cell types, Peripheral Blood Mononuclear Cells (PBMCs) have the greatest potential to reflect brain pathology, as these cells share a significant amount of their transcriptome with cells in the CNS.In a recent study by our group, six circRNAs with high brain expression were significantly downregulated in PBMCs from idiopathic PD patients compared with healthy controls. Using a receiver operating characteristic curve analysis, we determined the utility of peripheral blood mononuclear cell circRNA levels for differentiating subjects with idiopathic PD from healthy control subjects. The diagnostic sensitivity and specificity of a four-circRNA panel (SLAIN1_circ_0000497, SLAIN2_circ_0126525, ANKRD12_circ_0000826, and PSEN1_circ_0003848) were 75.3 and 78%, respectively, and the area under the curve was 0.84. These findings indicate that the four-circRNA panel had acceptable sensitivity and specificity for idiopathic PD [106].-

Another recent study discovered elevated levels of circ_0017204, circ_0085869, circ_0004381, and circ_0090668 in plasma samples taken from people with PD. Correlation analysis revealed that the circ_0017204 and circ_0004381 panels may be able to accurately differentiate individuals with early-stage PD from healthy controls, whereas the circ_0085869, circ_0004381, circ_0017204, and circ_0090668 panels may be able to differentiate the late stages of PD from the early stages and thereby serve as a dynamic monitoring factor for PD progression [107].

Xiao et al. [108] used microarray analysis to investigate the global expression levels of circRNAs in total blood mRNA from PD patients and controls and then verified the candidate circRNAs in another PD cohort. Compared with controls, hsa_circRNA_101275, hsa_circRNA_103730, and hsa_circRNA_038416 had significantly higher expression in PD patients, and hsa_circRNA_102850 had lower expression in PD patients. A circRNA panel containing the four differentially expressed circRNAs had a strong diagnostic capacity (area under the curve = 0.938) for distinguishing PD patients from controls.

Compared to protein biomarkers as we described above, microRNAs and circRNAs have the advantage of being stable, tissue-specific molecules that can be easily and accurately measured by routine laboratory protocols (e.g. RT-qPCR).Further investigation is needed to validate their diagnostic utility. The aforementioned studies provide promise for the development of panels of high diagnostic accuracy, but also for the understanding of brain pathological processes related to PD.

7. LIMITATIONS, CHALLENGES AND DIRECTIONS FOR FUTURE RESEARCH

I. Prospective longitudinal studies assessing multiple inflammatory markers are sparse, specifically for CSF for patient stratification in future PD drug trials.
II. In order to enrich cohorts for maximized therapeutic effects in clinical trials, knowledge of the predictive/prognostic value of metabolomic profiles in relation to clinical trajectories is crucial.
III. the methods of RNA and exosome isolation, and downstream miRNA detection, quantification and normalization methods varied between studies such as enzyme-linked immunosorbent assays (ELISA), Western blotting, and mass spectrometry. S, leading to conflicting results.
IV. There is a paucity of comprehensive biofluids analyses assessing CSF levels of multiple inflammatory markers along with CSF levels of neurodegenerative/PD-specific biomarkers such as Amyloid-β_{1-42} ($A\beta_{1-42}$), total-Tau (t-Tau), phospho-Tau (p181-Tau), NFL, and α-syn.
V. Human studies in genetic forms of PD or prodromal PD are in their infancy, without longitudinal reports so far.
VI. CSF α-Syn SAAs need to be standardized, validated and developed quantitatively, so that they can possibly be used for assessment of disease progression and response to disease-modifying therapies, while peripheral α-Syn SAAs also need to be further developed and validated.

8. CONCLUSIONS

The identification and validation of biofluid biomarkers for PD represents a critical frontier in PD research and clinical practice. These biomarkers offer the prospect of a non-invasive and accessible means of diagnosing PD in its early stages, predicting disease progression, and monitoring treatment responses. While significant progress has been made in identifying potential biomarkers, rigorous validation and standardization efforts are essential to translate these findings into robust and clinically relevant tools. The integration of biofluid biomarkers into multimodal diagnostic algorithms, as well as the development of advanced technologies (e.g. aSyn-SAAs) for biomarker detection, are crucial steps in harnessing

the full potential of biomarker-based approaches in PD. Ultimately, the successful implementation of biofluid biomarkers in the clinical care of individuals with PD has the potential to transform disease management, improve patient outcomes, and accelerate the development of disease-modifying therapies.

REFERENCES

[1] Dorsey, E.R.; Constantinescu, R.; Thompson, J.P.; Biglan, K.M.; Holloway, R.G.; Kieburtz, K.; Marshall, F.J.; Ravina, B.M.; Schifitto, G.; Siderowf, A.; et al. Projected number of people with Parkinson disease in the most populous nations, 2005 through 2030. *Neurology* **2007**, *68*, 384-386, doi:10.1212/01.wnl.0000247740.47667.03.

[2] Cardoso, F.; Goetz, C.G.; Mestre, T.A.; Sampaio Rocha-Filho, P.A.; Adler, C.H.; Berg, D.; Bloem, B.R.; Burn, D.J.; Fitts, M.S.; Gasser, T.; et al. A Statement of the MDS on Biological Definition, Staging, and Classification of Parkinson's Disease. *Movement disorders : official journal of the Movement Disorder Society* **2023**, doi:10.1002/mds.29683.

[3] Hughes, A.J.; Daniel, S.E.; Kilford, L.; Lees, A.J. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. *Journal of neurology, neurosurgery, and psychiatry* **1992**, *55*, 181-184.

[4] Jellinger, K.A. How valid is the clinical diagnosis of Parkinson's disease in the community? *Journal of neurology, neurosurgery, and psychiatry* **2003**, *74*, 1005-1006, doi:10.1136/jnnp.74.7.1005.

[5] Biomarkers. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clinical pharmacology and therapeutics* **2001**, *69*, 89-95, doi:10.1067/mcp.2001.113989.

[6] Berg, D.; Postuma, R.B.; Bloem, B.; Chan, P.; Dubois, B.; Gasser, T.; Goetz, C.G.; Halliday, G.M.; Hardy, J.; Lang, A.E.; et al. Time to redefine PD? Introductory statement of the MDS Task Force on the definition of Parkinson's disease. *Movement disorders : official journal of the Movement Disorder Society* **2014**, *29*, 454-462, doi:10.1002/mds.25844.

[7] Bougea, A. New markers in Parkinson's disease. *Advances in clinical chemistry* **2020**, *96*, 137-178, doi:10.1016/bs.acc.2019.12.001.

[8] Spillantini, M.G.; Schmidt, M.L.; Lee, V.M.; Trojanowski, J.Q.; Jakes, R.; Goedert, M. Alpha-synuclein in Lewy bodies. *Nature* **1997**, *388*, 839-840, doi:10.1038/42166.

[9] Polymeropoulos, M.H.; Lavedan, C.; Leroy, E.; Ide, S.E.; Dehejia, A.; Dutra, A.; Pike, B.; Root, H.; Rubenstein, J.; Boyer, R.; et al. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science (New York, N.Y.)* **1997**, *276*, 2045-2047.

[10] Bendor, J.T.; Logan, T.P.; Edwards, R.H. The function of α-synuclein. *Neuron* **2013**, *79*, 1044-1066, doi:10.1016/j.neuron.2013.09.004.

[11] Spillantini, M.G.; Crowther, R.A.; Jakes, R.; Hasegawa, M.; Goedert, M. alpha-Synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with lewy bodies. *Proceedings of the National Academy of Sciences of the United States of America* **1998**, *95*, 6469-6473.

[12] Bengoa-Vergniory, N.; Roberts, R.F.; Wade-Martins, R.; Alegre-Abarrategui, J. Alpha-synuclein oligomers: a new hope. **2017**, *134*, 819-838, doi:10.1007/s00401-017-1755-1.

[13] Giasson, B.I.; Duda, J.E.; Quinn, S.M.; Zhang, B.; Trojanowski, J.Q.; Lee, V.M. Neuronal alpha-synucleinopathy with severe movement disorder in mice expressing A53T human alpha-synuclein. *Neuron* **2002**, *34*, 521-533.

[14] Simonsen, A.H.; Kuiperij, B.; El-Agnaf, O.M.; Engelborghs, S.; Herukka, S.K.; Parnetti, L.; Rektorova, I.; Vanmechelen, E.; Kapaki, E.; Verbeek, M.; et al. The utility of alpha-synuclein as biofluid marker in neurodegenerative diseases: a systematic review of the literature. *Biomarkers in medicine* **2016**, *10*, 19-34, doi:10.2217/bmm.14.105.

[15] Mollenhauer, B.; Cullen, V.; Kahn, I.; Krastins, B.; Outeiro, T.F.; Pepivani, I.; Ng, J.; Schulz-Schaeffer, W.; Kretzschmar, H.A.; McLean, P.J.; et al. Direct quantification of CSF alpha-synuclein by ELISA and first cross-sectional study in patients with neurodegeneration. *Experimental neurology* **2008**, *213*, 315-325, doi:10.1016/j.expneurol.2008.06.004.

[16] Gao, L.; Tang, H.; Nie, K.; Wang, L.; Zhao, J.; Gan, R.; Huang, J.; Zhu, R.; Feng, S.; Duan, Z.; et al. Cerebrospinal fluid alpha-synuclein as a biomarker for Parkinson's disease diagnosis: a systematic review and meta-analysis. *The International journal of neuroscience* **2015**, *125*, 645-654, doi:10.3109/00207454.2014.961454.

[17] Eusebi, P.; Giannandrea, D.; Biscetti, L.; Abraha, I.; Chiasserini, D.; Orso, M.; Calabresi, P.; Parnetti, L. Diagnostic utility of cerebrospinal fluid alpha-synuclein in Parkinson's disease: A systematic review and meta-analysis. *Movement disorders : official journal of the Movement Disorder Society* **2017**, *32*, 1389-1400, doi:10.1002/mds.27110.

[18] Zhou, B.; Wen, M.; Yu, W.F.; Zhang, C.L.; Jiao, L. The Diagnostic and Differential Diagnosis Utility of Cerebrospinal Fluid alpha -Synuclein

Levels in Parkinson's Disease: A Meta-Analysis. *Parkinson's disease* **2015**, *2015*, 567386, doi:10.1155/2015/567386.
[19] Hall, S.; Surova, Y.; Ohrfelt, A.; Zetterberg, H.; Lindqvist, D.; Hansson, O. CSF biomarkers and clinical progression of Parkinson disease. *Neurology* **2015**, *84*, 57-63, doi:10.1212/wnl.0000000000001098.
[20] Stewart, T.; Liu, C.; Ginghina, C.; Cain, K.C.; Auinger, P.; Cholerton, B.; Shi, M.; Zhang, J. Cerebrospinal fluid alpha-synuclein predicts cognitive decline in Parkinson disease progression in the DATATOP cohort. *The American journal of pathology* **2014**, *184*, 966-975, doi:10.1016/j.ajpath.2013.12.007.
[21] Forland, M.G.; Ohrfelt, A.; Dalen, I.; Tysnes, O.B.; Blennow, K.; Zetterberg, H.; Pedersen, K.F.; Alves, G.; Lange, J. Evolution of cerebrospinal fluid total alpha-synuclein in Parkinson's disease. *Parkinsonism & related disorders* **2018**, *49*, 4-8, doi:10.1016/j.parkreldis.2018.01.018.
[22] Mollenhauer, B.; Caspell-Garcia, C.J.; Coffey, C.S.; Taylor, P.; Singleton, A.; Shaw, L.M.; Trojanowski, J.Q.; Frasier, M.; Simuni, T.; Iranzo, A.; et al. Longitudinal analyses of cerebrospinal fluid alpha-Synuclein in prodromal and early Parkinson's disease. *Movement disorders : official journal of the Movement Disorder Society* **2019**, doi:10.1002/mds.27806.
[23] Majbour, N.K.; Vaikath, N.N.; Eusebi, P.; Chiasserini, D.; Ardah, M.; Varghese, S.; Haque, M.E.; Tokuda, T.; Auinger, P.; Calabresi, P.; et al. Longitudinal changes in CSF alpha-synuclein species reflect Parkinson's disease progression. *Movement disorders : official journal of the Movement Disorder Society* **2016**, *31*, 1535-1542, doi:10.1002/mds.26754.
[24] Majbour, N.K.; Abdi, I.Y.; Dakna, M.; Wicke, T.; Lang, E.; Ali Moussa, H.Y.; Thomas, M.A.; Trenkwalder, C.; Safieh-Garabedian, B.; Tokuda, T.; et al. Cerebrospinal α-Synuclein Oligomers Reflect Disease Motor Severity in DeNoPa Longitudinal Cohort. *Movement disorders : official journal of the Movement Disorder Society* **2021**, *36*, 2048-2056, doi:10.1002/mds.28611.
[25] Kasai, T.; Tokuda, T.; Ishii, R.; Ishigami, N.; Tsuboi, Y.; Nakagawa, M.; Mizuno, T.; El-Agnaf, O.M. Increased alpha-synuclein levels in the cerebrospinal fluid of patients with Creutzfeldt-Jakob disease. *Journal of neurology* **2014**, *261*, 1203-1209, doi:10.1007/s00415-014-7334-7.
[26] Blennow, K.; Biscetti, L.; Eusebi, P.; Parnetti, L. Cerebrospinal fluid biomarkers in Alzheimer's and Parkinson's diseases-From pathophysiology to clinical practice. *Movement disorders : official journal of the Movement Disorder Society* **2016**, *31*, 836-847, doi:10.1002/mds.26656.
[27] Mackin, R.S.; Insel, P.; Zhang, J.; Mohlenhoff, B.; Galasko, D.; Weiner, M.; Mattsson, N. Cerebrospinal fluid alpha-synuclein and Lewy body-like symptoms in normal controls, mild cognitive impairment, and Alzheimer's disease. *Journal of Alzheimer's disease : JAD* **2015**, *43*, 1007-1016, doi:10.3233/jad-141287.
[28] Bougea, A.; Stefanis, L.; Paraskevas, G.P.; Emmanouilidou, E.; Vekrelis, K.; Kapaki, E. Plasma alpha-synuclein levels in patients with Parkinson's disease: a systematic review and meta-analysis. *Neurological sciences : official journal of the Italian Neurological Society and of the Italian Society of Clinical Neurophysiology* **2019**, doi:10.1007/s10072-019-03738-1.
[29] Foulds, P.G.; Diggle, P.; Mitchell, J.D.; Parker, A.; Hasegawa, M.; Masuda-Suzukake, M.; Mann, D.M.; Allsop, D. A longitudinal study on alpha-synuclein in blood plasma as a biomarker for Parkinson's disease. *Scientific reports* **2013**, *3*, 2540, doi:10.1038/srep02540.
[30] El-Agnaf, O.M.; Salem, S.A.; Paleologou, K.E.; Curran, M.D.; Gibson, M.J.; Court, J.A.; Schlossmacher, M.G.; Allsop, D. Detection of oligomeric forms of alpha-synuclein protein in human plasma as a potential biomarker for Parkinson's disease. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **2006**, *20*, 419-425, doi:10.1096/fj.03-1449com.
[31] Mullin, S.; Beavan, M.; Bestwick, J.; McNeill, A.; Proukakis, C.; Cox, T.; Hughes, D.; Mehta, A.; Zetterberg, H.; Schapira, A.H.V. Evolution and clustering of prodromal parkinsonian features in GBA1 carriers. *Movement disorders : official journal of the Movement Disorder Society* **2019**, doi:10.1002/mds.27775.
[32] Papagiannakis, N.; Koros, C.; Stamelou, M.; Simitsi, A.M.; Maniati, M.; Antonelou, R.; Papadimitriou, D.; Dermentzaki, G.; Moraitou, M.; Michelakakis, H.; et al. Alpha-synuclein dimerization in erythrocytes of patients with genetic and non-genetic forms of Parkinson's Disease. *Neuroscience letters* **2018**, *672*, 145-149, doi:10.1016/j.neulet.2017.11.012.
[33] Ju, Y.; Bai, H.; Ren, L.; Zhang, L. The Role of Exosome and the ESCRT Pathway on Enveloped Virus Infection. *International journal of molecular sciences* **2021**, *22*, doi:10.3390/ijms22169060.
[34] Stuendl, A.; Kunadt, M.; Kruse, N.; Bartels, C.; Moebius, W.; Danzer, K.M.; Mollenhauer, B.; Schneider, A. Induction of α-synuclein aggregate formation by CSF exosomes from patients with Parkinson's disease and dementia with Lewy bodies. *Brain : a journal of neurology* **2016**, *139*, 481-494, doi:10.1093/brain/

awv346.
[35] Yan, Y.Q.; Pu, J.L.; Zheng, R.; Fang, Y.; Gu, L.Y.; Guo, T.; Si, X.L.; Zhou, C.; Chen, Y.; Liu, Y.; et al. Different patterns of exosomal α-synuclein between Parkinson's disease and probable rapid eye movement sleep behavior disorder. *European journal of neurology* **2022**, *29*, 3590-3599, doi:10.1111/ene.15537.
[36] Niu, M.; Li, Y.; Li, G.; Zhou, L.; Luo, N.; Yao, M.; Kang, W.; Liu, J. A longitudinal study on α-synuclein in plasma neuronal exosomes as a biomarker for Parkinson's disease development and progression. *European journal of neurology* **2020**, *27*, 967-974, doi:10.1111/ene.14208.
[37] Shi, M.; Liu, C.; Cook, T.J.; Bullock, K.M.; Zhao, Y.; Ginghina, C.; Li, Y.; Aro, P.; Dator, R.; He, C.; et al. Plasma exosomal α-synuclein is likely CNS-derived and increased in Parkinson's disease. *Acta neuropathologica* **2014**, *128*, 639-650, doi:10.1007/s00401-014-1314-y.
[38] Zhao, Z.H.; Chen, Z.T.; Zhou, R.L.; Zhang, X.; Ye, Q.Y.; Wang, Y.Z. Increased DJ-1 and α-Synuclein in Plasma Neural-Derived Exosomes as Potential Markers for Parkinson's Disease. *Frontiers in aging neuroscience* **2018**, *10*, 438, doi:10.3389/fnagi.2018.00438.
[39] Jiang, C.; Hopfner, F.; Katsikoudi, A.; Hein, R.; Catli, C.; Evetts, S.; Huang, Y.; Wang, H.; Ryder, J.W.; Kuhlenbaeumer, G.; et al. Serum neuronal exosomes predict and differentiate Parkinson's disease from atypical parkinsonism. *Journal of neurology, neurosurgery, and psychiatry* **2020**, *91*, 720-729, doi:10.1136/jnnp-2019-322588.
[40] Si, X.; Tian, J.; Chen, Y.; Yan, Y.; Pu, J.; Zhang, B. Central Nervous System-Derived Exosomal Alpha-Synuclein in Serum May Be a Biomarker in Parkinson's Disease. *Neuroscience* **2019**, *413*, 308-316, doi:10.1016/j.neuroscience.2019.05.015.
[41] Kang, W.; Chen, W.; Yang, Q.; Zhang, L.; Zhang, L.; Wang, X.; Dong, F.; Zhao, Y.; Chen, S.; Quinn, T.J.; et al. Salivary total alpha-synuclein, oligomeric alpha-synuclein and SNCA variants in Parkinson's disease patients. *Scientific reports* **2016**, *6*, 28143, doi:10.1038/srep28143.
[42] Goldman, J.G.; Andrews, H.; Amara, A.; Naito, A.; Alcalay, R.N.; Shaw, L.M.; Taylor, P.; Xie, T.; Tuite, P.; Henchcliffe, C.; et al. Cerebrospinal fluid, plasma, and saliva in the BioFIND study: Relationships among biomarkers and Parkinson's disease Features. *Movement disorders : official journal of the Movement Disorder Society* **2018**, *33*, 282-288, doi:10.1002/mds.27232.
[43] Devic, I.; Hwang, H.; Edgar, J.S.; Izutsu, K.; Presland, R.; Pan, C.; Goodlett, D.R.; Wang, Y.; Armaly, J.; Tumas, V.; et al. Salivary alpha-synuclein and DJ-1: potential biomarkers for Parkinson's disease. *Brain : a journal of neurology* **2011**, *134*, e178, doi:10.1093/brain/awr015.
[44] Bougea, A.; Koros, C.; Stefanis, L. Salivary alpha-synuclein as a biomarker for Parkinson's disease: a systematic review. *Journal of neural transmission (Vienna, Austria : 1996)* **2019**, doi:10.1007/s00702-019-02062-4.
[45] Paciotti, S.; Bellomo, G.; Gatticchi, L.; Parnetti, L. Are We Ready for Detecting α-Synuclein Prone to Aggregation in Patients? The Case of "Protein-Misfolding Cyclic Amplification" and "Real-Time Quaking-Induced Conversion" as Diagnostic Tools. *Frontiers in neurology* **2018**, *9*, 415, doi:10.3389/fneur.2018.00415.
[46] Siderowf, A.; Concha-Marambio, L.; Lafontant, D.E.; Farris, C.M.; Ma, Y.; Urenia, P.A.; Nguyen, H.; Alcalay, R.N.; Chahine, L.M.; Foroud, T.; et al. Assessment of heterogeneity among participants in the Parkinson's Progression Markers Initiative cohort using α-synuclein seed amplification: a cross-sectional study. *The Lancet. Neurology* **2023**, *22*, 407-417, doi:10.1016/s1474-4422(23)00109-6.
[47] Cazzaniga, F.A.; Bistaffa, E.; De Luca, C.M.G.; Bufano, G.; Indaco, A.; Giaccone, G.; Moda, F. Sporadic Creutzfeldt-Jakob disease: Real-Time Quaking Induced Conversion (RT-QuIC) assay represents a major diagnostic advance. *European journal of histochemistry : EJH* **2021**, *65*, doi:10.4081/ejh.2021.3298.
[48] Grossauer, A.; Hemicker, G.; Krismer, F.; Peball, M.; Djamshidian, A.; Poewe, W.; Seppi, K.; Heim, B. α-Synuclein Seed Amplification Assays in the Diagnosis of Synucleinopathies Using Cerebrospinal Fluid-A Systematic Review and Meta-Analysis. *Movement disorders clinical practice* **2023**, *10*, 737-747, doi:10.1002/mdc3.13710.
[49] Shahnawaz, M.; Tokuda, T.; Waragai, M.; Mendez, N.; Ishii, R.; Trenkwalder, C.; Mollenhauer, B.; Soto, C. Development of a Biochemical Diagnosis of Parkinson Disease by Detection of α-Synuclein Misfolded Aggregates in Cerebrospinal Fluid. *JAMA neurology* **2017**, *74*, 163-172, doi:10.1001/jamaneurol.2016.4547.
[50] Kang, U.J.; Boehme, A.K.; Fairfoul, G.; Shahnawaz, M.; Ma, T.C.; Hutten, S.J.; Green, A.; Soto, C. Comparative study of cerebrospinal fluid α-synuclein seeding aggregation assays for diagnosis of Parkinson's disease. *Movement disorders : official journal of the Movement Disorder Society* **2019**, *34*, 536-544, doi:10.1002/mds.27646.
[51] Concha-Marambio, L.; Weber, S.; Farris, C.M.; Dakna, M.; Lang, E.; Wicke, T.; Ma, Y.; Starke,

M.; Ebentheuer, J.; Sixel-Döring, F.; et al. Accurate Detection of α-Synuclein Seeds in Cerebrospinal Fluid from Isolated Rapid Eye Movement Sleep Behavior Disorder and Patients with Parkinson's Disease in the DeNovo Parkinson (DeNoPa) Cohort. *Movement disorders : official journal of the Movement Disorder Society* **2023**, *38*, 567-578, doi:10.1002/mds.29329.

[52] Garrido, A.; Fairfoul, G.; Tolosa, E.; Marti, M.J.; Ezquerra, M.; Green, A.J.E. Brain and Cerebrospinal Fluid α-Synuclein Real-Time Quaking-Induced Conversion Identifies Lewy Body Pathology in LRRK2-PD. *Movement disorders : official journal of the Movement Disorder Society* **2023**, *38*, 333-338, doi:10.1002/mds.29284.

[53] Brockmann, K.; Quadalti, C.; Lerche, S.; Rossi, M.; Wurster, I.; Baiardi, S.; Roeben, B.; Mammana, A.; Zimmermann, M.; Hauser, A.K.; et al. Association between CSF alpha-synuclein seeding activity and genetic status in Parkinson's disease and dementia with Lewy bodies. *Acta Neuropathol Commun* **2021**, *9*, 175, doi:10.1186/s40478-021-01276-6.

[54] Wang, Y.; Hu, J.; Chen, X.; Wang, S.; Zhang, C.; Hu, J.; Guo, D.; Liu, X. Real-time quaking-induced conversion assay is accurate for Lewy body diseases: a meta-analysis. *Neurological sciences : official journal of the Italian Neurological Society and of the Italian Society of Clinical Neurophysiology* **2022**, *43*, 4125-4132, doi:10.1007/s10072-022-06014-x.

[55] De Luca, C.M.G.; Elia, A.E.; Portaleone, S.M.; Cazzaniga, F.A.; Rossi, M.; Bistaffa, E.; De Cecco, E.; Narkiewicz, J.; Salzano, G.; Carletta, O.; et al. Efficient RT-QuIC seeding activity for α-synuclein in olfactory mucosa samples of patients with Parkinson's disease and multiple system atrophy. *Translational neurodegeneration* **2019**, *8*, 24, doi:10.1186/s40035-019-0164-x.

[56] Stefani, A.; Iranzo, A.; Holzknecht, E.; Perra, D.; Bongianni, M.; Gaig, C.; Heim, B.; Serradell, M.; Sacchetto, L.; Garrido, A.; et al. Alpha-synuclein seeds in olfactory mucosa of patients with isolated REM sleep behaviour disorder. *Brain : a journal of neurology* **2021**, *144*, 1118-1126, doi:10.1093/brain/awab005.

[57] Bargar, C.; De Luca, C.M.G.; Devigili, G.; Elia, A.E.; Cilia, R.; Portaleone, S.M.; Wang, W.; Tramacere, I.; Bistaffa, E.; Cazzaniga, F.A.; et al. Discrimination of MSA-P and MSA-C by RT-QuIC analysis of olfactory mucosa: the first assessment of assay reproducibility between two specialized laboratories. *Molecular neurodegeneration* **2021**, *16*, 82, doi:10.1186/s13024-021-00491-y.

[58] Manne, S.; Kondru, N.; Jin, H.; Anantharam, V.; Huang, X.; Kanthasamy, A.; Kanthasamy, A.G. α-Synuclein real-time quaking-induced conversion in the submandibular glands of Parkinson's disease patients. *Movement disorders : official journal of the Movement Disorder Society* **2020**, *35*, 268-278, doi:10.1002/mds.27907.

[59] Chahine, L.M.; Beach, T.G.; Adler, C.H.; Hepker, M.; Kanthasamy, A.; Appel, S.; Pritzkow, S.; Pinho, M.; Mosovsky, S.; Serrano, G.E.; et al. Central and peripheral α-synuclein in Parkinson disease detected by seed amplification assay. *Annals of clinical and translational neurology* **2023**, *10*, 696-705, doi:10.1002/acn3.51753.

[60] Koga, S.; Sekiya, H.; Kondru, N.; Ross, O.A.; Dickson, D.W. Neuropathology and molecular diagnosis of Synucleinopathies. *Molecular neurodegeneration* **2021**, *16*, 83, doi:10.1186/s13024-021-00501-z.

[61] Luan, M.; Sun, Y.; Chen, J.; Jiang, Y.; Li, F.; Wei, L.; Sun, W.; Ma, J.; Song, L.; Liu, J.; et al. Diagnostic Value of Salivary Real-Time Quaking-Induced Conversion in Parkinson's Disease and Multiple System Atrophy. *Movement disorders : official journal of the Movement Disorder Society* **2022**, *37*, 1059-1063, doi:10.1002/mds.28976.

[62] Wang, Z.; Becker, K.; Donadio, V.; Siedlak, S.; Yuan, J.; Rezaee, M.; Incensi, A.; Kuzkina, A.; Orrú, C.D.; Tatsuoka, C.; et al. Skin α-Synuclein Aggregation Seeding Activity as a Novel Biomarker for Parkinson Disease. *JAMA neurology* **2020**, *78*, 1-11, doi:10.1001/jamaneurol.2020.3311.

[63] Mammana, A.; Baiardi, S.; Quadalti, C.; Rossi, M.; Donadio, V.; Capellari, S.; Liguori, R.; Parchi, P. RT-QuIC Detection of Pathological α-Synuclein in Skin Punches of Patients with Lewy Body Disease. *Movement disorders : official journal of the Movement Disorder Society* **2021**, *36*, 2173-2177, doi:10.1002/mds.28651.

[64] Zheng, Y.; Li, S.; Yang, C.; Yu, Z.; Jiang, Y.; Feng, T. Comparison of biospecimens for α-synuclein seed amplification assays in Parkinson's disease: A systematic review and network meta-analysis. *European journal of neurology* **2023**, *30*, 3949-3967, doi:10.1111/ene.16041.

[65] Dubois, B.; Hampel, H.; Feldman, H.H.; Scheltens, P.; Aisen, P.; Andrieu, S.; Bakardjian, H.; Benali, H.; Bertram, L.; Blennow, K.; et al. Preclinical Alzheimer's disease: Definition, natural history, and diagnostic criteria. *Alzheimer's & dementia : the journal of the Alzheimer's Association* **2016**, *12*, 292-323, doi:10.1016/j.jalz.2016.02.002.

[66] Parnetti, L.; Farotti, L.; Eusebi, P.; Chiasserini, D.; De Carlo, C.; Giannandrea, D.; Salvadori, N.; Lisetti, V.; Tambasco, N.; Rossi, A.; et al. Differential role of CSF alpha-synuclein species, tau, and Abeta42 in Parkinson's Disease. *Frontiers*

in aging neuroscience **2014**, *6*, 53, doi:10.3389/fnagi.2014.00053.
[67] Siderowf, A.; Xie, S.X.; Hurtig, H.; Weintraub, D.; Duda, J.; Chen-Plotkin, A.; Shaw, L.M.; Van Deerlin, V.; Trojanowski, J.Q.; Clark, C. CSF amyloid {beta} 1-42 predicts cognitive decline in Parkinson disease. *Neurology* **2010**, *75*, 1055-1061, doi:10.1212/WNL.0b013e3181f39a78.
[68] Terrelonge, M., Jr.; Marder, K.S.; Weintraub, D.; Alcalay, R.N. CSF beta-Amyloid 1-42 Predicts Progression to Cognitive Impairment in Newly Diagnosed Parkinson Disease. *Journal of molecular neuroscience : MN* **2016**, *58*, 88-92, doi:10.1007/s12031-015-0647-x.
[69] Liu, C.; Cholerton, B.; Shi, M.; Ginghina, C.; Cain, K.C.; Auinger, P.; Zhang, J. CSF tau and tau/Abeta42 predict cognitive decline in Parkinson's disease. *Parkinsonism & related disorders* **2015**, *21*, 271-276, doi:10.1016/j.parkreldis.2014.12.027.
[70] Mollenhauer, B.; Caspell-Garcia, C.J.; Coffey, C.S.; Taylor, P.; Shaw, L.M.; Trojanowski, J.Q.; Singleton, A.; Frasier, M.; Marek, K.; Galasko, D. Longitudinal CSF biomarkers in patients with early Parkinson disease and healthy controls. *Neurology* **2017**, *89*, 1959-1969, doi:10.1212/wnl.0000000000004609.
[71] Alirezaei, Z.; Pourhanifeh, M.H.; Borran, S.; Nejati, M.; Mirzaei, H.; Hamblin, M.R. Neurofilament Light Chain as a Biomarker, and Correlation with Magnetic Resonance Imaging in Diagnosis of CNS-Related Disorders. *Molecular neurobiology* **2020**, *57*, 469-491, doi:10.1007/s12035-019-01698-3.
[72] Parnetti, L.; Gaetani, L.; Eusebi, P.; Paciotti, S.; Hansson, O.; El-Agnaf, O.; Mollenhauer, B.; Blennow, K.; Calabresi, P. CSF and blood biomarkers for Parkinson's disease. *The Lancet. Neurology* **2019**, *18*, 573-586, doi:10.1016/s1474-4422(19)30024-9.
[73] Angelopoulou, E.; Bougea, A.; Papadopoulos, A.; Papagiannakis, N.; Koros, C.; Simitsi, A.M.; Georgakis, M.K.; Stefanis, L. CSF and Circulating NfL as Biomarkers for the Discrimination of Parkinson Disease From Atypical Parkinsonian Syndromes: Meta-analysis. *Neurology. Clinical practice* **2021**, *11*, e867-e875, doi:10.1212/cpj.0000000000001116.
[74] Mollenhauer, B.; Dakna, M.; Kruse, N.; Galasko, D.; Foroud, T.; Zetterberg, H.; Schade, S.; Gera, R.G.; Wang, W.; Gao, F.; et al. Validation of Serum Neurofilament Light Chain as a Biomarker of Parkinson's Disease Progression. *Movement disorders : official journal of the Movement Disorder Society* **2020**, *35*, 1999-2008, doi:10.1002/mds.28206.
[75] Hansson, O.; Janelidze, S.; Hall, S.; Magdalinou, N.; Lees, A.J.; Andreasson, U.; Norgren, N.; Linder, J.; Forsgren, L.; Constantinescu, R.; et al. Blood-based NfL: A biomarker for differential diagnosis of parkinsonian disorder. *Neurology* **2017**, *88*, 930-937, doi:10.1212/wnl.0000000000003680.
[76] Urso, D.; Batzu, L.; Logroscino, G.; Ray Chaudhuri, K.; Pereira, J.B. Neurofilament light predicts worse nonmotor symptoms and depression in Parkinson's disease. *Neurobiology of disease* **2023**, *185*, 106237, doi:10.1016/j.nbd.2023.106237.
[77] Havelund, J.F.; Heegaard, N.H.H.; Faergeman, N.J.K.; Gramsbergen, J.B. Biomarker Research in Parkinson's Disease Using Metabolite Profiling. *Metabolites* **2017**, *7*, doi:10.3390/metabo7030042.
[78] LeWitt, P.A.; Li, J.; Lu, M.; Guo, L.; Auinger, P. Metabolomic biomarkers as strong correlates of Parkinson disease progression. *Neurology* **2017**, *88*, 862-869, doi:10.1212/wnl.0000000000003663.
[79] Koros, C.; Simitsi, A.M.; Papagiannakis, N.; Bougea, A.; Prentakis, A.; Papadimitriou, D.; Pachi, I.; Beratis, I.; Stanitsa, E.; Angelopoulou, E.; et al. Serum Uric Acid as a Putative Biomarker in Prodromal Parkinson's Disease: Longitudinal Data from the PPMI Study. *Journal of Parkinson's disease* **2023**, *13*, 811-818, doi:10.3233/jpd-230007.
[80] Pellecchia, M.T.; Savastano, R.; Moccia, M.; Picillo, M.; Siano, P.; Erro, R.; Vallelunga, A.; Amboni, M.; Vitale, C.; Santangelo, G.; et al. Lower serum uric acid is associated with mild cognitive impairment in early Parkinson's disease: a 4-year follow-up study. *Journal of neural transmission (Vienna, Austria : 1996)* **2016**, *123*, 1399-1402, doi:10.1007/s00702-016-1622-6.
[81] Parnetti, L.P., S.Eusebi P, Dardis A, Zampieri S, Chiasserini D, Tasegian A, Tambasco N, Bembi B, Calabresi P, Beccari T. Cerebrospinal fluid beta-glucocerebrosidase activity is reduced in parkinson's disease patients. *Movement disorders : official journal of the Movement Disorder Society* **2017**, *32*, 1423-1431, doi:10.1002/mds.27136.
[82] Papagiannakis, N.; Xilouri, M.; Koros, C.; Stamelou, M.; Antonelou, R.; Maniati, M.; Papadimitriou, D.; Moraitou, M.; Michelakakis, H.; Stefanis, L. Lysosomal alterations in peripheral blood mononuclear cells of Parkinson's disease patients. *Movement disorders : official journal of the Movement Disorder Society* **2015**, *30*, 1830-1834, doi:10.1002/mds.26433.
[83] Hall, S.; Surova, Y.; Ohrfelt, A.; Blennow, K.; Zetterberg, H.; Hansson, O. Longitudinal Measurements of Cerebrospinal Fluid Biomarkers

in Parkinson's Disease. *Movement disorders : official journal of the Movement Disorder Society* **2016**, *31*, 898-905, doi:10.1002/mds.26578.
[84] Wijeyekoon, R.S.; Moore, S.F.; Farrell, K.; Breen, D.P.; Barker, R.A.; Williams-Gray, C.H. Cerebrospinal Fluid Cytokines and Neurodegeneration-Associated Proteins in Parkinson's Disease. *Movement disorders : official journal of the Movement Disorder Society* **2020**, *35*, 1062-1066, doi:10.1002/mds.28015.
[85] Brockmann, K.; Schulte, C.; Schneiderhan-Marra, N.; Apel, A.; Pont-Sunyer, C.; Vilas, D.; Ruiz-Martinez, J.; Langkamp, M.; Corvol, J.C.; Cormier, F.; et al. Inflammatory profile discriminates clinical subtypes in LRRK2-associated Parkinson's disease. *European journal of neurology* **2017**, *24*, 427-e426, doi:10.1111/ene.13223.
[86] Lerche, S.; Zimmermann, M.; Roeben, B.; Wurster, I.; Fries, F.L.; Deuschle, C.; Waniek, K.; Lachmann, I.; Jakobi, M.; Joos, T.O.; et al. Inflammatory CSF profiles and longitudinal development of cognitive decline in sporadic and GBA-associated PD. *NPJ Parkinson's disease* **2023**, *9*, 38, doi:10.1038/s41531-023-00476-2.
[87] Ahmadi Rastegar, D.; Ho, N.; Halliday, G.M.; Dzamko, N. Parkinson's progression prediction using machine learning and serum cytokines. *NPJ Parkinsons Dis.* **2019**, *5*, 14, doi:10.1038/s41531-019-0086-4.
[88] Guévremont, D.; Roy, J.; Cutfield, N.J.; Williams, J.M. MicroRNAs in Parkinson's disease: a systematic review and diagnostic accuracy meta-analysis. *Scientific reports* **2023**, *13*, 16272, doi:10.1038/s41598-023-43096-9.
[89] Marques, T.M.; Kuiperij, H.B.; Bruinsma, I.B.; van Rumund, A.; Aerts, M.B.; Esselink, R.A.J.; Bloem, B.R.; Verbeek, M.M. MicroRNAs in Cerebrospinal Fluid as Potential Biomarkers for Parkinson's Disease and Multiple System Atrophy. *Molecular neurobiology* **2017**, *54*, 7736-7745, doi:10.1007/s12035-016-0253-0.
[90] Xing, R.X.; Li, L.G.; Liu, X.W.; Tian, B.X.; Cheng, Y. Down regulation of miR-218, miR-124, and miR-144 relates to Parkinson's disease via activating NF-κB signaling. *The Kaohsiung journal of medical sciences* **2020**, *36*, 786-792, doi:10.1002/kjm2.12241.
[91] Starhof, C.; Hejl, A.M.; Heegaard, N.H.H.; Carlsen, A.L.; Burton, M.; Lilje, B.; Winge, K. The biomarker potential of cell-free microRNA from cerebrospinal fluid in Parkinsonian Syndromes. *Movement disorders : official journal of the Movement Disorder Society* **2019**, *34*, 246-254, doi:10.1002/mds.27542.
[92] He, S.; Huang, L.; Shao, C.; Nie, T.; Xia, L.; Cui, B.; Lu, F.; Zhu, L.; Chen, B.; Yang, Q. Several miRNAs derived from serum extracellular vesicles are potential biomarkers for early diagnosis and progression of Parkinson's disease. *Translational neurodegeneration* **2021**, *10*, 25, doi:10.1186/s40035-021-00249-y.
[93] Xie, S.; Niu, W.; Xu, F.; Wang, Y.; Hu, S.; Niu, C. Differential expression and significance of miRNAs in plasma extracellular vesicles of patients with Parkinson's disease. *The International journal of neuroscience* **2020**, 1-16, doi:10.1080/00207454.2020.1835899.
[94] Dong, H.; Wang, C.; Lu, S.; Yu, C.; Huang, L.; Feng, W.; Xu, H.; Chen, X.; Zen, K.; Yan, Q.; et al. A panel of four decreased serum microRNAs as a novel biomarker for early Parkinson's disease. *Biomarkers : biochemical indicators of exposure, response, and susceptibility to chemicals* **2016**, *21*, 129-137, doi:10.3109/1354750x.2015.1118544.
[95] Ding, H.; Huang, Z.; Chen, M.; Wang, C.; Chen, X.; Chen, J.; Zhang, J. Identification of a panel of five serum miRNAs as a biomarker for Parkinson's disease. *Parkinsonism & related disorders* **2016**, *22*, 68-73, doi:10.1016/j.parkreldis.2015.11.014.
[96] Hoss, A.G.; Labadorf, A.; Beach, T.G.; Latourelle, J.C.; Myers, R.H. microRNA Profiles in Parkinson's Disease Prefrontal Cortex. *Frontiers in aging neuroscience* **2016**, *8*, 36, doi:10.3389/fnagi.2016.00036.
[97] Ravanidis, S.; Bougea, A.; Papagiannakis, N.; Maniati, M.; Koros, C.; Simitsi, A.M.; Bozi, M.; Pachi, I.; Stamelou, M.; Paraskevas, G.P.; et al. Circulating Brain-enriched MicroRNAs for detection and discrimination of idiopathic and genetic Parkinson's disease. *Movement disorders : official journal of the Movement Disorder Society* **2020**, *35*, 457-467, doi:10.1002/mds.27928.
[98] Tatura, R.; Kraus, T.; Giese, A.; Arzberger, T.; Buchholz, M.; Höglinger, G.; Müller, U. Parkinson's disease: SNCA-, PARK2-, and LRRK2- targeting microRNAs elevated in cingulate gyrus. *Parkinsonism & related disorders* **2016**, *33*, 115-121, doi:10.1016/j.parkreldis.2016.09.028.
[99] Ma, W.; Li, Y.; Wang, C.; Xu, F.; Wang, M.; Liu, Y. Serum miR-221 serves as a biomarker for Parkinson's disease. *Cell biochemistry and function* **2016**, *34*, 511-515, doi:10.1002/cbf.3224.
[100] Bai, X.; Tang, Y.; Yu, M.; Wu, L.; Liu, F.; Ni, J.; Wang, Z.; Wang, J.; Fei, J.; Wang, W.; et al. Downregulation of blood serum microRNA 29 family in patients with Parkinson's disease. *Scientific reports* **2017**, *7*, 5411, doi:10.1038/s41598-017-03887-3.
[101] Soto, M.; Iranzo, A.; Lahoz, S.; Fernúndez, M.; Serradell, M.; Gaig, C.; Melún, P.; Martú M.J.; Santamarúa, J.; Camps, J.; et al. Serum

MicroRNAs Predict Isolated Rapid Eye Movement Sleep Behavior Disorder and Lewy Body Diseases. *Movement disorders : official journal of the Movement Disorder Society* **2022**, *37*, 2086-2098, doi:10.1002/mds.29171.

[102] Jiang, Y.; Chen, J.; Sun, Y.; Li, F.; Wei, L.; Sun, W.; Deng, J.; Yuan, Y.; Wang, Z. Profiling of Differentially Expressed MicroRNAs in Saliva of Parkinson's Disease Patients. *Frontiers in neurology* **2021**, *12*, 738530, doi:10.3389/fneur.2021.738530.

[103] Chen, Y.; Zheng, J.; Su, L.; Chen, F.; Zhu, R.; Chen, X.; Ye, Q. Increased Salivary microRNAs That Regulate DJ-1 Gene Expression as Potential Markers for Parkinson's Disease. *Frontiers in aging neuroscience* **2020**, *12*, 210, doi:10.3389/fnagi.2020.00210.

[104] Cressatti, M.; Juwara, L.; Galindez, J.M.; Velly, A.M.; Nkurunziza, E.S.; Marier, S.; Canie, O.; Gornistky, M.; Schipper, H.M. Salivary microR-153 and microR-223 Levels as Potential Diagnostic Biomarkers of Idiopathic Parkinson's Disease. *Movement disorders : official journal of the Movement Disorder Society* **2020**, *35*, 468-477, doi:10.1002/mds.27935.

[105] Lu, S.; Yang, X.; Wang, C.; Chen, S.; Lu, S.; Yan, W.; Xiong, K.; Liu, F.; Yan, J. Current status and potential role of circular RNAs in neurological disorders. *Journal of neurochemistry* **2019**, *150*, 237-248, doi:10.1111/jnc.14724.

[106] Ravanidis, S.; Bougea, A.; Karampatsi, D.; Papagiannakis, N.; Maniati, M.; Stefanis, L.; Doxakis, E. Differentially Expressed Circular RNAs in Peripheral Blood Mononuclear Cells of Patients with Parkinson's Disease. *Movement disorders : official journal of the Movement Disorder Society* **2021**, *36*, 1170-1179, doi:10.1002/mds.28467.

[107] Zhong, L.; Ju, K.; Chen, A.; Cao, H. Circulating CircRNAs Panel Acts as a Biomarker for the Early Diagnosis and Severity of Parkinson's Disease. *Frontiers in aging neuroscience* **2021**, *13*, 684289, doi:10.3389/fnagi.2021.684289.

[108] Xiao, Y.; Chen, H.; Liao, J.; Zhang, Q.; He, H.; Lei, J.; Huang, J.; Ouyang, Q.; Shen, Y.; Wang, J. The Potential Circular RNAs Biomarker Panel and Regulatory Networks of Parkinson's Disease. *Frontiers in neuroscience* **2022**, *16*, 893713, doi:10.3389/fnins.2022.893713.

TISSUE BIOMARKERS IN PARKINSON'S DISEASE AND ATYPICAL PARKINSONISM.

Athanasia Alexoudi
1st Department of Neurosurgery, National & Kapodistrian University of Athens

Abstract
Phosphorylated a-synuclein (phosaSYN), the pathological signature of Parkinson's disease (PD), is not confined to the central nervous system, but have also been reported in peripheral tissues. However, the usefulness of aSYN/phosaSYN detection in tissues accessible to biopsies as a reliable biomarker for prodromal PD remains unclear. A systematic review of studies using biopsies of skin, olfactory and gastrointestinal (GI) tissues was conducted to evaluate the sensitivity and specificity of both aSYN and phosaSYN staining in PD and related disorders. In total 128 post-mortem and in vivo studies were reviewed. Tissue was obtained from GI tract/salivary glands, skin and olfactory mucosa/bulb. We concluded that skin biopsy is an easy, minimum invasive approach which provides high specificity and good sensitivity for the detection and differential diagnosis of synucleinopathies. GI biopsies remain attractive in the detection of synucleinopathies. However, a standardized methodology is essential to increase their diagnostic value. The new promising assays could be incorporated in future cohorts, towards identifying the combinations and relative contributions of the sensitivity amongst peripheral tissues.

Key words: peripheral tissue biopsies, synucleuinopathies, gastrointestinal tract, skin, olfactory mucosa/bulb.

ΟΙ ΒΙΟΔΕΙΚΤΕΣ ΙΣΤΩΝ ΣΤΗ ΝΟΣΟ ΠΑΡΚΙΝΣΟΝ ΚΑΙ ΣΤΑ ΑΤΥΠΑ ΠΑΡΚΙΝΣΟΝΙΚΑ ΣΥΝΔΡΟΜΑ.

Αθανασία Αλεξούδη
Α΄ Νευροχειρουργική Κλινική ΕΚΠΑ

Περίληψη
Η εντόπιση της φωσφορυλιωμένης α-συνουκλεΐνης (phosaSYN), της παθογνωμονικής πρωτεΐνης στη νόσο Πάρκινσον (PD), δεν περιορίζεται στο κεντρικό νευρικό σύστημα, αλλά επεκτείνεται και σε περιφερικούς ιστούς. Ωστόσο, η χρησιμότητα της ανίχνευσης aSYN/phosaSYN σε ιστούς (προσβάσιμους με βιοψίες) ως αξιόπιστου βιοδείκτη για τον προσδιορισμό του πρόδρομου σταδίου PD παραμένει ασαφής. Διεξήχθη μια συστηματική ανασκόπηση μελετών στις οποίες πραγματοποιήθηκαν βιοψίες δέρματος, οσφρητικού και εντερικού (GI) ιστού, ώστε να αξιολογηθεί η ευαισθησία και η ειδικότητα τόσο της χρώσης aSYN όσο και της phosaSYN στην PD και στις συναφείς διαταραχές. Συνολικά επανεξετάστηκαν 128 νεκροτομικές και in vivo μελέτες. Ο ιστός ελήφθη από τον γαστρεντερικό σωλήνα και σιελογόνους αδένες, το δέρμα και τον οσφρητικό βλεννογόνο/βολβό. Καταλήξαμε στο συμπέρασμα ότι η βιοψία δέρματος είναι μια εύκολη, ελάχιστη επεμβατική προσέγγιση, που παρέχει υψηλή ειδικότητα και καλή ευαισθησία για την ανίχνευση και τη διαφορική διάγνωση των συνουκλεϊνοπαθειών. Οι βιοψίες του γαστρεντερικού συστήματος παραμένουν ελκυστικές στην ανίχνευση των συνουκλεϊνοπαθειών. Ωστόσο, η κοινή αποδοχή μιας τυποποιημένης μεθοδολογίας είναι απαραίτητη για την αύξηση της διαγνωστικής τους αξίας. Οι νέες πολλά υποσχόμενες τεχνικές θα μπορούσαν να ενσωματωθούν σε μελλοντικές μελέτες. Ο προσδιορισμός ενός συνδυασμού διαφορετικών τεχνικών διαφορετικής ευαισθησίας στους περιφερικούς ιστούς, αποτελεί αντικείμενο μελλοντικής έρευνας.

Λέξεις κλειδιά: βιοψίες περιφερικών ιστών, συνουκλεϊνοπάθειες, γαστρεντερική οδός, δέρμα, οσφρητικός βολβός/βλεννογόνος

Introduction

In this review, we will present available studies suggesting reliable biomarkers in peripheral tissues for PD diagnosis and progression. We will focus on Lewy bodies (LB) and Lewy neurites (LN) pathology in skin, olfactory and GI tissues. Further, we will scrutinize the existing literature for various limitations of different studies on the potential of candidate biomarkers.

Parkinson's disease (PD) is a complex and progressive neurodegenerative disease, the second most common neurodegenerative disease of the elderly

population. The PD prevalence is 0.5 to 1% in the age group of 65–69 years, and it gradually rises with the increasing age.[1] The pathological hallmark of PD is the intraneuronal accumulation of abnormal -lamentous inclusions containing phosphorylated a-synuclein (phosaSYN) as the major component of Lewy bodies (LB) and Lewy neurites (LN).[2, 3] Besides CNS, LB/LN have also been detected in peripheral tissues, mainly in the autonomic nervous system. These findings allow in vivo minimally invasive procedures for PD based on peripheral tissue biopsies, towards providing a window to early potentially preclinical diagnosis of PD, differential diagnosis among parkinsonian syndromes and thus putative neuroprotective therapies during their prodromal phase.

Methods

We searched PubMed databases for publications published until August 2023 using the search terms Parkinson's disease (PD), Dementia with Lewy bodies (DLB), Multiple System Atrophy (MSA), Pure Autonomic Failure (PAF), isolated Rapid eye movement Behavior Disorder (iRBD), aSYN and phosaSYN pathology, peripheral biomarkers, biopsy, skin, olfactory system, salivary glands, gastric, enteric, esophagus, stomach, small intestine, colon, rectum. Both postmortem and in vivo studies were included. Studies which used techniques regarded as safe with an acceptable risk (i.e. absence of major adverse events), were selected. Only studies in English were considered. Case reports were also excluded.

Studies performed on bronchial/lung or pericardiac tissue were not included. The total number of cases analyzed, and the derived data are presented comprehensively in tables separately for each peripheral tissue system.

Results

Of 128 studies identified 34 were post-mortem, 89 in vivo investigations and 5 including both alive patients and cadavers. Whilst most of the studies included immunohistochemical detection of either aSYN or phosaSYN, a number of them were processed by other techniques (e.g. histological tinctorials, immunohisto- chemistry for nerve tissue epitopes/ neurotransmitters, transmission electron microscopy, seeding amplification assays).

Skin

55 studies on skin biopsies were identifed; 45 in vivo[4-48], 8 post-mortem[49-56] and 2 including both alive patients and cadavers [57, 58](table 1).

Detection of aSYN by western blot could not reveal any diferences between PD patients and controls aSYN.[4] It was confirmed by aSYN immunohistochemistry which showed immunoreactive signals both in PD patients and in controls, whilst specicity of phosaSYN was satisfactory, as there was absent staining in controls.[10] The same study demonstrated that PD patients with severe or longer disease duration or with autonomic dysfunction have a greater deposition. They also observed that the deposition was more in sympathetic adrenergic fibers than in cholinergic ones. Further studies confirmed that the deposition of phosaSYN was predominant in in the cutaneous autonomic nerve fibers.[7, 11, 12, 23, 25] Using the Proximity Ligation Assay (PLA) procedure Mazzetti et al., also detected the oligomeric form of a-synuclein in autonomic nerve terminals in skin biopsy for the first time.[53]

A number of studies evaluated the morphology and distributional pattern of different subtypes of cutaneous nerves (e.g. intraepidermal/dermal, sudomotor, pilomotor, vasomotor nerve bers).[5, 7-11, 15-17, 19, 21, 25, 26, 44, 52] Consistently, nerve ber density was decreased in PD patients suggesting that cutaneous nerve fiber loss may reflect both neuronal death and axonal degeneration, adjacent to neurodegenerative alterations observed in PD.[8, 14]. However, despite these structural deficits electrophysiological findings often appeared normal in these cases.[13] One of the afore mentioned studies was a 2- year longitudinal study, and estimated the progression of PD. The authors suggested the association of low intraepidermal nerve fibers density (IENFD) at baseline with an increased risk of developing a cognitive decline and motor impairment.[29, 41] Cervical cutaneous denervation has also been suggested as a potential biomarker of PD progression.[29]

Sleep disorders and dysautonomia are the most common non-motor features in synucleinopathies. 7 studies estimated Rapid eye movement Behavior Disorder (RBD)[19, 21, 22, 32, 34, 38, 42] and 5 pure autonomic failure (PAF) in skin respectively.[12, 16, 21, 35, 44] In the study of Doppler et al., patients with PD with or without RBD and individuals with isolated RBD (iRBD) were screened.[22] Dermal phosaSYN deposition was more frequently found (81.8% vs. 52.4%) in patients with PD and RBD compared to PD patients without RBD and was similar to patients with iRBD (79.1%). Two other studies which included iRBD population (without confirmed PD) showed that cutaneous phosaSYN aggregation was detected in most of them and was associated with greater autonomic dysfunction.[32, 34] Therefore, dermal phosaSYN can be considered a peripheral histopathological marker of synucleinopathy representing prodromal PD.

Table 1. Skin biopsy studies.

	Reference	PD (n)	DLB (n)	MSA (n)	PAF (n)	iRBD (n)	CTR (*n*)	Skin: lower limb	Skin: upper limb	Skin: trunk	Skin: finger	Scalp	Brain	Oth-er tis-sues	aSYNIR somata(LB) neurites (LN) %	phosaSYNIR somata(LB) neurites (LN) %	Other techniques: [1] H&E [2]Azan—Mallory [3]Bodian [4]Electron microscopy [5]VIP,TH (IHC) [6]Luxol fastblue [7]IHC [8]PET blot [9]PMCA [10]RT-QuIC [11]PLA	Comments
1	Ikemura et al. 2008[49]	*	*				194		•	•	•		•	•	n.d	PD& PDD; 40% DLB:70% CTR:0%	1,2	*Prospective study: 142 retro-spective cases, 279 prospective cases with LBD
2	Beach et al. 2010[50]	17	9				23			•			•	•	n.d	PD: 0% LBD: CTR: 0%		
3	Michell et al. 2005[4]	16					5			•						PD: 19%* CTR: 20%*		*Protein extraction from skin biopsy
4	Dabby et al. 2006[5]	17–21					15–19	•							n.d	n.d	3*	*Nerve ũbers of blood vessels, sweat glands, erector pili muscles PD: decreased nerve ũber density
5	Rossi et al. 2007[8]	n.s					n.s	*		*					n.d	n.d	3	PD: decreased nerve ũber density
6	Nolano. et al. 2008[9]	18					30	*		*	*				n.d	n.d	3, 4*†	*Epidermal/ intrapapillar nerve ũbers, Meissner corpuscles †Abnormal nerve sprouting/ altered Neurotransmitters PD: decreased nerve ũber density increased nerve regeneration
7	Miki et al. 2010[6]	20					-	•		•					n.d	PD: 0%–10%*	?	*10% in skin biopsy from chest wall 0% in skin biopsyfrom lower limb
8	Wang et al. 2013[10]	20					14	•							PD: 100%* CTR: 100%	n.d	2,3,4 *	* PD: decreased nerve fiber density *Increased aSYN decreased nerve deposition and ratio ũber density compared to CTR in Intraepidermal nerve ũbers

9	Doppler et al. 2014[7]	31				35	●	●				n.d	PD: 52% CTR: 0%	2,3,4*	*Intraepidermal nerve decreased nerve fibers (length-fiber density dependent), SP-intraepidermal nerve fibers non-length-dependent) PD: decreased nerve fiber density
10	Donadio et al. 2014[11]	21				30	●	●				n.d	PD:14%–100%* CTR: 0%	2,3,4*	*14%–24% in leg, 42%–52% thigh, 75%–100% in trunk autonomic and somatic nerves PD: decreased nerve fiber density
11	Rodríguez-Leyva et al. 2014[27]	34	12	5		20		●*				PD:57,9%–62,1%	n.d	7	16 with Tauopathies were included in the study. *Retro auricular area
12	Gelpi et al. 2014[51]	10*	5			None	●				●	PD:0% DLB: 0%	PD: 0% DLB:0%	7	*Four with PD, six with PDD †Varying presence of LB/LN due to rostro-caudal gradient
13	Doppler et al. 2015[18]	30		12		39	●	●				n.d	PD: 67% MSA:67% CTR: 0%	7	Tauopathies were included in the study
14	Zange et al. 2015[23]	10		10		6		●				n.d	PD: 17–75% MSA: 0% CTR: 0%	7	
15	Haga et al. 2015[24]	38		13			●					n.d	PD: 5,3% MSA: 0	7	sensory IENFD was reduced in patients with PD compared with those with MSA
16	Gibbons et al. 2016[25]	28		23			●	●				n.d	n.d	7*	*PD: reduction in the sudomotor nerve and pilomotor fiber density a-Synuclein is deposited prominently in sympathetic adrenergic nerve fibers innervating the arrector pili muscles, but is also present in sudomotor (sympathetic 1cholinergic) nerve fibers, but is not detected in sensory fibers.
17	Rodríguez-Leyva et al. 2016[47]	17				17		●		●*		n.d†	n.d		10 PSP were included in the study *Occipital PD: co-occurrence of both tau and a-syn †Higher αSN immunopositivity in PD

18	Donadio et al. 2016[12]	16			14		15	•		•					n.d	*PD:31%-100% PAF:31-52% CTR:0%	7	*PD: 100% cervical, 75% thigh, 31% leg Differences in the innervation pattern and spatial distribution of neuritic p-syn inclusions in IPD and PAF
19	Gibbons et al. 2017[52]	11					5			•		•			*PD: 100% CTR:100%	n.d	7	*greater deposition of alpha-synuclein within pilomotor, sudomotor and vasomotor nerve fibers of PD compared to CTR
20	Donadio et al. 2017[45]		18				25	•		•					n.d	LBD:71%-100% CTR:0%	7	23 patients with non-synucleinopathy dementia were included in the study *LBD: p-syn 100%, 86% thigh, 71-94% leg
21	Donadio et al. 2017[28]	28								•*					n.d	†PD:100%	7	*Paravertebral C7&Th12 † C7: 100%, Th12: 62% Unilateral PD: 20% p-syn deposits in affected &non-affected site, 60% in both sites
22	Doppler et al. 2017[19]	25				18	20	•		•					n.d	PD: 80% iRBD:55,6% CTR:0%	7	
23	Doppler et al. 2018[20]	10*						•		•					n.d	PD: 60%†	7	*10 PD patients with 3 different GBA1 mutations †P-syn deposition was mainly detected in autonomic nerve fibers, but also in somatosensory fibers
24	Melli et al. 2018[29]	19					17	•		•					*PD:81,3% APtau:0% APsyn:57,1% CTR:14%	†PD:56% APtau:0% APsyn:0% CTR:0%	7	13 patients with atypical parkinsonism(AP) (7 syn, 6 tau) were included. immunofluorescence for: *5G4 †p-aSyn,
25	Donadio et al. 2018[13]	15	12	12	5		10	•		•					n.d	PD: 100% LBD:100% MSA:67% PAF:100% CTR:0%	7	localization and load differences of aggregates among synucleinopathies.
26	Donadio et al. 2019[14]	21	1	4		11		•		•					n.d	n.s*	7	Intra-laboratory analysis showed an excellent reproducibility in 2cemters. Inter-laboratory analysis showed reproducibility (90%; K = 0.8; P < 0.001). Different classification was mainly due to fragmented skin samples or weak fluorescent signals.
27	Donadio et al. 2018[46]	28*						•		•					n.d	n.s†	7	*14PD with & 14PD without neurogenic OH. †PD + OH showed a higher p-syn deposition

28	Kuzkinaet al. 2019[30]	27*		8			21	●		●					n.d	PD: 82% MSA:75% CTR:0%	7†	*Early PD †Immunostaining of nerve fibers with different conformation specific antibodies and digestion with PK gave comparable results.
29	Carmona-Abellan et al. 2019[44]	3*	3*	-	2*		3*			●					n.d	†PD: n.s LBD:n.s PAF:n.s CTR:0%	7	*PD:1E46K-SNCA, 2PARK2 LBD:3E46K-SNCA PAF:2E46K-SNCA CTR:1E46K-SNCA asymptomatic carrier &2 healthy controls †E46K-SNCA carriers: moderate to severe p-synuclein deposits-correlated with sudomotor dysfunction
30	Donadio et al. 2020[15]	25*		25*				●		●					n.d	†PD: 100% MSA:72%	7	*All patients with OH. †MSA-P: p-syn deposits mainly found in somatic fibers of subepidermal plexi
31	Giannoccaro et al. 2020[35]	21	7	13	13			●		●					n.d	*PD: 95,2% LBD:100% MSA:69,2% PAF:100%	7	*p-α-syn deposits rarely affected the autonomic fibers in MSA
32	Liu et al. 2020[39]	90				30		●	●	●					n.d	PD: 83,3% CTR:0%	7	
33	Wang et al. 2020[57]	47	7	3			43	●		●					n.d	*PD:71,6% LBD:53,1% MSA:51% PSP:17,8% CBD:13,3% AD:23,2% CTR:12,4%	10	30 patients with PSP(8), CBD(5),AD(17)were also included.
																*PD:48,2% LBD:51,6% MSA:54,6% PSP:26,2% CBD:1,5% AD: n.d CTR:11,9%	9	
		20					21	●		●					n.d	*PD:52,2% CTR:24,7%	10	
																*PD: 56,6% CTR:8,3%	9	
34	Wang et al. 2020[40]	29					21	●							n.d	*PD:100% in 50 μm 90% in 20 μm 73% in 10 μm CTR:0%	7*	*50-μm-thick tissue sections performed better than 20 or 10 μm tissue sections.
35	Chahine et al. 2020[48]	59					21	●		●				●*	n.d	PD; 24,1% CTR:0%	7	*Submandibular glands, colon

36	Yang et al. 2021[42]	59*					30	•		•					n.d	PD:79.7% CTR:0%	7	*LRRK2 G2385R carriers:12 LRRK2 G2385R non-carriers:47 †Different p-syn distribution pattern LRRK2 G2385R carrier vs non-carriers. LRRK2 G2385R carrier:increased prevalence of autonomic symptoms or RBD
37	Mazzetti et al. 2020[53]	57 (38+19) *					48 (39+19) *	•					•		PD:82% CTR:14% PD twins: 89% CTR twins: 47%	n.d	7,11	*Including 19 monozygotic twins discordant for PD. a-synuclein oligomers within synaptic terminals of autonomic fibres
38	Manne et al.2020[54]	25					25					•			n.d	PD:96% CTR:4%	10 (frozen skin tissues)	96% sensitivity and 96% specificity
		12					12									PD:75% CTR:16,7%	10 (formalin-fixed paraffin-embedded skin sections)	75% sensitivity and 83% specificity
39	Tanei et al. 2021[56]							•	•				•	•*	n.d	†	[7]	*Submandibular glands, esophagus, adrenal gland LB pathology in 34%(178/518) of autopsird cases. † LB pathology in skin: 18%
40	Vacchi et al. 2021[41]	30		12			22	•		•					n.d	n.s	7,11	2 years longitudinal study 11 patients with tauopathies were also included. *Immunofluorescence for αSyn-PLA, P-αSyn, αSyn-5G4 PD and MSA showed a significant reduction of IENFD compared to CTR. A linear discrimination analysis model of αSyn-PLA, P-αSyn, αSyn-5G4, and IENFD, stratified patients with accuracy (77.8%). discrimination between PD and MSA (84.6%).

41	Kuzkina et al. 2021[31]	34					30	•		•					n.d	†PD: 82,4% CTR:10%	10	Higher α-synuclein seeding activity was shown in PD with longer disease duration and more advanced disease
42	Donadio et al. 2021[16]	6	4	6	3		24*								n.d	†PD: 100% LBD:100% MSA:67% PAF:100% CTR:0%	7	†p-syn deposits mainly found inautonomic fibers of PD, DLB, and PAF, but detected in somatic fibers of the upper dermis in MSA
																††PD: 100% LBD:75% MSA:60% PAF:67% CTR:12,5%	10	††The mean skin α-synuclein ThT fluorescence reaction from patients with synucleinopathies was significantly higher than that from patients with non-synucleinopathies. 23,6% of patients with non-synucleinopathies showing positive α-synuclein ThT fluorescence reaction.
																		* CTR: patients with peripheral neuropathies Additionally, 23 non-synucleinopathieswere included in the study
43	Doppler et al. 2021[21]	0*				33*		•		•					n.d	iRBD:60,6%	7	*2-4year clinical and skin biopsy follow-up data of 33 iRBD (baseline)
		4*	1*			28*									n.d	PD: 100% (2/2) LBD:100% (1/1) iRBD:72,7%		*Phenoconvertion in 5/33 patients (follow-up)
44	Isonaka et al. 2021.	*19 iPD+ 25genetic PD					16+ 5with mutations			•					†iPD:95% SNCA:100% PRKN biallelic:0% LRRK2: 100% GBA:83% LRRK2/ GBA:95%	n.d	7	*SNCA:3 PRKN biallelic:7 PRKN monoallelic:3 LRRK2: 7 GBA:7 PARK7/DJ1 biallelic:1 PARK7/DJ1 monoallelic:2 †SNCA, DJ-1, LRRK2, GBA mutations have substantial intra-neuronal α-syn deposition in sympathetic noradrenergic nerves. Biallelic PRKN PD may have mildly increased α-synuclein deposition compared to CTR.
45	Mammana et al. 2021[58]	n.s*	n.s*				40*	•		•					n.d	PD+LBD:100% incidental Lewy body:85,7% CTR:2,5%	10	*Total: 49 (PD+LBD:2 incidental Lewy body:7 neurological CTR:40)
		13	15				41	•		•					n.d	PD: 76,9% LBD:100% CTR:4,9%	10	

46	Migliset al. 2021[34]	28				25	18	•		•					n.d	PD; 96% iRBD:64% CTR:0%		7	
47	Bargar et al. 2021	2					1	•				•		•		PD; + (n.s) CTR:0%		10	
48	Giannoc-caro et al. 2022[43]	26					26	•		•						*PD; 100% CTR:0%		7	26 patients fulfilling clinical diagnostic criteria of tauopathies (PSP, CBS) were included. *p-syn deposits found in 2/26 (7,7%) patients with tauopathies.
49	Oizumi et al. 2022	10					4		•	•			•			PD; 100% n.s		1, 7	PD: p-aSyn deposits in in dermal macrophages in skin
50	Doppler et al. 2022[22]	43*				43										PD with REM:81,8% PD withou-tREM:52,4% iRBD:79,1%		7	*PD with REM: PD without REM:
51	Nolano et al. 2022[37]	57		43				•			•							7	Higher p-α-syn deposits in autonomic nerves differentiated PD from MSA-p. p-α-syn
52	Gibbons et al. 2023[26]	54		31			24	•		•						*PD: 94,4% MSA:100% CTR:0%		7	*MSA: p-syn deposits in the subepidermal plexus region
53	Iranzo et al. 2023[38]					91	41	•		•						iRBD:76,9% CTR:2,4%		10	
54	Donadio et al. 2023[17]	34	46	16			50	•		•					n.d	PD: 100% LBD:100% MSA:787% CTR:0%		7	p-syn in RSCs, 74% in MSA 0% in PD, 0% in DLB patients.
55	Kuzkina et al. 2023[32]	27		3		18	30	•		•				•*	n.d	Olfactory	Skin		3 patients fulfilling clinical diagnostic criteria of PSP were also included. *Olfactory epithelium
																PD:48% MSA:67% iRBD:67% CTR:10%	PD:78,9% MSA:100% iRBD:100% CTR:n.d	10	
																n.d	n.d	7	

Post-mortem studies with accompanying brain pathology are shown in dark grey shaded cells, in vivo studies are shown in light grey shaded cells. aSYN, alpha synuclein; iPD, idiopathic PD; CTR, control; DLB, dementia with Lewy bodies; MSA, Multiple System Atrophy; PAF, pure autonomic failure; iRBD, Isolated Rapid eye movement Behavior Disorder; PSP, progressive supranuclear palsy; CBD, Corticobasal Degeneration; AD, Alzheimer disease; GBA1, glucocerebrosidase gene; GI, gastrointestinal; H&E, hematoxylin—eosin staining; IHC, immunohistochemistry; IR, immunoreactive; PMCA, protein misfolding cyclic amplification; RT-QuIC assay, real-time quaking induced conversion; PLA, Proximity Ligation Assay; LB, Lewy bodies; LN, Lewy neurites; n/a, not applicable; n.d., not determined; n.s., not stated; RSCs, Remak non-myelinating Schwann cells; PD, Parkinson's disease; PDD, PD dementia; IENF, intraepidermalnerve fibers; OH, orthostatic hypotension; phosaSYN, phosphorylated alpha synuclein; TH, tyrosine hydroxylase; VIP, vasoactive intestinal polypeptide; asterisks and daggers refer to the same table row. The percentages correspond to the sensitivity (PD%). The speci[4] city equals 100% – CTR%.

Donadio et al. were the first to publish that there are differences in the innervation pattern and spatial distribution of neuritic phosaSYN inclusions in idiopathic PD and PAF.[7] They further proved that besides the different pattern distribution there is higher phosaSYN load in PD patients with orthostatic hypotension.[14] The same authors who added patients suffering from DLB and MSA in later studies, stated that the distribution of phosaSYN deposits was more homogenous for PD patients with orthostatic hypotension compared to those without.[13, 15] The localization and load differences of aggregates led them to speculate that specific diagnostic traits identify different pathogenesis among synucleinopathies.[24] More specifically, phosaSYN positivity differed among patients with synucleinopathies, being mainly detected in autonomic fibers of PD, DLB, and PAF, but detected in somatic fibers of the upper dermis with relatively preserved autonomic innervation in MSA.[16, 26, 35] DLB-PAF showed the highest load of deposits among synucleinopathies with a widespread involvement of autonomic annexes.[13, 45] In MSA there was noticed a distal-to-proximal gradient of αSyn aggregates.[13, 41] A reliable clinical biomarker for MSA came up recently, by the detection of phosaSYN in skin Remak non-myelinating Schwann cells (RSCs) as Schwann cell cytoplasmic inclusions (SCCi), resembling brain and suggesting that non-myelinated glial cells are also involved in the MSA pathogenesis.[17]

5 in vivo studies which included people with tauopathies were identified.[18, 27, 29, 41, 43, 47]

Rodriguez et al. using immunohistochemical technique and the antibodies against p-tau (PHF and AT8) and a-syn reported that PHF values were similar among the PD, PSP, and controls.[47] AT8 was significantly higher in both PSP and PD groups as compared to controls whereas, a-syn values were significantly higher in the PD group as compared with both control and PSP groups.In line with their previous work,they found the presence of both a-syn and p-tau in the skin of PD patients not only in the nervous tissue, but also in the keratinocytes of the epidermis.[27] In a recent study, the use of PLA revealed that αSyn oligomers (αSyn-PLA) were more expressed in PD and MSA patients compared to Tau ones and controls.[41] Another study analyzed skin biopsies of patients with PD and atypical parkinsonism (synucleinopathies and tauopathies) by immunofluorescence for p-aSyn, 5G4. PD and atypical parkinsonism -synucleinopathies shared the features of marked cervical denervation and the presence of 5G4. In contrast atypical parkinsonism-tauopathies were normal.[29] Similar results were described by two other studies which described abundant phosaSYN deposition in patients with PD and MSA but 0% and 7,7% in patients with tauopathies respectively.[18, 43]

We found 4 studies dealing with genetic factors in PD.[20, 33, 42, 44] Doppler et al. screened 10 PD patients with 3 different glucocerebrosidase gene (GBA1) mutations (six N370S, three E326K, and one L444P).[20] phosaSYN deposition was mainly detected in autonomic nerve fibers, but also in somatosensory fibers with N370S and E326K mutations. Nevertheless, seems to offer the distribution and the frequency was the same observed in patients without a known mutation. Contrariwise, Isonaka et al. found that 83% of patients with GBA variants had higher total a-syn deposition (phosaSYN was not detected) in the skin noradrenergic nerves compared to controls.[33] In the same study he investigated the deposition of a-syn in PD patients with pathogenic mutations in SNCA, PRKN, LRRK2, and DJ1, in PD patients without known mutations and healthy controls. According to the researchers, SNCA, DJ-1, LRRK2, and GBA mutations had substantial intra-neuronal α-syn deposition in sympathetic noradrenergic nerves, but this finding was not observed in biallelic PRKN mutations. However, biallelic PRKN PD had mildly increased α-synuclein deposition compared to controls. The same year Yang et al. performed skin biopsy in 59 PD patients (12 LRRK2 G2385R carriers and 47 LRRK G2385R noncarriers) and 30 healthy controls.[42] He reported that that the distribution of skin phosaSYN in PD LRRK2 G2385R carriers had an homogeneous pattern and this variant was linked with increased prevalence of autonomic symptoms or RBD. PARK2 and SNCA E46K mutations were studied by Carmona-Abellan et al in cohort including people with PD, DLB, PAF, asymptomatic carriers and healthy controls. The results of the skin biopsies revealed moderate to severe phosaSYN deposits in E46K-SNCA carriers which were correlated with sudomotor dysfunction.

Interestingly, Oizumi et al. performing an immunohistological analysis of skin biopsy specimens from PD patients and controls suggested dermal macrophages with phosaSYN deposits as useful biomarkers for PD diagnosis. They also found that the total number of macrophages was significantly positively correlated with the number of macrophages with phosaSYN deposits.[36]

Gastrointestinal tract

Most studies (66 out of 129) have been performed in the gastrointestinal tract (GI) and it was the first peripheral tissue to be evaluated towards identifying PD pathology in 1960.[59] Amongst the 66 studies on the GI; 42 were in vivo, 21 post-mortem[59] and 3 including both alive patients and cadavers[60] respectively (table 2).

Salivary glands

Salivary glands appear as an attractive target for

biopsies as the highest amount of aSYN aggregates in the first autopsy studies was found in the submandibular gland.[50, 61] Incisional biopsy of the submandibular gland which is one of the major salivary glands (parotid, submandibular, sublingual gland)is associated with an increased risk of adverse events. Instead, numerous minor salivary glands are easily accessible at the vestibular site of the lower lip.[62]

After the introduction of phosaSYN immunohistochemistry techniques, Beach et al. reported submandibular specimen stained positive in 39% of all cases screened (i.e. dementia DLB, incidental LB disease (ILBD) and Alzheimer's disease with LB (ADLB)).[50] When the methodology of the process was improved with multiple sections phosaSYN staining rate raised to 93% in PD. When the same researchers replaced the needle core biopsies with large submandibular gland sections, phosaSYN immunoreactivity in nerve increased from 90% to 100% in PD.[63] In contrast, in an in vivo study, a better sensitivity was reported using needle core biopsies of the submandibular gland detecting phosaSYN immunoreactivity in 75% of PD (compared to 7% minor salivary gland biopsies).[64] Biopsies of minor salivary glands in alive patients demonstrate a great variability in phosaSYN staining rates (7% to 100%).[64-69] The in vivo and post mortem studies which explored the LB pathology in submandibular gland reported controversial results (sensitivity rates from 42% to 75%).[64, 70-73]

Table 2. GI tract biopsy studies.

	Reference	PD (n)	DLB (n)	MSA (n)	iRBD (n)	CTR (n)	Salivary glands	Esoph-agus	Stomach	Small intes-tine	Col. asc.	Col. desc. / Sigma	Rec-tum	Other tissues	Localization	aSYNIR somata(LB) neurites (LN) %	phosaSYNIR somata(LB) neurites (LN) %	Other techniques: [1] H&E [2]Azan—Mallory [3]Bodian [4]Electron microscopy [5]VIP;TH (IHC) [6]Luxol fastblue [7]IHC [8]PET blot [9]PMCA [10]RT-QuIC	Comments
1	DenHar-tog1960[59]	5				None		•	•	•	•			•		n.d.	n.d.	1 PD: 0%	
2	Qualman-*etal.*1984[74]	22				50		•	•	•	•	•	•	•		n.d.	n.d.	1,2,5,6 PD:9%LB* CTR:0%	*Esophagus, colon
3	Wakabayash-i*etal.* 1988[75]	7				24		•	•	•	•	•	•			n.d.	n.d.	1,2,3,4 PD:100%LB* CTR:33%LB*	*Across all GI tract segments
4	Wakabayashi et al. 1990[76]	3				3		•	•	•	•	•	•			n.d.	n.d.	PD: 100% LB+LN CTR: 0%LB+LN	
5	Bloch et al. 2006[78]	2				98		•						•		PD:50% CTR:6-14%	n.d.		
6	Braak*et-al.*2006[77]	5				5		•	•					•	Gastric ENS	PD:100% CTR:0%	n.d.		
7	DelTredici*etal.* 2010[61]	9		2		19	•							•		n.d.	PD: 100% MSA: 0% CTR: 0%		*Variable staining intensity
8	Beach *et al.* 2010[50]	17				23	•	•	•	•		•	•	•	submandibular gland (serial slides)	n.d.	PD:65%* n.d. (93%†) CTR: n.s.	7	*Across all GI tract segments †Esophagus,

9	Del Tredici and Duda 2011	3			None	•	•			•		PD:100%	n.d.	7	
10	Annerino et al. 2012	13			12		•	•		•	•	PD:100–25%† CTR: 0%	n.d.	7	*Transverse colon †Stomach to rectum (three slides)
11	Beach et al. 2013[63]	28		2	50	•						n.d.	PD: 0% (somata) PD:100%/ 90%* (neurites) MSA:0% CTR: 0%	7	AD, PSP, CBD needle core biopsy
12	Gold-*etal.*2013[93]	10			77				•	•	colon myenteric and submucosal ganglia	PD: 100% *AD: 0% CTR: 0% †	n.d	7	*8 patients with AD were also included †aSYN detection:PD: 100% AD: 0% CTR: 52%
13	Gelpi et al. 2014	10*	5		None	•	•	•		•	•	PD:80%† DLB: 100%	PD: 80%† DLB:100%	7	*Four with PD, six with PDD †Varying presence of LB/LN due to rostro-caudal gradient
14	Mu et al. 2015	10			4	•						n.s.	PD:100% CTR:0%	7	*tongue-pharynx-larynx-upper esophagus
15	Beach et al. 2016[119]	46	28	2	79	•					sigmoid colon mucosa and submucosa	PD: 91% DLB:71% MSA:0% CTR:0%	PD: 89% DLB:71% MSA:n.s CTR:0%	7	
16	Kupsky et al. 1987[141]	1			None				•	•		n.d.	n.d.	n.d.	1 PD: 100% LB*
17	Lebouvier et al. 2008[142]	5			5+3*				•		Submucosa of ascending colon (submucosal plexus)	n.d.	PD: 0% (somata) PD:80% (neurites) CTR: 0%	7	*Five healthy, three constipated subjects
18	Lebouvier et al. 2010[98]	29			10				•	•	Submucosa neurites	n.d.	PD: 0% (somata) PD:72% (neurites) CTR: 0%	7	

19	Forsyth et al. 2011[92]	9				10						•			n.a	PD: 100%* CTR: n.s.	n.d.		*Patients from study [41]
20	Cersosimo et al. 2011[124]	3				3	•								labial minor salivary	PD: 66% CTR: 0%	n.d.	n.d.	
21	Pouclet et al. 2012[91]	9				10					•	•	•		*Mucosa/ submucosa	PD: 0% CTR: 0%	PD: 33%/45%* CTR: 0%	7	
22	Pouclet et al. 2012[105]	9		6								•			submucosa and mucosa	n.d.	PD: 55,5% MSA:16,7%	7	
23	Shannon et al. 2012[95]	9				24						•			submucosa	PD: 100%* CTR: 8%†	n.d	7	*Variable staining intensity †Cells of unknown origin
24	Shannon et al. 2012[94]	3*				23						•			mucosa and two benign polyps	PD: 100% CTR: 0%	n.d	7	*prodromal PD
25	Devos et al. 2013[143]	18				14					•					n.d	PD: 67% CTR: n.s.		
26	Folgoas et al. 2013[69]	16				11	•								Labial salivary glands		PD: 19% CTR: 18%*	7	*Weak staining
27	Hilton et al. 2014[81]	62*				161		•	•	•	•	•	•	•†	mucosal and submucosal	PD: 0–13%** CTR: 0%.	PD: 0–13%** CTR: 0%	7	*62PD&Progromal PD †Gall bladder **Progromal PD 0% esophagus, 13% small/ large intestine, phosaSYN with better staining
28	Adler et al. 2014[64]	15				-	•								Submandibular gland*/labial salivary glands† (serial slides)	n.d	PD:75%*/7%†	7	
29	S⁴ nchez-Ferro et al. 2015[85]	34*				23			•						Gastric and duodenal mucosa	PD:60,7% prodromal PD: 33.3% CTR:4,3%	†PD: 60.7% prodromal PD:16,6% CTR:4,3%	7	*28 PD &6 prodromal PD †67% agreement between phosaSYN and aSYN
30	Gao et al. 2015[65]	13				13	•								Minor salivary glands	n.d	PD: 69% CTR: 0	7	
31	Aldecoa et al. 2015[144]	6				8					•	•			Stomach, ileum, jejunum, and colon myenteric plexus	PD:100%	PD:67%	7	

ΕΛΛΗΝΙΚΗ ΝΕΥΡΟΛΟΓΙΚΗ ΕΤΑΙΡΕΙΑ

32	Sprenger et al. 2015[104]	21			17	16					•	•	•		submucosa	PD:100% CTR:100% iRBD:100%	PD:5,3% CTR:0% iRBD:23,5%	7	
33	Visanji et al. 2015[126]	22*				11						•	•		mucosa	PD:100% CTR:81,8%	PD:100% CTR:90,1%	7	*15 early PD &7 late PD
														Early PD:80% Late PD:100% CTR:100%	Early PD:80% Late PD:100% CTR:100%	8			
34	Vilas et al. 2016[71]	24			9	26	•								Submandibular glands	n.d	PD:67% iRBD:89% CTR:0%		
35	Adler et al. 2016[70]	25*				10	•								Submandibular glands	n.d.	PD:74% CTR:22%	7	*early PD
36	Antunes et al. 2016[96]	19				8					•	•			mucosa	PD:94,7% CTR:100%	PD:100% CTR:100%	7	
37	Stokholm et al 2016[82]	57*				98	•	•	•	•	•	•			Gastrointestinal mucosa, submucosa and myenteric minor/submandibular glands	PD:61% CTR:59%	PD:44,4% Prodromal PD:56% CTR:25,5%	7	*18 PD & 39 prodromal PD
38	Chung et al.2015[87]	38		13		53			•		•	•			gastric and colonic mucosa	Stomach: PD:31.6% MSA:40% CTR:33.3% colon: PD:10.4% MSA:8% CTR:18.%	n.d	7	
39	Corbille et al. 2016[120]	9				3						•			mucosa-submucosa	n.s	n.s	7*	*Variable stainingmethods none of the tested method or staining pattern had a specificity and sensitivity more than 80 % regarding to PD
40	Iranzo et al. 2017[145]	-			20	32	•				•	•			Submandibular glands	n.d	iRBD: 50%* CTR: n.d	7	*colon: 2/4, salivary glands:1/2 †
41	Barrenschee et al. 2017[99]	12				11							•		submucosal ganglia	PD:100% CTR:100%	PD:25% CTR: 36%	7	
42	Shin et al. 2017[89]	33				21			•		•	•			Mucosa and myenteric plexus	n.d.	PD: 9.1% CTR: 18.2%	7	

43	Corbille et al. 2017[121] 1	17				13						•			n.s	n.s*	n.s*	7	*no differences between PD and for phosaSYN and aSYN levels
					1						•			n.s	n.s*	n.s*			
44	Iranzo et al. 2018[68]	13	10		62	33	•								minor salivary glands	n.d	PD:54% LBD: 50% iRBD:50% CTR:3%	7*	*phosaSYN 5G4 antibody. Only 1 patient with iRBD was positive with 5G4 and negative for phosaSYN
45	Yan et al. 2018[86]	31				32*			•		•	•	•		n.s	PD:54,8% CTR:21,8%	n.d	7	*CTR included patients with atypical parkinsonism
46	Carletti et al. 2018[123]	7				7									Labial minor salivary gland	n.s.	PD:71% CTR: 0%	7	
47	Chung et al. 2018[107]	38*				46					•	•			gastric and colonic mucosa	PD:92,3% CTR: 76,2%	n.d	7	*2 PD-SNCA-SNP rs11931074 variants were included. PD SNCA SNP-rs11931074 associated with aSYN staining
48	Ruffmann et al. 2018[83]	51*				21		•	•	•	•	•			mucosa and submucosa	PD: 35% Prodromal PD:42% CTR: 24%	PD: 15% Prodromal PD:13% CTR: 24%	7	*31 PD & 20 prodromal PD
49	Shin et al. 2018[146]	16				14	•								submandibular glands	n.d	PD:56.2% CTR:0%	7	
50	Ma et al. 2019[66]	8				7	•								minor salivary gland	n.d	PD:100% CTR:0%	7*	*Immunohistochemical 3-NT-Syn expression
51	Adler et al. 2019[147]	7*				-											PD: 100%†	7	*6 advanced PD& 1 early PD in previous study positive phos-a-syn patients (Adler et al. 2016) †increase density over time
52	Leclair-Visonneau et al. 2019[148]	43				-							•			n.s	PD: 47%		Correlation with dysautonomia with questionnaires and tests

ΕΛΛΗΝΙΚΗ ΝΕΥΡΟΛΟΓΙΚΗ ΕΤΑΙΡΕΙΑ

53	Fenyi et al. 2019[88]	18				11			•			•	•			PD: 35,7% (5/14) CTR: 0% (0/3)	PD: 55,8% CTR: 09% □ *	9	Advanced PD
54	Punsoni et al. 2019[84]	16				298		•	•	•	•	•	•			PD:100% CTR:100%	PD:25% (somata) CTR: n.s *6 pediatric and 4 adults	7	*15 pediatric and 14 adults
	17				10*											7			
55	Shin et al. 2020[106]	3*					•†							subman-dibular glands		n.d	PD:0%	7	*PRKN †core needle biopsy
56	Harapan et al. 2020[97]	25				20				•	•	•			submucosa and myenteric plexus	n.s*	n.s*	7	*similar rates of aSYN and phosaSYN in PD and CTR
57	Manne et al. 2020[73]	13				16	•								submandibular glands	n.s	*PD:100% CTR:0%	10	*paS seeding activity during the 24-h period
58	Beck et al. 2020[60]	7*				18			•		•	•			Mucosa, sub-mucosa, myenteric plexus	PD:57% prodromal PD:100% CTR:82-100%	PD:14% prodromal PD:100% CTR:0%	7	*3 prodromal PD were included
					4									n.a					
59	Chahine et al. 2020[48]	59				21	•				•	•		•*	submandibu-lary glands	†PD:56,1% CTR:7,1%	n.d	7	*skin †submandibulary glands
60	Fern4 ndez-Espejo et al. 2021[122]	6				6	•								submandibu-lary glands	n.d	PD:100% CTR:0%	7*	*Immunohisto-chemical 3-NT-Syn expression
61	Tanei et al. 2021[56]						•	•						•*	Mucosa, sub-mucosa, myenteric plexus	n.d	† PD: 75% PDD:92.1% LBD: 43.8% CTR:0%	7	*skin, adrenal gland, brain † LB pathology in esophagus LB pathology in 34%(178/518) of autopsird cases:

62	Sakashita et al. 2021[72]						•							•*	Submandibular glands	n.d	PD:89,1% DLB;75,4%	[7]	*brain Salivary gland biopsy in 64 autopsied patients who had prodromal or clinical LBD LB pathology in 32,8% (21/64) of cases LB pathology in 42,8% (9/21 of them. No CNS-LBD was found in all patients without LBD in submandibular glands
bo	Bargar et al. 2021[55]	2				1	•					•		•*	submandibular glands		PD; + (n.s) CTR:0%	[10]	*scalp skin, CSF
64	Jotanovic et al. 2022[103]	74*									•	•			Mucosa, submucosa	Subject with cerebral a-synucleinopathy: 81%†	n.d		*subjects with cerebral a-synucleinopathy †19% no GI a-syn 14,9%: GI a-syn , scarce a-syn in brain
65	Mangone et al. 2022[67]	27			16	18	•								Labial salivary glands	PD: 14.8% iRBD:18.8% CTR:0%	*PD:55.6% iRBD:43.8% CTR:38.9%	[7]	*anti-aggregated -Syn clone 5G4 antibody
66	Emmi et al. 2023[100]	18								•						n.d.	PD:100% CTR: + (n.s)	[7]	*14 advanced PD & 4 early PD

Post-mortem studies with accompanying brain pathology are shown in dark grey shaded cells, in vivo studies are shown in light grey shaded cells. aSYN, alpha synuclein; CTR, control; DLB, dementia with Lewy bodies; MSA, Multiple System Atrophy; LBD, Lewy body disease; PD, Parkinson's disease; PDD, PD dementia; iRBD, Isolated Rapid eye movement Behavior Disorder; UC, Ulcerative colitis; GI, gastrointestinal; H&E, hematoxylin—eosin staining; IHC,immunohistochemistry; IR, immunoreactive; PMCA, protein misfolding cyclic amplification; RT-QuIC assay, real-time quaking induced conversion; LB, Lewy bodies; LN, Lewy neurites; n/a, not applicable; n.d., not determined; n.s., not stated; phosaSYN, phosphorylated alpha synuclein; TH, tyrosine hydroxylase; SMP, submucosal plexus; VIP, vasoactive intestinal polypeptide; asterisks and daggers refer to the same table row. The percentages correspond to the sensitivity (PD%). The specificity equals 100% – CTR%. Col.desc, descending colon; col. asc, ascending colon.

Esophagus, Stomach, Small intestine, Colon and Rectum

The studies which evaluated the esophageal involvement in LB pathology are 15 and the 3 of them are post-mortem.[50, 59, 61, 63, 74-84] Qualman et al.in 1984 were the

first to report esophageal LB in 9% and in 25% of PD cadavers with dysphagia and achalasia respectively.[74] Staining for aSYN was positive in a range between 50% and 100% in 3 studies.[77-79] Comparing different studies of the last 4 decades, phosaSYN positivity reached 93% in PD, when multiple slides of paraffin-embedded and 80 lm frozen sections of esophagus were examined, in a survey which obtained different GI tract segments from patients with different synucleinopathies.[50] In the esophagus tissue most LB were found in neurons immunoreactive for vasoactive intestinal polypeptide (VIP).

The reports which explore stomach and small intestine are 19.[50, 51, 59, 60, 74-77, 79-89] aSYN staining was more abundant in stomach (from 80% to 100%) compared to other GI tract specimens.[51, 80, 90] The rostro-caudal gradient distribution in PD is confirmed by this finding.[50, 75, 91] However, the phosaSYN pathology ranged from 9,1% to 80%.[51, 80, 89]

It is out of any question that colon and rectum are the most studied segments of the GI tract.[59]

The accumulation and aggregation of aSYN in the gut mucosa of PD patient has been confirmed by several studies (table 2). Most of them reported that aSYN aggregates are more frequent in PD patients (54,8%-100%) compared to age-matched healthy controls (4,3%-21,8%).[85, 86, 92-95] In contrast, Harapan et al. and Antunes et al. argued that aSYN and phosaSYN rates did not differ between PD and controls.[96-97] In PD patients phosaSYN detection in the mucosa and submucosa of colon and rectum rates from 14%-100% in different studies.[56, 60, 98-100] Apparently, this variability could be partly attributed to the rostro-caudal gradient of the colorectal GI segment. A rather low sensitivity of rectal biopsies (23%) is noticed compared to biopsies taken from the ascending colon (65%).[101] It is noteworthy that deep submucosal biopsies increase the chance to discover phosaSYN neurites (45%) compared to conventional mucosal biopsies (33%).[91] Regardless the high heterogeneity, a systematic review and meta-analysis of 16 studies claimed a high degree of association between gut α-synuclein species and PD.[102]

5 in vivo studies estimated that LB pathology is present in the GI tissue of people up to 20 years prior to the onset of motor symptoms.[60, 81-83, 85, 94]. In contrast, one post-mortem study highlighted the absence of aSYN in 19% of people with LB pathology in brain.[103] GI phosaSYN deposition was frequently found in patients with iRBD, providing histopathological evidence that iRBD represents a synucleinopathy. Colonic and submandibular, revealed moderate sensitivity (23,5%-89%) to identify phosaSYN and very high specificity (97%-100%) to distinguish iRBD subjects from controls.[68, 71, 104] Nevertheless, Mangone et al. reported the that minor salivary gland biopsies lack sufficient accuracy to detect SYN species in salivary glands in PD and in iRBD. In a survey performed and using the anti-aggregated-Syn clone 5G4 antibody, they found oligomeric aSYN deposits in 55.6% in PD, 7 in iRBD, and 7 in 38.9% controls.[67]

In a post-mortem study included pathologically confirmed PD, MSA patients and controls, aSYN immunoreactivity was observed in tissue samples in nearly all cases of PD, but none in the control or MSA subjects.[61] aSYN immunoreactivity was less frequent in MSA (16,7%) than in PD (55,5%) in an in vivo study which examined submucosa and mucosa in colonic biopsies.[105] Chung et al. revealed similar rates of aSYN deposits when examined colon and stomach specimens of PD and MSA patients.[87] 2 studies explored the presence of phosaSYN in DLB. Iranzo et al. demonstrated deposits of deposits of phosaSYN in 50% of DLB patients (vs 54% of PD), and Gelpi et al. in 100% of cases with clinicopathological diagnoses of DLB (vs 54% of PD).[51, 68]

In a recent study it was described negative phosaSYN staining in the submandibular gland of patients carrying PRKN pathogenic variants.[106] In another survey in which gastric and colonic mucosa biopsies were obtained from PD patients and healthy controls, it was shown that PD SNCA variants (SNCA SNP-rs11931074) were associated with aSYN staining.[107]

Olfactory (mucosa & bulb)

Evaluation of the olfactory bulb is necessarily restricted to post-mortem examination in contrast to olfactory mucosa which can be investigated in vivo. We went through 11 post-mortem studies[78, 108-114] and three in vivo[115], which used immunochemistry techniques and seeding amplification assays (table 3). Post-mortem studies revealed a positive staining rate for aSYN between 0%-100%, and for phosaSYN between 75%-100%. In the only in vivo study using olfactory mucosa phosaSYN was not detectable in patients with PD.[115]

We also identified 5 recent studies which explored α-synuclein seeding activity using the RT-QuIC assay.[31, 55, 116-118] Herein, the findings were less heterogenous, as the positive aSYN RT-QuIC was from 46,5% to 67,4% for PD and 10% to 10,2% for controls in both in vivo and post-mortem studies. In another study was shown for the first time that RT-QuIC could detect aSYN aggregates in olfactory mucosa of DLB patients with sensitivity reaching 86,4%.[116] However, the rates of positive results were reduced in two other studies who included people with iRBD. The sensitivity for iRBD versus controls was between 44,4% and 67%, while the specificity was high (90%).[32, 117]

Table 3. Biopsy studies of olfactory mucosa and olfactory bulb.

	Reference	PD (n)	DLB (n)	MSA (n)	PAF (n)	iRBD (n)	CTR (*n*)	Olfactory mucosa	Olfactory bulb	Brain	Other tissues	aSYNIR somata(LB) neurites (LN) %	phosaSYNIR somata(LB) neurites (LN) %	Other techniques: [1] H&E [2]Azan—Mallory [3]Bodian [4]Electron microscopy [5]VIP,TH (IHC) [6]Luxol fastblue [7]IHC [8]PET blot [9]PMCA [10]RT-QuIC [11]PLA	Comments
1	Duda et al. 1999[108]							•		•		PD: 85%* CTR:90%*	PD: 85%* CTR:90%*	1,3	*In ORN
2	Bloch et al.2006[78]	2					98		•	•	•	PD: 100%* CTR:17%*, †	PD: 100%* CTR:17%*, †		*IR neurites predominate over IR somata †aSYN pathology was found in 17 (17.3%) out of 98 neurologically asymptomatic subjects. All of these had olfactory bulb involvement
3	Beach et al. 2009[109]	58					69		•	•		PD: 95%* CTR:8%	PD: 95%* CTR:8%		*LB-like perikaryal inclusions in ≈10%
4	Jellinger et al. 2009[110]	25					-	•	•	•		PD: 8%*	PD: 8%		*In olfacotry epithelium (exact site n.s.)
5	Witt et al. 2009[115]	7					25	•				PD: 0%* CTR: †	PD: 0%* CTR: 100%	1,4	*Loss of OMP IR in ORN †In some ORN
6	Arnold et al. 2010[111]	7					45	•		•		PD: 14% CTR:2%			Occasional intra-cytoplasmic inclusion
7	Funabe et al. 2013[112]	4					62	•	•	•	•	PD: 0% CTR: 0%	PD: 75%* CTR:0%	1,2	Nerve fibers in lamina propria
8	Toledo et al. 2016[149]	141	13						•	•	•*	PD:31,2%† PDD:30% DLB:38,4% AD/LB:22,7%	n.d	7	PDD:80, AD/LB pathology:308, were also included. *Submandibular gland, esophagus †LB pathology in AD/LB shows a distribution that differs from PD, with different patterns of spreading.
9	Saito et al. 2016[113]	8	2	2				•	•	•		n.d	PD:75%* iLB:9,1%	7	CBS:2, PSP:5, SCA:4, ALS:6, iLB:11 were .also included. *olfactory epithelium

10	Stevenson et al. 2020[114]	11					11		•			PD:100%*	PD:100%*	7	*AON
11	Perra et al. 2021[116]		37*					•				DLB:86,4%†		10	*Prodromal or propable DLB AD/LB pathology:6, non-LB related pathology:36 were also included †93.8% for CSF
12	Stefani et al. 2021[117]	41				63	59	•				PS: 46,5% iRBD:44,4% CTR:10,2%		10	
13	Bargar et al. 2021[55]	2					1			•	•*		PD; + (n.s) CTR:0%	10	*skin, submandibular glands
14	Bongianni et al. 2022[118]	66					29	•				PD:67,4% CTR:10%	PD:+ CTR:0%*	10, 7	*PhosaSYN: n.s
15	Kuzkina et al. 2023[32]	27		3		18	30	•			•*	Olfactory	Skin		3 patients fulfilling clinical diagnostic criteria of PSP. *skin
												PD:48% MSA:67% iRBD:67% CTR:10%	PD:78,9% MSA:100% iRBD:100% CTR:n.d	10	
												n.d	n.d	7	

Post-mortem studies with accompanying brain pathology are shown in dark grey shaded cells, in vivo studies are shown in light grey shaded cells. aSYN, alpha synuclein; iPD, idiopathic PD; PD, Parkinson's disease; PDD, PD dementia; CTR, control; DLB, dementia with Lewy bodies; MSA, Multiple System Atrophy; PAF, pure autonomic failure; iRBD, Isolated Rapid eye movement Behavior Disorder; ALS, Amyotrophic Lateral Sclerosis; PSP, progressive supranuclear palsy; CBD, Corticobasal Degeneration; AD, Alzheimer disease; SCA: Spinocerebellar Ataxia; GBA1, glucocerebrosidase gene; GI, gastrointestinal; H&E, hematoxylin—eosin staining; IHC, immunohistochemistry; IR, immunoreactive; PMCA, protein misfolding cyclic amplification; RT-QuIC assay, real-time quaking induced conversion; PLA, Proximity Ligation Assay; LB, Lewy bodies; LN, Lewy neurites; n/a, not applicable; n.d., not determined; n.s., not stated; RSCs, Remak non-myelinating Schwann cells;; orthostatic hypotension; phosaSYN, phosphorylated alpha synuclein; OMP, olfactory marker protein; ORN, olfactory receptor neurons; AON, anterior olfactory nucleus, TH, tyrosine hydroxylase; VIP, vasoactive intestinal polypeptide; asterisks and daggers refer to the same table row. The percentages correspond to the sensitivity (PD%). The specificity equals 100% – CTR%.

Discussion

Technical Issues: Which process? Which immunohistochemical marker?

In the majority of the skin biopsy studies, samples were derived from the trunk (C7-C8 C8 paravertebral area) and lower limb (i.e. thigh;15 cm above the patella and distal leg;10 cm above the lateral malleolus) which are considered the optimal biopsy-taking sites. But what happens with people with prodromal disease or unilateral motor symptoms? An unsolved question concerns phosaSYN aggregates and their preferential side of distribution. Does deposition reflect the site of motor dysfunction? It was found that in PD patients with unilateral disease 20% had abnormal deposits only in the affected motor side, 60% in both sides and 20% only in the non-affected side respectively. Regarding the spine topographical distribution of skin phosaSYN, it seems that deposits displayed a uniform distribution between both sides (and not following the motor dysfunction) in unilateral patients. It was also demonstrated a spine gradient with the cervical site expressing the highest positivity compared to Th12.[28] Furthermore, study findings on phosaSYN in skin biopsies revealed that the range of sensitivity depends on the biopsy site (ranging from 31% in distal leg, to 100% in cervical site).[12] However, according to other authors, the biopsy site does not affect the potency of total aSYN detection (90% sensitivity and specificity).[25]

Obviously, the detection rate of phosaSYN depends not only on the exact biopsy site taken but also on methodological differences using sections of different tissue thickness. It was demonstrated that double-immunostained 50 μm skin biopsy tissue sections are superior to 20 and 10 μm tissue sections for the detection of phosaSYN. Apparently, the greater volume of tissue analyzed and the improved visualization of nerve fiber architecture increases the sensitivity of the procedure.[39, 40]

Similarly, the amount of nervous tissue is usually insufficient, in conventional colonic biopsies. Therefore, the discovery of LB pathology is increased by obtaining full-thickness sections of colon. Beach et al. reported that the submucosa has the highest prevalence of pathological LB staining, followed by the muscularis and mucosa.[119] Notably, the distribution of aSYN/phosaSYN varies between different gut tissues, following a rostro-caudal gradient pattern, resembling skin topographical allocation. aSYN/phosaSYN burden shows highest levels in the esophagus and lowest involvement of the distal colon and rectum.[50, 51-77] Most of the in vivo studies which obtained tissue from the gastrointestinal tract have used immunohistochemistry techniques for the detection of LB pathology. The vast majority of them had a specificity and sensitivity less than 80 % regarding to PD. Moreover, the biochemical methods tested were not adequate for the prediction of PD.[120, 121] More specifically, salivary glands, studies showed higher sensitivity for needle core biopsies obtained from the submandibular gland (56,2%-100%)[73, 89, 122], whereas biopsies from minor salivary glands resulted in largely varying rates of positive phosaSYN/aSYN staining (7%–100%) in PD.[64, 66, 69, 123, 124] The heterogeneity of findings obtained from these studies is complicated not only by differences in immunocytochemical staining techniques, dissection protocols, and subjects included (accuracy of clinical diagnosis), but also by study design (cohort sampling size and stratification, retrospective vs. longitudinal, in vivo vs post-mortem).

The early and persistent accumulation of phosaSYN/aSYN in the GI of patients with prodromal PD supports the hypothesis that disease originates from the colon. On the other hand, LB pathology is present in colon in people who never developed the disease when alive.[103] Borghammer et al. conducted a focused re-analysis of two postmortem datasets, which included large numbers of mild LB disease cases. They observed that the pathologic process starts in either the olfactory bulb or the ENS, but rarely in the olfactory bulb and GI simultaneously. The above findings revise the dual-hit hypothesis of PD which postulates that the pathologic process starts from the olfactory bulb and dorsal motor nucleus of the vagus nerve.[125]

The first neuropathological attempts towards identifying a reliable biomarker in synucleinopathies in peripheral tissues started with the use of antibodies against aSYN. We already know that aSYN is also detectable in healthy people. Several studies have shown that the frequency of positive aSYN staining is varies reaching even 100% in healthy subjects and is seems to be unlikely that all controls included were affected by synuclein associated disease. (Tables 1–3).[10, 52, 60, 78, 82-85, 93, 96, 99, 104, 126] It was underlined by Beach et al. in 2013 that aSYN is one of the most abundant proteins in neural tissue, and therefore positive aSYN staining cannot be abnormal.[63] They suggested the use of antibodies against phosaSYN. In the same research work, they also proposed proteinase K pretreatment towards digesting normal aSYN and allowing affected pathological phosaSYN inclusions to be revealed.[127] The most reliable immunohistochemistry marker which distinguishes pathological deposits from physiological aSYN is phosphorylated αSyn (phosaSYN) at serine 129.Amongst the phosaSYN antibodies tested, many researchers retrieved the best results with the use of the monoclonal antibody directed against peptide 124–134 including phosphorylated Ser129 (Wako Pure Chemical Industries Ltd., Neuss, Germany).[128, 129]

The significance of aSYN phosphorylation is a mat-

ter of debate. In vitro studies reported that phosaSYN impels the formation of inclusions. Only a small amount of aSYN is phosphorylated in healthy human brain and aSYN appears to be phosphorylated as disease progresses.[130] More interestingly, aSYN oligomerization has been described as an early event in the pathologic process, independent of the phosphorylation.[131] Recently, aSYN targeting antibody, αSyn-5G4 showed high conformational specificity and strong immunoreactivity for all forms of αSyn aggregates, reliability in identifying aSYN deposits and was also able to detect astrocytic and oligodendroglial aSYN inclusions across synucleinopathies.[100, 132, 133] It is suggested that 5G4 deposits appear at an early stage of the disease and they are less detectable after the spread of neurodegeneration. Additionally, they have a different distribution among skin biopsy sites, compared to phosaSYN.[29] Another marker of the early stage of the pathology is PLA which recognizes the oligomeric form of aSYN. PLA does not reveal physiological aSYN and detects pathology in the form of extensive diffuse deposition of aSYN oligomers which are often localized, in the absence of Lewy bodies.[134] With the use of PLA, Mazzetti et al. first described, that aSYN oligomers accumulate within synaptic terminals of autonomic fibers of the skin in PD.[53] A combination of tests run with phosaSYN, aSYN, aSYN-5G4A, aSYN-PLA, and IENFD, will increase the diagnostic yield and open new windows in understanding the temporal events of aSYN spread.[41]

Seeding amplification assays (SAAs) as the Protein misfolding cyclic amplification (PMCA) and the real-time quaking-induced conversion (RT-QuIC), were originally developed to mimic prion replication.[135, 136] A meta-analysis study revealed that skin aSYN-SAAs exhibited the highest sensitivity (0.92), which was not different from that of cerebrospinal fluid (CSF) (0.90),[137] and therefore, skin biopsies could represent a valid alternative to CSF analysis.[58] Olfactory mucosa aSYN-SAAs exhibited a lower sensitivity compared to CSF and skin.[137] However, RT-QuIC sensitivity is significantly increased when nasal swab is performed at different areas covered by olfactory epithelium indicating that aSYN aggregates are preferentially detected in olfactory areas with higher concentration of olfactory neurons.[118] Additionally, applying the method in diverse tissues (i.e. olfactory as part of the central nervous system and skin as peripheral nervous system), diagnostic accuracy could increase.[32] The high sensitivity, specificity of RT-QuIC assay in skin specimens was confirmed by isolated in vivo and post-mortem studies.[31, 32, 38, 53, 57] Higher α-synuclein seeding activity in RT-QuIC was shown in patients with longer disease duration and more advanced stage of disease and was correlated with non-motor symptoms (i.e. RBD, cognitive decline, constipation).[31, 38] Therefore, the method could be useful not only for diagnostic reasons, but also for monitoring disease progression.[54, 57]

Classical LB are defined as round eosinophilic inclusions located in neuronal somata with hyaline appearance.[138] The pathological signature of LB diseases has broaden with the advent of immunohistochemistry. Additional morphological features have been described for LB/LN in the CNS (i.e. diffuse, granular or pleomorphic intraneuronal structures or intra-neuritic dot-like structures and axonal spheroids).[139] The question which arises is if positive aSYN/phosaSYN staining of neuronal somata or processes in peripheral tissues can be regarded as and termed LB or LN, respectively. Thus, studies on peripheral tissues should not only describe the absence or presence of aSYN/phosaSYN immunoreactivity but also precisely depict the morphological features resembling LB/LN-like structures.

Conclusions and future perspectives

This review of a combination of postmortem and in vivo studies redefines the remarks of previous evaluations regarding optimal tissue source, technique and immunohistochemical marker.[140] Skin biopsy is an easy, minimum invasive approach which provides high specificity and good sensitivity for the detection and differential diagnosis of synucleinopathies. GI biopsies remain attractive in the detection of synucleinopathies. However, a standardized methodology is essential to increase their diagnostic value. The new promising assays could be incorporated in future cohorts, towards identifying the combinations and relative contributions of the sensitivity amongst peripheral tissues.

Review highlights.

• Phosphorylated a-synuclein (phosaSYN) is the pathological signature of Parkinson's disease.
• phosaSYN is confined to the central nervous system, but also to peripheral tissues.
• Studies on peripheral tissues should not only describe the absence or presence of aSYN/phosaSYN immunoreactivity but also depict the morphological features resembling LB/LN-like structures.
• The most reliable immunohistochemistry marker which distinguishes pathological deposits from physiological aSYN is phosphorylated αSyn (phosaSYN) at serine 129.
• Cutaneous phosaSYN aggregation is detected in most of iRBD population.

• Dermal phosaSYN can be considered a peripheral histopathological marker of synucleinopathy representing prodromal PD.
• phosaSYN is mainly detected in autonomic fibers of PD and DLB.
• In MSA phosaSYN is detected in skin Remak non-myelinating Schwann cells (RSCs).
• The in vivo and postmortem studies in submandibular gland report controversial results.
• The distribution of aSYN/phosaSYN varies between different gut tissues, following a rostrocaudal gradient pattern.
• In GI tract, the submucosa has the highest prevalence of pathological staining, followed by the muscularis and mucosa.
• RT-QuIC can detect aSYN aggregates in olfactory mucosa in synucleinopathies with high sensitivity.
• A combination of tests run will increase the diagnostic yield.

Useful points to clinical practice

		PD diagnosis	Differential diagnosis among synucleinopathies	Early diagnosis	Disease progression
Tissues	skin	+	+	-	-
	GI			?	
	Olfactory mucosa	-	-	-	-
Techniques & markers	aSYN-5G4A			+	
	aSYN-PLA			+	
	aSYN-SAAs (RT-QuIC)	+			+

References

[1] Kouli A, Torsney KM, Kuan WL. Parkinson's Disease: Etiology, Neuropathology, and Pathogenesis. In: Stoker TB, Greenland JC, eds. Parkinson's Disease: Pathogenesis and Clinical Aspects. Brisbane AU: : The Authors., 2018.

[2] Jellinger KA. Neuropathological spectrum of synucleinopathies. Movement disorders : official journal of the Movement Disorder Society 2003;18 Suppl 6:S2-12.

[3] Braak H, Del Tredici K. Invited Article: Nervous system pathology in sporadic Parkinson disease. Neurology 2008;70:1916-1925.

[4] Michell AW, Luheshi LM, Barker RA. Skin and platelet alpha-synuclein as peripheral biomarkers of Parkinson's disease. Neuroscience letters 2005;381:294-298.

[5] Dabby R, Djaldetti R, Shahmurov M, et al. Skin biopsy for assessment of autonomic denervation in Parkinson's disease. Journal of neural transmission (Vienna, Austria : 1996) 2006;113:1169-1176.

[6] Miki Y, Tomiyama M, Ueno T, et al. Clinical availability of skin biopsy in the diagnosis of Parkinson's disease. Neuroscience letters 2010;469:357-359.

[7] Doppler K, Ebert S, Uçeyler N, et al. Cutaneous neuropathy in Parkinson's disease: a window into brain pathology. Acta neuropathologica 2014;128:99-109.

[8] Rossi A, Giovenali P, Benvenuti M, Di Iorio W, Calabresi P. Skin biopsy: a new diagnostic tool for autonomic dysfunctions in Parkinson's disease? The Lancet Neurology 2007;6:848-849; author reply 849.

[9] Nolano M, Provitera V, Estraneo A, et al. Sensory deficit in Parkinson's disease: evidence of a cutaneous denervation. Brain : a journal of

neurology 2008;131:1903-1911.
[10] Wang N, Gibbons CH, Lafo J, Freeman R. α-Synuclein in cutaneous autonomic nerves. Neurology 2013;81:1604-1610.
[11] Donadio V, Incensi A, Leta V, et al. Skin nerve α-synuclein deposits: a biomarker for idiopathic Parkinson disease. Neurology 2014;82:1362-1369.
[12] Donadio V, Incensi A, Piccinini C, et al. Skin nerve misfolded α-synuclein in pure autonomic failure and Parkinson disease. Annals of neurology 2016;79:306-316.
[13] Donadio V, Incensi A, El-Agnaf O, et al. Skin α-synuclein deposits differ in clinical variants of synucleinopathy: an in vivo study. Scientific reports 2018;8:14246.
[14] Donadio V, Doppler K, Incensi A, et al. Abnormal α-synuclein deposits in skin nerves: intra- and inter-laboratory reproducibility. European journal of neurology 2019;26:1245-1251.
[15] Donadio V, Incensi A, Rizzo G, et al. Skin Biopsy May Help to Distinguish Multiple System Atrophy-Parkinsonism from Parkinson's Disease With Orthostatic Hypotension. Movement disorders : official journal of the Movement Disorder Society 2020;35:1649-1657.
[16] Donadio V, Wang Z, Incensi A, et al. In Vivo Diagnosis of Synucleinopathies: A Comparative Study of Skin Biopsy and RT-QuIC. Neurology 2021;96:e2513-e2524.
[17] Donadio V, Incensi A, Rizzo G, et al. Phosphorylated α-synuclein in skin Schwann cells: a new biomarker for multiple system atrophy. Brain : a journal of neurology 2023;146:1065-1074.
[18] Doppler K, Weis J, Karl K, et al. Distinctive distribution of phospho-alpha-synuclein in dermal nerves in multiple system atrophy. Movement disorders : official journal of the Movement Disorder Society 2015;30:1688-1692.
[19] Doppler K, Jentschke HM, Schulmeyer L, et al. Dermal phospho-alpha-synuclein deposits confirm REM sleep behaviour disorder as prodromal Parkinson's disease. Acta neuropathologica 2017;133:535-545.
[20] Doppler K, Brockmann K, Sedghi A, et al. Dermal Phospho-Alpha-Synuclein Deposition in Patients With Parkinson's Disease and Mutation of the Glucocerebrosidase Gene. Frontiers in neurology 2018;9:1094.
[21] Doppler K, Antelmi E, Kuzkina A, et al. Consistent skin α-synuclein positivity in REM sleep behavior disorder - A two center two-to-four-year follow-up study. Parkinsonism & related disorders 2021;86:108-113.
[22] Doppler K, Mammadova S, Kuzkina A, et al. Association between probable REM sleep behavior disorder and increased dermal alpha-synuclein deposition in Parkinson's disease. Parkinsonism & related disorders 2022;99:58-61.
[23] Zange L, Noack C, Hahn K, Stenzel W, Lipp A. Phosphorylated α-synuclein in skin nerve fibres differentiates Parkinson's disease from multiple system atrophy. Brain : a journal of neurology 2015;138:2310-2321.
[24] Haga R, Sugimoto K, Nishijima H, et al. Clinical Utility of Skin Biopsy in Differentiating between Parkinson's Disease and Multiple System Atrophy. Parkinson's disease 2015;2015:167038.
[25] Gibbons CH, Garcia J, Wang N, Shih LC, Freeman R. The diagnostic discrimination of cutaneous α-synuclein deposition in Parkinson disease. Neurology 2016;87:505-512.
[26] Gibbons C, Wang N, Rajan S, et al. Cutaneous α-Synuclein Signatures in Patients With Multiple System Atrophy and Parkinson Disease. Neurology 2023;100:e1529-e1539.
[27] Rodríguez-Leyva I, Calderón-Garcidueñas AL, Jiménez-Capdeville ME, et al. α-Synuclein inclusions in the skin of Parkinson's disease and parkinsonism. Annals of clinical and translational neurology 2014;1:471-478.
[28] Donadio V, Incensi A, Rizzo G, et al. Spine Topographical Distribution of Skin α-Synuclein Deposits in Idiopathic Parkinson Disease. Journal of neuropathology and experimental neurology 2017;76:384-389.
[29] Melli G, Vacchi E, Biemmi V, et al. Cervical skin denervation associates with alpha-synuclein aggregates in Parkinson disease. Annals of clinical and translational neurology 2018;5:1394-1407.
[30] Kuzkina A, Schulmeyer L, Monoranu CM, Volkmann J, Sommer C, Doppler K. The aggregation state of α-synuclein deposits in dermal nerve fibers of patients with Parkinson's disease resembles that in the brain. Parkinsonism & related disorders 2019;64:66-72.
[31] Kuzkina A, Bargar C, Schmitt D, et al. Diagnostic value of skin RT-QuIC in Parkinson's disease: a two-laboratory study. NPJ Parkinson's disease 2021;7:99.
[32] Kuzkina A, Rößle J, Seger A, et al. Combining skin and olfactory α-synuclein seed amplification assays (SAA)-towards biomarker-driven phenotyping in synucleinopathies. NPJ Parkinson's disease 2023;9:79.
[33] Isonaka R, Goldstein DS, Zhu W, et al. α-Synuclein Deposition in Sympathetic Nerve Fibers in Genetic Forms of Parkinson's Disease. Movement disorders : official journal of the Movement Disorder Society 2021;36:2346-2357.
[34] Miglis MG, Zitser J, Schneider L, et al. Cutaneous α-synuclein is correlated with autonomic impairment in isolated rapid eye movement sleep behavior disorder. Sleep 2021;44.

[35] Giannoccaro MP, Donadio V, Giannini G, et al. Comparison of 123I-MIBG scintigraphy and phosphorylated α-synuclein skin deposits in synucleinopathies. Parkinsonism & related disorders 2020;81:48-53.
[36] Oizumi H, Yamasaki K, Suzuki H, et al. Phosphorylated alpha-synuclein in Iba1-positive macrophages in the skin of patients with Parkinson's disease. Annals of clinical and translational neurology 2022;9:1136-1146.
[37] Nolano M, Caporaso G, Manganelli F, et al. Phosphorylated α-Synuclein Deposits in Cutaneous Nerves of Early Parkinsonism. Journal of Parkinson's disease 2022;12:2453-2468.
[38] Iranzo A, Mammana A, Muñoz-Lopetegi A, et al. Misfolded α-Synuclein Assessment in the Skin and CSF by RT-QuIC in Isolated REM Sleep Behavior Disorder. Neurology 2023;100:e1944-e1954.
[39] Liu X, Yang J, Yuan Y, et al. Optimization of the Detection Method for Phosphorylated α-Synuclein in Parkinson Disease by Skin Biopsy. Frontiers in neurology 2020;11:569446.
[40] Wang N, Garcia J, Freeman R, Gibbons CH. Phosphorylated Alpha-Synuclein Within Cutaneous Autonomic Nerves of Patients With Parkinson's Disease: The Implications of Sample Thickness on Results. The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society 2020;68:669-678.
[41] Vacchi E, Senese C, Chiaro G, et al. Alpha-synuclein oligomers and small nerve fiber pathology in skin are potential biomarkers of Parkinson's disease. NPJ Parkinson's disease 2021;7:119.
[42] Yang J, Wang H, Yuan Y, et al. Peripheral synucleinopathy in Parkinson disease with LRRK2 G2385R variants. Annals of clinical and translational neurology 2021;8:592-602.
[43] Giannoccaro MP, Avoni P, Rizzo G, et al. Presence of Skin α-Synuclein Deposits Discriminates Parkinson's Disease from Progressive Supranuclear Palsy and Corticobasal Syndrome. Journal of Parkinson's disease 2022;12:585-591.
[44] Carmona-Abellan M, Gabilondo I, Murueta-Goyena A, et al. Small fiber neuropathy and phosphorylated alpha-synuclein in the skin of E46K-SNCA mutation carriers. Parkinsonism & related disorders 2019;65:139-145.
[45] Donadio V, Incensi A, Rizzo G, et al. A new potential biomarker for dementia with Lewy bodies: Skin nerve α-synuclein deposits. Neurology 2017;89:318-326.
[46] Donadio V, Incensi A, Del Sorbo F, et al. Skin Nerve Phosphorylated α-Synuclein Deposits in Parkinson Disease With Orthostatic Hypotension. Journal of neuropathology and experimental neurology 2018;77:942-949.
[47] Rodríguez-Leyva I, Chi-Ahumada EG, Carrizales J, et al. Parkinson disease and progressive supranuclear palsy: protein expression in skin. Annals of clinical and translational neurology 2016;3:191-199.
[48] Chahine LM, Beach TG, Brumm MC, et al. In vivo distribution of α-synuclein in multiple tissues and biofluids in Parkinson disease. Neurology 2020;95:e1267-e1284.
[49] Ikemura M, Saito Y, Sengoku R, et al. Lewy body pathology involves cutaneous nerves. Journal of neuropathology and experimental neurology 2008;67:945-953.
[50] Beach TG, Adler CH, Sue LI, et al. Multi-organ distribution of phosphorylated alpha-synuclein histopathology in subjects with Lewy body disorders. Acta neuropathologica 2010;119:689-702.
[51] Gelpi E, Navarro-Otano J, Tolosa E, et al. Multiple organ involvement by alpha-synuclein pathology in Lewy body disorders. Movement disorders : official journal of the Movement Disorder Society 2014;29:1010-1018.
[52] Gibbons CH, Wang N, Freeman R. Cutaneous Alpha-Synuclein From Paraffin Embedded Autopsy Specimens in Parkinson's Disease. Journal of Parkinson's disease 2017;7:503-509.
[53] Mazzetti S, Basellini MJ, Ferri V, et al. α-Synuclein oligomers in skin biopsy of idiopathic and monozygotic twin patients with Parkinson's disease. Brain : a journal of neurology 2020;143:920-931.
[54] Manne S, Kondru N, Jin H, et al. Blinded RT-QuIC Analysis of α-Synuclein Biomarker in Skin Tissue From Parkinson's Disease Patients. Movement disorders : official journal of the Movement Disorder Society 2020;35:2230-2239.
[55] Bargar C, Wang W, Gunzler SA, et al. Streamlined alpha-synuclein RT-QuIC assay for various biospecimens in Parkinson's disease and dementia with Lewy bodies. Acta neuropathologica communications 2021;9:62.
[56] Tanei ZI, Saito Y, Ito S, et al. Lewy pathology of the esophagus correlates with the progression of Lewy body disease: a Japanese cohort study of autopsy cases. Acta neuropathologica 2021;141:25-37.
[57] Wang Z, Becker K, Donadio V, et al. Skin α-Synuclein Aggregation Seeding Activity as a Novel Biomarker for Parkinson Disease. JAMA neurology 2020;78:1-11.
[58] Mammana A, Baiardi S, Quadalti C, et al. RT-QuIC Detection of Pathological α-Synuclein in Skin Punches of Patients with Lewy Body Disease. Movement disorders : official journal of the Movement Disorder Society 2021;36:2173-2177.
[59] den HJW, Bethlem J. The distribution of Lewy

bodies in the central and autonomic nervous systems in idiopathic paralysis agitans. Journal of neurology, neurosurgery, and psychiatry 1960;23:283-290.
[60] Beck G, Hori Y, Hayashi Y, Morii E, Takehara T, Mochizuki H. Detection of Phosphorylated Alpha-Synuclein in the Muscularis Propria of the Gastrointestinal Tract Is a Sensitive Predictor for Parkinson's Disease. Parkinson's disease 2020;2020:4687530.
[61] Del Tredici K, Hawkes CH, Ghebremedhin E, Braak H. Lewy pathology in the submandibular gland of individuals with incidental Lewy body disease and sporadic Parkinson's disease. Acta neuropathologica 2010;119:703-713.
[62] Caporali R, Bonacci E, Epis O, Bobbio-Pallavicini F, Morbini P, Montecucco C. Safety and usefulness of minor salivary gland biopsy: retrospective analysis of 502 procedures performed at a single center. Arthritis and rheumatism 2008;59:714-720.
[63] Beach TG, Adler CH, Dugger BN, et al. Submandibular gland biopsy for the diagnosis of Parkinson disease. Journal of neuropathology and experimental neurology 2013;72:130-136.
[64] Adler CH, Dugger BN, Hinni ML, et al. Submandibular gland needle biopsy for the diagnosis of Parkinson disease. Neurology 2014;82:858-864.
[65] Gao L, Chen H, Li X, Li F, Ou-Yang Q, Feng T. The diagnostic value of minor salivary gland biopsy in clinically diagnosed patients with Parkinson's disease: comparison with DAT PET scans. Neurological sciences : official journal of the Italian Neurological Society and of the Italian Society of Clinical Neurophysiology 2015;36:1575-1580.
[66] Ma LY, Gao LY, Li X, Ma HZ, Feng T. Nitrated alpha-synuclein in minor salivary gland biopsies in Parkinson's disease. Neuroscience letters 2019;704:45-49.
[67] Mangone G, Houot M, Gaurav R, et al. Relationship between Substantia Nigra Neuromelanin Imaging and Dual Alpha-Synuclein Labeling of Labial Minor in Salivary Glands in Isolated Rapid Eye Movement Sleep Behavior Disorder and Parkinson's Disease. Genes 2022;13.
[68] Iranzo A, Borrego S, Vilaseca I, et al. α-Synuclein aggregates in labial salivary glands of idiopathic rapid eye movement sleep behavior disorder. Sleep 2018;41.
[69] Folgoas E, Lebouvier T, Leclair-Visonneau L, et al. Diagnostic value of minor salivary glands biopsy for the detection of Lewy pathology. Neuroscience letters 2013;551:62-64.
[70] Adler CH, Dugger BN, Hentz JG, et al. Peripheral Synucleinopathy in Early Parkinson's Disease: Submandibular Gland Needle Biopsy Findings. Movement disorders : official journal of the Movement Disorder Society 2016;31:250-256.
[71] Vilas D, Iranzo A, Tolosa E, et al. Assessment of α-synuclein in submandibular glands of patients with idiopathic rapid-eye-movement sleep behaviour disorder: a case-control study. The Lancet Neurology 2016;15:708-718.
[72] Sakashita Y, Matsubara T, Takata T, et al. Lewy pathology of the submandibular gland in Lewy body disease: A report of autopsy cases. Neuropathology : official journal of the Japanese Society of Neuropathology 2021;41:476-483.
[73] Manne S, Kondru N, Jin H, et al. α-Synuclein real-time quaking-induced conversion in the submandibular glands of Parkinson's disease patients. Movement disorders : official journal of the Movement Disorder Society 2020;35:268-278.
[74] Qualman SJ, Haupt HM, Yang P, Hamilton SR. Esophageal Lewy bodies associated with ganglion cell loss in achalasia. Similarity to Parkinson's disease. Gastroenterology 1984;87:848-856.
[75] Wakabayashi K, Takahashi H, Takeda S, Ohama E, Ikuta F. Parkinson's disease: the presence of Lewy bodies in Auerbach's and Meissner's plexuses. Acta neuropathologica 1988;76:217-221.
[76] Wakabayashi K, Takahashi H, Ohama E, Ikuta F. Parkinson's disease: an immunohistochemical study of Lewy body-containing neurons in the enteric nervous system. Acta neuropathologica 1990;79:581-583.
[77] Braak H, de Vos RA, Bohl J, Del Tredici K. Gastric alpha-synuclein immunoreactive inclusions in Meissner's and Auerbach's plexuses in cases staged for Parkinson's disease-related brain pathology. Neuroscience letters 2006;396:67-72.
[78] Bloch A, Probst A, Bissig H, Adams H, Tolnay M. Alpha-synuclein pathology of the spinal and peripheral autonomic nervous system in neurologically unimpaired elderly subjects. Neuropathology and applied neurobiology 2006;32:284-295.
[79] Del Tredici K, Duda JE. Peripheral Lewy body pathology in Parkinson's disease and incidental Lewy body disease: four cases. Journal of the neurological sciences 2011;310:100-106.
[80] Annerino DM, Arshad S, Taylor GM, Adler CH, Beach TG, Greene JG. Parkinson's disease is not associated with gastrointestinal myenteric ganglion neuron loss. Acta neuropathologica 2012;124:665-680.
[81] Hilton D, Stephens M, Kirk L, et al. Accumulation of α-synuclein in the bowel of patients in the pre-clinical phase of Parkinson's disease. Acta neuropathologica 2014;127:235-241.
[82] Stokholm MG, Danielsen EH, Hamilton-Dutoit SJ, Borghammer P. Pathological α-synuclein in gastrointestinal tissues from prodromal Parkinson disease patients. Annals of neurology

2016;79:940-949.
[83] Ruffmann C, Bengoa-Vergniory N, Poggiolini I, et al. Detection of alpha-synuclein conformational variants from gastro-intestinal biopsy tissue as a potential biomarker for Parkinson's disease. Neuropathology and applied neurobiology 2018;44:722-736.
[84] Punsoni M, Friedman JH, Resnick M, Donahue JE, Yang DF, Stopa EG. Enteric Pathologic Manifestations of Alpha-Synucleinopathies. Applied immunohistochemistry & molecular morphology : AIMM 2019;27:543-548.
[85] Sánchez-Ferro Á, Rábano A, Catalán MJ, et al. In vivo gastric detection of α-synuclein inclusions in Parkinson's disease. Movement disorders : official journal of the Movement Disorder Society 2015;30:517-524.
[86] Yan F, Chen Y, Li M, et al. Gastrointestinal nervous system α-synuclein as a potential biomarker of Parkinson disease. Medicine 2018;97:e11337.
[87] Chung SJ, Kim J, Lee HJ, et al. Alpha-synuclein in gastric and colonic mucosa in Parkinson's disease: Limited role as a biomarker. Movement disorders : official journal of the Movement Disorder Society 2016;31:241-249.
[88] Fenyi A, Leclair-Visonneau L, Clairembault T, et al. Detection of alpha-synuclein aggregates in gastrointestinal biopsies by protein misfolding cyclic amplification. Neurobiology of disease 2019;129:38-43.
[89] Shin C, Park SH, Yun JY, et al. Fundamental limit of alpha-synuclein pathology in gastrointestinal biopsy as a pathologic biomarker of Parkinson's disease: Comparison with surgical specimens. Parkinsonism & related disorders 2017;44:73-78.
[90] Chung CY, Seo H, Sonntag KC, Brooks A, Lin L, Isacson O. Cell type-specific gene expression of midbrain dopaminergic neurons reveals molecules involved in their vulnerability and protection. Human molecular genetics 2005;14:1709-1725.
[91] Pouclet H, Lebouvier T, Coron E, Des Varannes SB, Neunlist M, Derkinderen P. A comparison between colonic submucosa and mucosa to detect Lewy pathology in Parkinson's disease. Neurogastroenterology and motility 2012;24:e202-205.
[92] Forsyth CB, Shannon KM, Kordower JH, et al. Increased intestinal permeability correlates with sigmoid mucosa alpha-synuclein staining and endotoxin exposure markers in early Parkinson's disease. PloS one 2011;6:e28032.
[93] Gold A, Turkalp ZT, Munoz DG. Enteric alpha-synuclein expression is increased in Parkinson's disease but not Alzheimer's disease. Movement disorders : official journal of the Movement Disorder Society 2013;28:237-240.
[94] Shannon KM, Keshavarzian A, Dodiya HB, Jakate S, Kordower JH. Is alpha-synuclein in the colon a biomarker for premotor Parkinson's disease? Evidence from 3 cases. Movement disorders : official journal of the Movement Disorder Society 2012;27:716-719.
[95] Shannon KM, Keshavarzian A, Mutlu E, et al. Alpha-synuclein in colonic submucosa in early untreated Parkinson's disease. Movement disorders : official journal of the Movement Disorder Society 2012;27:709-715.
[96] Antunes L, Frasquilho S, Ostaszewski M, et al. Similar α-Synuclein staining in the colon mucosa in patients with Parkinson's disease and controls. Movement disorders : official journal of the Movement Disorder Society 2016;31:1567-1570.
[97] 9Harapan BN, Frydrychowicz C, Classen J, et al. No enhanced (p-) α-synuclein deposition in gastrointestinal tissue of Parkinson's disease patients. Parkinsonism & related disorders 2020;80:82-88.
[98] Lebouvier T, Neunlist M, Bruley des Varannes S, et al. Colonic biopsies to assess the neuropathology of Parkinson's disease and its relationship with symptoms. PloS one 2010;5:e12728.
[99] Barrenschee M, Zorenkov D, Böttner M, et al. Distinct pattern of enteric phospho-alpha-synuclein aggregates and gene expression profiles in patients with Parkinson's disease. Acta neuropathologica communications 2017;5:1.
[100] Emmi A, Sandre M, Russo FP, et al. Duodenal alpha-Synuclein Pathology and Enteric Gliosis in Advanced Parkinson's Disease. Movement disorders : official journal of the Movement Disorder Society 2023;38:885-894.
[101] Pouclet H, Lebouvier T, Coron E, Neunlist M, Derkinderen P. Lewy pathology in gastric and duodenal biopsies in Parkinson's Disease. Movement disorders : official journal of the Movement Disorder Society 2012;27:708.
[102] Bu J, Liu J, Liu K, Wang Z. Diagnostic utility of gut α-synuclein in Parkinson's disease: A systematic review and meta-analysis. Behavioural brain research 2019;364:340-347.
[103] Jotanovic J, Milin-Lazovic J, Alafuzoff I. Gastrointestinal Biopsy Obtained During Cancer Screening, a Biological Marker for α-Synucleinopathy? Journal of neuropathology and experimental neurology 2022;81:356-362.
[104] Sprenger FS, Stefanova N, Gelpi E, et al. Enteric nervous system α-synuclein immunoreactivity in idiopathic REM sleep behavior disorder. Neurology 2015;85:1761-1768.
[105] Pouclet H, Lebouvier T, Coron E, et al. Analysis of colonic alpha-synuclein pathology in multiple system atrophy. Parkinsonism & related disorders

2012;18:893-895.
[106] Shin JH, Park SH, Shin C, et al. Negative α-synuclein pathology in the submandibular gland of patients carrying PRKN pathogenic variants. Parkinsonism & related disorders 2020;81:179-182.
[107] Chung SJ, König IR, Lohmann K, et al. Association of SNCA variants with α-synuclein of gastric and colonic mucosa in Parkinson's disease. Parkinsonism & related disorders 2019;61:151-155.
[108] Duda JE, Shah U, Arnold SE, Lee VM, Trojanowski JQ. The expression of alpha-, beta-, and gamma-synucleins in olfactory mucosa from patients with and without neurodegenerative diseases. Experimental neurology 1999;160:515-522.
[109] Beach TG, White CL, 3rd, Hladik CL, et al. Olfactory bulb alpha-synucleinopathy has high specificity and sensitivity for Lewy body disorders. Acta neuropathologica 2009;117:169-174.
[110] Jellinger KA. Olfactory bulb alpha-synucleinopathy has high specificity and sensitivity for Lewy body disorders. Acta neuropathologica 2009;117:215-216; author reply 217-218.
[111] Arnold SE, Lee EB, Moberg PJ, et al. Olfactory epithelium amyloid-beta and paired helical filament-tau pathology in Alzheimer disease. Annals of neurology 2010;67:462-469.
[112] Funabe S, Takao M, Saito Y, et al. Neuropathologic analysis of Lewy-related α-synucleinopathy in olfactory mucosa. Neuropathology : official journal of the Japanese Society of Neuropathology 2013;33:47-58.
[113] Saito Y, Shioya A, Sano T, Sumikura H, Murata M, Murayama S. Lewy body pathology involves the olfactory cells in Parkinson's disease and related disorders. Movement disorders : official journal of the Movement Disorder Society 2016;31:135-138.
[114] Stevenson TJ, Murray HC, Turner C, Faull RLM, Dieriks BV, Curtis MA. α-synuclein inclusions are abundant in non-neuronal cells in the anterior olfactory nucleus of the Parkinson's disease olfactory bulb. Scientific reports 2020;10:6682.
[115] Witt M, Bormann K, Gudziol V, et al. Biopsies of olfactory epithelium in patients with Parkinson's disease. Movement disorders : official journal of the Movement Disorder Society 2009;24:906-914.
[116] Perra D, Bongianni M, Novi G, et al. Alpha-synuclein seeds in olfactory mucosa and cerebrospinal fluid of patients with dementia with Lewy bodies. Brain communications 2021;3:fcab045.
[117] Stefani A, Iranzo A, Holzknecht E, et al. Alpha-synuclein seeds in olfactory mucosa of patients with isolated REM sleep behaviour disorder. Brain : a journal of neurology 2021;144:1118-1126.
[118] Bongianni M, Catalan M, Perra D, et al. Olfactory swab sampling optimization for α-synuclein aggregate detection in patients with Parkinson's disease. Translational neurodegeneration 2022;11:37.
[119] Beach TG, Corbillé AG, Letournel F, et al. Multicenter Assessment of Immunohistochemical Methods for Pathological Alpha-Synuclein in Sigmoid Colon of Autopsied Parkinson's Disease and Control Subjects. Journal of Parkinson's disease 2016;6:761-770.
[120] Corbillé AG, Letournel F, Kordower JH, et al. Evaluation of alpha-synuclein immunohistochemical methods for the detection of Lewy-type synucleinopathy in gastrointestinal biopsies. Acta neuropathologica communications 2016;4:35.
[121] Corbillé AG, Preterre C, Rolli-Derkinderen M, et al. Biochemical analysis of α-synuclein extracted from control and Parkinson's disease colonic biopsies. Neuroscience letters 2017;641:81-86.
[122] Fernández-Espejo E, Rodríguez de Fonseca F, Suárez J, et al. Native α-Synuclein, 3-Nitrotyrosine Proteins, and Patterns of Nitro-α-Synuclein-Immunoreactive Inclusions in Saliva and Submandibulary Gland in Parkinson's Disease. Antioxidants (Basel, Switzerland) 2021;10.
[123] Carletti R, Campo F, Fusconi M, et al. Phosphorylated α-synuclein immunoreactivity in nerve fibers from minor salivary glands in Parkinson's disease. Parkinsonism & related disorders 2017;38:99-101.
[124] Cersósimo MG, Perandones C, Micheli FE, et al. Alpha-synuclein immunoreactivity in minor salivary gland biopsies of Parkinson's disease patients. Movement disorders : official journal of the Movement Disorder Society 2011;26:188-190.
[125] Borghammer P, Just MK, Horsager J, et al. A postmortem study suggests a revision of the dual-hit hypothesis of Parkinson's disease. NPJ Parkinson's disease 2022;8:166.
[126] Visanji NP, Marras C, Kern DS, et al. Colonic mucosal a-synuclein lacks specificity as a biomarker for Parkinson disease. Neurology 2015;84:609-616.
[127] Beach TG, White CL, Hamilton RL, et al. Evaluation of alpha-synuclein immunohistochemical methods used by invited experts. Acta neuropathologica 2008;116:277-288.
[128] Böttner M, Zorenkov D, Hellwig I, et al. Expression pattern and localization of alpha-synuclein in the human enteric nervous system. Neurobiology of disease 2012;48:474-480.
[129] V acchi E, Pinton S, Kaelin-Lang A, Melli G. Targeting Alpha Synuclein Aggregates in Cutaneous Peripheral Nerve Fibers by Free-floating

Immunofluorescence Assay. Journal of visualized experiments : JoVE 2019.
[130] Oueslati A. Implication of Alpha-Synuclein Phosphorylation at S129 in Synucleinopathies: What Have We Learned in the Last Decade? Journal of Parkinson's disease 2016;6:39-51.
[131] Bengoa-Vergniory N, Roberts RF, Wade-Martins R, Alegre-Abarrategui J. Alpha-synuclein oligomers: a new hope. Acta neuropathologica 2017;134:819-838.
[132] Skorvanek M, Gelpi E, Mechirova E, et al. α-Synuclein antibody 5G4 identifies manifest and prodromal Parkinson's disease in colonic mucosa. Movement disorders : official journal of the Movement Disorder Society 2018;33:1366-1368.
[133] Kovacs GG, Breydo L, Green R, et al. Intracellular processing of disease-associated α-synuclein in the human brain suggests prion-like cell-to-cell spread. Neurobiology of disease 2014;69:76-92.
[134] Roberts RF, Wade-Martins R, Alegre-Abarrategui J. Direct visualization of alpha-synuclein oligomers reveals previously undetected pathology in Parkinson's disease brain. Brain : a journal of neurology 2015;138:1642-1657.
[135] Saborio GP, Permanne B, Soto C. Sensitive detection of pathological prion protein by cyclic amplification of protein misfolding. Nature 2001;411:810-813.
[136] Atarashi R, Satoh K, Sano K, et al. Ultrasensitive human prion detection in cerebrospinal fluid by real-time quaking-induced conversion. Nature medicine 2011;17:175-178.
[137] Yoo D, Bang JI, Ahn C, et al. Diagnostic value of α-synuclein seeding amplification assays in α-synucleinopathies: A systematic review and meta-analysis. Parkinsonism & related disorders 2022;104:99-109.
[138] Engelhardt E, Gomes MDM. Lewy and his inclusion bodies: Discovery and rejection. Dementia & neuropsychologia 2017;11:198-201.
[139] Dickson DW, Braak H, Duda JE, et al. Neuropathological assessment of Parkinson's disease: refining the diagnostic criteria. The Lancet Neurology 2009;8:1150-1157.
[140] Schneider SA, Boettner M, Alexoudi A, Zorenkov D, Deuschl G, Wedel T. Can we use peripheral tissue biopsies to diagnose Parkinson's disease? A review of the literature. European journal of neurology 2016;23:247-261.
[141] Kupsky WJ, Grimes MM, Sweeting J, Bertsch R, Cote LJ. Parkinson's disease and megacolon: concentric hyaline inclusions (Lewy bodies) in enteric ganglion cells. Neurology 1987;37:1253-1255.
[142] Lebouvier T, Chaumette T, Damier P, et al. Pathological lesions in colonic biopsies during Parkinson's disease. Gut 2008;57:1741-1743.
[143] Devos D, Lebouvier T, Lardeux B, et al. Colonic inflammation in Parkinson's disease. Neurobiology of disease 2013;50:42-48.
[144] Aldecoa I, Navarro-Otano J, Stefanova N, et al. Alpha-synuclein immunoreactivity patterns in the enteric nervous system. Neuroscience letters 2015;602:145-149.
[145] Iranzo A, Stefani A, Serradell M, et al. Characterization of patients with longstanding idiopathic REM sleep behavior disorder. Neurology 2017;89:242-248.
[146] Shin J, Park SH, Shin C, et al. Submandibular gland is a suitable site for alpha synuclein pathology in Parkinson disease. Parkinsonism & related disorders 2019;58:35-39.
[147] Adler CH, Serrano GE, Zhang N, et al. Feasibility of repeat and bilateral submandibular gland needle biopsies in Parkinson's disease. Parkinsonism & related disorders 2019;68:69-72.
[148] Leclair-Visonneau L, Clairembault T, Volteau C, et al. Colonic neuropathology is not associated with autonomic dysfunction in Parkinson's disease. Parkinsonism & related disorders 2019;61:224-227.
[149] Toledo JB, Gopal P, Raible K, et al. Pathological α-synuclein distribution in subjects with coincident Alzheimer's and Lewy body pathology. Acta neuropathologica 2016;131:393-409.

BLOOD AND CEREBROSPINAL FLUID BIOMARKERS OF COGNITIVE IMPAIRMENT IN PARKINSON'S DISEASE

Kanellos C. Spiliopoulos[1], George D. Vavougios[1], Triantafyllos Doskas[1]

[1]Neurology Department, Athens Naval Hospital, Athens, Greece

Abstract

Parkinson's disease (PD) is the second most common neurodegenerative disease, characterized by dopaminergic neuronal loss in substantia nigra and α-synuclein accumulation in intraneuronal aggregates. Apart from the cardinal motor symptoms, non-motor features are also evident; among them, cognitive impairment is a consistent finding in PD patients, who are susceptible to an increased dementia risk. Progressing cognitive decline includes the stages of subjective cognitive decline, mild cognitive impairment (MCI) and dementia. Various mechanisms contribute to each of these stages, whereas responsible neuropathological correlates have been investigated in clinicopathological correlation studies. Longitudinal studies focus on the prognostic value of different molecules in assessments of cognitive decline over time. The composition of the cerebrospinal fluid (CSF) reflects brain metabolism and neuronal condition; hence, CSF proteins may be promising biomarkers of cognitive dysfunction mechanisms in PD. Plasma and serum studies have also revealed candidate biomarkers for assessing cognition in PD. Since MCI conversion to dementia is variable, biomarkers that enhance early identification of cognitive dysfunction factors and prediction of dementia risk are necessary. This review summarizes recent studies of promising blood and CSF biomarkers of PD-related cognitive impairment. Several correlates of neuronal damage have been shown indicative of poor cognitive performance and predicted cognitive deterioration, including amyloid-β and neurofilament light chain. Inflammatory factors, lysosomal dysfunction, oxidative stress and genetic variants could be also useful in assessing cognitive decline in PD. Future research is needed for the validation of the candidate biomarkers, recognizing the potential benefit of robust biomarkers in clinical practice and their implementation in clinical trials.

Key-words: Parkinson's disease; cognitive impairment; biomarkers; cerebrospinal fluid; blood

ΒΙΟΔΕΙΚΤΕΣ ΝΟΗΤΙΚΗΣ ΕΚΠΤΩΣΗΣ ΣΤΟ ΕΓΚΕΦΑΛΟΝΩΤΙΑΙΟ ΥΓΡΟ ΚΑΙ ΤΟ ΑΙΜΑ ΣΤΗ ΝΟΣΟ ΤΟΥ ΠΑΡΚΙΝΣΟΝ

Κανέλλος Σπηλιόπουλος[1], Γεώργιος Βαβουγιιός[1], Τριαντάφυλλος Ντόσκας[1]

[1]Νευρολογική Κλινική, Ναυτικό Νοσοκομείο Αθηνών, Αθήνα, Ελλάδα

Περίληψη

Η νόσος του Πάρκινσον (ΝΠ) αποτελεί τη δεύτερη συχνότερη νευροεκφυλιστική νόσο, η οποία χαρακτηρίζεται από την απώλεια ντοπαμινεργικών νευρώνων στην μέλαινα ουσία και την συσσώρευση α-συνουκλεΐνης σε ενδοκυττάρια νευρωνικά έγκλειστα. Πέραν των κινητικών συμπτωμάτων της νόσου, συνυπάρχουν επίσης μη-κινητικά συμπτώματα. Εξ αυτών, η νοητική έκπτωση είναι συνήθης σε ασθενείς με Πάρκινσον, οι οποίοι βρίσκονται σε αυξημένο κίνδυνο εμφάνισης άνοιας. Η προοδευτικά επιδεινούμενη νοητική έκπτωση περιλαμβάνει την υποκειμενική νοητική έκπτωση, την ήπια νοητική έκπτωση (MCI) και την άνοια. Νευροπαθολογικά υποστρώματα των υποκείμενων παθοφυσιολογικών μηχανισμών έχουν ερευνηθεί σε μελέτες κλινικοπαθολογικής συσχέτισης. Μελέτες παρακολούθησης επικεντρώνονται στην προγνωστική αξία δεικτών κατά την αξιολόγηση της προόδου της νοητικής έκπτωσης στον χρόνο. Ο μεταβολισμός του εγκεφάλου κι η υγεία του νευρώνα εκπροσωπούνται στο εγκεφαλονωτιαίο υγρό (ΕΝΥ), μέσω πρωτεϊνών οι οποίες συνεισφέρουν ως πιθανοί βιοδείκτες των μηχανισμών νοητικής δυσλειτουργίας στην ΝΠ. Επιπλέον, βιοδείκτες νοητικής έκπτωσης δύναται να ανευρεθούν στον ορό και το πλάσμα αίματος. Δεδομένης της ασταθούς μετάπτωσης από την MCI σε άνοια, οι βιοδείκτες είναι αναγκαίοι για την πρώιμη ανίχνευση γνωστικών ελλειμμάτων και την πρόβλεψη του κινδύνου άνοιας. Η παρούσα ανασκόπηση συνοψίζει πρόσφατες μελέτες υπό-διερεύνηση βιοδεικτών, στο αίμα και το ΕΝΥ, της νοητικής έκπτωσης στην ΝΠ. Διάφοροι δείκτες νευρωνικής βλάβης έχουν συσχετιστεί με πτωχή νοητική λειτουργία και προβλέπουν γνωστική επιδείνωση, όπως το β-αμυλοειδές και τα νευροϊνίδια. Φλεγμονώδεις παράγοντες, το οξειδωτικό στρες, η λυσοσωμιακή δυσλειτουργία και γενετι-

κές παραλλαγές συνεισφέρουν επίσης στην νοητική έκπτωση. Μελλοντικές μελέτες είναι αναγκαίες ώστε να εδραιωθούν οι υποψήφιοι βιοδείκτες, δεδομένου του οφέλους που θα συνεισφέρουν στην κλινική πράξη και τον σχεδιασμό κλινικών μελετών.

Λέξεις-κλειδιά:νόσος του Πάρκινσον, νοητική έκπτωση, βιοδείκτες, εγκεφαλονωτιαίο υγρό, αίμα

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder, which is characterized by the loss of dopaminergic neurons in the substantia nigra (SN) and the subsequent pathological accumulation of a-synuclein in intraneural cellular inclusion [1]. These pathological protein aggregates are known as Lewy bodies. Apart from proteinopathy, several mechanisms contribute to the pathogenesis of PD,including Alzheimer's disease (AD)- related pathology, neuroinflammation, oxidative stress and neurotransmitter deficiency, as well as genetic variations [2]. The cardinal motor features of PD are bradykinesia, tremor and rigidity, which are primarily associated with striatal dopaminergic degeneration in SN. Apart from these substantial entities, non-motor symptoms of PD are equally involved in the disease related burden and disability. Cognitive impairment is a highly prevalent clinical feature in PD, affecting even newly diagnosed patients with PD [1].

PD individuals are considered to be at a higher risk, up to 6 times, of developing dementia as compared to similar aged non-PD individuals [2]. The spectrum of cognitive dysfunction in PD ranges accordingly from subjective cognitive decline (SCD) to mild cognitive impairment (MCI) and finally to dementia. Although SCD refers to signs of memory deficits that are self-reported and for which patients complain, conversion to MCI requires a diagnostic validation process. Recently, PD-MCI diagnostic criteria were established, defining MCI as SCD reported by patients, caregivers or physicians which is evident by deficits in neuropsychological evaluation, but does not interfere with significant functional independence [3]. Despite the fact that MCI is considered a transitional stage between normal cognition and dementia, the MCI course is variable and time interval to dementia is not definite, since not all patients become demented. Around 60% of PD-MCI individuals have been reported to develop dementia during a 5-year follow-up, reflecting the natural history of cognitive decline in PD [4]. Conversion from MCI to dementia in PD (PDD) is in the majority of cases an inevitable clinical event, determined by certain diagnostic criteria [5] which substantiate cognitive decline in more than one cognitive domain, which should be significant enough in order to negatively influence daily life and lasting for at least 6 months [2].

Neuropsychological assessment is required in order to diagnose cognitive impairment in PD. Both instruments of global cognition evaluation and more detailed neuropsychological tools are also used in clinical practice in order to monitor cognitive dysfunction and to determine the affected domains. Commonly used cognitive screening in PD includes the Montreal Cognitive Assessment (MoCA) [6], Mini Mental State Examination (MMSE), Mattis Dementia Rating Scale Second Edition (MDRS-2), Parkinson's Disease- Cognitive Rating Scale (PD-CRS)[7] and Scales for Outcomes in Parkinson's Disease- Cognition (SCOPA-COG)[8]. More targeted neuropsychological tools are implemented in the assessment of the five core cognitive domains: attention and working memory, executive, language, memory and visuospatial function [3].

Identification of cognitive decline in early stages is crucial in order to predict future disease progression and to design therapeutic interventions, aiming to prevent rapid cognitive decline or even to stabilize the cognitive status over a period. For this purpose, the interest in identifying biomarkers of cognitive impairment in PD has been increased in the last decade [9, 10]. Valuable biomarkers, i.e. objective and quantifiable parameters, of cognitive function in PD should either associate with pathological processes of the disease and discriminate between cognitive impaired (PD-CI) and non- cognitive impaired (PD-NCI) PD patients, or predict cognitive decline and the conversion to dementia.

Methods

Herein, we investigated the current literature to review candidate molecular biomarkers of cognitive dysfunction in PD. Our PubMed search was based on articles published from January 2010 to September 2023, in English language. Specific terms were used in order to form accurate searching algorithms, including: ("Parkinson's disease" OR PD) AND biomarker* AND ("cerebrospinal fluid" OR CSF OR serum OR blood OR plasma) AND ("cognitive impairment" OR "cognitive decline").

α-synuclein

The aggregates of α-synuclein are definite hallmarks of PDD neuropathology via their deposition in brainstem and olfactory domains, resulting in synaptic dysfunction due to loss of monoaminergic and

cholinergic neurons. The infiltration of limbic system (parahippocampal) and neocortex, involving frontal and temporal structures, with α-synuclein aggregates is associated with the development of cognitive impairment. Furthermore, in PD, α-synuclein pathology interacts with DNA repair processes, affecting neuronal DNA. Involvement of α-synuclein in neuroinflammation has been also suggested, with recent evidence supporting the role of α-synuclein in the activation of type 1 interferon, promoting neurodegeneration [11]. α-Synuclein aggregation is a complicated procedure, involving multiple protein-protein interactions, of which phosphorylation seems to promote LB formation and neuronal degeneration [12]. Apart from idiopathic PD, genetic variations of the SNCA gene, the encoding gene of α-synuclein, are directly associated with pathological α-synuclein isoforms and the increased dementia risk. Other PD-related genes, including LRRK2 and GBA, have been related to α-synucleinpathology as well, promoting aggregation via phosphorylation (LRRK2) and stabilizing soluble oligomeric intermediates (GBA).

CSF levels of α-synuclein have been studied as biomarkers of cognitive decline in PD, with evidence by relevant studies being inconsistent. In CSF studies, levels of α-synuclein have been found comparable or lower in PD subjects than in healthy controls [13-15]. Early studies have shown that increased CSF concentrations of α-synuclein predicted the progression of cognitive decline over time, as shown, among other studies, by the DATATOP cohort, where PD subjects with higher CSF α-synuclein had a faster cognitive decline [16-18]. Lower CSF α-synuclein was significantly associated with reduced performance on executive/attention domains and decreased composite cognitive score in the study by Skogseth et el. [15], as well as with deficits in phonetic fluency [19]. Higher baseline CSF α-synuclein concentrations were also related to worse performance in longitudinal assessments of affective and executive functioning domains [20]. In contrary, it was also showed that concentrations of α-synuclein were lower in PD-CI as compared to PD-NCI subjects [21]. Several studies revealed none significant relationship between CSF α-synuclein levels and cognitive decline, neither in single baseline measurements nor in longitudinal assessments, in PDD and PD-MCI subjects [13, 14, 18, 22-25]. Apart from total α-synuclein, posttranslational forms (i.e. ubiquinated, phosphorylated, nitrated or oligomeric forms) could indicate cognitive decline. Oligomeric α-synuclein levels in CSF have been found to be elevated in PDD subjects as compared to controls, yet no association was detected with cognitive deficits [19, 26]. Higher CSF phosphorylated α-synuclein and the ratio of phosphorylated-α-synuclein/total-α-synuclein were correlated with better executive functioning [27].

Elevated plasma levels of α-synuclein have been found in PD subjects as compared to healthy controls in several studies [28-31], while PD-CI subjects had also a higher plasma α-synuclein as compared to PD-NCI [29-31]. Performance in frontal lobe-mediated tasks was linked to plasma α-synuclein levels [31]. Higher plasma α-synuclein was associated with an increased risk of PD-MCI [30]. Plasma α-synuclein has been either positively or negatively correlated to MMSE score [29, 32], whereas lower plasma α-synuclein was indicative of cognitive decline in MoCA, FAB and RAVLT assessments [33]. Blommer et al. detected a lower neuronal extracellular vesicle α-synuclein in PD-CI as compared to PD-NCI subjects [34].

β-Amyloid

Apart from α-synuclein aggregates which constitute the hallmark of PD pathogenesis, it is well established that AD-related pathology contributes to cognitive impairment in PD, via extracellular β-amyloid (Aβ) and intracellular tau accumulation and deposition [2]. The overlap between the two neurodegenerative diseases involves Aβ plaques and tau tangles and was originally identified in post-mortem histological studies, which detected Aβ deposition in cortical and subcortical regions in about 50% of PDD subjects. Almost 1/3 of total PDD subjects had severe tau pathology in hippocampal and neocortical domains [35]. AD pathology is suggested to accelerate the progressing cognitive decline in PD via amyloid angiopathy and neuroinflammation, reflecting deficits in multiple cognitive domains [2, 35]. Given the established role of Aβ as biomarker in AD, several studies investigated the diagnostic and predictive value of this molecule in assessing cognition impairment in PD, both in cross-sectional and longitudinal studies. Several research groups investigating PDD individuals found that CSF $A\beta_{42}$ levels are commonly decreased in demented patients as compared to non-demented patients and/or healthy controls [19, 21, 36, 37]. A meta-analysis by Hu et al. suggested that CSF $A\beta_{42}$ was primarily associated with cases of PD-CI rather than PD-MCI individuals, since evidence from different studies also varied regarding comparisons of Aβ42 levels between mild cognitive impairment and normal cognition [38].

Studies have shown the association between CSF $A\beta_{42}$ and deficits both in global cognition and individual cognitive domains. Deficits in both MoCA and MMSE scores have been associated with low CSF $A\beta_{42}$ levels in PD [39, 40]. Decreased CSF $A\beta_{42}$ was related to deficits in attention [27, 41, 42], working memory [41], phonemic fluency [43], conceptualization [42], initiation/ preservation [42], memory [14, 42] and response inhibition [14]. Furthermore, Zarifkar et al. found significant correlations between low CSF $A\beta_{42}/A\beta_{40}$ ratio and impairment in attention/ executive function-

ing and language [44]. Higher prevalence of positive amyloidosis profile (low $A\beta_{42}/A\beta_{40}$) in CSF was also reported in PDD as compared to PD-MCI and healthy individuals [45]. $A\beta_{42}$/neurogranin ratio was described as a valuable marker to discriminate between PD-CI and PD-NCI patients, reflecting processes of synaptic dysfunction [46]. Evidence from studies investigating plasma $A\beta_{42}$ levels did not provide a definite association to impaired cognitive performance [30]. However, in the recent study by Lin et al., higher baseline $A\beta_{40}$ predicted a faster cognitive decline [47]. Plasma $A\beta_{40}$ has been associated with impaired cognition; yet, a disagreement between studies should be noted since plasma $A\beta_{40}$ concentrations have been described both increased and decreased in PD [48-50]. A potential advance in Aβ investigations as biomarker in PD was suggested by the findings in the novel study by Wang et al., where $A\beta_{42}$-containing platelet-derived extracellular vesicles (EV) were higher in PDD, as detected using a nano-scale flow cytometry assay [51]. Plasma EV $A\beta_{42}$ was previously shown increased in PD-CI as compared to PD-NCI patients [52].

The value of CSF $A\beta_{42}$ in predicting cognitive dysfunction in PD individuals has been the objective of many studies in the last decade. Overall, $A\beta_{42}$ should be considered an independent prognostic factor of cognitive decline in PD, since evidence from various studies, using different outcome measures and time-frames of the longitudinal assessments, showed that low CSF $A\beta_{42}$ in baseline measurements predicts cognitive impairment and progression to PDD, as well as time to dementia [22, 23, 25, 53-56]. In particular, findings by the Parkinson's Progression Markers Initiative (PPMI) cohort showed gradually decreased CSF $A\beta_{42}$ concentrations during disease progression and association between lower baseline CSF $A\beta_{42}$ and cognitive decline in a 3-year follow-up [53]. Baseline measures of CSF $A\beta_{42}$ were also found to predict memory deficits [22].

Total tau (t-tau) and Phosphorylated 181 tau (p-tau)

In contrast with the relatively definite role of Aβ in the development of cognitive dysfunction and dementia in PD, the contribution of tau in this process is yet to be clarified. Contradictory findings among individual studies demonstrate either increased or decreased [21, 37] levels of CSF t-tau and p-tau in cognitive impaired PD individuals. Notably, a meta-analysis including 590 PD-CI and 1182 PD-NCI patients detected elevated CSF t-tau and p-tau in presence of dementia [38]. Plasma t-tau has been related to cognitive dysfunction [49], including deficits in attention and executive functioning [15, 57] and visuospatial function [15]. Higher CSF p-tau related to worse language functioning in the study by Oosterveld et al. [27], whereas also predicted longitudinal impairments in memory and executive functioning [58]. Increased plasma p-tau in baseline measurements has been shown predictive of faster cognitive decline over time [47], while CSF p-tau increase during disease progression also related to faster cognitive decline [18]. However, other studies failed to identify significant relationships between t-tau, p-tau [55] and impaired cognition in the PD examined population [39, 50, 59]. A recent study revealed significant association of plasma EV tau with cognitive function, using the technological advances in immunoassay field [52].

The combination of AD-pathology markers, in terms of tau/Aβ ratios, seems to generate promising biomarkers of cognitive dysfunction in PD, as revealed by the findings of different studies. High baseline CSF p-tau/$A\beta_{42}$ was associated with faster cognitive decline [60] and subsequent memory and executive function deficits [58], whereas t-tau/$A\beta_{42}$ related to progression to dementia [61]. Plasma tau/$A\beta_{42}$ ratio has been correlated to posterior cortical-mediated tasks [31].

Neurofilament light chain (NfL)

Neurodegeneration and axonal damage result in the release of various subunits of neurofilaments in the interstitial space of CNS. NfL is a cytoskeletal protein, expressed in both central and peripheral neurons, whose injury and degeneration leads to increased CSF and blood concentrations of NfL [62].

Recently, advances were described in the investigation, both in CSF and plasma, of NfL as a biomarker of cognitive impairment in PD, utilizing the development of ultrasensitive techniques to measure these molecules. In the study by Bäckström et al., high CSF NfL in baseline measurements predicted progression to PDD in a 1-year follow-up assessment [23], a finding also observed in later longitudinal studies, which also associated faster cognitive decline to higher baseline CSF NfL [24, 63, 64]. Increased CSF NfL concentrations have been related to worse cognitive performance, in terms of worse MoCA score, as well as deficits in memory, attentional and executive functioning [13, 27, 65]. The value of plasma and serum NfL as a biomarker of cognitive decline has been also shown in recent studies, providing similar findings to that of CSF studies. High plasma NfL levels were associated with thinner temporal and insular cortical thickness, reflecting also posterior cortical neurodegeneration [28, 66]. Higher serum and plasma NfL was associated with worse cognitive performance, i.e. decreased MoCA and multiple cognitive domain scores, and was related to an increased risk of progression to PDD [28, 67-72]. Longitudinal studies of PD subjects showed that serum/plasma NfL increases over time during the disease progression and higher baseline NfL levels predict the cognitive decline in follow-up evaluations [66, 69, 71-73]. Serum/plasma NfL has been

associated not only to impaired global cognition but also to deficits in episodic memory, visuospatial functioning, executive functioning, processing speed, attention and language/verbal fluency [70, 72].

Neuroinflammation

Inflammatory markers

It has been hypothesized that inflammation contributes in the pathological processes of PD progression. Both central-nervous and peripheral, innate and adaptive immune system activation is considered to influence PD pathophysiology, promoted by activated microglia and α-synuclein-induced cytokine production [74]. Based on this hypothesis recent studies investigated the role inflammatory markers as biomarkers of impaired cognition in PD. Higher serum IL-6 levels were reported in PD subjects with cognitive deficits as compared to non-cognitive impaired patients, while significant negative correlations were shown between serum and CSF IL-6, MoCA score and cognitive speed [75-77]. Furthermore, IL-8 and IL-18were also associated with impaired cognitive performance [78-80]. Tumor necrosis factor-a (TNF-a) has been shown as a potential biomarker of cognitive impairment, since higher serum TNF-a related to worse MoCA scores and higher CSF TNF-a in baseline measurements could predict a faster cognitive decline [79, 80]. In investigations of various inflammatory mediators, lower MoCA scores were associated with higher CSF levels of ICAM-1, MCP-1, MIP-1 beta, FABP, SCF and higher serum levels of CA-125, while higher SAA and C-reactive-protein (CRP) were related to worse performance on global cognition, cognitive speed and attention assessments [76, 79, 80]. Interestingly, in the study by Shen et al. a predictive model of measuring three plasma proteins (melanoma inhibitory activity protein, CRP and albumin) identified accurately PD individuals of high risk to develop cognitive impairment [81]. Fibrinogen was also found upregulated in PD-CI subjects and correlated negatively to Wisconsin card sorting score [82].

Other proteins associated with cognitive impairment in PD

Glucoprotein Chitinase-3-like protein 1, also known as YKL-40, as marker of glial activation and inflammation expressed in microglia and astrocytes, was demonstrated to increase in CSF of PD patients in 2-year longitudinal measurements, whereas this increase was associated with a faster cognitive decline [13, 18]. Another microglial activation marker, the soluble fragment of triggering receptor expressed on myeloid cells 2 (sTREM2), could contribute as biomarker in the prediction of cognitive decline in PD, since higher baseline CSF sTREM2 predicted greater future impairment in global cognition [13, 24, 83]. Elevated concentrations of CSF sTREM2 were measured in subgroups of PD patients with a positive CSF tau profile, but not with Aβ [84]. Also derived from astrocytes, glial fibrillary acidic protein (GFAP) was recently investigated regarding its role in cognitive deterioration to dementia. High CSF GFAP at baseline predicted cognitive decline and dementia during longitudinal evaluations [13, 85]. Similarly, elevated plasma GFAP was associated with worse cognitive scores [86], independently contributed to PDD [67] and predicted PD-MCI to PDD conversion [87].

The inflammation-derived oxidative stress is postulated to play a significant role in PD pathogenesis and neurodegeneration, as well as to the potential development of cognitive impairment in PD, since studies also showed associations of worse cognitive performance with high CSF levels of hydroxyl radical (•OH) and low serum nitric oxide levels [75, 88]. As a consequence of cellular oxidative stress in PD, the activated phospholipases cause the lysis of cell membranes and the subsequent release of phospholipids (PL). Increased plasma PL were strongly associated with impaired cognitive performance [89]. Furthermore, abnormal lipid perioxidation may affect cognition in PD. Elevated serum high-density lipoprotein (HDL) levels were associated with worse cognitive performance in ACE-R and SCOPA-COG assessments in females PD patients [90]. Another study described higher levels of total cholesterol, triglyceride and apolipoprotein A1 as independent predictors of mild cognitive impairment in PD [91]. Moreover, potential predictive biomarkers, for which underlying mechanisms to develop dementia in PD are unclear, include low estimated glomerular filtration rate (eGFR) [92] and low serum uric acid [10, 93]. Higher serum homocysteine concentration at baseline also predicted declining MoCA scores in 54-month follow up [94]. Decreased levels of vitamin D were associated with impaired cognition in PD, being valuable in predicting development of mild cognitive impairment over a 48-month period [95, 96].

In the study by Martin-Ruiz et al. investigating senescence and inflammatory markers, higher p16 expression predicted cognitive decline in a 36-month follow-up, as well as shorter telomeres related to dementia over the same period [77]. Various other proteins have been recently associated with cognitive dysfunction in PD: increased acidic isoforms of CSF serpinA1, an acute phase protein, indicated a higher PDD risk [97]; plasma exosomal prion protein concentration was found elevated in PD-CI as compared to PD-NCI and healthy individuals, whereas a significant correlation to the MoCA score was detected [98].

Growth factors

Brain-derived neurotrophic factor (BDNF) is involved in the regulation of dopaminergic neuron

survival and preservation of synaptic plasticity, triggering researchers to explore its role in PD. BDNF plasma levels were positively correlated to cognitive performance assessed by MoCA/MMSE tests [99, 100], as well as in attention, executive, working memory and self-monitoring/inhibition domains [101]. Low concentration of plasma epidermal growth factor (EGF) has been linked to poor performance in cognitive tests, including domains of semantic fluency, verbal memory, attention/executive function and visuospatial abilities, while low baseline plasma EGF also predicted progression to cognitive impairment and dementia [102-104]. Furthermore, decreased plasma levels of glial cell line-derived neutrophic factor (GDNF), a protective neurotrophic factor for dopaminergic neurons, discriminated between PD-CI and PD-NCI patients [105] and could potentially used as biomarker of executive function in PD, including deficits in inhibitory control, cognitive flexibility, and attention performances [106]. Insulin-like growth factor 1 (IGF-1) was associated with poor performance on global cognition and executive tasks [107, 108], with low baseline IGF-1 showing also prognostic value for faster cognitive decline, including attention/executive and verbal memory performance [108].

Genetics

Genetic variations are involved in PD pathogenesis and the determination of the underlying genetic basis in PD is important in order to predict cognitive trajectories. Although Parkin gene- and LRRK2-related PD cases are considered to be associated with a lower risk of cognitive dysfunction, the opposite seems to apply to genetic polymorphisms that affect the encoding of α-synuclein (SNCA gene) and β-glucocerebrosidase (GBA gene) [9]. Apart from gene mutations, transcriptional and post-transcriptional products in plasma and CSF could provide novel insights in search of cognitive biomarkers in PD.

The ε4 allele of APOE, the encoding gene of apolipoprotein E, was associated with the incidence and progression of cognitive dysfunction in PD [10, 109]. Recently, in a genome-wide survival meta-analysis of 3923 PD individuals, APOE ε4 was characterized as a major risk factor of dementia development in PD, whereas a novel locus within APOE and LRP1B gene was also predictive of dementia in PD [110]. E4 allelle was associated with deficits of memory, attention/executive function and language, restricted to learning and semantic verbal fluency impairment in the non-demented subgroup [111], while APOE e4 was related to executive dysfunction in PDMCI patients [112]. A faster deterioration in visuospatial function was also detected for ε4+ PD individuals [22]. The APOE ε4 allele is considered to influence Aβ deposition and other AD-related changes, as suggested by evidence of prognostic correlations between higher baseline Aβ, p-tau and faster cognitive decline in ε4+ carriers [47].

Involved in the degradation of dopamine, genetic variations of the catechol O-methyltransferase (COMT) gene have been linked to cognitive function in PD [113]. Specifically, COMT Val/Val polymorphisms increase dopamine catabolism and predicted cognitive impairment in longitudinal assessments of de novo PD individuals [54], as well as a faster decline in executive function, verbal learning and memory [114]. Polymorphisms in the tau-related MAPT gene were also indicative of cognitive impairment in PD, with the H1/H1 being associated with an increased risk of dementia development [109]. SNCA gene is related to autosomal dominant PD, often characterized by prominent cognitive dysfunction [9]. In a recent study, non carrier status of SNCA rs6826785 single nucleotide polymorphism was identified as an increased risk parameter for mild cognitive impairment in PD [115].

Reduced α-synuclein degradation and pathological accumulation in the process of PD could be promoted by dysfunction of the lysosomal-autophagy system. This pathophysiological pathway is linked to mutations in the encoding gene of β-glucocerebrosidase, the GBA gene, whose pathological variants are regarded as genetic risk factors for cognitive impairment and dementia in PD [110, 116, 117], associated with deficits in working memory, visuospatial and executive functions [118]. Lysosomal dysfunction in PD was further supported by the findings of Parnetti et al., who reported that reduced activity of CSF β-glucocerebrosidase in PD independently of GBA carrier status, whereas dysfunction of CSF β-glucocerebrosidase and β-hexosaminidase associated with worse cognitive performance, supporting the potential of lysosomal enzymes as biomarkers of cognitive decline in PD [119]. Interestingly, a higher CSF glucocerebrosidase/ sphingomyelin ratio was able to predict faster cognitive decline in idiopathic PD individuals in longitudinal MoCA assessments [120].

The exponential research interest in genetic traits that implicate with disease progression led to the investigation of further genetic variants as predictors of cognitive impairment in PD. Klotho longevity gene was found to interfere with cognitive dysfunction, since in PD carriers of the KL-VS haplotype the interval between disease onset and the onset of cognitive impairment was shorter [121]. Single nucleotide polymorphisms (SNPs) of the aquaporin-4 gene (*AQP4*) were also associated to cognitive performance of PD patients; *AQP4* rs162009 SNP was found protective against cognitive decline, relating to better performance in letter-number sequencing test and SDMT; yet, PD patients with *AQP4* rs68006382 SNP demonstrated a faster progression to mild cognitive impairment and worse performance in letter-number sequencing, semantic fluency, and SDMT [122].

Conclusions

Our narrative review aimed to identify appropriate biomarkers of cognitive impairment in PD, detected in CSF and/or blood. Despite contradictory evidence regarding the role of α-synuclein as biomarker of cognitive dysfunction in PD, reported associations with MCI and dementia risk are evident. Research of posttranslational isoforms could provide insights in more appropriate markers of cognitive impairment, utilizing technological advances in immunoassays. Aβ is considered more established as indicator of worse cognition in PD and predictor of cognitive decline, while tau protein was inconsistent in corresponding associations. Ratios between CSF and plasma α-synuclein, Aβ and tau could further explored as cognitive biomarkers, since promising results has been reported [61]. Recently introduced in PD cognitive impairment course, studies of NfL demonstrated that increased CSF and plasma NfL concentrations could be considered reliable biomarker of worse cognitive performance as well as in predicting future cognitive deterioration. Furthermore, inflammatory mediators, oxidative stress markers and growth factors have been systematically related to impaired cognition in PD, reflecting pathophysiological properties in neuronal degeneration and injury.

Genetic predisposition is an independent risk factor for the progressing cognitive dysfunction in PD course, even in idiopathic PD cases. The *APOE* ε4 allele, *SNCA* and *GBA* mutations result in cognitive deficits in multiple domains, with a prognostic value regarding PDD development. *COMT* and *MAPT* polymorphisms could also indicate cognitive impairment, while recent studies revealed cognitive associations for *AQP4* and Klotho genes. Advances in genetic medicine are emerging and future studies could introduce new candidate biomarkers.

Future research is necessary for the validation of the various promising biomarkers, described in our review. Early detection of cognitive deficits as well as the prediction of cognitive decline are key elements for the development of targeted therapeutic interventions. Accordingly, robust biomarkers could be implemented in clinical trials as monitoring estimates.

References

[1] Fang, C., L. Lv, S. Mao, H. Dong, and B. Liu, *Cognition Deficits in Parkinson's Disease: Mechanisms and Treatment.* Parkinsons Dis, 2020. 2020: p. 2076942.doi:10.1155/2020/2076942

[2] Aarsland, D., L. Batzu, G.M. Halliday, G.J. Geurtsen, C. Ballard, K. Ray Chaudhuri, et al., *Parkinson disease-associated cognitive impairment.* Nat Rev Dis Primers, 2021. 7(1): p. 47.doi:10.1038/s41572-021-00280-3

[3] Litvan, I., J.G. Goldman, A.I. Tröster, B.A. Schmand, D. Weintraub, R.C. Petersen, et al., *Diagnostic criteria for mild cognitive impairment in Parkinson's disease: Movement Disorder Society Task Force guidelines.* Mov Disord, 2012. 27(3): p. 349-56.doi:10.1002/mds.24893

[4] Pedersen, K.F., J.P. Larsen, O.B. Tysnes, and G. Alves, *Natural course of mild cognitive impairment in Parkinson disease: A 5-year population-based study.* Neurology, 2017. 88(8): p. 767-774.doi:10.1212/wnl.0000000000003634

[5] Emre, M., D. Aarsland, R. Brown, D.J. Burn, C. Duyckaerts, Y. Mizuno, et al., *Clinical diagnostic criteria for dementia associated with Parkinson's disease.* Mov Disord, 2007. 22(12): p. 1689-707; quiz 1837.doi:10.1002/mds.21507

[6] Nasreddine, Z.S., N.A. Phillips, V. Bédirian, S. Charbonneau, V. Whitehead, I. Collin, et al., *The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment.* J Am Geriatr Soc, 2005. 53(4): p. 695-9. doi:10.1111/j.1532-5415.2005.53221.x

[7] Pagonabarraga, J., J. Kulisevsky, G. Llebaria, C. García-Sánchez, B. Pascual-Sedano, and A. Gironell, *Parkinson's disease-cognitive rating scale: a new cognitive scale specific for Parkinson's disease.* Mov Disord, 2008. 23(7): p. 998-1005. doi:10.1002/mds.22007

[8] Marinus, J., M. Visser, N.A. Verwey, F.R. Verhey, H.A. Middelkoop, A.M. Stiggelbout, et al., *Assessment of cognition in Parkinson's disease.* Neurology, 2003. 61(9): p. 1222-8. doi:10.1212/01.wnl.0000091864.39702.1c

[9] Kalia, L.V., *Biomarkers for cognitive dysfunction in Parkinson's disease.* Parkinsonism Relat Disord, 2018. 46 Suppl 1: p. S19-s23. doi:10.1016/j.parkreldis.2017.07.023

[10] Delgado-Alvarado, M., B. Gago, I. Navalpotro-Gomez, H. Jiménez-Urbieta, and M.C. Rodriguez-Oroz, *Biomarkers for dementia and mild cognitive impairment in Parkinson's disease.* Mov Disord, 2016. 31(6): p. 861-81.doi:10.1002/mds.26662

[11] Hinkle, J.T., J. Patel, N. Panicker, S.S. Karuppagounder, D. Biswas, B. Belingon, et al., *STING mediates neurodegeneration and neuroinflammation in nigrostriatal α-synucleinopathy.* Proc Natl Acad Sci U S A, 2022. 119(15): p. e2118819119.doi:10.1073/pnas.2118819119

[12] Fan, T.S., S.C. Liu, and R.M. Wu, *Alpha-Synuclein and Cognitive Decline in Parkinson Disease.* Life (Basel), 2021. 11(11).doi:10.3390/life11111239

[13] Bartl, M., M. Dakna, D. Galasko, S.J. Hutten, T. Foroud, M. Quan, et al., *Biomarkers of neurodegeneration and glial activation validated in Alzheimer's disease assessed in longitudinal cerebrospinal fluid samples of Parkinson's dis-*

ease. PLoS One, 2021. 16(10): p. e0257372. doi:10.1371/journal.pone.0257372
[14] Stav, A.L., D. Aarsland, K.K. Johansen, E. Hessen, E. Auning, and T. Fladby, *Amyloid-β and a-synuclein cerebrospinal fluid biomarkers and cognition in early Parkinson's disease.* Parkinsonism Relat Disord, 2015. 21(7): p. 758-64. doi:10.1016/j.parkreldis.2015.04.027
[15] Skogseth, R.E., K. Bronnick, J.B. Pereira, B. Mollenhauer, D. Weintraub, T. Fladby, et al., *Associations between Cerebrospinal Fluid Biomarkers and Cognition in Early Untreated Parkinson's Disease.* J Parkinsons Dis, 2015. 5(4): p. 783-92. doi:10.3233/jpd-150682
[16] Hall, S., Y. Surova, A. Öhrfelt, H. Zetterberg, D. Lindqvist, and O. Hansson, *CSF biomarkers and clinical progression of Parkinson disease.* Neurology, 2015. 84(1): p. 57-63.doi:10.1212/wnl.0000000000001098
[17] Stewart, T., C. Liu, C. Ginghina, K.C. Cain, P. Auinger, B. Cholerton, et al., *Cerebrospinal fluid a-synuclein predicts cognitive decline in Parkinson disease progression in the DATATOP cohort.* Am J Pathol, 2014. 184(4): p. 966-975.doi:10.1016/j.ajpath.2013.12.007
[18] Hall, S., Y. Surova, A. Öhrfelt, K. Blennow, H. Zetterberg, and O. Hansson, *Longitudinal Measurements of Cerebrospinal Fluid Biomarkers in Parkinson's Disease.* Mov Disord, 2016. 31(6): p. 898-905.doi:10.1002/mds.26578
[19] Compta, Y., T. Valente, J. Saura, B. Segura, ú Iranzo, M. Serradell, et al., *Correlates of cerebrospinal fluid levels of oligomeric- and total-a-synuclein in premotor, motor and dementia stages of Parkinson's disease.* J Neurol, 2015. 262(2): p. 294-306.doi:10.1007/s00415-014-7560-z
[20] Emdina, A., P. Hermann, D. Varges, S. Nuhn, S. Goebel, T. Bunck, et al., *Baseline Cerebrospinal Fluid a-Synuclein in Parkinson's Disease Is Associated with Disease Progression and Cognitive Decline.* Diagnostics (Basel), 2022. 12(5). doi:10.3390/diagnostics12051259
[21] Tao, M., K. Dou, Y. Xie, B. Hou, and A. Xie, *The associations of cerebrospinal fluid biomarkers with cognition, and rapid eye movement sleep behavior disorder in early Parkinson's disease.* Front Neurosci, 2022. 16: p. 1049118.doi:10.3389/fnins.2022.1049118
[22] Myers, P.S., J.L. O'Donnell, J.J. Jackson, C.N. Lessov-Schlaggar, R.L. Miller, E.R. Foster, et al., *Proteinopathy and Longitudinal Cognitive Decline in Parkinson Disease.* Neurology, 2022. 99(1): p. e66-e76.doi:10.1212/wnl.0000000000200344
[23] Bäckström, D.C., M. Eriksson Domellöf, J. Linder, B. Olsson, A. Öhrfelt, M. Trupp, et al., *Cerebrospinal Fluid Patterns and the Risk of Future Dementia in Early, Incident Parkinson Disease.* JAMA Neurol, 2015. 72(10): p. 1175-82. doi:10.1001/jamaneurol.2015.1449
[24] Paolini Paoletti, F., L. Gaetani, G. Bellomo, E. Chipi, N. Salvadori, C. Montanucci, et al., *CSF neurochemical profile and cognitive changes in Parkinson's disease with mild cognitive impairment.* NPJ Parkinsons Dis, 2023. 9(1): p. 68.doi:10.1038/s41531-023-00509-w
[25] Terrelonge, M., Jr., K.S. Marder, D. Weintraub, and R.N. Alcalay, *CSF β-Amyloid 1-42 Predicts Progression to Cognitive Impairment in Newly Diagnosed Parkinson Disease.* J Mol Neurosci, 2016. 58(1): p. 88-92.doi:10.1007/s12031-015-0647-x
[26] Hansson, O., S. Hall, A. Ohrfelt, H. Zetterberg, K. Blennow, L. Minthon, et al., *Levels of cerebrospinal fluid a-synuclein oligomers are increased in Parkinson's disease with dementia and dementia with Lewy bodies compared to Alzheimer's disease.* Alzheimers Res Ther, 2014. 6(3): p. 25.doi:10.1186/alzrt255
[27] Oosterveld, L.P., T.I. Kuiper, N.K. Majbour, I.M.W. Verberk, K.D. van Dijk, J.W.R. Twisk, et al., *CSF Biomarkers Reflecting Protein Pathology and Axonal Degeneration Are Associated with Memory, Attentional, and Executive Functioning in Early-Stage Parkinson's Disease.* Int J Mol Sci, 2020. 21(22).doi:10.3390/ijms21228519
[28] Chen, C.H., B.C. Lee, and C.H. Lin, *Integrated Plasma and Neuroimaging Biomarkers Associated with Motor and Cognition Severity in Parkinson's Disease.* J Parkinsons Dis, 2020. 10(1): p. 77-88. doi:10.3233/jpd-191766
[29] Lin, C.H., S.Y. Yang, H.E. Horng, C.C. Yang, J.J. Chieh, H.H. Chen, et al., *Plasma a-synuclein predicts cognitive decline in Parkinson's disease.* J Neurol Neurosurg Psychiatry, 2017. 88(10): p. 818-824.doi:10.1136/jnnp-2016-314857
[30] Ren, J., C. Pan, Y. Wang, C. Xue, H. Lin, J. Xu, et al., *Plasma a-synuclein and phosphorylated tau 181 as a diagnostic biomarker panel for de novo Parkinson's disease.* J Neurochem, 2022. 161(6): p. 506-515.doi:10.1111/jnc.15601
[31] Li, C.H., T.F. Chen, P.L. Peng, and C.H. Lin, *A task-specific cognitive domain decline is correlated with plasma and neuroimaging markers in patients with Parkinson's disease.* J Neurol, 2022. 269(12): p. 6530-6543.doi:10.1007/s00415-022-11301-w
[32] Chan, D.K.Y., J. Chen, R.F. Chen, J. Parikh, Y.H. Xu, P.A. Silburn, et al., *Plasma biomarkers inclusive of a-synuclein/amyloid-beta40 ratio strongly correlate with Mini-Mental State Examination score in Parkinson's disease and predict cognitive impairment.* J Neurol, 2022. 269(12): p. 6377-6385.doi:10.1007/s00415-022-11287-5

[33] Song, Z., J. Shen, Y. Liu, X. Du, Y. Wu, X. Liu, et al., *Lower Plasma a-Synuclein Levels are Associated with Cognitive Impairment in Parkinson's Disease.* Clin Lab, 2021. 67(5).doi:10.7754/Clin.Lab.2020.200852

[34] Blommer, J., T. Pitcher, M. Mustapic, E. Eren, P.J. Yao, M.P. Vreones, et al., *Extracellular vesicle biomarkers for cognitive impairment in Parkinson's disease.* Brain, 2023. 146(1): p. 195-208. doi:10.1093/brain/awac258

[35] Smith, C., N. Malek, K. Grosset, B. Cullen, S. Gentleman, and D.G. Grosset, *Neuropathology of dementia in patients with Parkinson's disease: a systematic review of autopsy studies.* J Neurol Neurosurg Psychiatry, 2019. 90(11): p. 1234-1243.doi:10.1136/jnnp-2019-321111

[36] Compta, Y., M. Ezquerra, E. Muñoz, E. Tolosa, F. Valldeoriola, J. Rios, et al., *High cerebrospinal tau levels are associated with the rs242557 tau gene variant and low cerebrospinal ß-amyloid in Parkinson disease.* Neurosci Lett, 2011. 487(2): p. 169-73.doi:10.1016/j.neulet.2010.10.015

[37] Montine, T.J., M. Shi, J.F. Quinn, E.R. Peskind, S. Craft, C. Ginghina, et al., *CSF Aß(42) and tau in Parkinson's disease with cognitive impairment.* Mov Disord, 2010. 25(15): p. 2682-5. doi:10.1002/mds.23287

[38] Hu, X., Y. Yang, and D. Gong, *Changes of cerebrospinal fluid Aß(42), t-tau, and p-tau in Parkinson's disease patients with cognitive impairment relative to those with normal cognition: a meta-analysis.* Neurol Sci, 2017. 38(11): p. 1953-1961.doi:10.1007/s10072-017-3088-1

[39] Parnetti, L., L. Farotti, P. Eusebi, D. Chiasserini, C. De Carlo, D. Giannandrea, et al., *Differential role of CSF alpha-synuclein species, tau, and Aß42 in Parkinson's Disease.* Front Aging Neurosci, 2014. 6: p. 53.doi:10.3389/fnagi.2014.00053

[40] Goldman, J.G., H. Andrews, A. Amara, A. Naito, R.N. Alcalay, L.M. Shaw, et al., *Cerebrospinal fluid, plasma, and saliva in the BioFIND study: Relationships among biomarkers and Parkinson's disease Features.* Mov Disord, 2018. 33(2): p. 282-288.doi:10.1002/mds.27232

[41] Leverenz, J.B., G.S. Watson, J. Shofer, C.P. Zabetian, J. Zhang, and T.J. Montine, *Cerebrospinal fluid biomarkers and cognitive performance in non-demented patients with Parkinson's disease.* Parkinsonism Relat Disord, 2011. 17(1): p. 61-4. doi:10.1016/j.parkreldis.2010.10.003

[42] Siderowf, A., S.X. Xie, H. Hurtig, D. Weintraub, J. Duda, A. Chen-Plotkin, et al., *CSF amyloid {beta} 1-42 predicts cognitive decline in Parkinson disease.* Neurology, 2010. 75(12): p. 1055-61. doi:10.1212/WNL.0b013e3181f39a78

[43] Compta, Y., J.B. Pereira, J. Ríos, N. Ibarretxe-Bilbao, C. Junqué, N. Bargalló, et al., *Combined dementia-risk biomarkers in Parkinson's disease: a prospective longitudinal study.* Parkinsonism Relat Disord, 2013. 19(8): p. 717-24. doi:10.1016/j.parkreldis.2013.03.009

[44] Zarifkar, P., J. Kim, C. La, K. Zhang, S. York-Williams, T.F. Levine, et al., *Cognitive impairment in Parkinson's disease is associated with Default Mode Network subsystem connectivity and cerebrospinal fluid Aß.* Parkinsonism Relat Disord, 2021. 83: p. 71-78.doi:10.1016/j.parkreldis.2021.01.002

[45] Bellomo, G., F. Paolini Paoletti, E. Chipi, M. Petricciuolo, S. Simoni, N. Tambasco, et al., *A/T/(N) Profile in Cerebrospinal Fluid of Parkinson's Disease with/without Cognitive Impairment and Dementia with Lewy Bodies.* Diagnostics (Basel), 2020. 10(12).doi:10.3390/diagnostics10121015

[46] Sancesario, G.M., G. Di Lazzaro, M. Alwardat, B. Biticchi, V. Basile, C. Salimei, et al., *Amyloid-ß42/Neurogranin Ratio as a Potential Index for Cognitive Impairment in Parkinson's Disease.* J Alzheimers Dis, 2020. 76(3): p. 1171-1178.doi:10.3233/jad-200344

[47] Lin, J., R. Ou, C. Li, Y. Hou, L. Zhang, Q. Wei, et al., *Evolution and Predictive Role of Plasma Alzheimer's Disease-related Pathological Biomarkers in Parkinson's Disease.* J Gerontol A Biol Sci Med Sci, 2023.doi:10.1093/gerona/glad189

[48] Tsai, H.H., L.K. Tsai, Y.L. Lo, and C.H. Lin, *Amyloid related cerebral microbleed and plasma Aß40 are associated with cognitive decline in Parkinson's disease.* Sci Rep, 2021. 11(1): p. 7115. doi:10.1038/s41598-021-86617-0

[49] Chen, N.C., H.L. Chen, S.H. Li, Y.H. Chang, M.H. Chen, N.W. Tsai, et al., *Plasma Levels of a-Synuclein, Aß-40 and T-tau as Biomarkers to Predict Cognitive Impairment in Parkinson's Disease.* Front Aging Neurosci, 2020. 12: p. 112. doi:10.3389/fnagi.2020.00112

[50] Alves, G., K. Brønnick, D. Aarsland, K. Blennow, H. Zetterberg, C. Ballard, et al., *CSF amyloid-beta and tau proteins, and cognitive performance, in early and untreated Parkinson's disease: the Norwegian ParkWest study.* J Neurol Neurosurg Psychiatry, 2010. 81(10): p. 1080-6. doi:10.1136/jnnp.2009.199950

[51] Wang, Z., Y. Zheng, H. Cai, C. Yang, S. Li, H. Lv, et al., *Aß1-42-containing platelet-derived extracellular vesicle is associated with cognitive decline in Parkinson's disease.* Front Aging Neurosci, 2023. 15: p. 1170663.doi:10.3389/fnagi.2023.1170663

[52] Chung, C.C., L. Chan, J.H. Chen, O.A. Bamodu, H.W. Chiu, and C.T. Hong, *Plasma extracellular vesicles tau and ß-amyloid as biomarkers of*

cognitive dysfunction of Parkinson's disease. Faseb j, 2021. 35(10): p. e21895.doi:10.1096/fj.202100787R

[53] Irwin, D.J., J. Fedler, C.S. Coffey, C. Caspell-Garcia, J.H. Kang, T. Simuni, et al., *Evolution of Alzheimer's Disease Cerebrospinal Fluid Biomarkers in Early Parkinson's Disease.* Ann Neurol, 2020. 88(3): p. 574-587.doi:10.1002/ana.25811

[54] Caspell-Garcia, C., T. Simuni, D. Tosun-Turgut, I.W. Wu, Y. Zhang, M. Nalls, et al., *Multiple modality biomarker prediction of cognitive impairment in prospectively followed de novo Parkinson disease.* PLoS One, 2017. 12(5): p. e0175674. doi:10.1371/journal.pone.0175674

[55] Gramotnev, D.K., G. Gramotnev, A. Gramotnev, and M.J. Summers, *Path analysis of biomarkers for cognitive decline in early Parkinson's disease.* PLoS One, 2022. 17(5): p. e0268379. doi:10.1371/journal.pone.0268379

[56] Bäckström, D., G. Granåsen, S.J. Mo, K. Riklund, M. Trupp, H. Zetterberg, et al., *Prediction and early biomarkers of cognitive decline in Parkinson disease and atypical parkinsonism: a population-based study.* Brain Commun, 2022. 4(2): p. fcac040.doi:10.1093/braincomms/fcac040

[57] Lin, W.T., J.S. Shaw, F.Y. Cheng, and P.H. Chen, *Plasma total tau predicts executive dysfunction in Parkinson's disease.* Acta Neurol Scand, 2022. 145(1): p. 30-37.doi:10.1111/ane.13517

[58] Liu, C., B. Cholerton, M. Shi, C. Ginghina, K.C. Cain, P. Auinger, et al., *CSF tau and tau/Aß42 predict cognitive decline in Parkinson's disease.* Parkinsonism Relat Disord, 2015. 21(3): p. 271-6.doi:10.1016/j.parkreldis.2014.12.027

[59] Chojdak-Łukasiewicz, J., M. Małodobra-Mazur, A. Zimny, L. Noga, and B. Paradowski, *Plasma tau protein and Aß42 level as markers of cognitive impairment in patients with Parkinson's disease.* Adv Clin Exp Med, 2020. 29(1): p. 115-121.doi:10.17219/acem/112058

[60] Lee, Y.G., S.H. Jeong, M. Park, S.W. Kang, K. Baik, S. Jeon, et al., *Effects of Alzheimer's genetic risk scores and CSF biomarkers in de novo Parkinson's Disease.* NPJ Parkinsons Dis, 2022. 8(1): p. 57.doi:10.1038/s41531-022-00317-8

[61] Delgado-Alvarado, M., R. Dacosta-Aguayo, I. Navalpotro-Gómez, B. Gago, A. Gorostidi, H. Jiménez-Urbieta, et al., *Ratios of proteins in cerebrospinal fluid in Parkinson's disease cognitive decline: prospective study.* Mov Disord, 2018. 33(11): p. 1809-1813.doi:10.1002/mds.27518

[62] Zetterberg, H., *Neurofilament Light: A Dynamic Cross-Disease Fluid Biomarker for Neurodegeneration.* Neuron, 2016. 91(1): p. 1-3. doi:10.1016/j.neuron.2016.06.030

[63] Sheng, Z.H., L.Z. Ma, J.Y. Liu, Y.N. Ou, B. Zhao, Y.H. Ma, et al., *Cerebrospinal fluid neurofilament dynamic profiles predict cognitive progression in individuals with de novo Parkinson's disease.* Front Aging Neurosci, 2022. 14: p. 1061096. doi:10.3389/fnagi.2022.1061096

[64] Aamodt, W.W., T. Waligorska, J. Shen, T.F. Tropea, A. Siderowf, D. Weintraub, et al., *Neurofilament Light Chain as a Biomarker for Cognitive Decline in Parkinson Disease.* Mov Disord, 2021. 36(12): p. 2945-2950.doi:10.1002/mds.28779

[65] Lerche, S., I. Wurster, B. Röben, M. Zimmermann, G. Machetanz, S. Wiethoff, et al., *CSF NFL in a Longitudinally Assessed PD Cohort: Age Effects and Cognitive Trajectories.* Mov Disord, 2020. 35(7): p. 1138-1144.doi:10.1002/mds.28056

[66] Sampedro, F., R. Pérez-González, S. Martínez-Horta, J. Marín-Lahoz, J. Pagonabarraga, and J. Kulisevsky, *Serum neurofilament light chain levels reflect cortical neurodegeneration in de novo Parkinson's disease.* Parkinsonism Relat Disord, 2020. 74: p. 43-49.doi:10.1016/j.parkreldis.2020.04.009

[67] Mao, S., X. Teng, Z. Li, J. Zu, T. Zhang, C. Xu, et al., *Association of serum neurofilament light chain and glial fibrillary acidic protein levels with cognitive decline in Parkinson's disease.* Brain Res, 2023. 1805: p. 148271.doi:10.1016/j.brainres.2023.148271

[68] Dong, L., Q. Chang, J. Ma, C. Liu, D. Guo, X. Li, et al., *Associations of blood UCH-L1 and NfL levels with cognitive dysfunction in Parkinson's disease patients.* Neurosci Lett, 2023. **804**: p. 137219.doi:10.1016/j.neulet.2023.137219

[69] Niemann, L., S. Lezius, A. Maceski, D. Leppert, C. Englisch, E. Schwedhelm, et al., *Serum neurofilament is associated with motor function, cognitive decline and subclinical cardiac damage in advanced Parkinson's disease (MARK-PD).* Parkinsonism Relat Disord, 2021. 90: p. 44-48. doi:10.1016/j.parkreldis.2021.07.028

[70] Pagonabarraga, J., R. Pérez-González, H. Bejr-Kasem, J. Marín-Lahoz, A. Horta-Barba, S. Martinez-Horta, et al., *Dissociable contribution of plasma NfL and p-tau181 to cognitive impairment in Parkinson's disease.* Parkinsonism Relat Disord, 2022. 105: p. 132-138.doi:10.1016/j.parkreldis.2022.05.020

[71] Batzu, L., S. Rota, A. Hye, A. Heslegrave, D. Trivedi, L.L. Gibson, et al., *Plasma p-tau181, neurofilament light chain and association with cognition in Parkinson's disease.* NPJ Parkinsons Dis, 2022. 8(1): p. 154.doi:10.1038/s41531-022-00384-x

[72] Ma, L.Z., C. Zhang, H. Wang, Y.H. Ma, X.N. Shen, J. Wang, et al., *Serum Neurofilament Dynamics Predicts Cognitive Progression in de novo Parkinson's Disease.* J Parkinsons Dis, 2021.

11(3): p. 1117-1127.doi:10.3233/jpd-212535
[73] Mollenhauer, B., M. Dakna, N. Kruse, D. Galasko, T. Foroud, H. Zetterberg, et al., *Validation of Serum Neurofilament Light Chain as a Biomarker of Parkinson's Disease Progression.* Mov Disord, 2020. 35(11): p. 1999-2008.doi:10.1002/mds.28206
[74] Gelders, G., V. Baekelandt, and A. Van der Perren, *Linking Neuroinflammation and Neurodegeneration in Parkinson's Disease.* J Immunol Res, 2018. 2018: p. 4784268.doi:10.1155/2018/4784268
[75] Yu, S.Y., L.J. Zuo, F. Wang, Z.J. Chen, Y. Hu, Y.J. Wang, et al., *Potential biomarkers relating pathological proteins, neuroinflammatory factors and free radicals in PD patients with cognitive impairment: a cross-sectional study.* BMC Neurol, 2014. 14: p. 113.doi:10.1186/1471-2377-14-113
[76] Hall, S., S. Janelidze, Y. Surova, H. Widner, H. Zetterberg, and O. Hansson, *Cerebrospinal fluid concentrations of inflammatory markers in Parkinson's disease and atypical parkinsonian disorders.* Sci Rep, 2018. 8(1): p. 13276.doi:10.1038/s41598-018-31517-z
[77] Martin-Ruiz, C., C.H. Williams-Gray, A.J. Yarnall, J.J. Boucher, R.A. Lawson, R.S. Wijeyekoon, et al., *Senescence and Inflammatory Markers for Predicting Clinical Progression in Parkinson's Disease: The ICICLE-PD Study.* J Parkinsons Dis, 2020. 10(1): p. 193-206.doi:10.3233/jpd-191724
[78] Kúçúk, A., E. Tüzün, E. Erdoúdu, B. Búgúç, Z. Tüfekçúoúu, E. Öztürk-Iúk, et al., *Neuroinflammation Mediators are Reduced in Sera of Parkinson's Disease Patients with Mild Cognitive Impairment.* Noro Psikiyatr Ars, 2020. 57(1): p. 15-17.doi:10.29399/npa.23624
[79] Lerche, S., M. Zimmermann, B. Roeben, I. Wurster, F.L. Fries, C. Deuschle, et al., *Inflammatory CSF profiles and longitudinal development of cognitive decline in sporadic and GBA-associated PD.* NPJ Parkinsons Dis, 2023. 9(1): p. 38.doi:10.1038/s41531-023-00476-2
[80] Lerche, S., M. Zimmermann, I. Wurster, B. Roeben, F.L. Fries, C. Deuschle, et al., *CSF and Serum Levels of Inflammatory Markers in PD: Sparse Correlation, Sex Differences and Association With Neurodegenerative Biomarkers.* Front Neurol, 2022. 13: p. 834580.doi:10.3389/fneur.2022.834580
[81] Shen, J., N. Amari, R. Zack, R.T. Skrinak, T.L. Unger, M. Posavi, et al., *Plasma MIA, CRP, and Albumin Predict Cognitive Decline in Parkinson's Disease.* Ann Neurol, 2022. 92(2): p. 255-269.doi:10.1002/ana.26410
[82] Naskar, A., A. Stezin, A. Dharmappa, S. Hegde, M. Philip, N. Kamble, et al., *Fibrinogen and Complement Factor H Are Promising CSF Protein Biomarkers for Parkinson's Disease with Cognitive Impairment-A Proteomics-ELISA-Based Study.* ACS Chem Neurosci, 2022. 13(7): p. 1030-1045.doi:10.1021/acschemneuro.2c00019
[83] Qin, Q., H. Wan, D. Wang, J. Li, Y. Qu, J. Zhao, et al., *The Association of CSF sTREM2 With Cognitive Decline and Its Dynamic Change in Parkinson's Disease: Analysis of the PPMI Cohort.* Front Aging Neurosci, 2022. 14: p. 892493.doi:10.3389/fnagi.2022.892493
[84] Wilson, E.N., M.S. Swarovski, P. Linortner, M. Shahid, A.J. Zuckerman, Q. Wang, et al., *Soluble TREM2 is elevated in Parkinson's disease subgroups with increased CSF tau.* Brain, 2020. 143(3): p. 932-943.doi:10.1093/brain/awaa021
[85] Liu, T., H. Zuo, D. Ma, D. Song, Y. Zhao, and O. Cheng, *Cerebrospinal fluid GFAP is a predictive biomarker for conversion to dementia and Alzheimer's disease-associated biomarkers alterations among de novo Parkinson's disease patients: a prospective cohort study.* J Neuroinflammation, 2023. 20(1): p. 167.doi:10.1186/s12974-023-02843-5
[86] Mizutani, Y., R. Ohdake, H. Tatebe, A. Higashi, S. Shima, A. Ueda, et al., *Associations of Alzheimer's-related plasma biomarkers with cognitive decline in Parkinson's disease.* J Neurol, 2023. 270(11): p. 5461-5474.doi:10.1007/s00415-023-11875-z
[87] Tang, Y., L. Han, S. Li, T. Hu, Z. Xu, Y. Fan, et al., *Plasma GFAP in Parkinson's disease with cognitive impairment and its potential to predict conversion to dementia.* NPJ Parkinsons Dis, 2023. 9(1): p. 23.doi:10.1038/s41531-023-00447-7
[88] Rathnayake, D., T. Chang, and P. Udagama, *Selected serum cytokines and nitric oxide as potential multi-marker biosignature panels for Parkinson disease of varying durations: a case-control study.* BMC Neurol, 2019. 19(1): p. 56.doi:10.1186/s12883-019-1286-6
[89] Li, Z., J. Zhang, and H. Sun, *Increased plasma levels of phospholipid in Parkinson's disease with mild cognitive impairment.* J Clin Neurosci, 2015. 22(8): p. 1268-71.doi:10.1016/j.jocn.2015.02.013
[90] Bakeberg, M.C., A.M. Gorecki, J.E. Kenna, A. Jefferson, M. Byrnes, S. Ghosh, et al., *Elevated HDL Levels Linked to Poorer Cognitive Ability in Females With Parkinson's Disease.* Front Aging Neurosci, 2021. 13: p. 656623.doi:10.3389/fnagi.2021.656623
[91] Deng, X., S.E. Saffari, S.Y.E. Ng, N. Chia, J.Y. Tan, X. Choi, et al., *Blood Lipid Biomarkers in Early Parkinson's Disease and Parkinson's Disease with Mild Cognitive Impairment.* J Parkinsons

Dis, 2022. 12(6): p. 1937-1943.doi:10.3233/jpd-213135
[92] Qu, Y., Q.X. Qin, D.L. Wang, J.T. Li, J.W. Zhao, K. An, et al., *Estimated glomerular filtration rate is a biomarker of cognitive impairment in Parkinson's disease.* Front Aging Neurosci, 2023. 15: p. 1130833.doi:10.3389/fnagi.2023.1130833
[93] Pellecchia, M.T., R. Savastano, M. Moccia, M. Picillo, P. Siano, R. Erro, et al., *Lower serum uric acid is associated with mild cognitive impairment in early Parkinson's disease: a 4-year follow-up study.* J Neural Transm (Vienna), 2016. 123(12): p. 1399-1402.doi:10.1007/s00702-016-1622-6
[94] Sleeman, I., R.A. Lawson, A.J. Yarnall, G.W. Duncan, F. Johnston, T.K. Khoo, et al., *Urate and Homocysteine: Predicting Motor and Cognitive Changes in Newly Diagnosed Parkinson's Disease.* J Parkinsons Dis, 2019. 9(2): p. 351-359.doi:10.3233/jpd-181535
[95] Santangelo, G., S. Raimo, R. Erro, M. Picillo, M. Amboni, M.T. Pellecchia, et al., *Vitamin D as a possible biomarker of mild cognitive impairment in parkinsonians.* Aging Ment Health, 2021. 25(11): p. 1998-2002.doi:10.1080/13607863.2020.1839860
[96] Wu, H., H. Khuram Raza, Z. Li, Z. Li, J. Zu, C. Xu, et al., *Correlation between serum 25(OH) D and cognitive impairment in Parkinson's disease.* J Clin Neurosci, 2022. 100: p. 192-195. doi:10.1016/j.jocn.2022.04.015
[97] Halbgebauer, S., M. Nagl, H. Klafki, U. Haußmann, P. Steinacker, P. Oeckl, et al., *Modified serpinA1 as risk marker for Parkinson's disease dementia: Analysis of baseline data.* Sci Rep, 2016. 6: p. 26145.doi:10.1038/srep26145
[98] Leng, B., H. Sun, J. Zhao, Y. Liu, T. Shen, W. Liu, et al., *Plasma exosomal prion protein levels are correlated with cognitive decline in PD patients.* Neurosci Lett, 2020. 723: p. 134866. doi:10.1016/j.neulet.2020.134866
[99] Khalil, H., M.A. Alomari, O.F. Khabour, A. Al-Hieshan, and J.A. Bajwa, *Relationship of circulatory BDNF with cognitive deficits in people with Parkinson's disease.* J Neurol Sci, 2016. 362: p. 217-20.doi:10.1016/j.jns.2016.01.032
[100] Quan, Y., J. Wang, S. Wang, and J. Zhao, *Association of the Plasma Long Non-coding RNA MEG3 With Parkinson's Disease.* Front Neurol, 2020. 11: p. 532891.doi:10.3389/fneur.2020.532891
[101] Costa, A., A. Peppe, G.A. Carlesimo, S. Zabberoni, F. Scalici, C. Caltagirone, et al., *Brain-derived neurotrophic factor serum levels correlate with cognitive performance in Parkinson's disease patients with mild cognitive impairment.* Front Behav Neurosci, 2015. 9: p. 253.doi:10.3389/fnbeh.2015.00253
[102] Chen-Plotkin, A.S., W.T. Hu, A. Siderowf, D. Weintraub, R. Goldmann Gross, H.I. Hurtig, et al., *Plasma epidermal growth factor levels predict cognitive decline in Parkinson disease.* Ann Neurol, 2011. 69(4): p. 655-63.doi:10.1002/ana.22271
[103] Pellecchia, M.T., G. Santangelo, M. Picillo, R. Pivonello, K. Longo, C. Pivonello, et al., *Serum epidermal growth factor predicts cognitive functions in early, drug-naive Parkinson's disease patients.* J Neurol, 2013. 260(2): p. 438-44. doi:10.1007/s00415-012-6648-6
[104] Lim, N.S., C.R. Swanson, H.R. Cherng, T.L. Unger, S.X. Xie, D. Weintraub, et al., *Plasma EGF and cognitive decline in Parkinson's disease and Alzheimer's disease.* Ann Clin Transl Neurol, 2016. 3(5): p. 346-55.doi:10.1002/acn3.299
[105] Shi, M.Y., C.C. Ma, F.F. Chen, X.Y. Zhou, X. Li, C.X. Tang, et al., *Possible role of glial cell line-derived neurotrophic factor for predicting cognitive impairment in Parkinson's disease: a case-control study.* Neural Regen Res, 2021. 16(5): p. 885-892.doi:10.4103/1673-5374.297091
[106] Tong, S.Y., R.W. Wang, Q. Li, Y. Liu, X.Y. Yao, D.Q. Geng, et al., *Serum glial cell line-derived neurotrophic factor (GDNF) a potential biomarker of executive function in Parkinson's disease.* Front Neurosci, 2023. 17: p. 1136499.doi:10.3389/fnins.2023.1136499
[107] Ma, J., Q. Jiang, J. Xu, Q. Sun, Y. Qiao, W. Chen, et al., *Plasma insulin-like growth factor 1 is associated with cognitive impairment in Parkinson's disease.* Dement Geriatr Cogn Disord, 2015. 39(5-6): p. 251-6.doi:10.1159/000371510
[108] Pellecchia, M.T., G. Santangelo, M. Picillo, R. Pivonello, K. Longo, C. Pivonello, et al., *Insulin-like growth factor-1 predicts cognitive functions at 2-year follow-up in early, drug-naïve Parkinson's disease.* Eur J Neurol, 2014. 21(5): p. 802-7.doi:10.1111/ene.12137
[109] Guo, Y., F.T. Liu, X.H. Hou, J.Q. Li, X.P. Cao, L. Tan, et al., *Predictors of cognitive impairment in Parkinson's disease: a systematic review and meta-analysis of prospective cohort studies.* J Neurol, 2021. 268(8): p. 2713-2722.doi:10.1007/s00415-020-09757-9
[110] Real, R., A. Martinez-Carrasco, R.H. Reynolds, M.A. Lawton, M.M.X. Tan, M. Shoai, et al., *Association between the LRP1B and APOE loci and the development of Parkinson's disease dementia.* Brain, 2023. 146(5): p. 1873-1887. doi:10.1093/brain/awac414
[111] Mata, I.F., J.B. Leverenz, D. Weintraub, J.Q. Trojanowski, H.I. Hurtig, V.M. Van Deerlin, et al., *APOE, MAPT, and SNCA genes and cognitive performance in Parkinson disease.* JAMA Neurol, 2014. 71(11): p. 1405-12.doi:10.1001/

jamaneurol.2014.1455
[112]Samat, N.A., N.A. Abdul Murad, K. Mohamad, M.R. Abdul Razak, and N. Mohamed Ibrahim, *Apolipoprotein Eε4: A Biomarker for Executive Dysfunction among Parkinson's Disease Patients with Mild Cognitive Impairment.* Front Neurosci, 2017. 11: p. 712.doi:10.3389/fnins.2017.00712
[113]Mollenhauer, B., L. Rochester, A. Chen-Plotkin, and D. Brooks, *What can biomarkers tell us about cognition in Parkinson's disease?* Mov Disord, 2014. 29(5): p. 622-33.doi:10.1002/mds.25846
[114]Chung, J., A. Ushakova, M. Doitsidou, C. Tzoulis, O.B. Tysnes, I. Dalen, et al., *The impact of common genetic variants in cognitive decline in the first seven years of Parkinson's disease: A longitudinal observational study.* Neurosci Lett, 2021. 764: p. 136243.doi:10.1016/j.neulet.2021.136243
[115]Deng, X., Y. Ning, S.E. Saffari, B. Xiao, C. Niu, S.Y.E. Ng, et al., *Identifying clinical features and blood biomarkers associated with mild cognitive impairment in Parkinson disease using machine learning.* Eur J Neurol, 2023. 30(6): p. 1658-1666.doi:10.1111/ene.15785
[116]Brockmann, K., K. Srulijes, S. Pflederer, A.K. Hauser, C. Schulte, W. Maetzler, et al., *GBA-associated Parkinson's disease: reduced survival and more rapid progression in a prospective longitudinal study.* Mov Disord, 2015. 30(3): p. 407-11.doi:10.1002/mds.26071
[117]Phongpreecha, T., B. Cholerton, I.F. Mata, C.P. Zabetian, K.L. Poston, N. Aghaeepour, et al., *Multivariate prediction of dementia in Parkinson's disease.* NPJ Parkinsons Dis, 2020. 6: p. 20.doi:10.1038/s41531-020-00121-2
[118]Mata, I.F., J.B. Leverenz, D. Weintraub, J.Q. Trojanowski, A. Chen-Plotkin, V.M. Van Deerlin, et al., *GBA Variants are associated with a distinct pattern of cognitive deficits in Parkinson's disease.* Mov Disord, 2016. 31(1): p. 95-102. doi:10.1002/mds.26359
[119]Parnetti, L., S. Paciotti, P. Eusebi, A. Dardis, S. Zampieri, D. Chiasserini, et al., *Cerebrospinal fluid β-glucocerebrosidase activity is reduced in parkinson's disease patients.* Mov Disord, 2017. 32(10): p. 1423-1431.doi:10.1002/mds.27136
[120]Huh, Y.E., H. Park, M.S.R. Chiang, I. Tuncali, G. Liu, J.J. Locascio, et al., *Glucosylceramide in cerebrospinal fluid of patients with GBA-associated and idiopathic Parkinson's disease enrolled in PPMI.* NPJ Parkinsons Dis, 2021. 7(1): p. 102. doi:10.1038/s41531-021-00241-3
[121]Zimmermann, M., L. Köhler, M. Kovarova, S. Lerche, C. Schulte, I. Wurster, et al., *The longevity gene Klotho and its cerebrospinal fluid protein profiles as a modifier for Parkinson-s disease.* Eur J Neurol, 2021. 28(5): p. 1557-1565. doi:10.1111/ene.14733
[122]Fang, Y., S. Dai, C. Jin, X. Si, L. Gu, Z. Song, et al., *Aquaporin-4 Polymorphisms Are Associated With Cognitive Performance in Parkinson's Disease.* Front Aging Neurosci, 2021. 13: p. 740491.doi:10.3389/fnagi.2021.740491

Vignettes

1. Highlights

- The present review aims to present recent studies regarding valuable biomarkers of cognitive impairment in PD
- Amyloid β has been established as biomarker of cognitive dysfunction in PD, reliably predicting cognitive decline in longitudinal assessments
- Neurofilament light chain is a promising biomarker
- Markers of neuroinflammation, lysosomal activity and growth factors have been associated with cognitive deficits in multiple domains
- Genetic variants contribute to a higher risk of cognitive dysfunction development

2. Potential of biomarkers in clinical practice

- Robust markers of cognitive function could be used as monitoring biomarkers, assessing the natural disease course as well as the effect of therapeutic interventions
- Prognostic biomarkers could enable earlier interventions in order to hinder the progressing cognitive decline
- Implementation of reliable biomarkers in clinical trials with investigational drugs could provide reliable outcome measures
- A combination of various biomarkers, reflecting different pathogenic processes of cognitive impairment in PD, could facilitate diagnosis and prognostic evaluation of cognitive decline

CSF AND BLOOD BIOMARKERS IN ATYPICAL PARKINSONISM

Vasileios C. Constantinides[1,2], Fotini Boufidou [2], Leonidas Stefanis [1], Elisabeth Kapaki [1,2]

[1] *First Department of Neurology, School of Medicine, National and Kapodistrian University of Athens, Eginition Hospital, Vas. Sophias Ave. 72-74, P.C.: 11528 Athens, Greece.*

[2] *Neurochemistry and Biological Markers Unit, First Department of Neurology, School of Medicine, National and Kapodistrian University of Athens, Eginition Hospital, Vas. Sophias Ave. 72-74, P.C.: 11528 Athens, Greece.΄*

Abstract

Atypical Parkinsonism is a collective term used to describe three rare neurodegenerative disorders which manifest with diverse phenotypes. It includes progressive supranuclear palsy (PSP), multiple system atrophy (MSA) and corticobasal degeneration (CBD). Despite the presence of specific clinical features in typical cases, many oligosymptomatic or atypical presentations are difficult to diagnose based on established clinical diagnostic criteria. To this end, one or more biomarkers, preferably with molecular specificity is paramount for the ***in vivo*** recognition of the underlying pathology in these patients. In this descriptive review we present the most important studies on cerebrospinal fluid (CSF) and plasma biomarkers in these disorders, with a particular focus on established Alzheimer's disease CSF biomarkers as well as alpha-synuclein.

Keywords: biomarkers; CSF; plasma; atypical parkinsonism; progressive supranuclear palsy; corticobasal degeneration; multiple system atrophy

ΒΙΟΔΕΙΚΤΕΣ ΕΝΥ ΚΑΙ ΠΛΑΣΜΑΤΟΣ ΣΕ ΑΤΥΠΑ ΠΑΡΚΙΝΣΟΝΙΚΑ ΣΥΝΔΡΟΜΑ

Βασίλειος Κ. Κωνσταντινίδης[1,2], Φωτεινή Μπουφίδου[2], Λεωνίδας Στεφανής[1], Ελισσάβετ Καπάκη[1,2]

[1] *Α' Νευρολογική Κλινική ΕΚΠΑ, Ιατρική Σχολή, Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αιγινήτειο Νοσοκομείο,*

[2] *Μονάδα Νευροχημείας και Βιολογικών Δεικτών, Α' Νευρολογική Κλινική ΕΚΠΑ, Ιατρική Σχολή, Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αιγινήτειο Νοσοκομείο*

Περίληψη

Ο όρος άτυπος παρκινσονισμός χρησιμοποιείται για να περιγράψει τρία σπάνια νευροεκφυλιστικά νοσήματα που χαρακτηρίζονται από ειδικούς φαινοτύπους. Περιλαμβάνει την προοδευτική υπερπυρηνική παράλυση (ΠΥΠ), ατροφία πολλαπλών συστημάτω (ΑΠΣ) και φλοιοβασική εκφύλιση (ΦΒΕ). Παρά την παρουσία ειδικών κλινικών χαρακτηριστικών σε τυπικές περιπτώσεις, πολλές ολιγοσυμπτωματικές ή άτυπες εκδηλώσεις των νοσημάτων αυτών δεν μπορούν να διαγνωσθούν αξιόπιστα βάσει των υπαρχόντων κλινικών διαγνωστικών κριτηρίων. Για το σκοπό αυτό, η ανάπτυξη βιοδεικτών με μοριακή ειδικότητα είναι υψίστης σημασίας για την αναγνώριση in vivo της υποκείμενης παθολογίας στους ασθενείς αυτούς. Στην παρούσα ανασκόπηση παρουσιάζουμε την υπάρχουσα βιβλιογραφία σε σχέση με τους βιοδείκτες στο εγκεφαλονωτιαίο υγρό και πλάσμα στα ανωτέρω νοσήματα, με ιδιαίτερη έμφαση στους καθιερωμένους βιοδείκτες της νόσου Alzheimer και στην α-συνουκλεΐνη

Λέξεις κλειδιά: βιοδείκτες; ΕΝΥ; πλάσμα; άτυπος παρκινσονισμός; προοδευτική υπερπυρηνική παράλυση; φλοιοβασική εκφύλιση; ατροφία πολλαπλών συστημάτων

Introduction

Atypical Parkinsonism is a termed used to include three distinct neurodegenerative disorders, with both movement and cognitive manifestations. These include progressive supranuclear palsy (PSP), a 4R-tauopathy characterized neuropathologically by tufted astrocytes, consisting of aggregated hyperphosphorylated tau protein. PSP presents with great phenotypical variability, with Richardson's syndrome being the most common manifestation. Corticobasal degeneration is an exceedingly rare 4R-tauopathy, with distinct neuropathological lesions, termed astrocytic plaques, which are also formed by aggregation of hyperphosphorylated tau protein. As is the case with PSP, CBD also presents with great clinical variability, most commonly manifesting as

corticobasal syndrome (CBS), primary progressive aphasia (PPA), Richardson's syndrome or a predominantly frontal-behavioural-visuospatial syndrome. Multiple system atrophy (MSA) is a synucleinopathy, characterized by glial cytoplasmic inclusions, which contain aggregates of misfolded, hyperphosphorylated alpha-synuclein protein (a-syn). Depending on the topographical distribution of neuropathological lesions (olivo-ponto-cerebellar vs. striato-nigral), MSA is divided into MSA-cerebellar and MSA-parkinsonian variant respectively. Despite the presence of distinct clinical and imaging features, these three disorders are often difficult to diagnose clinically, particularly in atypical cases and in oligosymptomatic patients. In an effort to enhance the accuracy of clinical diagnosis, various biomarkers have been tested. In this overview, we present the most relevant studies on biofluid markers in atypical Parkinonism, with a particular focus on established AD biomarkers and a-syn in cerebrospinal fluid (CSF) and plasma.

Established AD biomarkers

Amyloid beta with 42 amino-acids ($A\beta_{42}$), total tau (t-tau) and phosphorylated tau protein (p-tau), most commonly at threonine 181 are considered classical AD biomarkers. A typical AD CSF profile includes a decrease in $A\beta_{42}$ with a concomitant elevation in t-tau and p-tau levels. CSF t-tau is considered a non-specific marker of neurodegeneration and neuronal death. $A\beta_{42}$ is a marker of amyloidosis, whereas p-tau a marker of neurofibrillary tangle pathology. The primary use of these biomarkers lies in the in vivo recognition of patients with underlying AD pathology, either with typical (amnestic) or atypical (non-amnestic) presentations. Due to their availability, and the fact that the CBS phenotype in particular may have underlying AD pathology in a significant proportion of cases, these biomarkers have also been applied in patients with atypical Parkinsonism.

a) Total tau protein

Regarding CSF t-tau in PSP, the majority of relevant studies could not establish any differences between PSP patients and control subjects[1-5]. Along the same lines, no difference in t-tau was evident between PSP and other parkinsonian disorders, including CBS [1, 3-5], MSA [1, 3, 4] and PD [3, 4]. Two studies have reported decreased t-tau levels in PSP patients compared to controls [6, 7], and another study compared to CBS (with increased t-tau levels compared to PD) [8].

Regarding CBS, several initial studies have supported that CBS exhibits inherently elevated levels of CSF t-tau protein compared to controls [4, 8-11], PSP [8, 10, 11] and PD [1, 8]. These differences in some studies reached clinically meaningful significance: CSF t-tau could differentiate CBS from PSP with high (~80%) specificity and sensitivity in one study [11], and CBS from PD in another study (sensitivity 75%; specificity 90%) [8]. In contrast to these positive studies, several other studies have been negative in establishing differences in t-tau between CBS and controls [2, 3, 5], PSP [1, 3, 4, 12], MSA[3, 4] and PD [3, 4]. The initial positive relevant studies could be attributed to the admixture of AD patients in the CBS cohorts (see "Classical CSB biomarker profiling" for a detailed discussion on the subject).

Results regarding t-tau in MSA are conflicting, with most studies showing an increase in CSF t-tau in MSA compared either to controls [12-14] or other parkinsonian disorders [1, 12, 14-16]. However, several other studies did not establish any difference among MSA and other parkinsonian disorders [4, 17, 18] with a single study supporting that MSA patients present with decreased CSF t-tau levels compared to control subjects [18].

b) Phosphorylated tau protein

Most studies have focused on tau protein phosphorylated at threonine 181 (p-tau), as a surrogate marker of tau pathology in AD. CSF p-tau does not seem to be useful in the differentiation of PSP from other parkinsonian disorders or controls [2-6, 8], with two studies reporting decreased p-tau levels compared to controls [1, 7].

Likewise, in CBS most studies could not establish any meaningful difference between CBS patients and other parkinsonian disorders or controls with regards to CSF p-tau levels[1-5]. In a single study, CSF p-tau was elevated in CBS compared to PD [8], and in another study to MSA patients [1].

Accordingly, most relevant studies on p-tau did not find any meaningful difference between MSA and other study groups [3, 4, 12, 18], with the exception of a single study that identified decreased levels of CSF p-tau in MSA compared to PD and controls [1].

c) Amyloid beta with 42 amino acids

Most studies do not report any differences in CSF amyloid beta with 42 amino acids ($A\beta_{42}$) between PSP and other parkinsonian disorders or controls [1-4, 8, 19, 20]. There have been some reports indicating lower $A\beta_{42}$ values in PSP compared to controls [5-7]. A single study reported lower $A\beta_{42}$ levels in PSP vs. PD [7].

Along the same lines, the majority of CSF $A\beta_{42}$ studies in CBS do not report significant differences compared to other parkinsonian disorders [1, 3, 5, 8] with few studies reporting decreased CSF $A\beta_{42}$ levels in CBS patients compared to controls [2, 5] and PD [4].

As is the case with PSP and CBS, CSF $A\beta_{42}$ levels do not seem to be useful in differentiating MSA from other parkinsonian disorders or controls [1, 3, 4, 12, 17, 18, 20]. A single study reported decreased levels of CSF $A\beta_{42}$ compared to PD, PSP and controls [19]

and a different study group reported decreased $A\beta_{42}$ levels in MSA compared only to controls [18].

d) Established AD CSF biomarkers ratios

Established AD CSF biomarker ratios include the t-tau/$A\beta_{42}$, p-tau/$A\beta_{42}$ and p-tau/t-tau ratios. These ratios are composite markers incorporating data on two of the three biomarker categories of the AT(N) classification system. These ratios have been extensively used in the literature as surrogate markers of AD, with neuropathological studies supporting high biochemical/neuropathological correlations in AD. On a practical level, using biomarker ratios decreases the importance of confounding pre-analytical factors in biomarker measurement among different sites.

Regarding PSP, in an initial study including PSP and CBS patients, the p-tau/$A\beta_{42}$ ratio values could not differentiate between PSP and CBS [5], although it was useful in differentiating PSP patients from control subjects in another study [8]. A decreased p-tau/t-tau ratio has been reported to be useful in differentiating patients with atypical Parkinsonism (PSP and MSA) from PD [12], as well as PSP from CBS [8] and PSP patients from control subjects [4].

Few studies have included relevant data in CBS. A single study has reported increased t-tau/$A\beta_{42}$ ratio in CBS compared to PD patients and controls, and decreased p-tau/t-tau ratio compared to control subjects [8]. Another study posited that CBS patients have elevated t-tau/$A\beta_{42}$ and p-tau/$A\beta_{42}$ compared to PD patients [4].

Regarding MSA, a single study reported that MSA patients present with significantly lower p-tau/t-tau ratios compared to PD [12]. Another study suggested that higher values of t-tau/$A\beta_{42}$ ratio could differentiate MSA from PD with high specificity but suboptimal sensitivity [4].

e) Established AD CSF biomarker profiling

In lack of a single biomarker with molecular specificity for AD, researchers in the field have focused on establishing classification systems for categorizing biomarkers into different groups, in an effort to create biochemical profiles with data on all molecular aspects of AD (amyloidosis, tau pathology, and neurodegeneration). Initial attempts on this approach included CSF biomarkers ratios, such as t-tau/$A\beta_{42}$, p-tau/$A\beta_{42}$ and p-tau/t-tau, as discussed previously.

A more refined approach was the introduction of classification systems such as the BIOMARKAPD/ABSI and AT(N) systems. The implementation of biomarker profiling based on these classification systems is of paramount importance in cohorts of patients with typical (amnestic) and atypical (non-amnestic) presentations of AD, including frontal-executive predominant dementia, primary progressive aphasias, posterior cortical atrophy and corticobasal syndrome. Biomarker profiling in these cases will assist in recognizing patients with an AD underlying pathology and an atypical phenotype (e.g. corticobasal syndrome), which is of pivotal importance both clinically (for individualized management of symptoms) and on a research level (for accurate patient allocation in clinical trials).

Few studies have included CSF AD biomarker profiling data in PSP. In a large cohort, including diverse neurodegenerative disorders, 10% of PSP patients had a CSF-AD profile, as defined by an index incorporating CSF $A\beta_{42}$ and p-tau values [2]. In another study, a single PSP patient (~5%) had a typical CSF AD profile, as determined by abnormal $A\beta_{42}$, t-tau and p-tau values, in a cohort of patients with Parkinsonism [4]. These cases are more likely to represent instances with dual pathology, as neuropathological-clinical correlation studies have not described AD manifesting with Richardson's syndrome.

CSF biomarker profiling is of pivotal importance in CBS. An initial study concluded that 20% of CBS patients harboured a CSF AD profile (as defined by abnormal t-tau, $A\beta_{42}$ and t-tau/$A\beta_{42}$ ratio values) [21]. In another study, 38% of CBS patients harboured a CSF AD profile, based on an p-tau and Aβ42 derived index [2]. Along the same lines, a third study concluded that ~30% of CBS patients had a typical CSF-AD profile (abnormal values in all three AD biomarkers [4]. This study initially reported an increase in t-tau and a decrease in $A\beta_{42}$ in the CBS group, in accordance to previous studies. When the CBS patients with an AD CSF profile were excluded, these differences disappeared, indicating that the admixture of AD patients was driving these differences. CSF profiling was implemented in a follow-up study by the same study group, to investigate possible differences between AD and non-AD pathology in a CBS cohort [22].

The problems arising from implementing different classification criteria in cohorts of atypical or mixed cases of AD [23-25] have been systematically studied in CBS [26]. Depending on the classification criterion used, classification of a CBS patient varied from 39% to 46% in a study including 40 patients with a probable CBD diagnosis based on established diagnostic criteria (28 of these patients fulfilled criteria for probable CBS).

α-synuclein

A-syn is a mainly synaptic, 140 amino acid protein, expressed by neurons. Aggregated a-syn is the main constituent of Lewy bodies and Lewy neurites, the main neuropathological features of PD, PDD and DLB, as well as of glial cytoplasmic inclusions (GCIs), the neuropathological hallmark of MSA [27]. To this end, several studies have focused on total CSF a-syn as a candidate biomarker for MSA. Researchers

initially focused on CSF total a-syn as a candidate biomarker of synucleinopathies. However, over time focus has shifted on phosphorylated and oligomeric forms of a-synuclein, as post-translational alterations in a-syn seem to be driving neurodegeneration [28]. A breakthrough in the field of biomarkers in synucleinopathies has been achieved over the past 5 years with seeding amplification assays (SAAs), a technique currently applied in Creutzfeldt-Jacob disease. These SAAs are being tested for a-syn, due to the demonstration of prion-like properties of a-syn experimentally ***in vitro*** and ***in vivo*** [29, 30]. An overview of the a-syn studies in synucleinopathies is presented in the following section.

a) Total CSF and plasma a-synuclein

Several studies have measured total CSF a-syn levels in MSA. Most of these studies have reported a mild decrease in total a-syn levels in MSA, compared to control subjects [17, 18]. However, there is significant overlap in a-syn levels between MSA and healthy subjects [4, 17, 31]. For this reason CSF a-syn is not a clinically useful biomarker for MSA identification. Likewise, a similar decrease in CSF a-syn is evident in other synucleinopathies (i.e. PD and DLB). Thus, no significant differences were reported when comparing MSA with other synucleinopathies [4, 17, 18, 31, 32] or with other atypical parkinsonian syndromes (i.e.CBS, PSP) [4, 31]. A single study reported that CSF a-syn provided high positive predictive value for synucleinopathies, and could thus be used as a means for patient stratification in clinical trials [17].

Another approach is measuring panels of multiple biomarkers, in an effort to identify composite biomarkers. In accordance to this approach, CSF total a-syn was measured alongside four other biomarkers (Aβ42, t-tau, p-tau, NFL) in a large cohort comprising heterogeneous neurodegenerative disorders [1]. In this cohort, a-syn was significantly decreased in all synucleinopathies but could not independently differentiate among different synucleinopathies. A similar approach was implemented by another study group by assessing a panel of nine CSF biomarkers (including total a-syn) in a cohort of synucleinopathies (PD, MSA), tauopathies (PSP, CBS) as well as FTD and AD patients [3]. Contrary to most studies in the field, total a-syn in this cohort was decreased in the MSA group compared to PD patients, providing suboptimal diagnostic accuracy in differentiating MSA from PD. A subsequent study applied a panel of ten biomarkers, including CSF total and phosphorylated a-syn and plasma total a-syn in a cohort with diverse neurodegenerative disorders, including MSA [33]. MSA patients exhibited a non-specific decrease in CSF and plasma total a-syn levels, whereas phosphorylated a-syn was also decreased in MSA compared to the control groups. However, these a-syn forms were not useful in the differentiation of MSA from PD.

Several studies have measured plasma a-syn by ELISA. Most of these studies report a non-significant increase in plasma total a-syn in MSA compared to control groups, with significant between-group overlap [34, 35]. Another study reported significant plasma a-syn elevation in MSA patients compared to control subjects [36]. This increase was particularly pronounced in the MSA-P compared to the MSA-C group. Scatterplots of individual values of plasma a-syn indicated a large variability within the MSA group, with only a subset of MSA patients exhibiting significant a-syn elevation.

b) Phosphorylated and oligomeric CSF a-synuclein

Wang et al. measured total and phosphorylated at serine 129 CSF a-syn in a cohort of MSA, PD, PSP, AD patients and control subjects[37]. Total a-syn was decreased in the PD and MSA groups compared to controls. Phosphorylated a-syn was exclusively increased in the PD group, while MSA patients exhibited a decrease in phosphorylated a-syn compared to the control group. The phosphorylated/total a-syn ratio was significantly increased in both the PD and MSA groups compared to other study groups in both the discovery and validation cohorts of this study. These a-syn forms were not useful in differentiating PD from MSA.

Another study measured total, phosphorylated and oligomeric CSF a-syn in a cohort of 135 patients with diverse neurodegenerative disorders. Although numerical differences among differences emerged, synucleinopathies as a group (PD and MSA) presented with lower total a-syn and higher phosphorylated to total a-syn ratios compared to tauopathies (PSP and CBS)[38].

Foulds et al. measured total, oligomeric, phosphorylated and phosphorylated oligomeric a-syn in post-mortem ventricular CSF of a cohort of synucleinopathies [39]. MSA presented with numerically higher mean values of total, oligomeric and phosphorylated a-syn levels, whereas phosphorylated oligomeric a-syn levels were significantly higher (~20fold) compared to PD, DLB and PSP groups, indicating that this a-syn form may be a candidate biomarker for MSA. This finding has not been validated to date by follow-up studies.

c) A-synuclein in erythrocytes, exosomes

Zhang et al. measured haemoglobin-binding a-syn (Hb-a-syn) in erythrocytes in a large cohort of MSA patients (n=149), compared to healthy controls (n=149) [40]. Hb-a-syn could be considered a good surrogate marker of brain a-syn accumulation, but this requires further study. By use of ELISA, the authors concluded that Hb-a-syn in erythrocytes is

significantly increased in MSA compared to healthy subjects, with adequate specificity (~80%) but suboptimal sensitivity (~70%).

Another approach on a-synuclein quantification is isolation of exosomes from blood via immunoprecipitation. By using neuronal and oligodendroglial markers, Dutta et al. measured total a-syn in neuronal and oligodendroglial exosomes [41]. MSA patients exhibited significantly increased a-syn, particularly in oligodendroglial exosomes, compared to PD patients and control subjects. An elevated oligodendroglial / neuronal exosome a-syn ratio was highly indicative of MSA. This marker, established in a discovery cohort, was validated in a validation cohort.

A novel approach is measuring a-syn in erythrocyte membranes. A-synuclein is abundant in both erythrocyte membrane and cytoplasm. Liu et al. quantified total and oligomeric a-syn in erythrocytes membrane and cytoplasm through electrochemiluminescence immunoassays [42]. Both total and oligomeric, as well as the ratio of oligomeric/total a-syn were elevated in erythrocyte membranes in MSA patients compared to controls. These differences were not evident for cytoplasmic a-syn. The ratio provided suboptimal combined sensitivity and specificity for the differentiation of MSA from controls.

A study by Li et al. focused on erythrocyte phosphorylated a-syn (at serine 129) as a candidate biomarker for MSA[43]. In this study, the MSA group (n=107) exhibited significantly higher values of p-a-syn compared to control subjects (n=220), producing a~70% sensitivity and ~90% specificity for an MSA diagnosis. MSA-P patients presented elevated p-a-syn values compared to MSA-C.

Along the same lines, Wang et al. focused on the oligomeric a-syn quantification in red blood cells (RBC), as a candidate marker of synucleinopathies [44]. The oligomeric a-syn to total RBC protein ratio differentiated PD from control subjects, with suboptimal specificity. This ratio was also elevated in the MSA group compared to the control subjects, but did not produce adequate diagnostic accuracy for the differentiation of MSA from PD or the control group.

Folke et al. studied possible differences in CSF and plasma anti-a-syn IGM and IgG naturally occurring antibodies (nAbs) in MSA vs. PD [45]. This study reported an elevation of total CSF IgG nAbs, as well as IgG subclasses in MSA and PD compared to controls, with MSA presenting with increased CSF anti-a-syn IgG1, IgG3 and IgG4 nAbs levels compared to PD. The same pattern was evident for plasma IgG subgroups, with PD and MSA exhibiting lower levels of anti-a-syn IgM nAbs compared to controls in CSF and plasma. The utility of nAbs quantification as a surrogate biomarker of synucleinopathies need further validation.

A small study by Cao et al. measured total, phosphorylated and oligomeric a-syn in extracellular vesicles from saliva of MSA (n=16) and PD patients (n=26)[46]. The two groups did not exhibit significant differences in any of the aforementioned a-syn forms.

d) Seeding assays of a-synuclein

Shahnawaz et al. implemented a seeding assay (protein misfolding cyclic amplification – PMCA) in CSF of MSA and PD patients[47]. Using different amyloid-conformation-specific dyes, the authors concluded that a-syn PMCA in CSF samples can readily differentiate between PD and MSA, due to differences in a-syn conformational strains in these disorders. The overall sensitivity of this methodology approached 95%.

Likewise, Rossi et al. applied RT-QuIC in a large cohort (n=439) of CSF samples of diverse neurodegenerative disorders [48]. Only two of the 31 MSA patients exhibited seeding activity with this assay, indicating inherent differences in the conformational strains underlying MSA compared to Lewy body disease (LBD).

Poggiolini et al. applied an RT-QuIC assay in a cohort of synucleinopathies, in an effort to look into the possible value of this assay in predicting disease progression of synucleinopathies [49]. Sensitivity for MSA was 75%, with differences in reaction kinetics compared to PD (longer T_{50} and lower V_{max}). Reaction kinetics correlated with disease progression only in the MSA group.

Another approach in identifying biomarkers is the use of composite markers, which include >1 biomarkers. Using this approach, Singer et al. implemented an a-syn PMCA alongside CSF NFL in an effort to differentiate MSA patients from control subjects and PD/DLB [50]. CSF NFL was markedly increased compared to control subjects, whereas a-syn PMCA was reactive in almost all MSA, but with distinct reaction kinetics (MSA exhibited earlier but significantly lower fluorescence compared to LBD). This dual approach differentiated MSA from controls (NFL) as well as LBD (PMCA).

The same approach was followed by another study group, by combining a-syn RT-QuIC with CSF/plasma NFL [51]. RT-QuIC produced a positive seeding reaction in 3/65 MSA patients. The kinetic curves of RT-QuIC in MSA patients differed significantly from the respective curves in PD (significantly lower relative fluorescent units). Combined use of a-syn RT-QuIC and NFL further optimized the differentiation of MSA from PD.

Okuzumi et al. combined immunoprecipitation (IP), in a attempt to concentrate a-syn seeds from serum, followed by real-time quaking-induced conversion (RT-QuIC) assay (IP/RT-QuIC), in a cohort of synucleinopathies [52]. This method provided moderate diagnostic performance for differentiation of MSA

from control subjects, in two discovery cohorts and an external blinded validation cohort (AUCs: 0.64 to 0.80). Interestingly, amplified seeds from different synucleinopathies, maintained their morphological features of fibrils, as evidenced by transmission electron microscopy.

Other biomarkers

Neurofilament light chain (NFL) is a non-specific marker of neuroaxonal damage. CSF NFL was quantified by ELISA in an study comparing 19 PD patients, 12 PSP and 10 MSA patients. Mean NFL levels were significantly elevated in the MSA and PSP groups compared to PD patients, with some overlap between PSP/MSA and PD. NFL levels correlated with disease progression in atypical parkinsonian groups [53]. A subsequent study including a CBS group validated these results, further establishing NFL as a useful marker in the differentiation of PD from atypical Parkinsonism. Moreover, NFL remained unaltered in consecutive analyses, indicating a stable rate of axonal damage within the atypical parkinsonian disorders [54]. These findings were supported by follow-up studies comparing MSA with PD [14, 55] and PSP with synucleinopathies [56]. Baseline CSF and plasma NFL has been used as a predictor of disease progression in atypical parkinsonian disorders [55, 57, 58]. Plasma NFL has also been reported to assist in the differential diagnosis of PD from atypical Parkinsonism [55, 59].

MicroRNAs (miRNAs) participate in protein translation and are present in CSF. Several studies have focused on miRNAs profiles in CSF, as candidate biomarkers of neurodegenerative disorders. To this end, Marques et al. reported differences in miRNAs between PD and MSA patients from control subjects. Combinations of miRNAs could discriminate both MSA and PD from healthy subjects [60]. The same approach was implemented in plasma miRNA profiles in cohorts of PD and MSA, with differences emerging between groups regarding the expression of various miRNAs[61]. This concept was extended in CSF samples in two PSP cohorts, identifying an upregulation of multiple miRNAs in this disease group[62, 63], as well as in plasma samples in PSP[64].

Glial fibrillary acidic protein (GFAP) is a monomeric protein located in the astroglial cytoskeleton. It has been tested as a candidate biomarker for the differentiation of MSA from spinocerebellar ataxias and in the differentiation of PD from DLB and MSA [65-67]. Although mild elevations of CSF GFAP as measured by ELISA have been reported, GFAP did not assist in the differential diagnosis among synucleinopathies.

Coenzyme Q10 is a key component of the mitochondrial respiratory chain, and has been tried in clinical trials of PSP [68]. To this end, CSF and plasma Q10 levels have been measured in cohorts of synucleinopathies, particularly MSA. In these studies, a non-significant decrease in Q10 levels was reported, with significant overlap between PD and MSA groups [69-72].

A multitude of diverse candidate biomarkers in CSF and plasma, including YKL-40 [73, 74], myelin basic protein – MBP [65], various neurotransmitters [75-78] have been tested in the past. These studies have largely yielded negative results. The exhaustive review of all these studies is beyond the scope of the present overview.

Conclusions

Over the past three decades, studies on candidate biomarkers for atypical parkinsonian disorders have increased exponentially, thus providing us with valuable insight into the pathophysiological mechanisms underlying their complex neurodegenerative disorders. Despite these efforts, a clinically applicable CSF or plasma biomarker for neurodegenerative parkinsonian disorders is currently lacking.

However, from a practical standpoint, established AD CSF biomarker profiling is recommended in all instances of typical or atypical manifestations of AD, including corticobasal syndrome. This should be incorporated into everyday clinical practice where available, since the recognition of CBS-AD patients ***in vivo*** greatly enhances their individualized pharmacological management and assists in providing more accurate information regarding prognosis. Additionally, studies on CSF and plasma NFL levels have supported its use as a biomarker for the differentiation of PD from atypical Parkinsonism. Additional studies are needed to further validate the use of NFL in parkinsonian neurodegenerative disorders.

CSF AD biomarkers are routinely used in the setting of clinical trials of candidate disease-modifying, protein-targeting treatments in AD, and are starting to be implemented in a clinical setting. We are hopeful that the paradigm of AD will be followed in other proteinopathies, such as tauopathies, TDP-43 proteinopathies and synucleinopathies. The emergence of a-syn seeding assays has provided us with encouraging results regarding the development of a clinically relevant biomarker for synucleinopathies in the near future. If similar assays were developed also for Tauopathies, this would greatly aid in the differential diagnosis of atypical Tauopathies leading to Parkinsonism, such as PSP and CBS.

References

[1] Hall, S., et al., *Accuracy of a panel of 5 cerebrospinal fluid biomarkers in the differential diagnosis of patients with dementia and/or parkinsonian disorders.* Arch Neurol, 2012. **69**(11):

p. 1445-52.
[2] Schoonenboom, N.S., et al., *Cerebrospinal fluid markers for differential dementia diagnosis in a large memory clinic cohort.* Neurology, 2012. **78**(1): p. 47-54.
[3] Magdalinou, N.K., et al., *A panel of nine cerebrospinal fluid biomarkers may identify patients with atypical parkinsonian syndromes.* J Neurol Neurosurg Psychiatry, 2015. **86**(11): p. 1240-7.
[4] Constantinides, V.C., et al., *CSF biomarkers beta-amyloid, tau proteins and a-synuclein in the differential diagnosis of Parkinson-plus syndromes.* J Neurol Sci, 2017. **382**: p. 91-95.
[5] Noguchi, M., et al., *Decreased beta-amyloid peptide42 in cerebrospinal fluid of patients with progressive supranuclear palsy and corticobasal degeneration.* J Neurol Sci, 2005. **237**(1-2): p. 61-5.
[6] Wagshal, D., et al., *Divergent CSF tau alterations in two common tauopathies: Alzheimer's disease and progressive supranuclear palsy.* J Neurol Neurosurg Psychiatry, 2015. **86**(3): p. 244-50.
[7] Schirinzi, T., et al., *Clinical value of CSF amyloid-beta-42 and tau proteins in Progressive Supranuclear Palsy.* J Neural Transm (Vienna), 2018. **125**(9): p. 1373-1379.
[8] Aerts, M.B., et al., *Cerebrospinal fluid tau and phosphorylated tau protein are elevated in corticobasal syndrome.* Mov Disord, 2011. **26**(1): p. 169-73.
[9] Mitani, K., et al., *Increased CSF tau protein in corticobasal degeneration.* J Neurol, 1998. **245**(1): p. 44-6.
[10] Urakami, K., et al., *A comparison of tau protein in cerebrospinal fluid between corticobasal degeneration and progressive supranuclear palsy.* Neurosci Lett, 1999. **259**(2): p. 127-9.
[11] Urakami, K., et al., *Diagnostic significance of tau protein in cerebrospinal fluid from patients with corticobasal degeneration or progressive supranuclear palsy.* J Neurol Sci, 2001. **183**(1): p. 95-8.
[12] Sussmuth, S.D., et al., *Differential pattern of brain-specific CSF proteins tau and amyloid-beta in Parkinsonian syndromes.* Mov Disord, 2010. **25**(9): p. 1284-8.
[13] Abdo, W.F., et al., *CSF neurofilament light chain and tau differentiate multiple system atrophy from Parkinson's disease.* Neurobiol Aging, 2007. **28**(5): p. 742-7.
[14] Herbert, M.K., et al., *CSF Neurofilament Light Chain but not FLT3 Ligand Discriminates Parkinsonian Disorders.* Front Neurol, 2015. **6**: p. 91.
[15] Abdo, W.F., et al., *Cerebrospinal fluid analysis differentiates multiple system atrophy from Parkinson's disease.* Mov Disord, 2004. **19**(5): p. 571-9.
[16] Herbert, M.K., et al., *CSF levels of DJ-1 and tau distinguish MSA patients from PD patients and controls.* Parkinsonism Relat Disord, 2014. **20**(1): p. 112-5.
[17] Mollenhauer, B., et al., *alpha-Synuclein and tau concentrations in cerebrospinal fluid of patients presenting with parkinsonism: a cohort study.* Lancet Neurol, 2011. **10**(3): p. 230-40.
[18] Shi, M., et al., *Cerebrospinal fluid biomarkers for Parkinson disease diagnosis and progression.* Ann Neurol, 2011. **69**(3): p. 570-80.
[19] Holmberg, B., et al., *Cerebrospinal fluid Abeta42 is reduced in multiple system atrophy but normal in Parkinson's disease and progressive supranuclear palsy.* Mov Disord, 2003. **18**(2): p. 186-90.
[20] Verbeek, M.M., et al., *Cerebrospinal fluid Abeta42 levels in multiple system atrophy.* Mov Disord, 2004. **19**(2): p. 238-40; author reply 240-1.
[21] Borroni, B., et al., *CSF Alzheimer's disease-like pattern in corticobasal syndrome: evidence for a distinct disorder.* J Neurol Neurosurg Psychiatry, 2011. **82**(8): p. 834-8.
[22] Constantinides, V.C., et al., *Clinical, neuropsychological and imaging characteristics of Alzheimer's disease patients presenting as corticobasal syndrome.* J Neurol Sci, 2019. **398**: p. 142-147.
[23] Paraskevas, G.P., et al., *Cerebrospinal Fluid Biomarkers as a Diagnostic Tool of the Underlying Pathology of Primary Progressive Aphasia.* Journal of Alzheimers Disease, 2017. **55**(4): p. 1453-1461.
[24] Paraskevas, G.P., et al., *In vivo Prevalence of Alzheimer Biomarkers in Dementia with Lewy Bodies.* Dement Geriatr Cogn Disord, 2019. **47**(4-6): p. 289-296.
[25] Constantinides, V.C., et al., *Application of the AT(N) and Other CSF Classification Systems in Behavioral Variant Frontotemporal Dementia.* Diagnostics (Basel), 2023. **13**(3).
[26] Constantinides, V.C., et al., *Cerebrospinal fluid biomarker profiling in corticobasal degeneration: Application of the AT(N) and other classification systems.* Parkinsonism Relat Disord, 2021. **82**: p. 44-49.
[27] Spillantini, M.G., et al., *Filamentous alpha-synuclein inclusions link multiple system atrophy with Parkinson's disease and dementia with Lewy bodies.* Neurosci Lett, 1998. **251**(3): p. 205-8.
[28] Beyer, K. and A. Ariza, α-Synuclein posttranslational modification and alternative splicing as a trigger for neurodegeneration. Mol Neurobiol, 2013. **47**(2): p. 509-24.
[29] Volpicelli-Daley, L. and P. Brundin, *Prion-like propagation of pathology in Parkinson disease.* Handb Clin Neurol, 2018. **153**: p. 321-335.
[30] Bernis, M.E., et al., *Prion-like propagation of hu-*

man brain-derived alpha-synuclein in transgenic mice expressing human wild-type alpha-synuclein. Acta Neuropathol Commun, 2015. **3**: p. 75.
[31] Aerts, M.B., et al., *CSF alpha-synuclein does not differentiate between parkinsonian disorders.* Neurobiol Aging, 2012. **33**(2): p. 430 e1-3.
[32] Tateno, F., et al., *Alpha-synuclein in the cerebrospinal fluid differentiates synucleinopathies (Parkinson Disease, dementia with Lewy bodies, multiple system atrophy) from Alzheimer disease.* Alzheimer Dis Assoc Disord, 2012. **26**(3): p. 213-6.
[33] Schulz, I., et al., *Systematic Assessment of 10 Biomarker Candidates Focusing on alpha-Synuclein-Related Disorders.* Mov Disord, 2021. **36**(12): p. 2874-2887.
[34] Lee, P.H., et al., *The plasma alpha-synuclein levels in patients with Parkinson's disease and multiple system atrophy.* J Neural Transm (Vienna), 2006. **113**(10): p. 1435-9.
[35] Seino, Y., et al., *Cerebrospinal Fluid and Plasma Biomarkers in Neurodegenerative Diseases.* J Alzheimers Dis, 2019. **68**(1): p. 395-404.
[36] Sun, Z.F., et al., *Increase of the plasma alpha-synuclein levels in patients with multiple system atrophy.* Mov Disord, 2014. **29**(3): p. 375-9.
[37] Wang, Y., et al., *Phosphorylated alpha-synuclein in Parkinson's disease.* Sci Transl Med, 2012. **4**(121): p. 121ra20.
[38] Constantinides, V.C., et al., *Cerebrospinal Fluid a-synuclein species in cognitive and movement disorders.* Brain Sci, 2021. **11**(1).
[39] Foulds, P.G., et al., *Post mortem cerebrospinal fluid alpha-synuclein levels are raised in multiple system atrophy and distinguish this from the other alpha-synucleinopathies, Parkinson's disease and Dementia with Lewy bodies.* Neurobiol Dis, 2012. **45**(1): p. 188-95.
[40] Zhang, X., et al., *Hemoglobin-binding alpha-synuclein levels in erythrocytes are elevated in patients with multiple system atrophy.* Neurosci Lett, 2022. **789**: p. 136868.
[41] Dutta, S., et al., *alpha-Synuclein in blood exosomes immunoprecipitated using neuronal and oligodendroglial markers distinguishes Parkinson's disease from multiple system atrophy.* Acta Neuropathol, 2021. **142**(3): p. 495-511.
[42] Liu, G., et al., *Alpha-synuclein in erythrocyte membrane of patients with multiple system atrophy: A pilot study.* Parkinsonism Relat Disord, 2019. **60**: p. 105-110.
[43] Li, X.Y., et al., *Phosphorylated Alpha-Synuclein in Red Blood Cells as a Potential Diagnostic Biomarker for Multiple System Atrophy: A Pilot Study.* Parkinsons Dis, 2020. **2020**: p. 8740419.
[44] Wang, X., et al., *Detection of alpha-synuclein oligomers in red blood cells as a potential biomarker of Parkinson's disease.* Neurosci Lett, 2015. **599**: p. 115-9.
[45] Folke, J., et al., *Cerebrospinal fluid and plasma distribution of anti-alpha-synuclein IgMs and IgGs in multiple system atrophy and Parkinson's disease.* Parkinsonism Relat Disord, 2021. **87**: p. 98-104.
[46] Cao, Z., et al., *Differential Diagnosis of Multiple System Atrophy-Parkinsonism and Parkinson's Disease Using alpha-Synuclein and External Anal Sphincter Electromyography.* Front Neurol, 2020. **11**: p. 1043.
[47] Shahnawaz, M., et al., *Discriminating alpha-synuclein strains in Parkinson's disease and multiple system atrophy.* Nature, 2020. **578**(7794): p. 273-277.
[48] Rossi, M., et al., *Ultrasensitive RT-QuIC assay with high sensitivity and specificity for Lewy body-associated synucleinopathies.* Acta Neuropathol, 2020. **140**(1): p. 49-62.
[49] Poggiolini, I., et al., *Diagnostic value of cerebrospinal fluid alpha-synuclein seed quantification in synucleinopathies.* Brain, 2022. **145**(2): p. 584-595.
[50] Singer, W., et al., *Alpha-Synuclein Oligomers and Neurofilament Light Chain in Spinal Fluid Differentiate Multiple System Atrophy from Lewy Body Synucleinopathies.* Ann Neurol, 2020. **88**(3): p. 503-512.
[51] Quadalti, C., et al., *Neurofilament light chain and alpha-synuclein RT-QuIC as differential diagnostic biomarkers in parkinsonisms and related syndromes.* NPJ Parkinsons Dis, 2021. **7**(1): p. 93.
[52] Okuzumi, A., et al., *Propagative alpha-synuclein seeds as serum biomarkers for synucleinopathies.* Nat Med, 2023. **29**(6): p. 1448-1455.
[53] Holmberg, B., et al., *Increased cerebrospinal fluid levels of neurofilament protein in progressive supranuclear palsy and multiple-system atrophy compared with Parkinson's disease.* Mov Disord, 1998. **13**(1): p. 70-7.
[54] Constantinescu, R., et al., *Consecutive analyses of cerebrospinal fluid axonal and glial markers in Parkinson's disease and atypical Parkinsonian disorders.* Parkinsonism Relat Disord, 2010. **16**(2): p. 142-5.
[55] Chelban, V., et al., *Neurofilament light levels predict clinical progression and death in multiple system atrophy.* Brain, 2022. **145**(12): p. 4398-4408.
[56] Oliveira Hauer, K., et al., *Performance of [(18) F]RO948 PET, MRI and CSF neurofilament light in the differential diagnosis of progressive supranuclear palsy.* Parkinsonism Relat Disord, 2023. **106**: p. 105226.
[57] Rojas, J.C., et al., *Plasma neurofilament light*

chain predicts progression in progressive supranuclear palsy. Ann Clin Transl Neurol, 2016. **3**(3): p. 216-25.
[58] Bacioglu, M., et al., *Neurofilament Light Chain in Blood and CSF as Marker of Disease Progression in Mouse Models and in Neurodegenerative Diseases.* Neuron, 2016. **91**(1): p. 56-66.
[59] Marques, T.M., et al., *Serum NFL discriminates Parkinson disease from atypical parkinsonisms.* Neurology, 2019. **92**(13): p. e1479-e1486.
[60] Marques, T.M., et al., *MicroRNAs in Cerebrospinal Fluid as Potential Biomarkers for Parkinson's Disease and Multiple System Atrophy.* Mol Neurobiol, 2017. **54**(10): p. 7736-7745.
[61] Uwatoko, H., et al., *Identification of plasma microRNA expression changes in multiple system atrophy and Parkinson's disease.* Mol Brain, 2019. **12**(1): p. 49.
[62] Nonaka, W., et al., *A cerebrospinal fluid microRNA analysis: Progressive supranuclear palsy.* Mol Med Rep, 2022. **25**(3).
[63] Simoes, F.A., et al., *Potential of Non-Coding RNA as Biomarkers for Progressive Supranuclear Palsy.* Int J Mol Sci, 2022. **23**(23).
[64] Ramaswamy, P., et al., *Plasma microRNAs as a Potential Biomarker for Identification of Progressive Supranuclear Palsy.* Diagnostics (Basel), 2022. **12**(5).
[65] Santaella, A., et al., *Cerebrospinal fluid myelin basic protein is elevated in multiple system atrophy.* Parkinsonism Relat Disord, 2020. **76**: p. 80-84.
[66] Canaslan, S., et al., *Detection of Cerebrospinal Fluid Neurofilament Light Chain as a Marker for Alpha-Synucleinopathies.* Front Aging Neurosci, 2021. **13**: p. 717930.
[67] Brouillette, A.M., G. Oz, and C.M. Gomez, *Cerebrospinal Fluid Biomarkers in Spinocerebellar Ataxia: A Pilot Study.* Dis Markers, 2015. **2015**: p. 413098.
[68] Apetauerova, D., et al., *CoQ10 in progressive supranuclear palsy: A randomized, placebo-controlled, double-blind trial.* Neurol Neuroimmunol Neuroinflamm, 2016. **3**(5): p. e266.
[69] Kasai, T., et al., *Serum Levels of Coenzyme Q10 in Patients with Multiple System Atrophy.* PLoS One, 2016. **11**(1): p. e0147574.
[70] Mitsui, J., et al., *Plasma Coenzyme Q10 Levels in Patients With Multiple System Atrophy.* JAMA Neurol, 2016. **73**(8): p. 977-80.
[71] Compta, Y., et al., *Cerebrospinal fluid levels of coenzyme Q10 are reduced in multiple system atrophy.* Parkinsonism Relat Disord, 2018. **46**: p. 16-23.
[72] Du, J., et al., *Clinical correlates of decreased plasma coenzyme Q10 levels in patients with multiple system atrophy.* Parkinsonism Relat Disord, 2018. **57**: p. 58-62.
[73] Olsson, B., et al., *The glial marker YKL-40 is decreased in synucleinopathies.* Mov Disord, 2013. **28**(13): p. 1882-5.
[74] Hall, S., et al., *Cerebrospinal fluid concentrations of inflammatory markers in Parkinson's disease and atypical parkinsonian disorders.* Sci Rep, 2018. **8**(1): p. 13276.
[75] Polinsky, R.J., et al., *CSF acetylcholinesterase levels are reduced in multiple system atrophy with autonomic failure.* Neurology, 1989. **39**(1): p. 40-4.
[76] Martignoni, E., et al., *Cerebrospinal fluid norepinephrine, 3-methoxy-4-hydroxyphenylglycol and neuropeptide Y levels in Parkinson's disease, multiple system atrophy and dementia of the Alzheimer type.* J Neural Transm Park Dis Dement Sect, 1992. **4**(3): p. 191-205.
[77] Kanemaru, K., K. Mitani, and H. Yamanouchi, *Cerebrospinal fluid homovanillic acid levels are not reduced in early corticobasal degeneration.* Neurosci Lett, 1998. **245**(2): p. 121-2.
[78] Atack, J.R., et al., *Cerebrospinal fluid acetylcholinesterase in progressive supranuclear palsy: reduced activity relative to normal subjects and lack of inhibition by oral physostigmine.* J Neurol Neurosurg Psychiatry, 1991. **54**(9): p. 832-5.

GENETIC BIOMARKERS IN PARKINSON'S DISEASE

Georgia Xiromerisiou, Chrysoula Marogianni , Olga Sinani
University of Thessaly, Medical School

Summary

Parkinson's disease (PD) is a neurodegenerative condition that involves the gradual loss of dopaminergic neurons in the substantia nigra. The discovery of genetic biomarkers has greatly enhanced our comprehension of the etiology of Parkinson's disease (PD), providing valuable knowledge about the molecular pathways that drive the disease and creating opportunities for improved diagnosis, prognosis, and focused treatment. Genetic investigations have identified several crucial genes linked to Parkinson's disease (PD), such as LRRK2, SNCA, GBA, PARK2 (Parkin), PINK1, and PARK7 (DJ-1), among others. The presence of mutations in these genes emphasizes the significance of protein aggregation, mitochondrial dysfunction, lysosomal processing, and oxidative stress in the etiology of Parkinson's disease (PD). For example, mutations in the LRRK2 and SNCA genes are associated with both familial and sporadic cases of Parkinson's disease (PD), highlighting the importance of alpha-synuclein aggregation and kinase activity. GBA mutations, which are commonly linked to Gaucher's disease, have been found to be important risk factors for the development of PD. This highlights the role of lysosomal dysfunction in contributing to the disease.

Recent progress in the fields of genomics and bioinformatics has made it easier to identify more genetic variables and pathways that have a role in Parkinson's disease (PD). These genes encompass those associated with immunological response, cellular adhesion, dopamine production, and mitochondrial quality control. These findings not only improve our comprehension of the diverse genetic characteristics of PD but also emphasize the intricate interplay between genetic vulnerability and environmental factors in the progression of the disease.

Discovering genetic biomarkers for PD has potential for enhancing the therapeutic treatment of the disease. Genetic screening can assist in the early detection of diseases, enabling the implementation of neuroprotective treatments prior to the occurrence of substantial neurodegeneration. Moreover, comprehending the genetic foundation of PD facilitates the creation of individualized medical strategies that focus on certain pathways modified by genetic mutations in affected individuals.

Keywords:Parkinson's disease , biomarkes, genetic biomarkers, genes , mutations, genetic screening

Research on genetic indicators of PD is advancing quickly, with important implications for the diagnosis, prognosis, and therapy of the illness. Subsequent research should prioritize the examination of the functional nature The important step in understanding the complicated causes of Parkinson's disease (PD) and creating successful treatments is the thorough examination of discovered genetic variations and their interplay with environmental factors.

ΓΕΝΕΤΙΚΟΙ ΒΙΟΔΕΙΚΤΕΣ ΣΤΗ ΝΟΣΟ ΠΑΡΚΙΝΣΟΝ

Γεωργία Ξηρομερήσιου, Χρυσούλα Μαρογιάννη, Όλγα Σινάνη
Νευρολογική Κλινική Πανεπιστημίου Θεσσαλίας

Περίληψη

Η νόσος του Πάρκινσον (PD είναι μια νευροεκφυλιστική διαταραχή που χαρακτηρίζεται από την προοδευτική απώλεια ντοπαμινεργικών νευρώνων στη μέλαινα ουσία. Ο εντοπισμός γενετικών βιοδεικτών έχει βελτιώσει σημαντικά την κατανόησή μας για την παθογένεια της PD, προσφέροντας γνώσεις για τους μοριακούς μηχανισμούς που βρίσκονται στη βάση της νόσου και ανοίγοντας νέους δρόμους για διάγνωση, πρόγνωση και στοχευμένη θεραπεία.

Γενετικές μελέτες έχουν αποκαλύψει πολλά βασικά γονίδια που σχετίζονται με την PD, συμπεριλαμβανομένων των LRRK2, SNCA, GBA, PARK2 (Parkin), PINK1 και PARK7 (DJ-1) μεταξύ άλλων. Οι μεταλλάξεις σε αυτά τα γονίδια υπογραμμίζουν τη σημασία της συσσώρευσης πρωτεϊνών, της μιτοχονδριακής δυσλειτουργίας, της λυσοσωμικής επεξεργασίας και του οξειδωτικού στρες στην παθοφυσιολογία της PD. Για παράδειγμα, οι μεταλλάξεις LRRK2 και SNCA εμπλέκονται σε οικογενείς και σποραδικές περιπτώσεις PD, δίνοντας έμφαση στο ρόλο της συσσωμάτωσης άλφα-συνουκλεΐνης και της δραστηριότητας κινάσης της LRRK2. Ομοίως, οι μεταλλάξεις GBA, γνωστές για τη συσχέτισή τους με τη νόσο του Gaucher, έχουν αναγνωριστεί ως σημαντικοί παράγοντες κινδύνου για την ανάπτυξη PD, υπογραμμίζοντας τη συμβολή της λυσοσωμικής δυσλειτουργίας στη νόσο.

Οι πρόσφατες εξελίξεις στη γονιδιωματική και τη βιοπληροφορική έχουν διευκολύνει την ανακάλυψη πρόσθετων γενετικών παραγόντων και οδών που εμπλέκονται στην PD. Αυτό περιλαμβάνει γονίδια που σχετίζονται με την ανοσολογική απόκριση, την κυτταρική προσκόλληση, τη βιοσύνθεση ντοπαμίνης και τον ποιοτικό έλεγχο των μιτοχονδρίων. Αυτές οι ανακαλύψεις όχι μόνο ενισχύουν την κατανόησή μας για τη γενετική ετερογένεια της PD αλλά τονίζουν επίσης την περίπλοκη αλληλεπίδραση μεταξύ της γενετικής ευαισθησίας και των περιβαλλοντικών παραγόντων στην ανάπτυξη της νόσου.
Ο εντοπισμός γενετικών βιοδεικτών για την PD υπόσχεται τη βελτίωση της κλινικής διαχείρισης της νόσου. Ο γενετικός έλεγχος μπορεί να βοηθήσει στην έγκαιρη διάγνωση, επιτρέποντας την έναρξη νευροπροστατευτικών θεραπειών πριν εμφανιστεί σημαντική νευροεκφύλιση. Επιπλέον, η κατανόηση της γενετικής βάσης της PD επιτρέπει την ανάπτυξη εξατομικευμένων ιατρικών προσεγγίσεων, στοχεύοντας συγκεκριμένα μονοπάτια που μεταβάλλονται από γενετικές μεταλλάξεις σε προσβεβλημένα άτομα.
Συμπερασματικά, η έρευνα για τους γενετικούς βιοδείκτες της PD εξελίσσεται ταχέως, με σημαντικές επιπτώσεις στη διάγνωση, την πρόγνωση και τη θεραπεία της νόσου. Μελλοντικές μελέτες που επικεντρώνονται στον λειτουργικό χαρακτηρισμό των αναγνωρισμένων γενετικών παραλλαγών και την αλληλεπίδρασή τους με περιβαλλοντικούς παράγοντες θα είναι κρίσιμες για την αποκάλυψη της πολύπλοκης αιτιολογίας της PD και την ανάπτυξη αποτελεσματικών θεραπευτικών στρατηγικών.

Λέξεις κλειδιά: Νόσος Parkinson, βιοδείκτες, γενετικοί βιοδείκτες, γονίδια, μεταλλάξεις, γενετικός έλεγχος

1.Introduction

Parkinson's disease is a neurodegenerative condition that gradually worsens over time. It is characterized by the gradual death of specific brain cells called dopaminergic neurons in a region of the brain called the substantia nigra. This cell death leads to the development of symptoms such as tremors, stiffness, and slowness of movement. Although the precise etiology of PD is still uncertain, it is widely accepted that a mix of genetic, environmental, and lifestyle variables play a role in its pathogenesis. Recent advancements in genetic research have provided valuable information on certain genetic markers that heighten the vulnerability to PD, providing fresh understanding of its development and prospective targets for treatment. Genetic factors contribute to over 25% of the risk linked to Parkinson's disease, and the genetic variations related with it differ in terms of both occurrence and risk level. Although uncommon, mutations occurring within specific genes (referred to as monogenic causes) can potentially contribute to the development of the condition. However, these mutations are typically identified through linkage analysis in families affected by the condition. Genome-wide association studies (GWASs) have identified several common genetic variations that have a minor impact on risk. These variants, such as GBA or LRRK2 variants, contribute to intermediate risk.

Familial Parkinson's disease, also known as monogenic PD, is distinguished by infrequent yet very influential genetic variations that elevate the likelihood of developing the condition. Autosomal dominant (for example, SNCAA53T and VPS35D620N) and recessive types of familial Parkinson's disease have been detected using linkage analysis in families, with the use of next-generation sequencing technology. However, it is important to note that only a small percentage, specifically 5-10%, of cases can be classified under this disease group. Genome-wide association studies (GWASs) have discovered low-penetrance genetic variants that are more commonly associated with sporadic Parkinson's disease, as opposed to high-penetrance variants. Initially, differentiating between familial and sporadic disease can assist in the identification of the disease, prediction of its course, and providing genetic guidance for family members who are at risk. Nevertheless, this categorization may mask the shared genetic or biological pathways that underlie both conditions.

2. Genetic basis of Parkinson's disease

2.1 The PARK genes and their influence

The identification of mutations in certain PARK genes has played a crucial role in comprehending the genetic foundation of Parkinson's disease (PD). The designation "PARK" for genes linked to Parkinson's disease (PD) is derived from a systematic nomenclature employed to discover and classify genes that, when altered, result in the onset of PD. The purpose of this naming approach is to systematically categorize the expanding roster of genetic variables that have been identified as contributors to the disease. Every gene associated with Parkinson's disease is given a distinct numerical identifier with the prefix "PARK", for example PARK1, PARK2, and so forth. This measurement aids in differentiating between several genes and mutations that contribute to the development of Parkinson's disease. The numerical sequence frequently corresponds to the chronological order in which these genes were identified or persistently linked to Parkinson's disease, regardless of their significance or role in the disease mechanism.

The PARK genes encompass a diverse array of functions and pathways, which include, but are not limited to, alpha-synuclein aggregation (e.g., SNCA/PARK1), mitochondrial function (e.g., PINK1/PARK6, PARK2/parkin), lysosomal function (e.g., GBA), and protein degradation (e.g., LRRK2/PARK8). The discovery and classification of these genes using the PARK nomenclature has played a crucial role in enhancing our comprehension of Parkinson's disease. This has provided valuable information about its molecular foundation and identified prospective targets for therapeutic treatment. PARK8, or the LRRK2 gene, is responsible for a significant number of cases of Parkinson's disease due to autosomal dominant mutations. LRRK2 mutations play a role in both familial and sporadic types of the disease, underscoring its significance in the pathogenesis of Parkinson's disease. Mutations in PARK1/4 have a significant impact on the protein alpha-synuclein, leading to the buildup of alpha-synuclein, which is a crucial characteristic of Parkinson's disease.

Autosomal recessive variants of Parkinson's disease (PD) are mostly linked to mutations in the PARK2/parkin gene, which are connected to mitochondrial malfunction and oxidative stress. Additional recessive mutations, such as those found in PARK6, PARK7 and additional PARK genes, further highlight the genetic diversity and complexity of PD.

We will provide a more comprehensive analysis of each gene and its specific contribution to the development of the disease.

The SNCA gene, which codes for the alpha-synuclein protein, has a key function in the development of Parkinson's disease (PD), a neurodegenerative condition marked by the gradual decline of dopaminergic neurons in the substantia nigra. This paper examines the intricate correlation between SNCA and PD, emphasizing the gene's structure, function, and the molecular mechanisms that explain its involvement in the disease.

2.2 Genetic variations and the risk of Parkinson's disease

2.2.1. SNCA

Alpha-synuclein, a protein produced by the SNCA gene, plays a crucial role in Parkinson's disease (PD) through both uncommon and frequent genetic variations. Autosomal dominant types of familial Parkinson's disease are linked to infrequent mutations, including p.A53T, p.A30P, and p.E46K. On the other hand, the SNCA rs356168 mutation, which is present in around 40% of people with European ancestry, has a less significant impact on the likelihood of developing the condition. These findings emphasize the significance of alpha-synuclein in the development of PD, namely its involvement in disease processes caused by the buildup of alpha-synuclein. The SNCA gene codes for a protein that weighs 14.5 kilodaltons and consists of 140 amino acids. This protein is produced from five exons and has a transcript length of 3,041 base pairs. SNCA is situated on chromosome 4q21.3-q22 and is a member of the synuclein protein family, which also contains beta-synuclein (SNCB) and gamma-synuclein (SNCG). The alpha-synuclein protein contains an area at the beginning called the N-terminal region, which has repetitive motifs known as KXKEGV. It also has a region called the non-Aβ component (NAC) region, which is very hydrophobic. Lastly, there is a portion at the end called the C-terminal region, which is acidic. The structural flexibility of this entity enables it to exist in two different forms: either as a disordered single unit or as a folded arrangement of four helices. This challenges the previously accepted notion that its single unit form is inherently hazardous. Alpha-synuclein is predominantly located in the brain, although it can also be detected in the heart, skeletal muscle, and pancreas. The precise biological role of it is not well understood, however various possibilities have been suggested. These functions encompass the regulation of dopamine, the promotion of tau protein fibrillation, and the protection of non-dopaminergic neurons through the modulation of p53 expression and apoptosis-related genes. The characteristic of Parkinson's disease is the abnormal buildup of alpha-synuclein within neurons, which then spreads to other regions of the brain. This process encompasses multiple methods of intraneuronal transmission, resulting in extensive neurotoxicity. SNCA plays a vital role in the development of PD, making it a key focus for therapeutic intervention and research. Gaining a clear understanding of the specific pathways via which SNCA mutations cause PD can help in the development of focused treatments that attempt to reduce the harmful consequences of alpha-synuclein buildup. Moreover, a deeper understanding of the evolutionary adaptations of SNCA could provide valuable knowledge for manipulating its function in a therapeutic setting, perhaps presenting novel approaches for the treatment or prevention of Parkinson's disease.

Ultimately, the SNCA gene and its corresponding protein, alpha-synuclein, are crucial to the investigation of Parkinson's disease. The presence of genetic variations in the SNCA gene has a considerable impact on the chance of developing Parkinson's disease, indicating the important role of the protein in the processes of the disease. Ongoing study into the genetic, structural, and functional features of alpha-synuclein holds the potential to reveal new

therapeutic solutions, despite the obstacles in completely understanding its roles and hazardous forms.

LRRK2

The presence of LRRK2 gene mutations has a significant role in both familial and sporadic cases of Parkinson's disease (PD), making it a crucial field of study for understanding the genetic variables that contribute to this neurodegenerative disorder. LRRK2 mutations are linked to around 5-12% of familial PD cases and 1-5% of sporadic PD cases, underscoring their significance in the genetic makeup of the disease. So far, scientists have discovered seven missense variants in LRRK2 that are known to cause disease. These mutations are R1441G, R1441C, R1441H, Y1699C, G2019S, R1628P, G2385R, and I2020T. These mutations occur in different functional regions of the protein, and some, like the G2019S mutation, cause the kinase activity of LRRK2 to become activated.

The G2019S mutation, in particular, stands out due to the high prevalence in certain populations, accounting for 36% of familial and sporadic PD cases among North African Arabs and about 30% in Ashkenazi Jewish communities. Conversely, it is significantly less common in European and North American populations and nearly non-existent in Asian ethnicities. Additional variants, such as G2385R and R1628P, demonstrate a strong correlation with the risk of Parkinson's disease (PD) in Asian populations. This emphasizes the influence of ethnicity and geographic location on the likelihood of developing LRRK2-related PD. Although there are genetic variations, the clinical and neurochemical characteristics of Parkinson's disease (PD) linked to LRRK2 mutations are remarkably comparable to those observed in idiopathic PD. Patients exhibit degeneration of dopaminergic neurons and inflammation in the substantia nigra compacta (SNpc), decreased levels of dopamine in the caudate pole, and the distinctive presence of Lewy bodies in the brainstem. Pathogenic mutations in LRRK2 impact many functional domains of the protein, causing changes in its normal enzymatic activity, specifically its kinase function. The G2019S mutation results in excessive stimulation of the kinase activity of LRRK2, indicating that the heightened activity of this enzyme may have a key function in the progression of PD. The specific molecular processes by which these mutations lead to neurodegeneration are currently being actively researched. Current ideas propose that these mutations affect neuronal autophagy, mitochondrial function, and cytoskeletal dynamics.

Gaining insight into the function of LRRK2 in Parkinson's disease (PD) provides new opportunities for therapeutic intervention. Targeting the kinase activity of LRRK2 offers a hopeful strategy for controlling the advancement of the disease. Multiple compounds that limit the kinase activity of LRRK2 are now being studied, providing potential for therapeutic interventions that may decelerate or halt the advancement of Parkinson's disease in individuals with these genetic abnormalities.

2.3 Genes that are inherited in an autosomal recessive manner

PINK1

The PINK1 gene, originally discovered by Unoki and Nakamura in 2001, consists of eight exonic regions that encode a serine/threonine protein kinase. This enzyme plays a vital part in the functioning of mitochondria and the metabolism of cellular energy, emphasizing the important role of mitochondria in maintaining the health of neuronal cells. According to the MDSGene database, there are 151 individuals worldwide who have been found to have PINK1 mutations. These mutations consist of 62 distinct variations. The PINK1 protein is mostly found in mitochondria, where it has a crucial function in maintaining mitochondrial quality control (mitoQC). This process entails the maintenance of robust mitochondrial networks and the removal of impaired mitochondria through mitophagy, an essential autophagic mechanism for maintaining cellular balance. The presence of mitochondrial malfunction is a defining characteristic of Parkinson's disease (PD), and the involvement of PINK1 in alleviating this dysfunction is crucial to its connection with the disease. In addition to its role in mitoQC, PINK1 demonstrates neuroprotective characteristics in response to different stressors, promoting cell survival and reducing neuronal demise. The dual role of PINK1 emphasizes the significance of this protein in preserving the overall well-being and ability to recover of cells, especially in the nervous system. The PINK1 protein possesses several key structural elements that play a crucial role in its localization and activity within mitochondria. These include an N-terminal mitochondrial targeting region, a transmembrane sequence, and a C-terminal domain.

Discovering PINK1 mutations in people with early-onset disease offers important understanding of how the disease develops and possible ways to intervene. Due to the enzyme's involvement in maintaining and protecting mitochondria from cell death, medications that try to improve PINK1 function or imitate its activity show potential as effective ways for slowing

down or stopping the progression of diseases.

Gaining a comprehensive understanding of the precise processes via which PINK1 mutations contribute to the onset of Parkinson's disease is of utmost importance in order to facilitate the development of medicines that specifically target these mechanisms. Contemporary studies concentrate on the restoration of mitochondrial function, the enhancement of mitophagy, and the protection of neuronal cells from damage caused by stress. Although there have been notable breakthroughs in comprehending the function of PINK1 in Parkinson's disease (PD), there are still various obstacles that need to be addressed. These include elucidating the full range of PINK1 mutations and their specific effects on mitochondrial function, developing effective therapies that targeting these molecular pathways and understanding the interaction between PINK1 and other genes involved in PD.

Parkin

Parkin is an E3 ubiquitin protein ligase that has a crucial function in directing proteins for destruction through the ubiquitin-proteasome system (UPS). The primary role of this function is crucial in preserving cellular homeostasis by the elimination of impaired or incorrectly folded proteins. The accumulation of such proteins might result in cellular malfunction and demise.

PRKN gene mutations are a prevalent hereditary cause of early-onset Parkinson's disease. Frequently, these genetic alterations cause a decrease in the functional ability of parkin enzymes, leading to the buildup of the substances that parkin normally acts upon and the specific demise of dopaminergic neurons in the substantia nigra, a characteristic feature of Parkinson's disease.

Parkin plays a crucial function not only in breaking down proteins but also in maintaining the quality of mitochondria through a process called mitophagy. Mitophagy is the process by which damaged or defective mitochondria are selectively broken down through autophagy, in order to effectively eliminate them and preserve the overall health of the cell. Parkin dysfunction, caused by genetic abnormalities, hinders the cell's capacity to eliminate dysfunctional mitochondria. The interruption can result in mitochondrial impairment, marked by decreased ATP synthesis, heightened oxidative stress, and the release of pro-apoptotic substances. These variables collectively contribute to the development of Parkinson's disease. Furthermore, parkin's function goes beyond mitochondria to encompass the control of other cellular mechanisms, such as inflammation, apoptosis, and certain components of the immunological response. Parkin's various functions emphasize its significance in maintaining cellular balance.

Gaining knowledge about the roles of parkin and the underlying mechanisms that cause its malfunction in Parkinson's disease (PD) provides opportunities for future therapeutic approaches. Potential treatment options could be explored through approaches that try to enhance parkin function, facilitate the clearance of damaged mitochondria, or duplicate its activities using pharmaceutical techniques. Furthermore, the field of research also includes gene therapy that focuses on rectifying PRKN mutations or enhancing its expression, which has great potential.

2.4 GBA

Glucocerebrosidase is an essential lysosomal enzyme responsible for the degradation and reutilization of glucocerebrosides, which are a specific form of glycolipid. GBA is essential for regulating cellular homeostasis and lipid metabolism by converting glycocerebrosides into ceramide and glucose. The presence of genetic abnormalities in the GBA gene leads to a dysfunctional GBA enzyme. This dysfunction causes glycocerebrosides to build up in lysosomes, which in turn impairs cellular function and contributes to the development of neurodegenerative diseases.

GBA gene mutations are connected to Gaucher disease, a type of lysosomal storage disorder, and have also been related with a higher likelihood of developing PD. The precise pathways via which GBA mutations contribute to Parkinson's disease (PD) are not well comprehended, but various possibilities have been put forward. These factors consist of altered autophagy-lysosomal pathways, heightened alpha-synuclein buildup caused by decreased lysosomal degradative ability, and intensified neuroinflammation and oxidative stress. The connection between GBA mutations and Parkinson's disease was discovered through studies made in families affected by Gaucher disease, which is a genetic illness that affects the lysosomes and is caused by mutations in the GBA gene. Researchers noted a higher occurrence of Parkinson's disease (PD) among individuals with Gaucher disease mutations, which prompted further investigations that confirmed GBA as a significant risk factor for developing PD. The discovery, occurring during the late 1990s and early 2000s, marked a significant change in scientists' understanding of the genetic makeup of Parkinson's disease.

GBA mutations are prevalent genetic risk factors for Parkinson's disease. Studies indicate that the prevalence of GBA gene mutations among individuals with Parkinson's disease in Western nations ranges from roughly 5% to 10%. The in-

cidence of this occurrence may be elevated in specific populations, particularly among Ashkenazi Jews, where the prevalence of GBA mutations among the general population is much higher. The discovery of GBA mutations as a risk factor for Parkinson's disease has had significant consequences for both research and clinical practice. The importance of lysosomal dysfunction and autophagy in the development of Parkinson's disease (PD) has been emphasized, broadening the scope of research in this area of PD beyond the dopaminergic system and alpha-synuclein pathology. This recognition has prompted the development of new therapeutic strategies aimed at enhancing lysosomal function, reducing alpha-synuclein accumulation, and improving mitochondrial health.

Clinically, the discovery of the GBA-PD link has led to increased interest in genetic testing and counseling services for people with PD and their families. It has also prompted research into the natural history and phenotype of GBA-associated PD, which is often characterized by earlier onset, faster progression, and greater likelihood of cognitive decline compared with PD without GBA mutations.

Conclusions

Studying monogenic types of PD is driven by the desire to apply molecular knowledge to understand the development of idiopathic PD. According to our current knowledge of the pathophysiology of PD, the main illness mechanism in both the idiopathic and hereditary types is the buildup of α-synuclein. The histopathological results in genetic forms exhibit greater variability and consist of tau pathology in LRRK2 cases, while most autopsied PRKN mutation carriers do not show α-synuclein accumulation. Further exploration of disease pathways is expected to result in a fusion of customized disease-altering treatments for each individual with Parkinson's disease. This can be likened to the symptomatic treatment of people with Parkinson's disease, where a tailored combination of antiparkinsonian medications is utilized to effectively manage the specific illness symptoms experienced by each patient.

There are several plausible causes for the absence of disease modification in Parkinson's disease (PD), including extensive degeneration at the time of diagnosis, varying involvement of individual disease mechanisms in PD patients, and a very brief observation period. The inclusion of study cohorts consisting of individuals with well-defined genetic characteristics is essential for the advancement of targeted medicines. Currently, there is ongoing research on medications that are based on genotype.

Nevertheless, genetic testing is not yet commonly incorporated into the process of diagnosing patients or selecting participants for clinical trials. Early consideration of genetic testing is crucial in the diagnostic care of patients with Parkinson's disease in order to address the limited timeframe available for implementing disease-modifying treatments. In certain fields, such as cancer treatment, the analysis of genetic variations has already become an integral part of clinical practice and has resulted in the creation of more effective clinical trials. The utilization of genetic testing will grow in significance for the therapeutic management of individuals with neurological conditions. For instance, the oligonucleotide medication that has recently been approved by the FDA Genetic testing of patients with spinal muscular atrophy is necessary for Nusinersen in order to determine the genetic diagnosis and evaluate patients' eligibility for clinical trials.

In order to further develop the effective strategy of targeted therapeutics in Parkinson's disease, it is necessary to have biomarkers that can categorize patients according to the cause of the underlying disease (such as identifying those with significant mitochondrial damage). Managing Parkinson's disease with medications that change its progression is challenging because there are no proven and dynamic biomarkers based on the underlying mechanisms. Genetic findings in Parkinson's disease (PD) have enhanced our comprehension of the clinical symptoms, the fundamental causes, and the possibility of specific treatments. While our current comprehension of disease biology is continuously growing, it is imperative that we promptly tackle the existing gaps in knowledge in the future. Performing genetic testing on individuals with "idiopathic" or "sporadic" Parkinson's disease is necessary to identify those who are eligible for genotype-based treatments. Stratifying research participants based on their genotype will increase the potential use of targeted medications.

[1] Afaki E, Westbroek W, Sidransky E (2017) The complicated relationship between Gaucher disease and parkinsonism: insights from a rare disease. Neuron 93(4):737–746. https://doi.org/10.1016/j. neuron.2017.01.018

[2] Asakawa S, Abe I, Kudoh Y, Kishi N, Wang Y, Kubota R, Kudoh J, Kawasaki K, Minoshima S, Shimizu N (1997) Human BAC library: construction and rapid screening. Gene 191(1):69–79. **https://doi.org/10.1016/s0378-1119(97)00044-9**

[3] Bannwarth S, Ait-El-Mkadem S, Chaussenot A, Genin EC, Lacas-Gervais S, Fragaki K, Berg-Alonso L, Kageyama Y, Serre V, Moore DG,

Verschueren A, Rouzier C, Le Ber I, Auge G, Cochaud C, Lespinasse F, N'Guyen K, de Septenville A, Brice A, Yu-WaiMan P, Sesaki H, Pouget J, Paquis-Flucklinger V (2014) A mitochondrial origin for frontotemporal dementia and amyotrophic lateral sclerosis through CHCHD10 involvement. Brain 137(Pt 8):2329–2345. **https://doi.org/10.1093/brain/awu138**

[4] Barodia SK, McMeekin LJ, Creed RB, Quinones EK, Cowell RM, Goldberg MS (2019) PINK1 phosphorylates ubiquitin predominantly in astrocytes. NPJ Parkinsons Dis 5:29. https://doi.org/10. 1038/s41531-019-0101-9

[5] Cardoso F, Goetz CG, Mestre TA, Sampaio C, Adler CH, Berg D, Bloem BR, Burn DJ, Fitts MS, Gasser T, Klein C, de Tijssen MAJ, Lang AE, Lim SY, Litvan I, Meissner WG, Mollenhauer B, Okubadejo N, Okun MS, Postuma RB, Svenningsson P, Tan LCS, Tsunemi T, Wahlstrom-Helgren S, Gershanik OS, Fung VSC, Trenkwalder C (2023) A statement of the MDS on biological defnition, staging, and classifcation of Parkinson's disease. Mov Disord. **https://doi.org/10.1002/mds.29683**

[6] Castelo Rueda MP, Zanon A, Gilmozzi V, Lavdas AA, Raftopoulou A, Delcambre S, Del Greco MF, Klein C, Grunewald A, Pramstaller PP, Hicks AA, Pichler I (2023) Molecular phenotypes of mitochondrial dysfunction in clinically nonmanifesting heterozygous PRKN variant carriers. NPJ Parkinsons Dis 9(1):65. https://doi.org/10.1038/s41531-023-00499-9

[7] Cossu D, Yokoyama K, Sato S, Noda S, Sechi LA, Hattori N (2021) PARKIN modifes peripheral immune response and increases neuroinfammation in active experimental autoimmune encephalomyelitis (EAE). J Neuroimmunol 359:577694. https://doi.org/ 10.1016/j.jneuroim.2021.577694

[8] Daida K, Funayama M, Billingsley KJ, Malik L, Miano-Burkhardt A, Leonard HL, Makarious MB, Iwaki H, Ding J, Gibbs JR, Ishiguro M, Yoshino H, Ogaki K, Oyama G, Nishioka K, Nonaka R, Akamatsu W, Blauwendraat C, Hattori N (2023) Long-read sequencing resolves a complex structural variant in PRKN Parkinson's disease. Mov Disord 38(12):2249–2257. https://doi.org/10.1002/ mds.29610

[9] Funayama M, Ohe K, Amo T, Furuya N, Yamaguchi J, Saiki S, Li Y, Ogaki K, Ando M, Yoshino H, Tomiyama H, Nishioka K, Hasegawa K, Saiki H, Satake W, Mogushi K, Sasaki R, Kokubo Y, Kuzuhara S, Toda T, Mizuno Y, Uchiyama Y, Ohno K, Hattori N (2015) CHCHD2 mutations in autosomal dominant late-onset Parkinson's disease: a genome-wide linkage and sequencing study. Lancet Neurol 14(3):274–282. https://doi.org/10.1016/S1474- 4422(14)70266-2

[10] Hattori N, Kitada T, Matsumine H, Asakawa S, Yamamura Y, Yoshino H, Kobayashi T, Yokochi M, Wang M, Yoritaka A, Kondo T, Kuzuhara S, Nakamura S, Shimizu N, Mizuno Y (1998) Molecular genetic analysis of a novel Parkin gene in Japanese families with autosomal recessive juvenile parkinsonism: evidence for variable homozygous deletions in the Parkin gene in afected individuals. Ann Neurol 44(6):935–941. https://doi.org/10.1002/ ana.410440612

[11] He Y, Kaya I, Shariatgorji R, Lundkvist J, Wahlberg LU, Nilsson A, Mamula D, Kehr J, Zareba-Paslawska J, Biverstal H, Chergui K, Zhang X, Andren PE, Svenningsson P (2023) Prosaposin maintains lipid homeostasis in dopamine neurons and counteracts experimental parkinsonism in rodents. Nat Commun 14(1):5804. **https://doi.org/10.1038/s41467-023-41539-5**

[12] Hedrich K, Marder K, Harris J, Kann M, Lynch T, Meija-Santana H, Pramstaller PP, Schwinger E, Bressman SB, Fahn S, Klein C (2002) Evaluation of 50 probands with early-onset Parkinson's disease for Parkin mutations. Neurology 58(8):1239–1246. https:// doi.org/10.1212/wnl.58.8.1239

[13] Hristova VA, Beasley SA, Rylett RJ, Shaw GS (2009) Identifcation of a novel Zn2+-binding domain in the autosomal recessive juvenile Parkinson-related E3 ligase parkin. J Biol Chem 284(22):14978– 14986. **https://doi.org/10.1074/jbc.M808700200**

[14] Ikeda A, Matsushima T, Daida K, Nakajima S, Conedera S, Li Y, Yoshino H, Oyama G, Funayama M, Nishioka K, Hattori N (2017) A novel mutation of CHCHD2 p. R8H in a sporadic case of Parkinson's disease. Parkinsonism Relat Disord 34:66–68. https://doi. org/10.1016/j.parkreldis.2016.10.018

[15] Ikeda A, Nishioka K, Meng H, Takanashi M, Hasegawa I, Inoshita T, Shiba-Fukushima K, Li Y, Yoshino H, Mori A, Okuzumi A, Yamaguchi A, Nonaka R, Izawa N, Ishikawa KI, Saiki H, Morita M, Hasegawa M, Hasegawa K, Elahi M, Funayama M, Okano H, Akamatsu W, Imai Y, Hattori N (2019) Mutations in CHCHD2 cause alpha-synuclein aggregation. Hum Mol Genet 28(23):3895– 3911. **https://doi.org/10.1093/hmg/ddz241**

[16] Ikeda A, Imai Y, Hattori N (2022) Neurodegeneration-associated mitochondrial proteins, CHCHD2 and CHCHD10-what distinguishes the two? Front Cell Dev Biol 10:996061. https://doi.org/10.3389/ fcell.2022.996061

[17] Kano M, Takanashi M, Oyama G, Yoritaka A, Hatano T, ShibaFukushima K, Nagai M, Nishiyama K, Hasegawa K, Inoshita T, Ishikawa KI, Aka-

matsu W, Imai Y, Bolognin S, Schwamborn JC, Hattori N (2020) Reduced astrocytic reactivity in human brains and midbrain organoids with PRKN mutations. NPJ Parkinsons Dis 6(1):33. https://doi.org/10.1038/s41531-020-00137-8

[18] Khan NL, Graham E, Critchley P, Schrag AE, Wood NW, Lees AJ, Bhatia KP, Quinn N (2003) Parkin disease: a phenotypic study of a large case series. Brain 126(Pt 6):1279–1292. https://doi.org/ 10.1093/brain/awg142

[19] Kitada T, Asakawa S, Hattori N, Matsumine H, Yamamura Y, Minoshima S, Yokochi M, Mizuno Y, Shimizu N (1998) Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. Nature 392(6676):605–608. https://doi.org/10.1038/ 33416

[20] Matsuda N, Sato S, Shiba K, Okatsu K, Saisho K, Gautier CA, Sou YS, Saiki S, Kawajiri S, Sato F, Kimura M, Komatsu M, Hattori N, Tanaka K (2010) PINK1 stabilized by mitochondrial depolarization recruits Parkin to damaged mitochondria and activates latent N. Hattori et al. Parkin for mitophagy. J Cell Biol 189(2):211–221. https://doi.org/ 10.1083/jcb.200910140

IMAGING BIOMARKERS IN PARKINSON'S DISEASE

Georgios A. Tagaris, MD
Neurological Department, General Hospital of Athens "G. Gennimatas"

Abstract
Parkinson's Disease (PD) is a complex neurodegenerative disorder characterized by dopaminergic neuron loss and alpha-synuclein aggregates. Currently, PD diagnosis relies on clinical criteria. Imaging biomarkers have gained attention due to their ability to provide quantitative and localized information about both brain structure and function. Advanced MRI techniques, such as Volumetric MRI, Diffusion Tensor Imaging, Free Water Imaging, Susceptibility Weighted Imaging, and Neuromelanin-sensitive MRI, offer insights into brain structure, function, and molecular pathology. Molecular imaging techniques, including Positron Emission Tomography (PET) and Single-Photon Emission Computed Tomography (SPECT), use specific tracers to assess the integrity and function of dopaminergic, as well as serotonergic, noradrenergic, and cholinergic pathway. These techniques also evaluate neuroinflammation and visualize brain metabolism, identifying patterns associated with PD and related disorders. Additionally, alpha-synuclein and tau imaging have emerged as promising techniques for directly visualizing and quantifying the pathological proteins implicated in PD and other neurodegenerative conditions. These methods show significant potential for early diagnosis, differential diagnosis, disease staging, progression tracking, and assessing therapeutic responses in the context of clinical trials. This review underscores the evolving landscape of imaging biomarkers in PD, emphasizing their current status and integration into clinical practice.

Keywords: Biomarkers, Imaging; Parkinson's; Parkinsonism; Diagnosis; Disease staging; Progression tracking; MRI; PET; SPECT; cardiac scintigraphy; molecular imaging

ΑΠΕΙΚΟΝΙΣΤΙΚΟΙ ΒΙΟΔΕΙΚΤΕΣ ΣΤΗ ΝΟΣΟ ΠΑΡΚΙΝΣΟΝ

Γεώργιος Α. Τάγαρης
Νευρολογική Κλινική, Γενικό Νοσοκομείο Αθηνών "Γ. Γεννηματάς"

Περίληψη
Η νόσος του Πάρκινσον (ΝΠ) είναι μια σύνθετη νευροεκφυλιστική διαταραχή που χαρακτηρίζεται από απώλεια ντοπαμινεργικών νευρώνων και συσσωματώματα άλφα-συνουκλεΐνης. Σήμερα η διάγνωση της PD βασίζεται σε κλινικά κριτήρια. Οι απεικονιστικοί βιοδείκτες αποτελούν αντικείμενο προσοχής λόγω της δυνατότητας να παρέχουν ποσοτικές και τοπογραφικά εντοπισμένες πληροφορίες τόσο για την δομή όσο και για την λειτουργία του εγκεφάλου. Σύγχρονες τεχνικές μαγνητικής τομογραφίας, όπως η ογκομετρική μαγνητική τομογραφία, η απεικόνιση του τανυστή διάχυσης, η απεικόνιση των ελεύθερων μορίων νερού, η απεικόνιση μαγνητικής επιδεκτικότητας και οι ευαίσθητες στην νευρομελανίνη ακολουθίες προσφέρουν πληροφορίες για την δομή του εγκεφάλου, την λειτουργία αλλά και την παθολογία σε μοριακό επίπεδο. Οι τεχνικές μοριακής απεικόνισης, συμπεριλαμβανομένης της τομογραφίας εκπομπής ποζιτρονίων (PET) και της υπολογιστικής τομογραφίας εκπομπής ενός φωτονίου (SPECT), χρησιμοποιούν ειδικούς ιχνηθέτες για την αξιολόγηση της ακεραιότητας και της λειτουργίας όχι μόνο των ντοπαμινεργικών αλλά και σεροτονινεργικών, νοραδρενεργικών και χολινεργικών οδών. Έχουν επίσης την δυνατότητα να αξιολογήσουν την φλεγμονή του νευρικού ιστού και να δώσουν πληροφορίες για τον μεταβολισμό του εγκεφάλου, προσδιορίζοντας συγκεκριμένα πρότυπα που σχετίζονται με την νόσο του Πάρκινσον και άλλα παρκινσονικά σύνδρομα. Επιπλέον, η απεικόνιση άλφα-συνουκλεΐνης και πρωτεΐνης ταυ αποτελούν υποσχόμενες τεχνικές για την άμεση απεικόνιση και τον ποσοτικό προσδιορισμό των παθολογικών πρωτεϊνών που εμπλέκονται στην ΝΠ και σε άλλες νευροεκφυλιστικές καταστάσεις. Αυτές οι μέθοδοι φαίνεται να προσφέρουν σημαντικές δυνατότητες για την έγκαιρη διάγνωση, την διαφορική διάγνωση, την σταδιοποίηση της νόσου, την παρακολούθηση της εξέλιξης της νόσου αλλά και την αξιολόγηση της θεραπευτικής ανταπόκρισης στο πλαίσιο κλινικών δοκιμών. Η ανασκόπηση αυτή περιγράφει το εξελισσόμενο τοπίο των βιοδεικτών απεικόνισης στην ΝΠ, δίνοντας έμφαση στην σημερινή πραγματικότητα και την ενσωμάτωση των τεχνικών αυτών στην κλινική πράξη.

Parkinson's Disease (PD) is a complex neurodegenerative disorder that is primarily characterized by the loss of dopaminergic neurons in the substantia nigra and the accumulation of alpha synuclein protein aggregates. This neurodegeneration leads to a disruption of dopaminergic and other neurotrans-

mitter systems and the appearance of various motor and non-motor symptoms. Tremor, bradykinesia, rigidity, and postural instability are classic motor symptoms. As the disease progresses, patients may experience freezing of gait and other motor complications. Non-motor symptoms include cognitive impairment, mood disorders, sleep disturbances, autonomic dysfunction, and gastrointestinal issues, significantly impacting the quality of life.

Currently, the diagnosis of PD is based mainly on clinical criteria [1]. However, several fluid and imaging biomarkers have been proposed to aid in early and accurate diagnosis, especially in differentiating PD from other parkinsonian syndromes. Another potential use of biomarkers may be to track disease progression and provide insights into the spread of pathology and potential subtypes of PD. Finally, biomarkers, especially in clinical trials, may help assess the efficacy of therapeutic interventions. Fluid biomarkers include alpha -synuclein, DJ-1, tau protein, neurofilament light chain (NFL) as well as inflammatory markers in blood or cerebrospinal fluid (CSF)

Imaging biomarkers are gaining increasing attention due to their inherent capability to provide localized information regarding brain structure, function, and molecular pathology. Imaging biomarkers can be divided into structural and functional categories. Structural imaging (MRI) helps detect changes in brain structures, such as atrophy and alterations in white matter integrity, aiding in the differential diagnosis and tracking of disease progression. Most functional imaging techniques involve PET/SPECT and use specific molecules (tracers) to assess degeneration in various brain circuits, e.g., the dopaminergic circuit. Another potential use is the development of tracers that target specific molecules that are related to a specific pathologic process e.g., alpha-synuclein aggregates, potentially enabling early diagnosis and tracking of disease progression [2,3]. Thus, the term 'molecular imaging' is commonly used. In this review, we will examine the status and future perspectives of various imaging biomarkers.

I Structural imaging

Structural imaging techniques, especially MRI play a crucial role in identifying and characterizing changes in brain structure associated with Parkinsonism. Conventional MRI sequences provide detailed anatomical images of the brain and assist mainly in ruling out other structural pathologies e.g. vascular, inflammatory, or space-occupying lesions, metal depositions etc. Certain findings, referred to as "red flags," are associated with specific parkinsonian syndromes. These include midbrain atrophy in Progressive Supranuclear Palsy (PSP) and atrophy of the pons and cerebellum in Multiple System Atrophy (MSA) (see Figure 1). Additionally, typical images have been described in Wilson's disease, Pantothenate kinase-associated neurodegeneration (PKAN), and other syndromes such as vascular parkinsonism and hydrocephalus. Other potential findings comprise asymmetric cortical atrophy in Corticobasal Degeneration (CBD) and middle cerebellar peduncle hyperintensity in MSA (see Figure 2).

Advanced MRI techniques, in addition to differential diagnosis, may help understand disease progression, and monitor treatment effects. These techniques are described below.

- **Volumetric MRI** refers to the quantitative assessment of brain structures using magnetic resonance imaging. In the context of Parkinson's disease, volumetric analysis enables the measurement of specific brain regions affected by neurodegeneration. Region of Interest (ROI) analysis provides a quantitative assessment of the substantia nigra, putamen and globus pallidus, as well as cortical structures. Cortical thickness and volume, derived from high-resolution images, have been measures of particular interest [4]. Additionally, advanced software allows automated segmentation of brain structures, providing volumetric measurements of various structures across the entire brain. ***Voxel-Based Morphometry (VBM)*** is a technique used to analyze differences in brain anatomy, particularly in terms of local brain volume and tissue concentration [5]. In this technique, high resolution MRI scans are spatially normalized and registered to a common reference brain template. The normalized images are then segmented into different tissue types, distinguishing between gray matter, white matter, and cerebrospinal fluid using automated algorithms. This segmentation step creates maps that represent the distribution and concentration of different brain tissues. Statistical analyses are performed on the segmented brain images to identify and quantify differences in brain structure between groups or conditions. VBM has been widely used in neuroscience research and clinical studies to identify localized changes in various neurological conditions, including Parkinson's disease. ***Deformation-based morphometry (DBM)*** is another advanced technique. Similar to VBM, images are normalized and aligned to a common reference template. The analysis is based on the deformation fields needed to morph individual brain images onto the common template. Statistical analyses are performed to identify and quantify differences in brain structure between groups or conditions. This analysis can reveal localized differences in brain regions not easily identifiable with traditional measurements [2]. At

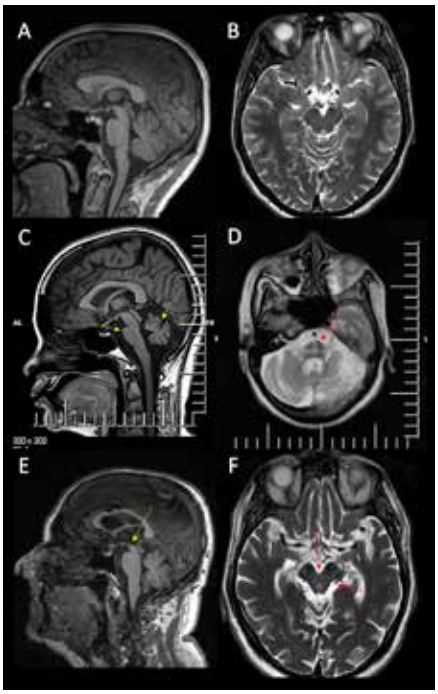

Figure 1. Classic MRI.in the differential diagnosis of idiopathic Parkinson's Disease A, B: Normal brain. C, D: Patient with Multiple System Atrophy. Cerebellar and pontine atrophy are visible in image C (yellow arrows). The "hot cross bun sign" can bre seen in image D. This sign is produced by a selective loss of myelinated transverse pontocerebellar fibers with preservation of the pontine tegmentum and corticospinal tracts. E, F: Patient with Progressive Supranuclear Palsy. Image E depicts midbrain atrophy with a flattening outline in the superior aspect instead of being upwardly convex (yellow arrow). Midbrain atrophy in axial imaging at the level of the superior colliculi produces the "Mickey Mouse" sign: reduction of the anteroposterior midbrain diameter and loss of the lateral convex margin of the midbrain tegmentum (Image F, red arrows)

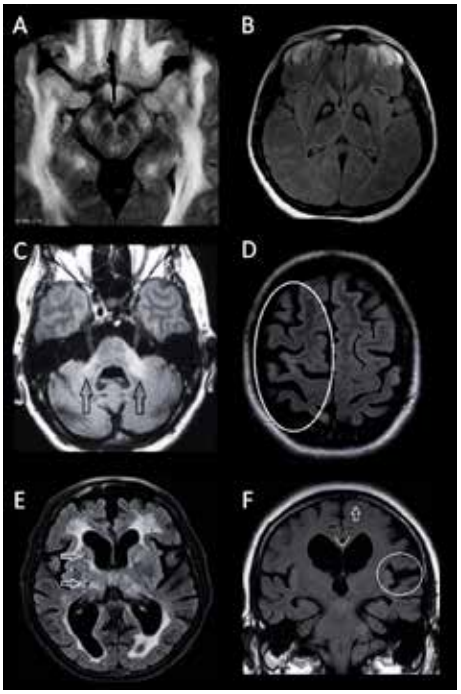

Figure 2. Typical images in various syndromes that share clinical features with Parkinson's disease.
Image A: The "face of the giant Panda" in Wilson's disease. This image, in T2 sequence, is a result of high signal intensity of the midbrain tegmentum with normal hypointense red nuclei forming the eyes, preserved signal intensity of the substantia nigra pars reticulata forming the ears and hypointensity of superior colliculi forming the chin.
Image B. The "eye of the tiger" in Pantothenate Kinase-Associated Neurodegeneration (PKAN) in T2 weighted imaging comprises a central region of signal hyperintensity due to gliosis and edema in the globus pallidus surrounded by a hypointense area caused by the accumulation of iron.
Image C. Increased signal in middle cerebellar peduncles (MCP) in a patient with MSA: The "bright MCP" sign indicating neuronal degeneration. Flair sequence.
Image D. Asymmetric cortical atrophy in a patient with corticobasal degeneration (CBD). T1 sequence.
Image E. Multiple ischemic lesions in white matter in a patient with vascular parkinsonism. Flair sequence.
Image F. Normal pressure hydrocephalus. Morphological changes include a callosal angle between 50-80 degrees, dilated Sylvian fissures and insular cisterns (circle) and effacement of the sulci and subarachnoid space at the vertex (arrow). T1 sequence.

present, these techniques do not have a practical impact on the daily diagnostic work-up of PD and are used more in research or in clinical trials.

- **Diffusion Tensor Imaging** (DTI) is an advanced MRI technique used to assess the microstructural integrity of white matter tracts within the brain. DTI measures the diffusion of water molecules within brain tissues. In white matter, water diffusion is directionally constrained by the microstructural organization of axonal fibers [6]. Metrics derived from DTI include ***Fractional Anisotropy (FA) and Mean Diffusivity (MD).*** Fractional Anisotropy reflects the directionality of water diffusion within white matter tracts. Reduced FA indicates disruptions in white matter integrity. Mean Diffusivity, as well as the conceptually

similar Apparent Diffusion Coefficient (ADC), represents the average rate of water diffusion. Increased MD suggests changes in tissue microstructure, such as axonal loss or demyelination. In the context of Parkinsonism, DTI serves as a structural biomarker by detecting alterations not evident on conventional MRI in the organization and integrity of white matter pathways, providing insights into the underlying pathology and disease progression. More specifically, DTI alterations, such as decreased FA or increased MD, may correlate with the degree of neurodegeneration in specific brain regions. Additionally, DTI patterns can differentiate between Parkinson's disease and atypical Parkinsonism by detecting distinct white matter changes characteristic of different syndromes. Specifically, DTI is useful for discriminating MSA from PD, particularly in the early stages due to increased putaminal diffusivity that is directly visible in the ADC or MD maps [7].

- **Free water imaging** is a specialized MRI method designed to isolate and quantify extracellular free water in brain tissues. It employs a new computational approach on diffusion MRI data, using a bitensor model instead of a single tensor model and being capable to differentiate between water contained within cells (intracellular) and free water, i.e., water found in the extracellular spaces [8]. Free water reflects neurodegeneration and neuroinflammation in cortical and subcortical regions. By specifically targeting extracellular water content in areas like the anterior or posterior substantia nigra, this technique allows a deeper view into the brain's microenvironment. Free water imaging is one of the most promising techniques in monitoring progression in Parkinson's disease,
- **Susceptibility weighted imaging (SWI)** is an advanced MRI technique that enhances the visualization of tissues with different magnetic susceptibilities, particularly in brain imaging. SW imaging is sensitive to magnetic inhomogeneity effects, mainly due to iron accumulation, hemorrhages, and/or slow venous blood flow, allowing for enhanced tissue contrast sensitive to variations in tissue composition. In Parkinson's disease research, various susceptibility-weighted imaging (SWI) techniques have been utilized, but the most commonly employed methods, aside from basic SWI contrast, include Quantitative Susceptibility Mapping and R2 star (R2*). ***Quantitative Susceptibility Mapping (QSM)*** has gained significant attention in PD research. It has been used to measure and map the magnetic susceptibility of tissues. QSM provides quantitative information about iron deposits particularly in regions like the substantia nigra where iron accumulation is observed in PD. ***R2 Star (R2*)*** is a measure used in susceptibility-weighted imaging to quantify the rate at which MRI signals decay due to susceptibility-induced effects in a magnetic field. In the context of Parkinson's disease, R2* mapping can also be employed to assess iron content changes in the substantia nigra. Increased R2* values indicate higher iron concentration in this region.
- The seminal anatomical work of Damier et al. has revealed that the Substantia Nigra Pars Compacta consist of a calbindin-rich matrix and five discrete calbindin-poor clusters of dopaminergic neurons identified as nigrosomes. Using high-field MRI, SWI is capable of visualizing the elements of the microstructure of the SN. Among these, nigrosome 1 stands out as a hyperintense area in the dorsal SN, (Dorsal Nigral Hyperintensity, DNH, or "Swallowtail sign.") Damier et al. also demonstrated that in Parkinson's disease, nigrosome 1 undergoes early and profound degeneration, resulting in the loss of the distinctive DNH sign early in the course of the disease [9, 10] (see Figure 3A, 3B).
- **Neuromelanin-sensitive MRI (NM-MRI)** utilizes T1-weighted MRI sequences optimized to enhance the contrast between neuromelanin-rich regions and surrounding brain tissues. By exploiting the paramagnetic properties of neuromelanin, these sequences can increase the signal intensity in areas rich in neuromelanin, making them more visible and aiding in the assessment of conditions associated with changes in neuromelanin-containing structures, such as Parkinson's disease [11] (see figure 3E, 3F).

II Molecular Imaging

As mentioned before, molecular imaging (PET/SPECT) uses tracers that bind specific receptors to assess dopaminergic function and aid in PD diagnosis by revealing reduced dopamine transporter or dopamine receptor binding. Also, tracers targeting alpha-synuclein aggregates may reveal the extent of the PD-related pathology and enable early diagnosis and tracking disease progression. Figure 4 summarizes most of the techniques described below [12].

- **Dopaminergic molecular imaging** is a powerful tool for studying the integrity and function of the dopaminergic system in the brain, particularly in the context of parkinsonian disorders. Techniques like Positron Emission Tomography (PET) and Single-Photon Emission Computed Tomography (SPECT) are commonly used to assess dopaminergic function. Briefly, PET imaging uses radiotracers i.e. compounds with a short-lived radioactive isotope.

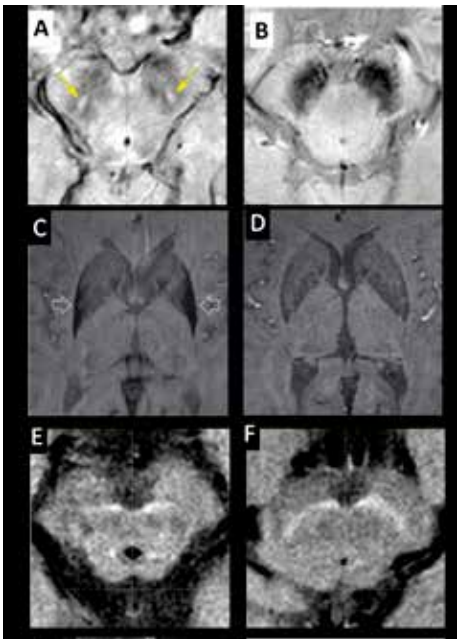

Figure 3. Advanced MRI techniques used in the diagnosis and follow up of parkinsonism.
Images A and B Iron sensitive SWI. The Dorsal Nigral Hyperintensity (DNH) is visible bilaterally in the brain of a healthy subject (Image A, arrows) but not in a patient with parkinsonism, due to accumulation of iron.
Image C. SWI of the putamen of a patient with MSA depicting bilateral signal hypointensity due to the accumulation of iron. Image D: Similar image of a healthy control subject for comparison.
Image E. Neuromelanin-sensitive MRI scan in a patient with Parkinson disease. The high-signal-intensity area is smaller compared to a healthy control subject (Image F)

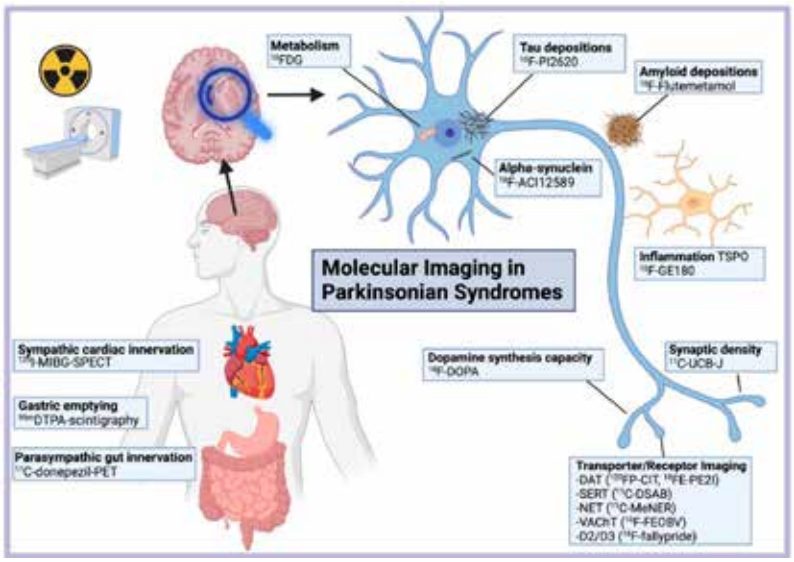

Figure 4. Overview of the targets and tracers for molecular imaging in movement disorders. Source: Prange et al. (2022) [12].

These molecules target specific molecules or biological processes in the body. The tracer is administered, usually through injection, and quickly travels to the area of interest. Once in the body, the tracer emits positrons which encounter electrons in body tissues, annihilate each other and produce two gamma rays that move in opposite directions. Special detectors in the PET scanner detect these gamma rays and measure their location and quantity. Advanced computer algorithms reconstruct the detected signals into detailed images that show the concentration and activity of the tracer. In the context of Parkinsonism, PET imaging utilizes [18F]-FDOPA (fluorodopa). This molecule is distributed in dopamine-producing cells. Therefore, [18F]-FDOPA PET scan depicts the distribution of dopaminergic cells. Another radiotracer, [11C]-raclopride, binds to D2 dopamine receptors in the brain. Both tracers can be used to evaluate the extent of the degeneration of the dopaminergic system and

the integrity of the D2 dopamine receptors. Another relevant technique is imaging of the Vesicular Monoamine Transporter Type 2 (VMAT2), a protein responsible for transporting monoamine neurotransmitters such as dopamine from the cytoplasm of neurons into synaptic vesicles. VMAT2 is significant in the context of various neurological and psychiatric conditions. Medications targeting VMAT2, like tetrabenazine, are used in the treatment of movement disorders such as Huntington's disease and other hyperkinetic movement disorders. Imaging techniques utilizing radioligands that bind to VMAT2, such as [18F]-fluoropropyl-dihydrotetrabena-zine (DTBZ) in PET imaging, have been developed to visualize and quantify VMAT2 density in the brain. These imaging methods offer insights into the integrity and density of dopamine nerve terminals. Similar to PET imaging, Single-Photon Emission Computed Tomography (SPECT) involves the use of a radioactive tracer. This tracer is typically a radiopharmaceutical, a compound tagged with a radioactive isotope such as technetium-99m, iodine-123, or thallium-201. The radiopharmaceutical is administered into the body, usually through injection, inhalation, or ingestion. Once inside the body, the radiopharmaceutical emits gamma rays as it decays. These gamma rays are single photons that are detected by a rotating gamma camera. Computer algorithms process the data collected by the detectors to reconstruct 3D images of the distribution of the radioactive tracer in the body. One of these tracers, [123-I]-CIT, also known as DaT-Scan, has been widely used as it binds to the dopamine transporter, providing an indirect measure of dopamine neuron integrity (Figure 7A,D). Also, SPECT imaging with iodobenzamide (IBZM) provides information about the distribution and density of the D2 dopaminergic receptors (Figure 7B,E)

- **Molecular imaging of other neurotransmitter systems** Specific tracers have been developed that capture serotonergic noradrenergic and cholinergic denervation, using PET Scan. [11C]-DASB is a benzonitrile that binds to serotonin transporters and reflects serotonergic denervation. Tracers that bind to acetylcholinesterase include [11C]-Methylpiperidin Propionate (PMP) and [11C]-Donepezil; both bind to acetylcholinesterase and reflect cholinergic activity. Another tracer, [18F] fluorobenzovesamicol (FBVM), binds to the vesicular acetylcholine transporter and may be a more reliable marker for cholinergic nerve terminal density. 11C-methylreboxetine PET (11C-MeNER, also known as 11C-MRB) binds to noradrenergic nerve terminals originating in the locus coeruleus. Of particular interest is the demonstration of sympathetic denervation of the heart using [131-I]-metaiodobenzylguanidine (MIBG-SPECT). This denervation can be demonstrated in early idiopathic Parkinson's disease but not in Parkinson Plus syndromes (i.e., MSA, PSP, CBD), providing a tool for the differential diagnosis (FIGURE 7C, F).
- **Imaging of neuroinflammation** Neuroinflammation in the brain can be visualized with PET imaging, using specific radiolabeled ligands that bind to the Translocator Protein (TSPO). TSPO is expressed in the outer mitochondrial membrane of various cell types, including microglia and astrocytes. This technique allows the visualization and quantification of the distribution of TSPO-expressing cells, which may indicate areas of neuroinflammation.
- **Metabolic imaging** using [18F]-fluorodeoxyglucose PET (FDG-PET) is a technique that enables the visualization and quantification of glucose metabolism in the brain. FDG is a radiolabeled glucose analog that, once administered, accumulates in cells similarly to glucose. Unlike glucose, FDG does not undergo further metabolism, allowing PET scanners to trace its distribution and accumulation in tissues. In the context of brain imaging, FDG-PET measures regional cerebral metabolic rates of glucose, providing insights into the brain's functional activity and identifying areas with altered glucose utilization. The basic hypothesis is that areas with consistently correlated metabolic activity are functionally interconnected. Thus, a ***PD-related pattern (PDRP)*** has been identified, with increased metabolic activity in brain areas such as the globus pallidus, putamen, thalamus, pons, cerebellum and motor cortex along with decreased activity in other regions, including the lateral cortex and parietooccipital association regions [13, 14]. [Figure 5] On the other hand, MSA is associated with impaired glucose metabolism in the putamen, pons, and cerebellum and PSP in the medial prefrontal cortex (PFC), the frontal eye fields, the ventrolateral prefrontal cortex (VLPFC), the caudate nuclei, the medial thalamus, and the upper brainstem [15].
- **Alpha-synuclein imaging** has emerged as one of the most promising areas in Parkinson's disease research. Alpha -synuclein imaging is basically PET imaging using specific radiotracers that bind to alpha-synuclein aggregates. It is common knowledge that alpha-synuclein is a key protein implicated in PD pathogenesis, forming aggregates (Lewy bodies) which are a hallmark of the disease. The detection of alpha-synuclein aggregates enables direct visualization and quantification of these pathological protein deposits in the brain. Thus, imaging techniques target-

ing alpha-synuclein aggregates offer potential as biomarkers for diagnosing the disease at the very early stages and tracking PD progression. For this purpose, various radiotracers have been used, and are being investigated for their ability to bind to aggregated alpha-synuclein. Still, alpha-synuclein imaging faces several technical challenges in terms of resolution, sensitivity and specificity. Also, the intracellular nature of alpha-synuclein aggregates poses additional challenges for imaging. Hopefully, the next generation of tracers will be able to overcome these obstacles [16]

- **Tau imaging.** The molecular imaging of misfolded and hyperphosphorylated tau proteins is a subject of great interest in Progressive Supranuclear Palsy (PSP) and Corticobasal Degeneration (CBD), though its current application remains primarily in research studies. Challenges arise from the diverse forms of tau pathology, complicating the development of tau-imaging tracers. First generation tracers have shown variable results in PSP studies, with concerns about specificity, particularly off-target binding. Second-generation tracers like [18F]-PI-2620 offer demonstrate improved specificity, offering promise in distinguishing PSP from other neurodegenerative conditions. While tau-PET holds potential as a supportive in vivo diagnostic tool for PSP, limited evidence on sensitivity and specificity against neuropathological standards restricts definitive conclusions.

The techniques mentioned above can be used alone or in combination with other imaging technique for the early diagnosis of Parkinson's Disease, the differential diagnosis from other parkinsonian syndromes, the staging and the progression of the disease as well as the therapeutic response, especially in the context of clinical trials. Next, we will examine specifically each of these uses both in the current status and in the foreseeable future.

Detection of Preclinical and Prodromal Parkinson's Disease

It is well known that the dopaminergic system starts declining several years prior to the onset of motor symptoms. PET/SPECT imaging of striatal membrane dopamine transporters (DaT) reveals a dopaminergic deficit in around 50% of individuals with idiopathic REM behavior disorder (RBD) [1]. Additionally, asymptomatic carriers of leucine-rich kinase 2 (LRRK2) variants exhibit reduced DaT binding compared to non-carriers, along with decreased fluorodopa uptake [17]. Also, imaging of the vesicular monoamine transporter type 2 (VMAT2) starts declining several years before the onset of motor symptoms [18]. Therefore, presynaptic dopaminergic denervation markers from PET/SPECT imaging exhibit potential as indicators of preclinical and prodromal disease states (see Table 1).

Non-dopaminergic PET modalities, including serotonergic and cholinergic systems, alongside markers of neuroinflammation, have shown sensitivity to disease states and association with non-motor pathophysiology in Parkinson's disease (PD). Notably, serotonin transporter is upregulated in asymptomatic LRRK2 variant carriers, contrasting with downregulation in symptomatic LRRK2 variant carriers and idiopathic PD. This finding may hint at a compensatory or protective mechanism [19]. In contrast, asymptomatic carriers of A53T α-synuclein (SNCA) exhibit reduced binding of serotonin transporters in brainstem and subcortical regions compared to controls [20]. Moreover, while cholinesterase activity increases in asymptomatic LRRK2 variant carriers in the cortex, reduced peripheral cholinesterase activity in the gastrointestinal tract has been observed in individuals with RBD. This is accompanied by evidence of cardiac sympathetic denervation and diminished central nervous system noradrenergic activity, even preceding the development of striatal dopamine deficiency [21]. Additionally, translocator protein (TSPO) PET has revealed microglial activation in asymptomatic LRRK2 variant carriers and individuals with RBD, suggesting a potential role of neuroinflammation in prodromal PD. However, these techniques face several challenges in terms of standardization and reproducibility and their potential as early disease-state biomarkers remains uncertain [22].

Metabolic imaging using fluorodeoxyglucose PET, as mentioned before, has unveiled a specific PD-related pattern (PDRP) with increased activity in specific brain areas. This pattern also appears in prodromal PD [20]. Furthermore, it is present in carriers of LRRK2 and GBA mutations as well as in patients with RBD (Figure 5). However, its capability to track progression in preclinical and prodromal stages remains undetermined.

Diffusion imaging in the substantia nigra (SN) enables early detection of neurodegeneration in prodromal PD. Single-tensor diffusion imaging indicates changes in nigral, midbrain, and pontine fractional anisotropy in RBD individuals. The implementation of advanced diffusion models, such as free-water imaging, demonstrates increased free water in the posterior SN of RBD subjects, indicating early neurodegenerative processes [23]. This could potentially serve as a target for disease-modifying clinical trials.

Numerous studies employing neuromelanin-sensitive MRI in RBD individuals have indicated reduced neuromelanin signal in the SN, particularly in the ventrolateral segment. Imaging of the locus coeruleus/subcoeruleus complex is another promising early nondopaminergic marker, with reduced signal demonstrating high sensitivity and specificity for RBD

Imaging Technique	Diagnosis	Differential from Parkinson Plus (PPlus)	Progression	Comments
MRI				
T1-weighted structural	Preclinical + Early ++ Advanced ++	**+++**	Preclinical - Early + Advanced ++	Diagnostic utility: Identification of "red flags". Monitoring the progression in advanced PD, used more in research
Iron-sensitive (SWI)	Preclinical ++ Early +++ Advanced +++	+	Preclinical - Early - Advanced ++	Potential biomarker in preclinical and early PD (loss of Dorsal Nigral Hyperintensity). **Potentially, clinically useful, especially with high field MRI** Biomarker for progression monitoring in advanced PD Questionable usefulness in the d.d. from PPlus
Neuromelanin-sensitive	Preclinical ++ Early +++ Advanced +++	+	Preclinical - Early ++ Advanced ++	**Potentially, clinically useful** biomarker in early to advanced PD. Progression biomarker in early PD (posterior SN) and in advanced PD (anterior SN) Questionable usefulness in the d.d. from PPlus
Free water imaging	Preclinical ++ Early ++ Advanced +++	**+++**	Preclinical - Early +++ Advanced +	**Potentially, clinically useful, especially with high field MRI**
Molecular				
Dopaminergic PET/SPECT	Preclinical + Early +++ Advanced +++	+++	Preclinical ++ Early +++ Advanced -	**Presynaptic PET/SPECT is the only approved technique for the diagnosis of early PD** **D2 PET/SPECT may be useful in the d.d. from PPlus** **Progression monitoring in early PD**
Non-Dopaminergic PET/SPECT	Preclinical ++ Early ++ Advanced ++	++	-	**Cardiac Scintigraphy (MIBG) useful in differential diagnosis from PPlus.** Other techniques require standardization and are considered experimental
Metabolic imaging	Preclinical +++ Early +++ Advanced +++	**+++**	Preclinical + Early + Advanced +++	Parkinson's Disease Related Pattern (PDRP). Promising technique for the diagnosis, the d.d. and the monitoring of the advanced stages. Its utility is limited by the fact that patients must be dopamine naive
Alpha-Synuclein Imaging	Preclinical +++ Early +++ Advanced +++	++	Preclinical +++ Early +++ Advanced +++	Promising technique, awaiting for next generation tracers

Table 1 Current Status of Neuroimaging Biomarkers in Parkinsons Disease. Techniques potentially useful in current clinical practice are emphasized in bold.

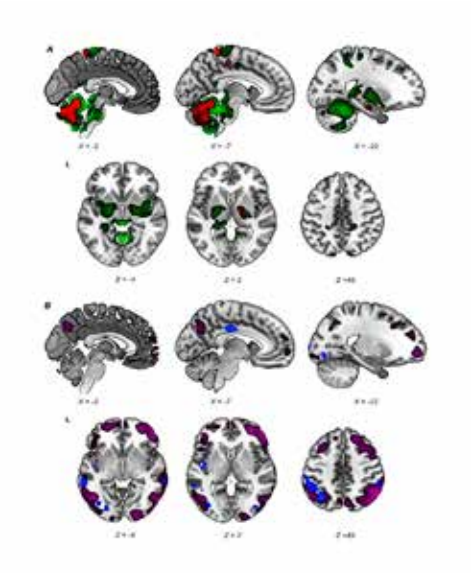

Figure 5. FDG-PET in PD, depicting brain metabolism. A specific Parkinson's Disease Related Pattern (PDRP) can be identified, with increased metabolic activity in the globus pallidus, putamen, thalamus, pons, cerebellum and motor cortex (green areas) along with decreased activity in the lateral cortex and parietooccipital association regions (purple areas). Notably, a similar pattern has been observed in non-parkinsonian patients with idiopathic REM Behavior Disorder (iRBD): Red and blue for hyper- and hypometabolic areas respectively. Source: Meles et al. 2021[14].

identification [24]. Both neuromelanin-sensitive MRI in the ventral or posterior SN and locus coeruleus imaging exhibit potential as prodromal markers of disease.

Iron-sensitive techniques, such as R2* relaxation imaging, susceptibility-weighted imaging (SWI), and quantitative susceptibility mapping (QSM), show promise in RBD. QSM reveals increased iron content bilaterally in the SN of individuals with RBD [25]. SWI, as mentioned before, highlights the loss of the dorsolateral nigral hyperintensity (DNH) in PD. This finding is detected in approximately 60% of RBD patients [26]. However, further studies are essential to confirm the utility of these imaging methods as prodromal disease-state biomarkers.

T1-based structural MRI methods, including cortical thickness and VBM, offer sensitive measures of disease state in individuals with prodromal PD. These methods have shown reduced hippocampal volume and cortical thickness alterations in patients with RBD. Further validation and investigation, especially in RBD patients without cognitive impairment, are warranted [27].

It should be noted that the Movement Disorders Society has established specific research criteria for the prodromal Parkinson's disease. These criteria, first published in 2015 and updated in 2019, include the abnormal tracer uptake of the presynaptic dopaminergic system (SPECT or PET) as the only acceptable imaging biomarker [28]. Several other techniques are labeled as "promising", requiring consensus on specific methods and analyses. These promising techniques include cholinergic gut innervation (PET/CT), cardiac sympathetic denervation (MIBG SPECT) susceptibility-weighted MRI (loss of dorsal nigral hyperintensity), neuromelanin-sensitive MRI and PET imaging of noradrenergic nerve terminals originating in the locus coeruleus.

Diagnosis of Early-Stage PD and differential diagnosis from other forms of parkinsonism

The diagnosis of early-stage PD is commonly encountered in everyday clinical practice. As mentioned before, dopaminergic imaging stands as the primary technique that is used to confirm the diagnosis of degenerative parkinsonism in individuals with early PD (i.e., Hoehn and Yahr Scale stage 1). The fact that parkinsonian symptoms occur with the loss of more than 50% of dopaminergic cells underscores the sensitivity of dopaminergic imaging, even when symptoms are exceptionally mild. DaT SPECT, in particular, is the most extensively employed technique. Its significance lies in its capability to essentially rule out degenerative parkinsonism when DaT SPECT results are normal. Typical cases where dopaminergic imaging helped in the diagnosis are presented in Figure 6. It should be kept in mind that in patients with suspected Parkinson's disease the initial diagnosis, even by movement disorders experts, is correct in about 80% of cases. Indeed, sometimes symptomatology that seems typical may be misleading. DaT SPECT may identify such patients, some of them carrying the wrong diagnosis of Parkinson's disease for years. Such cases, with no evidence of dopaminergic deficit on imaging despite the presence of clinical symptoms suggesting Parkinson's disease have been named SWEDDs (Scans Without Evidence of Dopaminergic Deficit). The follow up of these patients confirmed that they were not suffering from Parkinson's disease. One such case is displayed in Figure 6, images E and F. Alongside DaT imaging, VMAT2 imaging and fluoro-

dopa uptake can be used to detect presynaptic striatal dopaminergic denervation [29]. VMAT2 imaging is believed to be less affected by compensatory changes in expression than DaT or 6-[18F]-fluoro-L-DOPA [30]. However, it may be sensitive to large changes in dopamine content, e.g. in DOPA-responsive dystonia [31] while DaT binding tends to be more sensitive to dopamine denervation. Nevertheless, all dopaminergic tracers are limited to detecting nigrostriatal pathology, and it is well known that dopaminergic activity may be reduced in other parkinsonian syndromes like Progressive Supranuclear Palsy (PSP) and Multiple System Atrophy (MSA). Consequently, dopaminergic imaging cannot distinguish between idiopathic Parkinson' disease and Parkinson Plus syndromes, restricting its clinical utility to identifying dopaminergic deficit (Figure 6). Techniques employed for the differential diagnosis with Parkinson Plus syndromes include imaging with radiotracers that bind to D2 receptors like [11C]-raclopride PET and [123-I]-Iodobenzamide SPECT (IBZM).

Several other techniques have been studied in early PD. ***Non-dopaminergic imaging***, e.g serotonergic imaging demonstrates reduced binding in individuals with early PD (i.e. patients with disease duration less than 5 years); it does not correlate to disease severity or duration [32], however it may correlate with reduced levodopa response [33]. ***Cholinergic denervation*** also occurs in early PD (disease duration less than 3 years) but is more pronounced in PD with dementia [34]. The utility of these markers in the diagnosis of early PD or differential diagnosis of atypical parkinsonism is not yet clear. ***Metabolic imaging*** may detect early Parkinson's disease by revealing the Parkinson's Disease Related Pattern (PDRP) mentioned before and differentiate from atypical parkinsonism, however its utility is limited by the fact that patients must be dopamine naive. Finally, cardiac sympathetic neuroimaging with PET-scan using 11C-hydroxyephedrine or SPECT using [135I]-metaiodobenzylguanidine (MIBG) may reveal sympathetic denervation of the heart. Cardiac sympathetic denervation is an early finding in idiopathic Parkinson's disease but not in Parkinson Plus syndromes thus these methods have been used in clinical practice for the differentiation of Parkinson's Disease from atypical parkinsonism (Figure 7).

Diffusion Tensor Imaging (DTI) reveals a reduction in fractional anisotropy across the entire substantia nigra (SN) in early-stage PD. However, conflicting findings exist: A meta-analysis of 10 studies found no significant association between DTI-derived parameters in the SN and PD [35]

Free water imaging is a promising biomarker with potentially useful clinical applications. As mentioned before, it reflects neurodegeneration and/or neuroinflammation [36]. Multiple studies have demonstrated that free water is increased in the posterior SN in early PD [8,37]. In addition, free-water imaging in basal ganglia, midbrain, and cerebellum can differentiate PSP and MSA from PD [38].

Neuromelanin imaging in individuals with early-stage PD (disease duration of 1.5 years) reveals reduced signal in the posterior SN [39] and seems to be a robust early-stage marker of PD. Preliminary evidence suggests neuromelanin signal in the SN and locus coeruleus detects some differences in MSA and PSP, although sensitivity and specificity were not optimal compared with PD [40].

Susceptibility Weighted Imaging (SWI), as mentioned before, is another promising technique. R2* and Quantitative Susceptibility Mapping (QSM) in the Substantia Nigra are significantly different from healthy controls even in individuals with very early disease (i.e. disease duration less than 1 year) Looking at the absence of the dorsolateral nigral hyperintensity (DNH), mentioned before in the detection of prodromal PD, one study in individuals with early-stage PD (disease duration of 9 months) found signal loss of DNH was an excellent diagnostic marker with an accuracy of 94%[41]. Another SWI study in a larger cohort of patients with primarily de novo PD replicated this finding and found that 88% of patients had signal loss of DNH[42]. Moreover, in early-stage and de novo PD, R2* imaging, SWI, and QSM seem to be robust disease-state biomarkers. For differential diagnosis, SWI reveals a putaminal hypointensity that can be quantified to distinguish MSA [43] (see Figure 3C). Several studies also report SWI differences in PSP in various brain regions; however, more research is needed.

Tracking of disease progression and prediction of outcome

The evolution of imaging techniques significantly impacts the assessment of Parkinson's disease (PD) progression. Assessing disease progression at different stages is essential for understanding its course as well as monitoring therapeutic interventions [44]. For this purpose, various imaging techniques and biomarkers have been investigated. ***Dopaminergic PET/SPECT imaging*** can monitor progression in prodromal and early-stage PD but not in moderate to late-stage PD. A major problem with dopamine imaging in the striatum is that there is a poor correlation between changes in DA imaging and changes in clinical function over time [45]. It seems that striatal dopaminergic markers follow an exponential decline during the first two years followed by a slower decline in the next three years and a plateau five years after diagnosis [18] (Figure 8). Metabolic imaging is another promising technique in early PD as the Parkinson's disease related pattern (PDRP)

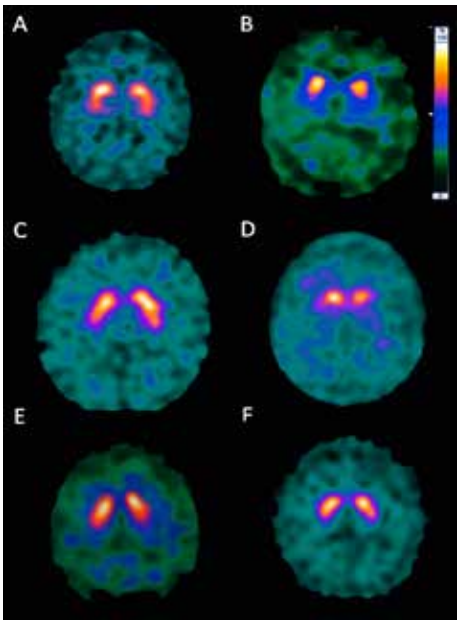

Figure 6. DaT scan for the diagnosis of degenerative parkinsonism
Image A. Man 56 years old, 15-y history of bilateral action tremor. Normal DaT scan. Diagnosis: Essential tremor (ET)
Image B. Man 72y, 1 year history of bradykinesia. Frequent falls. Poor response to levodopa. DaT scan: Bilateral degenerative parkinsonism. Final diagnosis: Progressive supranuclear palsy.
Image C. Woman 70y. Six-month history of rest tremor-bradykinesia-rigidity L>R. History of vertigo - takes flunarizine. DaT-scan normal. Diagnosis: Drug-induced parkinsonism
Image D. Man 67y. Six-month history of rest tremor-bradykinesia-rigidity L>R. Dat Scan: Degenerative parkinsonism. Excellent response to levodopa. Diagnosis: Parkinson's disease
Images E, F: Man 75y, misdiagnosed with Parkinson's Disease. The clinical diagnosis was based on typical features i.e. rest tremor, rigidity, mild bradykinesia R>L however the response to levodopa was poor. The DaT-scan was normal (Image E). Five years later, the DaT-scan was still normal (Image F)

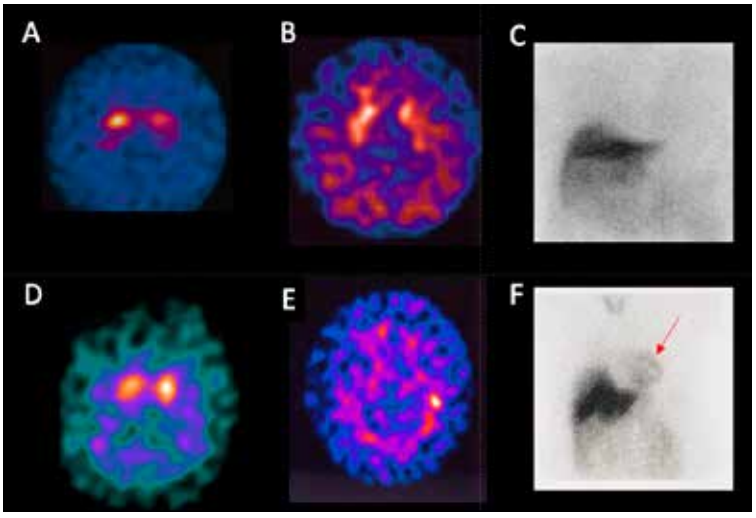

Figure 7. Differential diagnosis of idiopathic Parkinson's Disease from Parkinson Plus using D2 imaging (IBZM) and cardiac scintigraphy (MIBG) that identifies sympathetic aponeurosis of the heart.
Upper row: Patient with Parkinson's Disease. Lower row: Patient with Multiple System Atrophy (MSA). Images A and D: Abnormal DaT scan in both cases. Images B and E: D2 imaging (IBZM). The basal ganglia are visible in the patient with Parkinson's Disease (although the image is less clear compared to DaT-scan) but not in the patient with MSA. Images C and F: Cardiac scintigraphy (MIBG) reveals reduced uptake of the tracer from the heart in the patient with Parkinson's Disease (image C), reflecting sympathetic denervation due to the degeneration of the second (postganglionic) sympathetic neurons that arise in the superior sympathetic ganglion. In the patient with MSA, the first (preganglionic) sympathetic neuron degenerates, while the second neuron remains intact, thus the MIBG uptake remains unaffected.

seems to progress over 24 months [46]. As mentioned before, this technique bears the critical limitation of the effect of the dopaminergic treatment. Techniques like ***free-water imaging*** seem more suitable in monitoring disease progression up to 5 years. In particular, free-water imaging in the posterior substantia nigra stands as a robust progression marker in early-stage PD and may serve as a prediction marker, as the free water change over 1 year seems to predict a 4-year Hoehn and Yahr Scale change [8]. On the other hand, as mentioned before, free-water in the anterior substantia nigra may monitor progression in moderate to late-stage PD [47]. Also, ***diffusion imaging*** of the nucleus basalis of Meynert precedes and

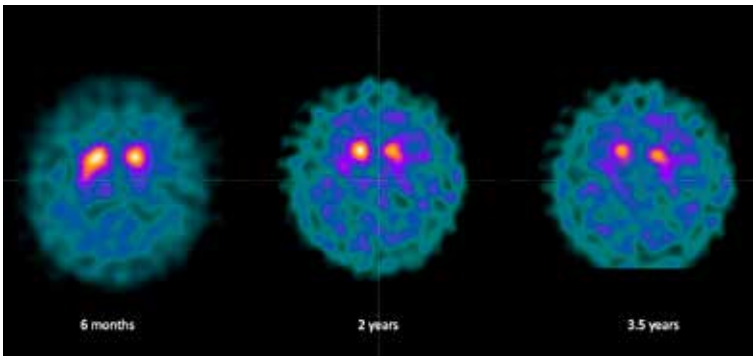

Figure 8. Serial DaT scans in a patient with Parkinson's Disease. Images are taken 6 months, 2 years and 3,5 years after the appearance of the symptoms. In has been observed that striatal dopaminergic markers follow an exponential decline during the first two years followed by a slower decline in the next years.

predicts cognitive impairment [48].

Neuromelanin-sensitive MRI of the SN has also demonstrated its ability to track progression in individuals with mild to moderate PD, making it a promising biomarker [49]. Other imaging modalities have also been used to track progression in early PD. T1-weighted structural MRI techniques like cortical thickness exhibit potential for tracking progression, particularly in advanced stages [50]. In summary, the imaging techniques mentioned above provide the potential to monitor the progression of Parkinson's disease. These biomarkers not only help track disease stages but also hold promise in predicting specific clinical outcomes. Continued research and validation efforts are essential to refine and establish these biomarkers as reliable tools in the management and understanding of Parkinson's disease. Regarding clinical trials, it is crucial to select the proper technique, depending on the stage of the disease. Table 1 summarizes the characteristics of all the techniques mentioned above.

Current clinical practice

The integration of imaging techniques with everyday clinical practice is an issue of great interest. As mentioned before, even though Parkinson's Disease is a clinical diagnosis, in the diagnostic workup, a conventional MRI is usually performed with the main purpose of ruling out an underlying secondary pathology for the symptoms of the patient [51]. The MRI protocol should include T1-weighted, T2 flair, diffusion-weighted imaging (DWI) and susceptibility weighted imaging (SWI), both in the sagittal and transversal planes. Also, conventional MRI might reveal signs indicating the presence of an Parkinson Plus syndrome: Atrophy of the putamen. T2-hyperintensity of the pons (the "hot cross bun" sign) and middle cerebellar peduncles in multiple system atrophy (MSA), midbrain atrophy (the "hummingbird" sign and the "mickey mouse" sign) in progressive supranuclear palsy (PSP), or asymmetric dorsal frontal or parietal atrophy in corticobasal degeneration (CBD) [52] (see figures 1, 2). However, the clinician should keep in mind that these signs will not be present early in the disease, in approximately half of the patients with Parkinson Plus syndromes [53].

For the confirmation of degenerative parkinsonism, presynaptic dopaminergic imaging (mainly DAT-Scan) is the technique most commonly used in clinical practice (see figure 6). DAT-Scan imaging has been approved by both the US Food and Drug Administration (FDA) and the European Medicines Agency for the differentiation of parkinsonism from essential tremor and, although it is not required for the diagnosis of Parkinson's Disease, an normal presynaptic dopaminergic imaging is an absolute exclusion criterion in the Movement Disorders Society (MDS) Clinical Diagnostic Criteria for PD [1].

It should be noted that the results of the DaT-Scan may be affected by technical issues, as well as by other pathologies revealed with conventional MRI, e.g., microvascular lesions. Of particular interest is the observation that patients with normal pressure hydrocephalus may present with an abnormal DaT-Scan that returns to normal after the surgical management of hydrocephalus. It has been hypothesized that the mechanical effect exerted on the striatum by ventriculomegaly leads to the downregulation of dopaminergic transporters, which may improve after surgery [54]. In such cases, or in other situations where the results of the initial DaT-Scan are not convincing, other imaging modalities might be considered to confirm degenerative parkinsonism. The most promising techniques are SWI for the demonstration of Dorsal Nigral Hyperintensity (DNH) and Neuromelanin MRI. As mentioned before, these techniques have excellent accuracy, especially in high-field MRI

The differential diagnosis of idiopathic Parkinson's disease from Parkinson Plus syndromes is based on clinical criteria and the response to levodopa treatment. Imaging techniques that may help the diagnosis include dopaminergic D2 imaging and cardiac sympathetic denervation using [123-I]-MIBG- scintigraphy (see figure 7); as mentioned before, the latter is included by the MDS in the supportive criteria for the diagnosis of Parkinson's Disease.

Origin of Figures

In this article, we have incorporated images that adhere to Open Access principles, promoting the free and open dissemination of knowledge. Additionally, the images included in this article are made available under various Creative Commons licenses, allowing for the respectful use, sharing, and adaptation of the content while respecting the rights of the original creators.

Readers are encouraged to refer to the figure captions and the references section for detailed information about the sources and licensing of the images used.

Figure 1.

Images C, D courtesy of Aruna Pallewatte, Radiopaedia.org, rID: 39232

Image E courtesy of Frank Gaillard, Radiopaedia.org, rID: 48610

Image F courtesy of Prashant Gupta, Radiopaedia.org, rID: 18863

Figure 2.

Image A courtesy of Frank Gaillard, Radiopaedia.org, rID: 4438

Image B courtesy of Abdallah Al Khateeb, Radiopaedia.org, rID: 45520

Images C-D from Meijer et al. 2017 [52].

Figure 3

Images A, B, E, F from Bae et al. 2021 [9].

Images C, D from Meijer et al. 2017 [52].

Figure 4

Image from Prange et al 2022 [12].

Figure 5

Image from Meles et al 2021 [14].

All other images, namely Figure 1, A, B, Figure 6. 7. 8 are from the archive of G. Gennimatas hospital

REFERENCES

[1] Postuma RB, Berg D, Stern M, Poewe W, Olanow CW, Oertel W, Obeso J, Marek K, Litvan I, Lang AE, Halliday G, Goetz CG, Gasser T, Dubois B, Chan P, Bloem BR, Adler CH, Deuschl G.(2015) MDS clinical diagnostic criteria for Parkinson's disease. ***Mov Disord***. 2015;30(12):1591-601. doi: 10.1002/mds.26424.

[2] Mitchell, T., Lehéricy, S., Chiu, S. Y., Strafella, A. P., Stoessl, A. J., & Vaillancourt, D. E. (2021). Emerging Neuroimaging Biomarkers Across Disease Stage in Parkinson Disease: A Review. ***JAMA Neurology***, 2021;78(10), 1262. https://doi.org/10.1001/jamaneurol.2021.1312

[3] Brooks DJ, Pavese N. (2011) Imaging biomarkers in Parkinson's disease. ***Prog Neurobiol***.,2011; 95(4):614-28. doi: 10.1016/j.pneurobio.2011.08.009.

[4] Pletcher, C., Dabbs, K., Barzgari, A., Pozorski, V., Haebig, M., Wey, S., Krislov, S., Theisen, F., Okonkwo, O., Cary, P., Oh, J., Illingworth, C., Wakely, M., Law, L., & Gallagher, C. L. (2023). Cerebral cortical thickness and cognitive decline in Parkinson's disease. ***Cerebral Cortex Communications***,2023; 4(1). https://doi.org/10.1093/texcom/tgac044

[5] Saeed U, Compagnone J, Aviv RI, Strafella AP, Black SE, Lang AE, Masellis M. (2017). Imaging biomarkers in Parkinson's disease and Parkinsonian syndromes: current and emerging concepts. ***Transl Neurodegener***. 2017;28;6:8. doi: 10.1186/s40035-017-0076-6.

[6] Meoded, A., Poretti, A., Mori, S., & Zhang, J. (2016). Diffusion Tensor Imaging (DTI). ***Reference Module in Neuroscience and Biobehavioral Psychology***. https://doi.org/10.1016/B978-0-12-809324-5.02472-X

[7] Bajaj, S., Krismer, F., Palma, A., Wenning, G. K., Kaufmann, H., Poewe, W., & Seppi, K (2017). Diffusion-weighted MRI distinguishes Parkinson disease from the parkinsonian variant of multiple system atrophy: A systematic review and meta-analysis. ***PLOS ONE***, 2017;12(12), e0189897. ttps://doi.org/10.1371/journal.pone.0189897

[8] Burciu RG, Ofori E, Archer DB, Wu SS, Pasternak O, McFarland NR, Okun MS, Vaillancourt DE. (2017) Progression marker of Parkinson's disease: a 4-year multi-site imaging study. ***Brain.*** 2017:1;140(8):2183-2192. doi: 10.1093/brain/awx146.

[9] Bae YJ, Kim JM, Sohn CH, Choi JH, Choi BS, Song YS, Nam Y, Cho SJ, Jeon B, Kim JH. (2021) Imaging the Substantia Nigra in Parkinson Disease and Other Parkinsonian Syndromes. ***Radiology.*** 2021;300(2):260-278. doi: 10.1148/radiol.2021203341..

[10] Cho SJ, Bae YJ, Kim JM, Kim HJ, Baik SH, Sunwoo L, Choi BS, Jung C, Kim JH. (2021) Iron-sensitive magnetic resonance imaging in Parkinson's disease: a systematic review and meta-analysis. ***J Neurol.*** 2021;268(12):4721-4736. doi: 10.1007/s00415-021-10582-x.

[11] Biondetti E, Gaurav R, Yahia-Cherif L, Mangone G, Pyatigorskaya N, Valabrègue R, Ewenczyk C, Hutchison M, François C, Arnulf I, Corvol JC, Vidailhet M, Lehéricy S. (2020). Spatiotemporal changes in substantia nigra neuromelanin content in Parkinson's disease. ***Brain.*** 2020;143(9):2757-2770. doi: 10.1093/brain/awaa216.

[12] Prange S, Theis H, Banwinkler M, van Eimeren T. (2022). Molecular Imaging in Parkinsonian Disorders-What's New and Hot? ***Brain Sci***. 2022;12(9):1146. doi: 10.3390/brainsci12091146.

[13] Schindlbeck, K. & Eidelberg, D. (2018). Network imaging biomarkers: insights and clinical applications in Parkinson's disease. ***The Lancet Neurology***. 2018;17. 629-640. 10.1016/S1474-4422(18)30169-8.

[14] Meles, S.K., Oertel, W.H. & Leenders, K.L. (2021) Circuit imaging biomarkers in preclinical and prodromal Parkinson's disease. ***Mol Med*** 2021;27, 111. https://doi.org/10.1186/s10020-021-00327-x

[15] Eckert, T., Tang, C., Ma, Y., Brown, N., Lin, T., Frucht, S., Feigin, A., & Eidelberg, D. (2008). Abnormal metabolic networks in atypical parkinsonism. Movement Disorders, 2008;23(5), 727-733. https://doi.org/10.1002/mds.21933

[16] Endo H., Ono M. et al: Imaging α-synuclein pathologies in animal models and patients with Parkinson's and related diseases. Published online August 23, 2023. https://doi.org/10.1101/2020.10.23.349860 https://www.biorxiv.org/content/10.1101/2020.10.23.349860v5.full.pdf

[17] Stokholm, M. G., Garrido, A., Tolosa, E., Serradell, M., Iranzo, A., Østergaard, K., Borghammer, P., Müller, A., Parbo, P., Stær, K., Brooks, D. J., Martí, M. J., & Pavese, N. (2020). Imaging dopamine function and microglia in asymptomatic LRRK2 mutation carriers. ***Journal of Neurology***, 2020;267(8), 2296-2300. https://doi.org/10.1007/s00415-020-09830-3

[18] Nandhagopal R, Kuramoto L, Schulzer M, Mak E, Cragg J, McKenzie J, McCormick S, Ruth TJ, Sossi V, de la Fuente-Fernandez R, Stoessl AJ. (2011). Longitudinal evolution of compensatory changes in striatal dopamine processing in Parkinson's disease. ***Brain.*** 2011;134(Pt 11):3290-8. doi: 10.1093/brain/awr233.

[19] Wile DJ, Agarwal PA, Schulzer M, Mak E, Dinelle K, Shahinfard E, Vafai N, Hasegawa K, Zhang J, McKenzie J, Neilson N, Strongosky A, Uitti RJ, Guttman M, Zabetian CP, Ding YS, Adam M, Aasly J, Wszolek ZK, Farrer M, Sossi V, Stoessl AJ. (2017) Serotonin and dopamine transporter PET changes in the premotor phase of LRRK2 parkinsonism: cross-sectional studies. Lancet Neurol. 2017;16(5):351-359. doi: 10.1016/S1474-4422(17)30056-X.

[20] Wilson H, Dervenoulas G, Pagano G, Koros C, Yousaf T, Picillo M, Polychronis S, Simitsi A, Giordano B, Chappell Z, Corcoran B, Stamelou M, Gunn RN, Pellecchia MT, Rabiner EA, Barone P, Stefanis L, Politis M. (2019) Serotonergic pathology and disease burden in the premotor and motor phase of A53T α-synuclein parkinsonism: a cross-sectional study. Lancet Neurol. 2019;18(8):748-759. doi: 10.1016/S1474-4422(19)30140-1.

[21] Liu SY, Wile DJ, Fu JF, Valerio J, Shahinfard E, McCormick S, Mabrouk R, Vafai N, McKenzie J, Neilson N, Perez-Soriano A, Arena JE, Cherkasova M, Chan P, Zhang J, Zabetian CP, Aasly JO, Wszolek ZK, McKeown MJ, Adam MJ, Ruth TJ, Schulzer M, Sossi V, Stoessl AJ. (2018) The effect of LRRK2 mutations on the cholinergic system in manifest and premanifest stages of Parkinson's disease: a cross-sectional PET study. ***Lancet Neurol***. 2018;17(4):309-316. doi: 10.1016/S1474-4422(18)30032-2.

[22] Varnäs K, Cselényi Z, Jucaite A, Halldin C, Svenningsson P, Farde L, Varrone A. (2019) PET imaging of [11C]PBR28 in Parkinson's disease patients does not indicate increased binding to TSPO despite reduced dopamine transporter binding. ***Eur J Nucl Med Mol Imaging***. 2019:46(2):367-375. doi: 10.1007/s00259-018-4161-6.

[23] Zhou L, Li G, Zhang Y, Zhang M, Chen Z, Zhang L, Wang X, Zhang M, Ye G, Li Y, Chen S, Li B, Wei H, Liu J. (2021) Increased free water in the substantia nigra in idiopathic REM sleep behaviour disorder. ***Brain***.2021; 144(5):1488-1497. doi: 10.1093/brain/awab039. .

[24] Ehrminger M, Latimier A, Pyatigorskaya N, Garcia-Lorenzo D, Leu-Semenescu S, Vidailhet M, Lehericy S, Arnulf I. (2016) The coeruleus/subcoeruleus complex in idiopathic rapid eye movement sleep behaviour disorder. ***Brain***. 2016;139(Pt 4):1180-8. doi: 10.1093/brain/aww006.

[25] Sun J, Lai Z, Ma J, Gao L, Chen M, Chen J, Fang J, Fan Y, Bao Y, Zhang D, Chan P, Yang Q, Ye C, Wu T, Ma T. (2020) Quantitative evaluation of iron content in idiopathic rapid eye movement sleep behavior disorder. ***Mov Disord***. 2020;35(3):478-485. doi: 10.1002/mds.27929.

[26] Marzi, R. D., Seppi, K., Högl, B., Müller, C., Scherfler, C., Stefani, A., Iranzo, A., Tolosa, E., Santamaría, J., Gizewski, E., Schocke, M., Skalla, E., Kremser, C., & Poewe, W. (2016). Loss of dorsolateral nigral hyperintensity on 3.0 tesla susceptibility-weighted imaging in idiopathic rapid eye movement sleep behavior disorder. ***Annals of Neurology***, 2016;79(6), 1026-1030. https://doi.org/10.1002/ana.24646

[27] Rahayel S, Postuma RB, Montplaisir J, Génier Marchand D, Escudier F, Gaubert M, Bourgouin PA, Carrier J, Monchi O, Joubert S, Blanc F, Gagnon JF. Cortical and subcortical gray matter bases of cognitive deficits

in REM sleep behavior disorder. ***Neurology***. 2018;15;90(20):e1759-e1770. doi: 10.1212/WNL.0000000000005523.

[28] Heinzel S, Berg D, Gasser T, Chen H, Yao C, Postuma RB (2019); MDS Task Force on the Definition of Parkinson's Disease. Update of the MDS research criteria for prodromal Parkinson's disease. ***Mov Disord***. 2019 Oct;34(10):1464-1470. doi: 10.1002/mds.27802.

[29] Lee CS, Samii A, Sossi V, Ruth TJ, Schulzer M, Holden JE, Wudel J, Pal PK, de la Fuente-Fernandez R, Calne DB, Stoessl AJ. (2000) In vivo positron emission tomographic evidence for compensatory changes in presynaptic dopaminergic nerve terminals in Parkinson's disease. ***Ann Neurol***.2000;47(4):493-503.

[30] Vander Borght T, Kilbourn M, Desmond T, Kuhl D, Frey K. The vesicular monoamine transporter is not regulated by dopaminergic drug treatments. ***Eur J Pharmacol***. 1995;294(2-3):577-83. doi: 10.1016/0014-2999(95)00594-3.

[31] De La Fuente-Fernández R, Furtado S, Guttman M, Furukawa Y, Lee CS, Calne DB, Ruth TJ, Stoessl AJ. (2003) VMAT2 binding is elevated in dopa-responsive dystonia: visualizing empty vesicles by PET. ***Synapse*** 2003;49(1):20-8. doi: 10.1002/syn.10199.

[32] Politis M, Wu K, Loane C, Kiferle L, Molloy S, Brooks DJ, Piccini P. (2010) Staging of serotonergic dysfunction in Parkinson's disease: an in vivo 11C-DASB PET study. ***Neurobiol Dis***. 2010;40(1):216-21. doi: 10.1016/j.nbd.2010.05.028.

[33] Fu JF, Matarazzo M, McKenzie J, Neilson N, Vafai N, Dinelle K, Felicio AC, McKeown MJ, Stoessl AJ, Sossi V. (2021) Serotonergic System Impacts Levodopa Response in Early Parkinson's and Future Risk of Dyskinesia. ***Mov Disord***. 2021;36(2):389-397. doi: 10.1002/mds.28340.

[34] Shimada H, Hirano S, Shinotoh H, Aotsuka A, Sato K, Tanaka N, Ota T, Asahina M, Fukushi K, Kuwabara S, Hattori T, Suhara T, Irie T. (2009) Mapping of brain acetylcholinesterase alterations in Lewy body disease by PET. ***Neurology***. 2009;73(4):273-8. doi: 10.1212/WNL.0b013e3181ab2b58.

[35] Schwarz ST, Abaei M, Gontu V, Morgan PS, Bajaj N, Auer DP. (2013) Diffusion tensor imaging of nigral degeneration in Parkinson's disease: A region-of-interest and voxel-based study at 3 T and systematic review with meta-analysis. ***Neuroimage Clin***. 2013;14;3:481-8. doi: 10.1016/j.nicl.2013.10.006.

[36] Febo M, Perez PD, Ceballos-Diaz C, Colon-Perez LM, Zeng H, Ofori E, Golde TE, Vaillancourt DE, Chakrabarty P. (2020) Diffusion magnetic resonance imaging-derived free water detects neurodegenerative pattern induced by interferon-γ. ***Brain Struct Funct***. 2020;225(1):427-439. doi: 10.1007/s00429-019-02017-1.

[37] Ofori E, Pasternak O, Planetta PJ, Burciu R, Snyder A, Febo M, Golde TE, Okun MS, Vaillancourt DE (2015). Increased free water in the substantia nigra of Parkinson's disease: a single-site and multi-site study. ***Neurobiol Aging***. 2015;36(2):1097-104. doi: 10.1016/j.neurobiolaging.2014.10.029..

[38] Archer, D. B., Bricker, J. T., Chu, W. T., Burciu, R. G., Mccracken, J. L., Lai, S., Coombes, S. A., Fang, R., Barmpoutis, A., Corcos, D. M., Kurani, A. S., Mitchell, T., Black, M. L., Herschel, E., Simuni, T., Parrish, T. B., Comella, C., Xie, T., Seppi, K., Vaillancourt, D. E. (2019). Development and Validation of the Automated Imaging Differentiation in Parkinsonism (AID-P): A Multi-Site Machine Learning Study. ***The Lancet. Digital Health***, 2019;1(5), e222. https://doi.org/10.1016/s2589-7500(19)30105-0

[39] Ohtsuka C, Sasaki M, Konno K, Kato K, Takahashi J, Yamashita F, Terayama Y. (2014) Differentiation of early-stage parkinsonisms using neuromelanin-sensitive magnetic resonance imaging. ***Parkinsonism Relat Disord***.2014;20(7):755-60. doi: 10.1016/j.parkreldis.2014.04.005.

[40] Noh Y, Sung YH, Lee J, Kim EY. (2015) Nigrosome 1 Detection at 3T MRI for the Diagnosis of Early-Stage Idiopathic Parkinson Disease: Assessment of Diagnostic Accuracy and Agreement on Imaging Asymmetry and Clinical Laterality. ***AJNR Am J Neuroradiol***. 2015;36(11):2010-6. doi: 10.3174/ajnr.A4412.

[41] Bae YJ, Kim JM, Kim E, Lee KM, Kang SY, Park HS, Kim KJ, Kim YE, Oh ES, Yun JY, Kim JS, Jeong HJ, Jeon B, Kim SE. (2016) Loss of Nigral Hyperintensity on 3 Tesla MRI of Parkinsonism: Comparison With (123) I-FP-CIT SPECT. ***Mov Disord.*** 2016;31(5):684-92. doi: 10.1002/mds.26584.

[42] Meijer FJ, van Rumund A, Fasen BA, Titulaer I, Aerts M, Esselink R, Bloem BR, Verbeek MM, Goraj B. (2015). Susceptibility-weighted imaging improves the diagnostic accuracy of 3T brain MRI in the work-up of parkinsonism. ***AJNR Am J Neuroradiol***. 2015;36(3):454-60. doi: 10.3174/ajnr.A4140..

[43] Sjöström H, Granberg T, Westman E, Svenningsson P. (2017). Quantitative susceptibility mapping differentiates between parkinsonian disorders. ***Parkinsonism Relat Disord***. 2017;44:51-57. doi: 10.1016/j.parkreldis.2017.08.029

[44] Merchant KM, Cedarbaum JM, Brundin P, Dave KD, Eberling J, Espay AJ, Hutten SJ, Javidnia M,

Luthman J, Maetzler W, Menalled L, Reimer AN, Stoessl AJ, Weiner DM; The Michael J. Fox Foundation Alpha Synuclein Clinical Path Working Group. (2019) A Proposed Roadmap for Parkinson's Disease Proof of Concept Clinical Trials Investigating Compounds Targeting Alpha-Synuclein. ***J Parkinsons Dis.*** 2019;9(1):31-61. doi: 10.3233/JPD-181471.

[45] Perlmutter JS, Norris SA. (2014) Neuroimaging biomarkers for Parkinson disease: facts and fantasy. ***Ann Neurol***. 2014;76(6):769-83. doi: 10.1002/ana.24291.

[46] Huang C, Tang C, Feigin A, Lesser M, Ma Y, Pourfar M, Dhawan V, Eidelberg D. (2007). Changes in network activity with the progression of Parkinson's disease***. Brain***. 2007;130(Pt 7):1834-46. doi: 10.1093/brain/awm086.

[47] Guttuso T Jr, Bergsland N, Hagemeier J, Lichter DG, Pasternak O, Zivadinov R.(2018). Substantia Nigra Free Water Increases Longitudinally in Parkinson Disease***. AJNR Am J Neuroradiol***. 2018;39(3):479-484. doi: 10.3174/ajnr.A5545.

[48] Schulz J, Pagano G, Fernández Bonfante JA, Wilson H, Politis M.(2018) Nucleus basalis of Meynert degeneration precedes and predicts cognitive impairment in Parkinson's disease. ***Brain.*** 2018;141(5):1501-1516. doi: 10.1093/brain/awy072. PMID: 29701787; PMCID: PMC6171218.

[49] Matsuura K, Maeda M, Tabei KI, Umino M, Kajikawa H, Satoh M, Kida H, Tomimoto H. (2016) A longitudinal study of neuromelanin-sensitive magnetic resonance imaging in Parkinson's disease. ***Neurosci Lett***. 2016;633:112-117. doi: 10.1016/j.neulet.2016.09.011

[50] Mak E, Su L, Williams GB, Firbank MJ, Lawson RA, Yarnall AJ, Duncan GW, Owen AM, Khoo TK, Brooks DJ, Rowe JB, Barker RA, Burn DJ, O'Brien JT. (2015) Baseline and longitudinal grey matter changes in newly diagnosed Parkinson's disease: ICICLE-PD study. ***Brain.*** 2015;138(Pt 10):2974-86. doi: 10.1093/brain/awv211.

[51] Peralta, C., Strafella, A. P., Ceravolo, R., Seppi, K., Kaasinen, V., Arena, J. E., & Lehericy, S. (2021). Pragmatic Approach on Neuroimaging Techniques for the Differential Diagnosis of Parkinsonisms. ***Movement Disorders Clinical Practice*** 2021;9(1), 6-19. https://doi.org/10.1002/mdc3.13354

[52] Meijer FJA, Goraj B, Bloem BR, Esselink RAJ (2017). Clinical Application of Brain MRI in the Diagnostic Work-up of Parkinsonism. J Parkinsons Dis. 2017;7(2):211-217. doi: 10.3233/JPD-150733.

[53] Mahlknecht P, Hotter A, Hussl A, Esterhammer R, Schocke M, Seppi K. (2010). Significance of MRI in diagnosis and differential diagnosis of Parkinson's disease. ***Neurodegener Dis.*** 2010;7(5):300-18. doi: 10.1159/000314495.

[54] Del Gamba C, Bruno A, Frosini D, Volterrani D, Migaleddu G, Benedetto N, Perrini P, Pacchetti C, Cosottini M, Bonuccelli U, Ceravolo R. (2021) Is DAT imaging abnormality in normal pressure hydrocephalus always suggestive of degeneration? ***Neurol Sci***. 2021;42(2):723-726. doi: 10.1007/s10072-020-04743-5

NEUROPHYSIOLOGICAL BIOMARKERS AT CORTICAL AND BASAL GANGLIA LEVELS IN PARKINSON'S DISEASE

Athanasios Leonardos[1], Pantelis Stathis[2], Stergios-Stylianos Gatzonis[3]
[1.] 1st Department of Neurology, National and Kapodistrian University of Athens
[2.] Department of Neurology, Hospital Mediterraneo
[3.] 1st Neurosurgery Department of the University of Athens, General Hospital of Athens "Evaggelismos"

Abstract
Parkinson's disease (PD) is a complex neurodegenerative disorder, associated with dopaminergic denervation of the basal ganglia (BG), resulting in aberrant activity patterns of surviving neurons. Early electrophysiological recordings in parkinsonian animals at both cortical and BG level helped in investigating cortico-thalamo-BG-cortical circuit dysfunction and developing the models of PD-related changes in neuronal activity (rate, rhythm, or synchronization). In addition, invasive recordings in PD patients during Deep Brain Stimulation (DBS) procedure, apart from verifying a lot of characteristics of the models, contributed to the identification of neurophysiological parameters that could play the role of a biomarker. In the field of DBS neurophysiology, the term biomarker is commonly used to describe a brain activity pattern that provides information, apart from the pathophysiological changes, for a specific clinical condition or a therapeutic effect. Local field potentials (LFPs) represent synchronized presynaptic and postsynaptic activity of large neuronal populations in direct vicinity to the implanted electrode. LFPs from DBS electrodes could give direct insight into electrophysiological dynamics of affected network nodes targeted by DBS. This review will discuss some potential biomarkers that characterize the neurophysiological changes in PD and their possible utility for monitoring and treatment of the corresponding PD symptoms.

Key Words: Parkinson's Disease, biomarkers, cerebral cortex, basal ganglia, neurophysiology, oscillatory activity, phase-amplitude coupling, local field potentials.

ΝΕΥΡΟΦΥΣΙΟΛΟΓΙΚΟΙ ΒΙΟΔΕΙΚΤΕΣ ΑΠΟ ΤΟΝ ΕΓΚΕΦΑΛΙΚΟ ΦΛΟΙΟ ΚΑΙ ΤΑ ΒΑΣΙΚΑ ΓΑΓΓΛΙΑ ΣΤΗΝ ΝΟΣΟ ΤΟΥ ΠΑΡΚΙΝΣΟΝ.

Αθανάσιος Λεονάρδος[1], Παντελής Στάθης[2] , Στέργιος-Στυλιανός Γκαζώνης[3]
[1] Α' Νευρολογική Κλινική ΕΚΠΑ
[2] Νευρολογική Κλινική Νοσοκομείο Mediterraneo
[3] Α' Ν/Χ Κλινική Πανεπιστημίου Αθηνών Νοσοκομείο «Ευαγγελισμός»

Περίληψη
Η νόσος του Πάρκινσον (ΝΠ) είναι μια σύνθετη νευροεκφυλιστική διαταραχή, συσχετιζόμενη με τη ντοπαμινεργική απονεύρωση των βασικών γαγγλίων (ΒΓ), που οδηγεί σε ανώμαλα μοτίβα δραστηριότητας των διασωθέντων νευρώνων των ΒΓ. Πρώιμες ηλεκτροφυσιολογικές καταγραφές σε παρκινσονικά ζωικά μοντέλα βοήθησαν στη διερεύνηση της δυσλειτουργίας των κυκλωμάτων φλοιού-θαλάμου-ΒΓ-φλοιού και πρότειναν μοντέλα που περιέγραφαν τις αλλαγές της νευρωνικής δραστηριότητας (συχνότητα, ρυθμός ή συγχρονισμός εκφορτίσεων) που σχετίζονται με την ΝΠ. Επιπλέον, οι επεμβατικές καταγραφές σε ασθενείς με PD κατά τη διάρκεια της διαδικασίας της Εν τω Βάθει Εγκεφαλικής Διέγερσης (DBS), επαλήθευσαν πολλά από τα χαρακτηριστικά των παραπάνω μοντέλων, συμβάλλοντας παράλληλα στην ανάδειξη νευροφυσιολογικών παραμέτρων, που θα μπορούσαν να παίξουν το ρόλο βιοδείκτη. Στη νευροφυσιολογία του DBS, ο όρος βιοδείκτης χρησιμοποιείται συχνά για να περιγράψει ένα πρότυπο εγκεφαλικής δραστηριότητας, που παρέχει πληροφορίες για συγκεκριμένα συμπτώματα ή θεραπευτικά αποτελέσματα. Τα τοπικά δυναμικά πεδίου (LFPs) αντιπροσωπεύουν την συγχρονισμένη προσυναπτική και μετασυναπτική δραστηριότητα μεγάλων πληθυσμών νευρώνων σε άμεση γειτνίαση με τα εμφυτευμένα ηλεκτρόδια. Η καταγραφή των LFPs από τα ηλεκτρόδια θα μπορούσε να προσφέρει άμεση πληροφόρηση για τα ηλεκτροφυσιολογικά δεδομένα των κυκλωμάτων και των πυρήνων – στόχων του DBS. Στην ανασκόπηση αυτή θα γίνει αναφορά σε πιθανούς βιοδείκτες που χαρακτηρίζουν τις εν λόγω νευροφυσιολογικές αλλαγές στην ΝΠ και τη χρησιμότητα τους για την παρακολούθηση και τη θεραπεία των συμπτωμάτων που σχετίζονται με αυτές.

Λέξεις κλειδιά: Νόσος Πάρκινσον; βιοδείκτες; βασικά γάγγλια; εγκεφαλικός φλοιός;νευροφυσιολογία; νευρωνική δραστηριότητα; τοπικά δυναμικά πεδία;

Introduction

Parkinson's disease (PD) is a common and disabling movement disorder owing to dopaminergic denervation in basal ganglia (BG). The core pathology is the degeneration of the dopaminergic neurons in the substantia nigra pars compacta (SNc) that project to the striatum. Striatum is the major gate of the basal ganglia, receiving inputs from thalamus and the cerebral cortex and projecting to the pallidonigral system.[1] Therefore, cortical mode and thalamocortical coupling, being the major carriers of information, could play a major role for basal ganglia function. An early raised question was how the basal ganglia translate cortical inputs under physiological conditions and consequently how is this transformed under abnormal conditions. This 'reading' of cortical activity has been extensively studied in both animals and humans, especially during operations for DBS treatment of PD patients. Physiological studies of simultaneously recorded neurons in basal ganglia, cortex and cerebellum gave us a window for brain dysfunction in PD.

Cortical Dynamics

The recording of neuronal signals directly from the cortical surface of the brain was first reported in rabbits and monkeys by the British physician Richard Caton in 1875[2] and in humans by the German psychiatrist Hans Berger in 1924.[3] Recording of brain potentials by means of electroencephalography and recordings by means of electromyography (mainly during surgical interventions for the treatment of epilepsy) were historically the first organized recordings of the electrical activity of the human cerebral cortex.

The early years

There have been several early reports concerning electroencephalography (EEG) in PD. In most of them, EEGs have been diagnosed as abnormal in up to 30-50% of the cases.[4,5,6,7] These early recordings were analogic and their analysis consisted of simple visual qualitive inspection of the signals. Therefore, only rough conclusions could be drawn, concerning obvious signal changes on a restricted time scale. Generally speaking, the most prominent and frequent findings of these early works were an increase in the sum of lower frequencies as well as a slowing of α rate. These findings were non-specific.[8]

In the following years, EEG studies in PD patients noticed that a general disturbance of the EEG, together with some other indicators of brain dysfunction, is related to an increased risk of a progressive dementing process.[9] In 1988, Neufeldt et al. reported a significant association between occipital background slowing and motor disability in non-demented patients.[10] In 1991, Soikkeli et al. suggested that the absolute and relative amplitudes of delta, theta, alpha and beta bands and the peak and mean frequency differed significantly in Parkinson's dementia patients. An interesting finding of the study was the increase of delta activity in Parkinsonian patients without dementia, and the theta activity. The frequencies were slower than in controls.[9]

Digital era

Since the 1990s, the use of digital devices for signal storage and the use of large-scale computational methods for data processing have led to the most profound and most extensive investigation of CNS electrical potentials. Apart from the development of computing power through digital devices, invasive recordings in PD patients, which occurred from the development of invasive treatments for the disease, provided direct information concerning PD neurophysiology. Since the mid-1990s, studies of electrical brain potentials in PD - and movement disorders in general - have been steadily increasing.[11,12,13]

Scalp EEG versus electrocorticography

Scalp EEG is limited by poor source localization and low signal amplitude, which is problematic for studying higher frequencies and is also poisoned by heaps of spurious potentials (movements, etc.).[14] The electrocorticography technique, in contrast, has high signal amplitude, excellent spatial localization, and, in the context of movement disorders surgery, does not require additional brain penetrations or surgical exposure. Furthermore, broadband spectral gamma power in cortical local field potentials is thought to reflect underlying pyramidal cell spiking activity, suggesting that electrocorticography may provide a new technique for assessment of underlying neuronal activation state in human movement disorders.[15] Although widely used in studies of the "normal" physiology of the sensorimotor cortex in humans with epilepsy, electrocorticography had not been applied extensively to the study of the most common movement disorders.[16,17,18,19]

Central oscillations

Neurophysiological recordings were used to highlight the role of central oscillators in tremor in Parkinson's disease. Oscillatory activities have been reported at a variety of frequencies between 4 and 60 Hz.[20] After a series of animal and human studies, especially during Deep Brain Stimulation (DBS) procedures, several discrete forms of oscillatory activity in the basal ganglia have been demonstrated.[21] In addition, electroencephalography (EEG) and magnetoencephalography (MEG) studies have shown oscillatory activity at the tremor frequency throughout the cerebellar-thalamic-cortical circuit.[22,23] These

oscillations play an important role in both normal function and the pathophysiology of movement disorders.[24,25,26] In 2003, Timermann et al. demonstrated tremor-related oscillatory activity within a cerebral network, with abnormal coupling in a cerebello-diencephalic-cortical loop and cortical motor and sensory areas contralateral to the tremor hand. The main frequency of cerebro-cerebellar coupling corresponded to double the tremor frequency.[22] The hypotheses for many of the above studies was that excessive oscillatory synchronization in the basal ganglia–thalamocortical motor network at or near 20 Hz is a clear and distinctive feature and may underlie bradykinesia.[1,27,28] Hammond et al. demonstrated in 2007 that in Parkinsonian patients an abnormally synchronized oscillatory activity occurs at multiple levels of the basal ganglia-cortical loop. Notably, this excessive synchronization correlates with motor deficit, and its suppression by dopaminergic therapies, ablative surgery, or DBS might provide the basic mechanism whereby diverse therapeutic strategies ameliorate motor impairment in PD patients.[1]

Increased cortical beta power

EEG and MEG studies suggest that advanced PD is associated with pathologically increased cortical beta power.[29] This association between beta cortical power and PD has also been demonstrated in animal models of PD with dopamine depletion.[30] However, increased cortical beta power has also been demonstrated in early PD, especially in bilateral primary sensorimotor cortices.[31] In particular, Crowell et al., using corticography during DBS surgery for PD, demonstrated that primary motor cortex broadband spectral power is increased in those patients.[20] This increase extended over a very broad frequency range, from as low as 20 Hz to >200 Hz, always taking into account the specific conditions under which the recordings were made ("off" state, during surgery etc.). Broadband spectral power changes are thought to reflect asynchronous spiking activity in the region underlying the recording electrode.[15] However, we should keep in mind that cortical broadband local field potential (LFP) power also correlates with the blood oxygen level-dependent (BOLD) signal on functional MRI studies.[32] Following this finding, the question raised is whether this is related to the metabolic disorder and not to a real change in neurophysiological pattern.[33]

Cortical desynchronization seems to be a consistent finding and could have different interpretations. Since recordings concern DBS surgeries, we are dealing with patients with advanced disease, and it is not therefore clear if cortical desynchronization reflects a primary abnormality or a compensatory mechanism. DeLong proposed from early 90's that the original 'rate model' of basal ganglia and cortical function in PD posited resting state cortical hypoactivity, driven by excessive inhibitory basal ganglia output.[34] Crowell et al., based on corticocortical recordings, proposed another hypothesis for the increased subthalamic nucleus single unit discharge that is characteristic of the parkinsonian state: subthalamic nucleus hyperactivity may be driven by an overactive cortical area, via the cortico-subthalamic 'hyperdirect' pathway.[20]

Corticography also carries some limitations since its findings depend on the underlying cortical signal generators. How much does brain atrophy affect outcomes in Parkinson's disease? How much are outcomes affected by levodopa administration or the existence or non-existence of tremor? Another disadvantage of corticography studies - not only in Parkinson's disease - is the lack of controls, i.e. healthy controls.

β-synchronization

At this point, it should be noted that in a series of studies with transcranial alternating current there were conflicting results. For example, Timmermann et al. demonstrated worsening of Unified Parkinson's Disease Rating Scale (UPDRS) scores with STN DBS at 10 Hz but not at 20 Hz, compared to no stimulation.[35] Chen et al., studying the increasing slope in a reaction time catch task, demonstrated a reduction with 20 Hz but not with 5 or 10 Hz STN DBS compared to no stimulation.[21] Eusebio et al. reported that finger tapping rate was reduced with STN DBS at 5 Hz and 20 Hz but not at 10 Hz.[36] The above findings suggest that no single pathological frequency may reflect all parkinsonian motor symptoms. It is possible that specific frequency bands are associated with specific motor performance parameters.[37] This led to the concept that measures of brain physiology reflecting β-synchronization could be potential "biomarkers" for the pathophysiology of PD. Such objective measures of PD symptoms would have enormous clinical potential for diagnosing, monitoring, and tailoring patient treatments. In particular, the cortical β-waveform shape may indicate the summation of synchronous inputs (perhaps from the basal ganglia via the thalamus) to cortical pyramidal neurons.[38]

Phase-Amplitude Coupling (PAC)

Another biomarker under investigation in movement disorders is the phase-amplitude coupling (PAC). PAC is the coupling of the phase of slower electrophysiological oscillations with the amplitude of faster oscillations and is thought to facilitate dynamic integration of neural activity in the brain.[39]While conventional signal processing measures, such as β power, have failed to reliably differentiate PD as a function of cortical severity or diagnosis, phase-amplitude coupling (PAC) between β and broadband γ (50–150 Hz) seems more promising.[40,41]Specifically,

phase-amplitude coupling (PAC) over the motor cortex, detected using electrocorticography (ECoG), is increased in PD compared to other groups and decreased with DBS in a clinically relevant manner.[42] Interestingly, after characterizing PAC with ECoG, it was shown that increased PAC can also be detected non-invasively with scalp electroencephalography (EEG).[43] In addition, PAC recorded with scalp EEG could differentiate PD patients on and off medication and differentiate PD patients off medication from healthy controls. Increased beta-gamma PAC in PD was first found with interventional electrocorticography (ECoG) studies,[40,41] and subsequently demonstrated in EEG studies.[44] Increased beta-gamma PAC in the sensorimotor cortex was found in untreated PD patients compared to healthy controls and those taking medication.[43]

Pattern of β-oscillations

The pattern of beta oscillations could be considered as another neurophysiological biomarker. PD patients' brain activity is characterized by beta oscillations with a non-sinusoidal shape. Furthermore, the pattern changes with medication status, as greater sharpness asymmetry and slope asymmetry of regular beta oscillations over sensorimotor cortex, were found in drug-free PD patients as opposed to those on medication. Specifically, β oscillations in areas above sensorimotor cortex in untreated PD patients had greater sharpness and slope asymmetry compared to patients on medication. These findings suggest that new ways of measuring β-synchrony incorporating waveform shape could improve the detection of PD pathophysiology in noninvasive recordings.[44]

Parkinson's Disease: Possible neurophysiological biomarkers at basal ganglia level.

The principal goal of Deep Brain Stimulation (DBS) of the subthalamic nucleus (STN) or internal globus pallidus (GPi) is the improvement of major clinical motor symptoms of Parkinson's disease (PD) such as tremor, bradykinesia and rigidity, along with improvement of motor response complications.[45,46] However, the success of DBS depends fundamentally in placing the DBS electrodes with high precision into the sensorimotor region of the STN corresponding to the dorsolateral posterior part of the nucleus, or the GPi corresponding to its posteroventral part.[47] To achieve a high precision implantation in this region, intraoperative microelectrode recordings (MER) of the neuronal electrical activity from targeted structures are widely used. The main principle underlying this procedure is that in hypo-dopaminergic (parkinsonian) state increased firing rates and discharge patterns of neurons both in the STN and the GPi are so characteristic that constitute the hallmark of the nuclei.[48,49]

The increased firing rates of STN and GPi are in concordance with the so-called "rate" or "classical" model of basal ganglia (BG). The classical model of basal ganglia function has critically helped understanding of how dopamine contributes to motor output and how loss of midbrain dopamine neurons leads to circuit-level changes underlying the motor symptoms of PD.[50,51] Its basic assumption, generating several (but not all) testable predictions regarding changes in firing rate throughout the basal ganglia in Parkinson's disease, is that information is encoded in the firing rate of individual neurons.[52,53] Moreover, evidence linking changes in basal ganglia neurophysiology with PD motor deficits, including both observational and interventional evidence obtained from PD patients, as well as parkinsonian nonhuman primate and rodent models, also revealed changes in firing patterns and synchrony.

Indeed, additional neurophysiological characteristic findings in BG in parkinsonian state, are the emergence of burst discharges, greater synchrony of firing between neighboring neurons, oscillatory activity patterns, and excessive coupling of oscillatory activities at different frequencies, which are in concordance with what is called "pattern" model.[1,54] Such oscillatory activity could be generated internally within the basal ganglia but also, and perhaps more likely, as part of a larger network involving the cortex and thalamus. All these alterations of neuronal activities in parkinsonism prevent the normal separation of the firing of individual neurons in the basal ganglia, limiting the space available for information coding through spatial selectivity and/or temporal patterning and thus impairing motor processing.[1] Consequently, the pattern model provides an attractive view of adaptive and maladaptive plasticity processes involved in PD.

Local Field Potentials (LFPs)

The resulting neuronal synchrony is also implied by the finding of increased amplitudes of local field potentials (LFPs) in the beta-band range of frequencies (10–30 Hz) in the basal ganglia and cortex.[55,56] LFPs are summations of extracellular electrophysiologic activity of a population of neurons occupying a small area, being recorded using intracerebral electrodes.

Further on, recording LFPs through macroelectrodes implanted in the STN or GPi for DBS in Parkinsonian patients has brought to light the following associations: (a) The 11–30 Hz (beta band) peak characterizes the "off" parkinsonian state and a 4–6

Hz peak appears in patients with tremor. (b) In the "On" pharmacological state there is a predominant 60–80 Hz gamma band peak, while beta rhythm is drastically attenuated. (c) In patients with levodopa-induced dyskinesias there is predominant 4–10 Hz activity. These findings indicate that the degree of neuronal synchronization and discharge pattern in PD change drastically within the BG in direct relation with the degree of dopaminergic deficit or replacement.[57,58,59] The same holds true for the antiparkinsonian effect of DBS. Recordings from STN during electrical stimulation at frequency of 130 Hz have revealed a tapering of beta band during "Stim ON" phase.[60,61]

Moreover, several other recording data suggest that: (a) Patterns of LFPs of GPi are different in Parkinson's disease from dystonia.[62] (b) The detection of dorsolateral posterior STN LFPs activity is considered as the electrophysiological "sweet spot" for effective clinical outcome.[63] These neurophysiological characteristics contribute to the definition of the optimal DBS implantation trajectory, as well as to the optimum adjustment of stimulation parameters.[64,65,66] (c) LFP recordings may also prove useful toward quantification of motor subtypes of Parkinson's disease[67] and severity of rigidity and bradykinesia in PD.[68]

Recent studies have further used various recordings using MER with the advent of sophisticated analysis and modeling for localizing dorsal–ventral border of STN[69] and predicting therapeutic volume of tissue activation.[70]

Phase-Amplitude Coupling (PAC)

As already mentioned earlier, changes in coupling between the phase of low-frequency and the amplitude of high-frequency oscillations [phase-amplitude coupling (PAC)] have also been proposed as biomarkers of PD. More recent studies hypothesize that PAC could be a robust biomarker of PD.[40,71] PD patients exhibited a reduction of PAC measured in the STN after levodopa administration[72]. The studies by de Hemptinne et al. showed that PD patients were more likely to exhibit significant measurements of PAC in the primary motor cortex (M1) compared to epilepsy and dystonia controls, and that cortical PAC was reduced during therapeutic STN DBS.[40,41] A study using LFPs from microelectrode recordings in the nonhuman primate MPTP PD model demonstrated that PAC in the pallidum progressively increased in concordance with parkinsonism severity.[71]

High Frequency Oscillations (HFOs)

Other researchers have suggested that changes in the high-frequency side of the spectrum (200-400 Hz) may also be associated with PD. For example, it was showed that levodopa administration elicited an increase in power at 320 Hz in the STN of PD patients implanted with DBS leads and demonstrated that the power between 200 and 300 Hz in the internal segment of the globus pallidus (GPi) of PD patients was movement dependent.[73,74] Both groups of investigators hypothesized that these high-frequency oscillations (HFOs) are required for normal information processing and motor control. On contrary, recent findings from GPi recordings provide evidence that exaggerated, movement-modulated HFOs in the GPi are pathophysiological features of PD. These findings suggest that the functional role(s) of HFOs may differ between the STN and GPi and motivate additional investigations regarding their relationship to motor control in normal and diseased states. The same group stress the possibility of the utility of HFOs in the development of electrophysiological-based adaptive DBS approaches, for example with HFOs in the GPi being a potential functional marker of motor state.[75]

Towards biomarkers for closed-loop (adaptive) DBS

Therefore, identifying neurophysiological biomarkers that correlate with motor symptoms or disease severity will be supportive in understanding the pathophysiology of PD and developing more effective treatments. As a matter of fact, there is particular interest in incorporating such biomarkers into devices that could deliver closed-loop deep brain stimulation (DBS) tailored to the clinical state of individual patients.[76,77] Biomarkers derived from LFPs seem attractive for closed-loop-sensing DBS because they can be recorded continuously from brain structures via permanently implanted electrodes.[78,79]

Along with its definition as a characteristic that is measured as an indicator of normal biological processes, pathogenic processes or responses to an exposure or intervention,[80] a biomarker should also fulfil three criteria of clinical usefulness. An ideal biomarker should be:

1. Indicative (Is the neurophysiological biomarker sufficiently linked to the severity of fluctuating symptoms?)
2. Individual (Is the neurophysiological biomarker detectable in every patient and patient-specific if needed?)
3. Implementable (Is the neurophysiological biomarker (technically) capable of automatically titrating stimulation?).[81]

Hence, current adaptive applications such as sensing-enabled DBS devices, would likely need to be improved addressing all three of the criteria, providing an optimum performance to the patients, through valid closed-loop algorithms, that can automatically detect relevant biomarkers for titrating stimulation with minimum clinical intervention.

Neurophysiological biomarkers to optimize DBS in Parkinson's disease

Implantation of DBS electrodes provides the unique opportunity to record in vivo deep brain activity. Local field potentials (LFPs) represent synchronized presynaptic and postsynaptic activity of large neuronal populations in direct vicinity to the target area. LFPs from DBS electrodes could give direct insight into electrophysiological dynamics of affected network nodes targeted by DBS, enabling therefore a systematic phenotyping of oscillatory patterns in patients undergoing DBS surgery.

In the field of DBS neurophysiology, the term biomarker is commonly used to describe a brain activity pattern that provides information on specific symptoms or therapeutic effects. An ideal biomarker should have a direct correlation to clinical symptoms, tracking disease state constantly and dynamically, with minimal sampling error. In the case of neurophysiological biomarkers, desirable characteristics include signal stability over time and across multiple conditions, as well as differentiation from ongoing spontaneous activity. Fulfillment of the aforementioned criteria is very important when we are dealing with adaptive DBS (aDBS), since adaptive control systems require a reliable and informative feedback signal to support appropriate therapeutic adaptations.[82]

LFPs in the beta band frequency (13-35 Hz)

LFP recordings from the STN or GPi consistently demonstrate excessively synchronized activity in the beta band frequency (13-35 Hz) in patients with PD during "off" periods or after withdrawal of dopaminergic medications. Beta power was first found by Peter Brown in 2001 to be abnormally high in the STN of untreated PD patients. Beta power decreases during movement preparation and execution, showing a significant rebound after movement termination. One should keep in mind that beta activity is not specific to PD or even pathological per se. Excessive beta activity in PD most likely reflects a pathological alteration of physiological synchronization involved in dynamic brain state transitions.[83]

Beta band and aDBS

Beta band is the most studied neurophysiological biomarker in the design of aDBS. Beta activity is considered to be the most suitable feedback sign for aDBS due to its clinical relevance and consistency. Beta activity correlates with the presence of contralateral rigidity and bradykinesia. Moreover, dopaminergic and DBS induced suppression of beta activity correlates with the improvement of motor impairment. Clinical implementation of sensing-enabled pulse generators provides the opportunity of safe, long-term recording of beta activity. Interestingly, the significant correlation between beta power and disease severity does not diminish over time. Since beta activity is measurable and consistently correlates with contralateral akinetic-rigid symptoms and their response to DBS therapy, it is currently regarded as a reliable neurophysiological biomarker to optimize DBS in patients with PD.[84]

Low beta (13-20 Hz) and high beta (21-35 Hz) bands

Beta activity is divided into two separate frequency components with rather distinct functions: low beta (13-20 Hz) and high beta (21-35 Hz). Low beta activity is more dominant within the STN and is generally considered as a pathological oscillation. Low beta activity is more sensitive to the beneficial effects of levodopa. Moreover, the power of low beta activity correlates with disease severity. In contrast to the low beta band, which plays a local (intraregional) role, high beta is related to long-distance (interregional) coupling. It has been suggested that high beta activity is a unique spectral signature of the hyperdirect pathway.[85]

Phase-amplitude coupling (PAC)

Phase-amplitude coupling (PAC) between STN high beta activity and cortical high-frequency oscillations (HFOs) is regarded as prokinetic and physiological. Higher high beta-cortical HFO coupling is in principle associated with significantly better motor performance. Hence, phase-amplitude coupling (PAC) between STN and motor cortex is regarded as a promising electrophysiological biomarker for aDBS.[86]

Beta bursts and aDBS

Physiological beta activity consists of some short-lived phasic bursts. Larger and longer duration bursts usually indicate pathological beta activity. In PD, the presence of abnormal beta bursts is significantly correlated with the degree of motor impairment and the severity of akinetic-rigid symptoms.[87,88] Diminishment of beta bursts amplitude and duration during movement, along with the levodopa and DBS effect on STN beta bursts distribution from long to short duration, make beta bursts a promising biomarker for aDBS. Having set a threshold to quantify and define beta bursts, aDBS could selectively trim larger and longer bursts, leading to a restoration of physiological STN beta activity, as well as a prevention of overtreatment that will cause dyskinesias.

Beta band and post-operative programming

Beta activity can also serve as a feedback signal to predict the optimal stimulation contacts. The contact pair with maximal STN beta power is very likely to provide the best symptom control and has the widest therapeutic window. If a particular contact pair has stronger beta activity, these two contacts are

more likely to be close to the pathological source. Accordingly, a lower amount of current is required to reach clinical improvement.[89]

Low frequency (LF) range: theta (4-7 Hz) and alpha (8-12 Hz) bands

Theta (4-7 Hz) and alpha (8-12 Hz) are usually discussed together, collectively referred to as the low frequency (LF) band. LF activity, recorded both from STN and GPi, showed a great increase after levodopa intake or DBS stimulation, in association with PD motor symptoms alleviation. LF has been also correlated to peak-dose as well as biphasic dyskinesias.[90] Therefore, it has been suggested that LF could be considered as biomarker for aDBS.

LF/beta power ratio

In particular, LF/beta power ratio has been introduced as a reliable feedback signal for triggering closed-loop DBS. In the "off" state, STN power is low in LF and high in beta band, triggering the stimulation to turn on. On the contrary, in the "on" – as well as in the "overtreatment" – state, STN power is in a way transferred from beta to the LF band, increasing the LF/beta ratio and consequently turning the stimulation off. It has been suggested that application of LF/beta ratio as a biomarker could also help reducing stimulation-related side effects.[58]

LF vs. beta power

Compared with beta power alone, STN LF activity may have two advantages as a supplementary feedback signal. First, beta activity is mainly located in the dorsolateral STN, while LF is usually more widespread, with its peak tracked in the ventromedial STN. In view of the fact that the stimulating electrode is constantly adjusted to achieve optimal efficacy, while simultaneous stimulating and recording cannot be performed in the same contact, it is obviously meaningful to be able to record feedback signals from both the dorsal and ventral STN. Second, beta activity is more correlated with rigidity and bradykinesia compared to rest tremor.[91] LF band, ranging from 4 to 12 Hz, normally includes tremor frequency band (4-8 Hz). Therefore, LF could potentially serve as a supplementary electrophysiological biomarker in tremor dominant patients. However, since LF band enhancement has been associated with rest tremor as well as levodopa-induced dyskinesias (LIDs), a lack of precise distinction between these two states could lead to undesirable side effects when applying DBS. Machine learning algorithms and multifeatured engineering have been proved helpful for the quick and accurate detection of rest tremor.[92,93]

Tremor frequency (4-8 Hz)

Oscillations within the tremor frequency (TF) band (4-8 Hz) are detected in the STN of tremor dominant PD patients. On the other hand, there is no correlation between beta power and rest tremor, likely implying that different pathophysiological mechanisms are involved in tremor generation and akinetic-rigid symptoms. In fact, in tremor dominant PD patients, beta power was found to significantly decrease along with tremor frequency enhancement. It has been hypothesized that rest tremor reduces STN beta power while simultaneously increasing TF. Hence, it has been suggested that a combination of TF and beta power could provide adequate information in the case of aDBS, releasing stimulation whenever STN power increases in the band of TF while decreasing in the beta band. However, it should be noted that TF oscillations normally occur shortly after tremor onset. This short latency period has been reported to vary between 150ms and several seconds. Therefore, the length of the aforementioned latency period should be taken under consideration when using TF as feedback signal for aDBS.[92]

Gamma band (35-200 Hz)

Gamma band activity is generally regarded as prokinetic, acting as a compensatory mechanism for the akinetic role played by beta activity. A positive correlation between movement velocity and an increase in the STN narrow gamma band activity (40-90 Hz) has been established. The synchronization of gamma power during movement occurs in bursts, with gamma burst rates significantly increasing in parallel with fast movements.[94] Movement-related gamma band augmentation is not restricted to the STN, being also present in the cortex. In contrast to beta activity response, levodopa administration leads to gamma power enhancement. Moreover, peak-dose dyskinesias are associated with gamma band overactivity. Nonetheless, studies have shown that low gamma activity (35-45 Hz) is associated with rest tremor severity. A possible explanation to this contradictory finding could be that tremor amplitude increases during stress when STN gamma oscillations become stronger.

High Frequency Oscillations (>200 Hz)

Similar to gamma band activity, high frequency oscillations (HFOs) are considered to be prokinetic. HFO power increases at movement onset, as well as after levodopa administration. Given the prokinetic nature of HFO, it has been postulated that high-frequency STN-DBS improves PD motor symptoms by evoking STN neural activities that are quite similar to HFOs.[95]

Slow HFOs (200-300Hz) and Fast HFOs (300-400Hz) – sHFO/fHFO ratio

HFOs are divided into two subgroups: slow (200-300 Hz) and fast (300-400 Hz) HFOs. These two subgroups have distinct functional roles with clinical relevance in the case of aDBS. Slow HFO (sHFO)

power is more pronounced in the "off" state and undergoes a significant decrease following levodopa administration. On the contrary, fast (fHFO) activity is remarkably enhanced after levodopa intake, resulting in a substantial power increase in the overall HFO band. Moreover, fHFO power is inversely correlated to akinesia. Power transition from sHFO to fHFO is regarded as an electrophysiological signal of shifting from the hypo-dopaminergic to the hyper-dopaminergic state.[96] In particular, power sHFO/fHFO ratio is significantly associated with akinesia/rigidity UPDRS-III scores. Additionally, power sHFO/fHFO is found to be significantly different between tremor and non-tremor states. As previously mentioned, tremor at rest does not reliably correlate with beta band activity. However, a frequency shift from sHFO toward fHFO may be a reliable biomarker of PD tremor. Furthermore, since HFOs are prone to change on a short timescale, a combination of TF, beta power and HFO could be valuable in detecting tremor in aDBS, allowing a much faster triggering of stimulation compared to beta activity.[97]

Conclusion

Deep brain stimulation (DBS) is nowadays considered as an effective neurosurgical treatment for Parkinson's disease (PD). Implanted electrodes provide the unique opportunity to record subcortical electrophysiological activity in vivo. Local field potentials (LFPs) is the term coined to describe the recorded discharges from a cluster of neurons surrounding the implanted electrode. Compared to cortical neural signals such as electroencephalography (EEG), electrocorticography (ECoG), and magnetoencephalography (MEG), LFPs can provide direct insight into basal ganglia function. In Parkinson's disease (PD), local field potentials (LFPs) that are abnormally synchronized in the beta frequency band (13-35 Hz) correlate with the severity of akinetic-rigid symptoms and their response to pharmacological and DBS therapy. Improvement of akinetic-rigid symptoms is associated with DBS suppression of abnormally synchronized LFPs in the low beta frequency band (13-20 Hz) and facilitation of high frequency gamma band (35-250 Hz).

DBS treatment could be optimized by adapting stimulation settings to the presence or absence of PD symptoms through closed-loop control. This critically relies on the use of biomarkers extracted from neurophysiological signals. This form of DBS is called "adaptive" (aDBS) or "closed-loop" DBS, and is currently available as clinical care in some countries. aDBS potentially reduces side-effects due to over-stimulation, saves battery power consumption, and holds promise for implementing symptom-specific stimulation settings. The success of aDBS applications critically depends on the quality and predictive value of the used biomarkers. Ideal biomarkers for adaptive DBS (aDBS) are indicative of symptom severity, detectable in every patient, and technically suitable for implementation. In the last decades, much effort has been put into the detection of local field potential (LFP) biomarkers and in their use in clinical practice. Out of the LFP signal features that have been linked to PD symptom severity so far, the most frequently reported associations are between UPDRS-III (motor) scores of rigidity and bradykinesia and measures of contralateral STN beta (13-35 Hz) oscillations. To date, most aDBS applications have used beta bursts with a minimum duration and amplitude as biomarker for triggering stimulation, with performance comparable but not superior to continuous DBS. It appears, though, that beta power alone is not sufficient to explain the full spectrum of Parkinsonian symptoms. The role of other frequency bands and the interaction between them needs to be further explored. Several strategies are being developed to overcome the limitations of current LFP biomarkers for aDBS. One promising avenue is the simultaneous use of multiple signal features to monitor different symptoms in parallel. In theory, monitoring of the tremor frequency range could be combined with the monitoring of beta and gamma oscillations to control stimulation. In this way, the amplitude of beta oscillations might act as a trigger for switching on or off the stimulation, while the stimulation amplitude can be controlled based on gamma band power.

Choosing the right biomarker(s) for aDBS can be challenging. With further development of hardware and neurophysiological understanding, it might be that additional biomarkers can be identified that are closer to true neurobiological causes. The ideal adaptive DBS system should be able to differentiate and individualize specific characteristics of the measured neurophysiological signals in real time, to then automatically deliver therapeutic electrical pulses of specific parameters for a specific amount of time. Neurophysiological biomarkers have great potential to optimize DBS and move the field toward adaptive DBS modalities.[81]

Review highlights

- Parkinson's disease dopaminergic denervation in BG results in aberrant activity patterns of surviving neurons both at cortical and BG levels due to cortico-thalamo-BG-cortical circuit dysfunction.
- Neurophysiological studies at cortical level in PD have revealed that both β-synchronization and Phase-Amplitude Coupling (PAC) are potential biomarkers.
- DBS intraoperative recordings from BG have detected oscillatory activity patterns at different frequencies that characterize different parkinsonian clinical states ("on" or "off").
- The resulting neuronal synchrony is also implied by the finding of increased amplitudes of local field potentials (LFPs) in the beta-band range of frequencies (10–30 Hz) at off (akinetic-rigid) state.
- Brain abnormal activity pattern configuration could provide valuable biomarkers that characterize not only a pathophysiological substrate, but also a corresponded specific clinical condition or a therapeutic effect.
- DBS treatment could be optimized by adapting stimulation settings according to LFPs recordings from the permanent electrode, combined with the presence or absence of PD symptoms, through closed-loop control.

Useful points to clinical practice

- Up to date, beta activity has shown the more consistent characteristics for an electrophysiological biomarker: it scales with hypokinetic motor symptoms (rigidity and bradykinesia).
- Certain types of permanent electrodes currently used for DBS in BG are able to monitor beta band activity (sensing) and provide the most effective configuration of stimulation parameters, optimizing the current delivery for best clinical results.
- Moreover, the so called "adaptive" (aDBS) or "closed-loop" DBS, is currently available as clinical care in some countries.

REFERENCES

[1] Hammond, C., Bergman, H., Brown, P. Pathological synchronization in Parkinson's disease: networks, models and treatments. Trends Neurosci. 2007;30(7):357–364.
[2] Caton R. The electric currents of the brain. British Medical Journal. 1875, Aug 28: 278
[3] Jung R, Berger W. Fiftieth anniversary of Hans Berger's publication of the electroencephalogram. His first records in 1924-1931. Arch Psychiatr Nervenkr. 1979: 227: 279-300.
[4] England AC, Schwab RS, Peterson E. The electroencephalogram in Parkinson's syndrome. Electroencephalogr Clin Neurophysiol. 1959 Nov;11(4): 723-731
[5] Ganglberger JA. The EEG in parkinsonism and its alteration by stereotaxically produced lesions in pallidum. Electroencephalogr Clin Neurophysiol. 1961; 13:82.
[6] Sirakov AA, Mezan IS. EEG findings in parkinsonism. Electroencephalogr Clin Neurophysiol. 1963 Apr; 15(2): 321-322.
[7] Yeager CL, Alberts WW, Delattre LD. Effect of stereotaxic surgery upon electroencephalographic status of Parkinsonian patients. Neurology. 1966; 16:904-910.
[8] Soikkeli R, Partanen J, Soininen H, Pääkkönen A, Riekkinen P Sr. Slowing of EEG in Parkinson's disease. Electroencephalogr Clin Neurophysiol. 1991 Sep;79(3):159-65.
[9] Portin R, Rinne UK. Predictive factors for cognitive deterioration and dementia in Parkinson's disease. Adv Neurol. 1987; 45:413-416.
[10] Neufeld MY, Inzelberg R, Korczyn AD. EEG in demented and non-demented parkinsonian patients. Acta Neurol Scand. 1988 Jul;78(1):1-5.
[11] Hirato M, Ishihara J, Horikoshi S, Shibazaki T, Ohye C. Parkinsonian rigidity, dopa-induced dyskinesia and chorea-dynamic studies on the basal ganglia-thalamocortical motor circuit using PET scan and depth microrecording. Acta Neurochir Suppl. 1995; 64:5-8.
[12] Rottach KG, Riley DE, DiScenna AO, Zivotofsky AZ, Leigh RJ. Dynamic properties of horizontal and vertical eye movements in parkinsonian syndromes. Ann Neurol. 1996 Mar;39(3):368-77.
[13] Lörincz A. Static and dynamic state feedback control model of basal ganglia-thalamocortical loops. Int J Neural Syst. 1997 Jun;8(3):339-57.
[14] Pfurtscheller G, Cooper R. Frequency dependence of the transmission of the EEG from cortex to scalp. Electroencephalogr Clin Neurophysiol. 1975 Jan;38(1):93-96.
[15] Manning JR, Jacobs J, Fried I, Kahana MJ. Broadband shifts in local field potential power spectra are correlated with single-neuron spiking in humans. J Neurosci. 2009 Oct28;29(43):13613-20.
[16] Toro C, Deuschl G, Hallett M. Movement-related electroencephalographic desynchronization in patients with hand cramps: evidence for motor cortical involvement in focal dystonia. Ann Neurol. 2000 Apr;47(4):456-461.
[17] Crone NE, Miglioretti DL, Gordon B, Sieracki JM, Wilson MT, Uematsu S, Lesser RP. Functional mapping of human sensorimotor cortex with electrocorticographic spectral analysis. I. Alpha and beta event-related desynchroniza-

tion. Brain. 1998 Dec:121 (Pt 12): 2271-2299.
[18] Miller KJ, Leuthardt EC, Schalk G, Rao RP, Anderson NR, Moran DW, Miller JW, Ojemann JG. Spectral changes in cortical surface potentials during motor movement. J Neurosci. 2007 Feb 28;27(9):2424-2432.
[19] Pfurtscheller G. Induced oscillations in the alpha band: functional meaning. Epilepsia. 2003;44 Suppl 12:2-8.
[20] Crowell AL, Ryapolova-Webb ES, Ostrem JL, Galifianakis NB, Shimamoto S, Lim DA, Starr PA. Oscillations in sensorimotor cortex in movement disorders: an electrocorticography study. Brain. 2012 Feb;135(Pt 2):615–630.
[21] Chen CC, Lin WY, Chan HL, Hsu YT, Tu PH, Lee ST, Chiou SM, Tsai CH, Lu CS, Brown P. Stimulation of the subthalamic region at 20 Hz slows the development of grip force in Parkinson's disease. Exp. Neurol. 2011 Sep:231(1):91-96.
[22] Timmermann L, Gross J, Dirks M, Volkmann J, Freund HJ, Schnitzler A. The cerebral oscillatory network of parkinsonian resting tremor. Brain. 2003 Jan;126(Pt 1):199-212.
[23] Muthuraman M, Raethjen J, Koirala N, Anwar AR, Mideksa KG, Elble R, Groppa S, Deuschl G. Cerebello-cortical network fingerprints differ between essential, Parkinson's and mimicked tremors. Brain. 2018 Jun 1;141(6):1770-1781.
[24] Hutchison WD, Lozano AM, Tasker RR, Lang AE, Dostrovsky JO. Identification and characterisation of neurons with tremor-frequency activity in human globus pallidus. Exp Brain Res. 1997 Mar;113(3):557-563.
[25] Levy R, Hutchison WD, Lozano AM, Dostrovsky JO. High-frequency synchronization of neuronal activity in the subthalamic nucleus of parkinsonian patients with limb tremor. J Neurosci. 2000 Oct 15; 20:7766-7775.
[26] Hutchison WD, Dostrovsky JO, Walters JR, Courtemanche R, Boraud T, Goldberg J, Brown P. Neuronal oscillations in the basal ganglia and movement disorders: evidence from whole animal and human recordings. J Neurosci. 2004 Oct 20; 24(42): 9240–9243.
[27] Engel AK, Fries P. Beta-band oscillations: signalling the status quo? Curr Opin Neurobiol. 2010 Apr;20(2):156-165.
[28] Eusebio A, Pogosyan A, Wang S, Averbeck B, Gaynor LD, Cantiniaux S, Witjas T, Limousin P, Azulay JP, Brown P. Resonance in subthalamocortical circuits in Parkinson's disease. Brain. 2009 Aug;132(Pt 8):2139-2150.
[29] Berendse HW, Stam CJ. Stage-dependent patterns of disturbed neural synchrony in Parkinson's disease. Parkinsonism Relat Disord. 2007;13 Suppl 3: S440-445.
[30] Mallet N, Pogosyan A, Sharott A, Csicsvari J, Bolam JP, Brown P, Magill PJ. Disrupted dopamine transmission and the emergence of exaggerated beta oscillations in subthalamic nucleus and cerebral cortex. J Neurosci. 2008 Apr 30;28(18):4795-4806.
[31] Pollok B, Krause V, Martsch W, Wach C, Schnitzler A, Südmeyer M. Motor-cortical oscillations in early stages of Parkinson's disease. J Physiol. 2012 Jul 1;590(13):3203-3212.
[32] Scheeringa R, Fries P, Petersson KM, Oostenveld R, Grothe I, Norris DG, Hagoort P, Bastiaansen MC. Neuronal dynamics underlying high- and low-frequency EEG oscillations contribute independently to the human BOLD signal. Neuron. 2011 Feb 10;69(3):572-583.
[33] Tang CC, Eidelberg D. Abnormal metabolic brain networks in Parkinson's disease from blackboard to bedside. Prog Brain Res. 2010; 184:161-176.
[34] Delong MR, Wichmann T. Circuits and circuit disorders of the basal ganglia. Arch. Neurol. 2007 Jan;64(1):20-24.
[35] Timmermann L, Wojtecki L, Gross J, Lehrke R, Voges J, Maarouf M, Treuer H, Sturm V, Schnitzler A. Ten-Hertz stimulation of subthalamic nucleus deteriorates motor symptoms in Parkinson's disease. Mov Disord. 2004 Nov;19(11):1328-1333.
[36] Eusebio A, Chen CC, Lu CS, Lee ST, Tsai CH, Limousin P, Hariz M, Brown P. Effects of low-frequency stimulation of the subthalamic nucleus on movement in Parkinson's disease. Exp Neurol. 2008 Jan;209(1):125-130.
[37] Timmermann L, Florin E. Parkinson's disease and pathological oscillatory activity: is the beta band the bad guy? - New lessons learned from low-frequency deep brain stimulation. Exp. Neurol. 2012 Jan;233(1):123-125.
[38] Sherman MA, Lee S, Law R, Haegens S, Thorn CA, Hämäläinen MS, Moore CI, Jones SR. Neural mechanisms of transient neocortical beta rhythms: Converging evidence from humans, computational modeling, monkeys, and mice. Proc Natl Acad Sci USA. 2016 Aug 16;113(33): E4885-4894.
[39] Mariscal MG, Levin AR, Gabard-Durnam LJ, Xie W, Tager-Flusberg H, Nelson CA. EEG Phase-Amplitude Coupling strength and phase preference: Association with age over the first three years after birth. eNeuro. 2021 Jun 24;8(3).
[40] de Hemptinne C, Ryapolova-Webb ES, Air EL, Garcia PA, Miller KJ, Ojemann JG, Galifianakis NB, Starr PA. Exaggerated phase-amplitude coupling in the primary motor cortex in Parkinson disease. Proc Natl Acad Sci USA, 2013 Mar 19;110(12):4780-4785.
[41] de Hemptinne C, Swann NC, Ostrem JL,

Ryapolova-Webb ES, San Luciano M, Galifianakis NB, Starr PA. Therapeutic deep brain stimulation reduces cortical phase-amplitude coupling in Parkinson's disease. Nat Neurosci. 2015 May;18(5):779-786.
[42] Malekmohammadi M, AuYong N, Ricks-Oddie J, Bordeleon Y, Pouratian N. Pallidal deep brain stimulation modulates excessive cortical high β phase amplitude coupling in Parkinson disease. Brain Stimul. 2018 May-Jun;11(3):607-617.
[43] Swann NC, de Hemptinne C, Aron AR, Ostrem JL, Knight RT, Starr PA. Elevated synchrony in Parkinson disease detected with electroencephalography. Ann Neurol. 2015 Nov;78(5):742-750.
[44] Jackson N, Cole SR, Voytek B, Swann CW. Characteristics of waveform shape in Parkinson's disease detected with scalp electroencephalography. eNeuro. 2019 Jun 5; 6(3).
[45] Miocinovic S, Somayajula S, Chitnis S, Vitek JL. History, applications, and mechanisms of Deep Brain Stimulation. JAMA Neurol. 2013 Feb;70(2):163–171.
[46] Deuschl G, Antonini A, Costa J, úmiúowska K, Berg D, Corvol JC, Fabbrini G, Ferreira J, Foltynie T, Mir P, Schrag A, Seppi K, Taba P, Ruzicka E, Selikhova M, Henschke N, Villanueva G, Moro E. European Academy of Neurology/ Movement Disorder Society-European Section guideline on the treatment of Parkinson's disease: I. Invasive Therapies. Mov Disord. 2022 Jul; 37:1360–1374.
[47] Maks CB, Butson CR, Walter BL, Vitek JL, McIntyre CC. Deep brain stimulation activation volumes and their association with neurophysiological mapping and therapeutic outcomes. J Neurol Neurosurg Psychiatry. 2009 Jun; 80:659–666.
[48] Bergman H, Wichmann T, Karmon B, DeLong MR. The primate subthalamic nucleus. II. Neuronal activity in the MPTP model of parkinsonism. J Neurophys. 1994 Aug;72(2):507-520.
[49] Wichmann T, Dostrovsky JO. Pathological basal ganglia activity in movement disorders. Neuroscience. 2011 Dec 15; 198:232-244.
[50] Albin RL, Young AB, Penney JB. The functional anatomy of basal ganglia disorders. Trends Neurosci. 1989 Oct;12(10):366-375.
[51] DeLong MR. Primate models of movement disorders of basal ganglia origin. Trends Neurosci. 1990 Jul;13(7):281-285.
[52] Bergman, H, Wichmann T, DeLong, MR. Reversal of experimental parkinsonism by lesions of the subthalamic nucleus. Science. 1990 Sep 21;249(4975):1436–1438.
[53] Filion M, Tremblay L. Abnormal spontaneous activity of globus pallidus neurons in monkeys with MPTP-induced parkinsonism. Brain Res. 1991 Apr 26;547(1):142–151.
[54] Wichmann T. Changing views of the pathophysiology of Parkinsonism. Mov Disord. 2019 Aug;34(8):1130-1143.
[55] Connolly AT, Muralidharan A, Hendrix C, Johnson L, Gupta R, Stanslaski S, Denison T, Baker KB, Vitek JL, Johnson MD. Local field potential recordings in a non-human primate model of Parkinsons disease using the Activa PC + S neurostimulator. J Neural Eng. 2015 Dec;12(6):066012.
[56] Goldberg JA, Rokni U, Boraud T, Vaadia E, Bergman H. Spike synchronization in the cortex/basal-ganglia networks of Parkinsonian primates reflects global dynamics of the local field potentials. J Neurosci. 2004 Jun 30;24(26):6003-6010.
[57] Lempka SF, McIntyre CC. Theoretical analysis of the local field potential in deep brain stimulation applications. PLoS One. 2013;8(3): e59839.
[58] Giannicola G, Rosa M, Servello D, Menghetti C, Carraba G, Pacchetti C, Zangaglia R, Cogiamanian F, Scelzo E, Marceglia S, Rossi L, Priori A. Subthalamic local field potentials after seven-year deep brain stimulation in Parkinson's disease. Exp Neurol. 2012 Oct;237(2):312-317.
[59] Weinberger M, Mahant N, Hutchison WD, Lozano AM, Moro E, Hodaie M, Lang AE, Dostrovsky JO. Beta Oscillatory Activity in the Subthalamic Nucleus and its relation to dopaminergic response in Parkinson's Disease. J Neurophysiol. 2006 Dec; 96(6):3248-3256.
[60] Brown P, Williams D. Basal ganglia local field potential activity: character and functional significance in the human. Clin Neurophysiol. 2005 Nov;116(11):2510-2519.
[61] Kühn AA, Kempf F, Brücke C, Gaynor Doyle L, Martinez-Torres I, Pogosyan A, Trottenberg T, Kupsch A, Schneider GH, Hariz MI, Vandenberghe W, Nuttin B, Brown P. High-frequency stimulation of the subthalamic nucleus suppresses oscillatory β activity in patients with Parkinson's Disease in parallel with improvement in motor performance. J Neurosci. 2008 Jun 11;28(24):6165– 6173.
[62] Silberstein P, Kühn AA, Kupsch A, Trottenberg T, Krauss JK, Wöhrle JC, Mazzone P, Insola A, Di Lazzaro V, Oliviero A, Aziz T, Brown P. Patterning of globus pallidus local field potentials differs between Parkinson's disease and dystonia. Brain. 2003 Dec; 126(Pt 12):2597-2608.
[63] Horn A, Neumann WJ, Degen K, Schneider GH, Kühn AA. Toward an electrophysiological "sweet spot" for deep brain stimulation in the subthalamic nucleus. Hum. Brain Mapp. 2017 Jul;38(7):3377–3390.

[64] Karamintziou SD, Tsirogiannis GL, Stathis PG, Tagaris GA, Boviatsis EJ, Sakas DE, Nikita KS. Supporting clinical decision making during deep brain stimulation surgery by means of a stochastic dynamical model. J Neural Eng. 2014 Oct;11(5):056019.
[65] Michmizos KP, Frangou P, Stathis P, Sakas D, Nikita KS. Beta-band frequency peaks inside the subthalamic nucleus as a biomarker for motor Improvement after deep brain stimulation in Parkinson's Disease. IEEE J Biomed Health Inform. 2015 Jan; 19(1):174-180.
[66] Kostoglou K, Michmizos KP, Stathis P, Sakas D, Nikita KS, Mitsis GD. Classification and Prediction of Clinical Improvement in Deep Brain Stimulation from Intraoperative Microelectrode Recordings. IEEE Trans Biomed Eng. 2017 May;64(5):1123-1130.
[67] Telkes I, Viswanathan A, Jimenez-Shahed J, Abosch A, Ozturk M, Gupte A, Jankovic J, Ince NF. Local field potentials of subthalamic nucleus contain electrophysiological footprints of motor subtypes of Parkinson's disease. Proc Natl Acad Sci USA. 2018 Sep 4; 115(36):E8567–E8576.
[68] Camalier CR, Konrad PE, Gill CE, Kao C, Remple MR, Nasr HM, Davis TL, Hedera P, Phibbs FT, Molinari AL, Neimat JS, Charles D. Methods for surgical targeting of the STN in early-stage Parkinson's disease. 2014 Front Neurol. 2014 Mar 19;5(25):1-6.
[69] Valsky D, Marmor-Levin O, Deffains M, Eitan R, Blackwell KT, Bergman H, Israel Z. Stop! Border Ahead: Automatic detection of subthalamic exit during deep brain stimulation surgery. Mov Disord. 2017 Jan;32(1):70–79.
[70] Lu CW, Malaga, KA, Chou KL, Chestek CA, Patil PG. High density microelectrode recording predicts span of therapeutic tissue activation volumes in subthalamic deep brain stimulation for Parkinson disease. Brain Stimul. 2020 Mar-Apr;13(2):412–419.
[71] Connolly AT, Jensen AL, Bello EM, Netoff TI, Baker KB, Johnson MD, Vitek JL. Modulations in oscillatory frequency and coupling in globus pallidus with increasing parkinsonian severity. J Neurosci. 2015 Apr 15;35(15): 6231–6240.
[72] López-Azcárate J, Tainta M, Rodríguez-Oroz MC, Valencia M, González R, Guridi J, Iriarte J, Obeso JA, Artieda J, Alegre M. Coupling between beta and high-frequency activity in the human subthalamic nucleus may be a pathophysiological mechanism in Parkinson's disease. J Neurosci. 2010 May 12;30(19):6667–6677.
[73] Foffani G, Priori A, Egidi M, Rampini P, Tamma F, Caputo E, Moxon KA, Cerutti S, Barbieri S. 300-Hz subthalamic oscillations in Parkinson's disease. Brain. 2003 Oct; 126(Pt 10): 2153–2163.
[74] Tsiokos C, Hu X, Pouratian N. 200–300Hz movement modulated oscillations in the internal globus pallidus of patients with Parkinson's Disease. Neurobiol Dis. 2013 Jun; 54:464–474.
[75] Johnson LA, Aman JE, Yu Y, Sanabria DE, Wang J, Hill M, Dharnipragada R, Patriat R, Fiecas M, Li L, Schrock LE, Cooper SE, Johnson MD, Park MC, Harel M, Vitek JL. High-Frequency Oscillations in the Pallidum: A Pathophysiological Biomarker in Parkinson's Disease? Mov Disord. 2021 Jun;36(6):1332–1341.
[76] Johnson LA, Nebeck SD, Muralidharan A, Johnson MD, Baker KB, Vitek JL. Closed-loop deep brain stimulation effects on Parkinsonian motor symptoms in a non-human primate - is beta enough? Brain Stimulat. 2016 Nov-Dec;9(6):892–896.
[77] Karamintziou SD, Custódio AL, Piallat B, Polosan M, Chabardès S, Stathis PG, Tagaris GA, Sakas DE, Polychronaki GE, Tsirogiannis GL, David O, Nikita KS. Algorithmic design of a noise-resistant and efficient closed-loop deep brain stimulation system: A computational approach. PLoS One. 2017 Feb 21;12(2): e0171458.
[78] Little S, Brown P. What brain signals are suitable for feedback control of deep brain stimulation in Parkinson's disease? Ann N Y Acad Sci. 2012 Aug;1265: 9–24.
[79] Abosch A, Lanctin D, Onaran I, Eberly L, Spaniol M, Ince NF. Long-term recordings of local field potentials from implanted deep brain stimulation electrodes. Neurosurgery. 2012 Oct;71(4):804-814.
[80] FDA-NIH Biomarker Working Group. BEST (Biomarkers, EndpointS, and other Tools) Resource. 2016. Silver Spring (MD): Food and Drug Administration (US).
[81] van Wijk BCM, de Bie RMA, Beudel M. A systematic review of local field potential physiomarkers in Parkinson's disease: from clinical correlations to adaptive deep brain stimulation algorithms. J Neurol. 2023 Feb;270(2):1162-1177.
[82] Hoang KB, Turner DA. The emerging role of biomarkers in adaptive modulation of clinical brain stimulation. Neurosurgery. 2019 Sep 1;85(3): E430-E439.
[83] Yin Z, Zhu G, Zhao B, Bai Y, Jiang Y, Neumann WJ, Kühn AA, Zhang J. Local field potentials in Parkinson's disease: A frequency-based review. Neurobiol Dis. 2021 Jul; 155:105372.
[84] Sirica D, Hewitt AL, Tarolli AG, Weber MT, Zimmerman C, Santiago A, Wensel A, Mink JW, Lizárraga KJ. Neurophysiological biomarkers to optimize deep brain stimulation in movement disorders. Neurodegener Dis Manag. 2012 Aug;11(4):315-328.

[85] Neumann WJ, Degen K, Schneider GH, Brücke C, Huebl J, Brown P, Kühn AA. Subthalamic synchronized oscillatory activity correlates with motor impairment in patients with Parkinson's disease. Mov Disord. 2016 Nov;31(11):1748-1751.
[86] Ozturk M, Abosch A, Francis D, Wu J, Jimenez-Shahed J, Ince NF. Distinct subthalamic coupling in the ON state describes motor performance in Parkinson's disease. Mov Disord. 2020 Jan;35(1):91-100.
[87] Tinkhauser G, Pogosyan A, Tan H, Herz DM, Kühn AA, Brown P. Beta burst dynamics in Parkinson's disease OFF and ON dopaminergic medication. Brain. 2017 Nov 1; 140(11):2968-2981.
[88] Tinkhauser G, Pogosyan A, Little S, Beudel M, Herz DM, Tan H, Brown P. The modulatory effect of adaptive deep brain stimulation on beta bursts in Parkinson's disease. Brain. 2017 Apr 1;140(4):1053-1067.
[89] Tinkhauser G, Pogosyan A, Debove I, Nowacki A, Shah SA, Seidel K, Tan H, Brittain JS, Pettermann K, di Biase L, Oertel M, Pollo C, Brown P, Schuepbach M. Directional field potentials: A tool to optimize deep brain stimulation. Mov Disord. 2018 Jan;33(1):159-164.
[90] Alonso-Frech F, Zamarbide I, Alegre M, Rodriguez-Oroz M, Guridi J, Manrique M, Valencia M, Artieda J, Obeso JA. Slow oscillatory activity and levodopa-induced dyskinesias in Parkinson's disease. Brain. 2006 Jul;129(Pt 7):1748-1757.
[91] Eusebio A, Thevathasan W, Doyle Gaynor L, Pogosyan A, Bye E, Foltynie T, Zrinzo L, Ashkan K, Aziz TZ, Brown P. Deep brain stimulation can suppress pathological synchronisation in parkinsonian patients. J Neurol Neurosurg Psychiatry. 2011 May; 82(5):569-573.
[92] Hirschmann J, Abbasi O, Storzer L, Butz M, Hartmann CJ, Wojtecki L, Schnitzler A. Longitudinal recordings reveal transient increase of alpha/low-beta power in the subthalamic nucleus associated with the onset of Parkinsonian rest tremor. Front Neurol. 2019 Mar 7; 10:145.
[93] Yao L, Brown P, Shoaran M. Improved detection of Parkinsonian rest tremor with feature engineering and Kalman filtering. Clin Neurophysiol. 2020 Jan;131(1):274-284.
[94] Lofredi R, Neumann WJ, Bock A, Horn A, Huebl J, Siegert S, Schneider GH, Krauss JK, Kühn AA. Dopamine-dependent scaling of subthalamic gamma bursts with movement velocity in patients with Parkinson's disease. Elife. 2018 Feb 1;7: e31895.
[95] Van Wijk BC, Beudel M, Jha A, Oswal A, Foltynie T, Hariz MI, Limousin P, Zrinzo L, Aziz TZ, Green AL, Brown P, Litvak V. Subthalamic nucleus phase-amplitude coupling correlates with motor impairment in Parkinson's disease. Clin Neurophysiol. 2016 Apr; 127(4):2010-2019.
[96] Özkurt TE, Butz M, Homburger M, Elben S, Vesper J, Wojtecki L, Schnitzler A. High frequency oscillations in the subthalamic nucleus: a neurophysiological marker of the motor state in Parkinson's disease. Exp Neurol. 2011 Jun;229(2):324-331.
[97] Little S, Beudel M, Zrinzo L, Foltynie T, Limousin P, Hariz M, Neal S, Cheeran B, Cagnan H, Gratwicke J, Aziz TZ, Pogosyan A, Brown P. Bilateral adaptive deep brain stimulation is effective in Parkinson's disease. J Neurol Neurosurg Psychiatry. 2016 Jul;87(7):717-721.

WEARABLE DEVICES AND SMARTPHONES FOR PARKINSON'S DISEASE DIAGNOSIS

Sevasti Bostantjopoulou, Zoe Katsarou, Ioannis Dagklis
3rd Department of Neurology, Aristotle University of Thessaloniki

Abstract
Parkinson's disease(PD) is a common neurodegenerative disorder with a prevalence that is expected to increase in the next decades. The implementation of digital health technology (wearable devices and smartphones) in PD is promising. Wearable devices can capture subtle motor symptoms (voice ,facial expression, fine finger movements) and non-motor symptoms (REM sleep behavior disorder, gastric motility)thus improving early diagnosis, identifying prodromal PD and enabling population screening for PD. Furthermore sensors are useful for accurately and objectively evaluate and monitor in real life the motor (bradykinesia, tremor, gait parameters, freezing of gait, balance) and the non-motor symptoms of the disease as well as the treatment response and the fluctuations. Touch technology with keystrokes dynamics during typing a computer offers another opportunity for studying motor symptoms in PD. However there are limitations, barriers and risks on the use of digital technology. Further studies involving patients and caregivers will help implement technology in PD.

Key words: wearables, smartwatch, smartphone, Parkinson disease, digital technology

ΦΟΡΗΤΕΣ ΣΥΣΚΕΥΕΣ ΚΑΙ ΕΞΥΠΝΑ ΤΗΛΕΦΩΝΑ ΣΤΗΝ ΔΙΑΓΝΩΣΗ ΤΗΣ ΝΟΣΟΥ ΠΑΡΚΙΝΣΟΝ

Σεβαστή Μποσταντζοπούλου ,Ζωή Κατσαρού ,Ιωάννης Δαγκλής
Γ–Νευρολογική Κλινική ,Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης

Περίληψη
Η νόσος του Parkinson (NP) είναι μία συχνή νευροεκφυλιστική διαταραχή της οποίας η συχνότητα αναμένεται να αυξηθεί τις επόμενες δεκαετίες. Η εφαρμογή της ψηφιακής τεχνολογίας (φορητοί αισθητήρες/συσκευές και έξυπνα τηλέφωνα) στην NP είναι πολλά υποσχόμενη. Οι φορητοί αισθητήρες/συσκευές και τα έξυπνα τηλέφωνα μπορούν να ανιχνεύσουν ελαφρά κινητικά συμπτώματα (μεταβολή της φωνής, της έκφρασης του προσώπου, των λεπτών κινήσεων των δακτύλων) και μη κινητικά συμπτώματα (διαταραχή συμπεριφοράς στον ύπνο REM,μεταβολή της γαστρικής κινητικότητας). Μας δίνουν έτσι την δυνατότητα να βελτιώσουμε την πρώιμη διάγνωση της νόσου, να προσδιορίσουμε την πρόδρομη φάση και να ελέγξουμε τον πληθυσμό για την παρουσία της νόσου. Επιπλέον βοηθούν στην ακριβή και αντικειμενική αξιολόγηση και παρακολούθηση στην καθημερινή ζωή των κινητικών (βραδυκινησία τρόμος, παράμετροι βάδισης, πάγωμα στην βάδιση, ισορ-ροπία) και μη κινητικών συμπτωμάτων, της απάντησης στην θεραπεία και των διακυμάνσεων της θεραπείας. Επίσης η τεχνολογία επαφής με την καταγραφή των δυναμικών των πλήκτρων καθώς γράφει ο ασθενής στο υπολογιστή αποτελεί μία άλλη ευκαιρία μελέτης των κινητικών συμπτωμάτων. Υπάρχουν βέβαια περιορισμοί και προβληματισμοί στην χρήση της τεχνολογίας. Περισσότερες μελέτες με την συμμετοχή ασθενών και φροντιστών θα βοηθήσουν στη ευρεία εφαρμογή της τεχνολογίας στην νόσο.

Λέξεις κλειδιά: φορητές συσκευές, έξυπνα κινητά ,έξυπνα τηλέφωνα, νόσος Πάρκινσον, ψηφιακή τεχνολογία

Introduction

Parkinson's disease (PD) is a common neurodegenerative disorder affecting 6.2 million people worldwide and this number is expected to reach 12 million by 2040[1]. PD is a multisystem disorder and although motor symptoms are the hallmarks of the disease, PD is associated with a variety of non-motor symptoms [2,3].PD is very heterogeneous regarding the age of onset, the motor symptoms, the non-motor symptoms, the rate of progression and the genetic background[3,4]. Furthermore, the natural history of PD has a prediagnostic phase (preclinical and prodromal) and a manifested phase (early stage and late stage)[3-5]. The prodromal phase is characterized by a range of non-motor symptoms

(constipation, REM sleep behavior disorder, smell loss e.t.c.) and a subtle motor signs (voice changes, decreased facial expression e.t.c.)[3-5]. This complexity of the disease make the implementation of precision medicine quite difficult.

Technology/wearables and Parkinson's disease

New technologies such as artificial intelligence, wearable sensors, smartphones, virtual reality ,machine learning e.t.c. that have been developed intend to generate accurate measurement of motor function[6-10]. The most widely used are inertial measurement units. These units have a triaxal accelerometer that measure inertia acceleration of a body, a triaxal gyroscope that measure angular accelerations , a global positioning technology and a magnetometer[8,11]. These inertial units have been embedded in wearable devices that can be attached to almost any part of the body (wrist, finger, trunk, foot). So, wearable sensors can record orientation, amplitude, frequency and speed of movements[11]. These sensors can also evaluate gait and give specific gait parameters. The wearable sensors are worn by the patient in the clinic and for remote monitoring in home setting, thus giving the opportunity for a continuous home monitoring during the activities of daily living[12,13]. The implementation of sensor based and wearable technologies is useful for the objectively evaluation and monitor patients with manifested PD, for the improvement of disease management and also for early disease diagnosis.

Evaluation of patients with manifested PD

The assessment of a patient with PD is challenging. Although clinical examination and MDS-UPDRS are the standards for PD evaluation there are some drawbacks. MDS-UPDRS and other scales are prone to subjectivity and they reflect the patient status at the in-person/clinic visit, that is in a precise moment. It is very important to be informed about the patient's symptoms (tremor, bradykinesia, gait disturbance, falls) and on/off states all day long during his daily routine. Therefore, wearable devices give the opportunity to continuously evaluate the patients' motor function in real time and thus objectively better manage PD symptoms and improve patients' quality of life.

Various digital technologies have been developed for the assessment of various aspects of motor function in PD, such as tremor, bradykinesia, gait disturbance, freezing of gait, falls and dyskinesias [6-15].In 2023 the UK National Institute for Health and Care Excellence (NICE) published their recommendations for the use of devices for remote monitoring of Parkinson's disease[16]. According to the Committee five wearable devices are conditionally recommended as options for remote monitoring of PD to inform treatment if further evidence is generated and cost impact is managed.These devices are: 1)***Kinesia360:*** the device has two sensors worn on the patient's wrist and ankle, a tablet and a charge pad. It measures tremor, bradykinesia, dyskinesia, body position and steps all day long (16 hours battery life) during daily activities, 2)***KinesiaU***: has a smartwatch and a smartphone for continuous recording or for recording specific active tasks. The device rates tremor, bradykinesia and dyskinesias (good, mild, moderate, severe),3)***PDMonitor***: the device comprises 5 sensors worn on both wrists, both ankles and waist, a SmartBox and a PDMonitor mobile application. PDMonitor measures arm/leg/body tremor, arm/leg/body bradykinesia, dyskinesia, off time, gait impairment as well as number of steps and gait analysis, freezing of gait and postural instability,4)***Personal KinetiGraph (PKG):***it consists of a PKG watch and a PKG report. The watch is worn on the wrist of the most affected side for continuous monitoring for 6-10 days. It measures tremor, bradykinesia, dyskinesias and motor fluctuations, and final 5)***STAT-ON:*** the wearable device worn on the patient's waist analyzes inertial signals with advanced machine learning algorithms and contains a communication unit that transfer the motor assessment to an external mobile device. STAT-ON measures gait parameters, freezing of gait, falls, posture, motor fluctuations, and dyskinesias but it does not measure tremor. Many other wearable devices/systems have reached a Technology Readiness Level (TRL) of 8-9 and have the FDA approval such as: Mobility Lab -APMD,DynaPort7-McRoberts and FeetMe Monitor Insoles[7,10]. Recently a new smartwatch based monitoring system- the Rune Labs Kinematics System- has been granted with FDA clearance [17]. This device uses an Apple smartwatch and special algorithms for detecting tremor and dyskinesias.

Non-motor symptoms in PD are common, they can precede the onset of motor symptoms and affect the patients' quality of life. Relatively few studies with digital health technology focus on non-motor symptoms of PD. Van Wamelen et al [18] identified eight studies using triaxial wrist-worn devices to monitor sleep quality and quantity in PD. The results of the devices correlated with the PD Sleep Scale, the patient's sleep diaries and the polysomnography measures.

Technology for Parkinson's disease diagnosis

The diagnosis of Parkinson's disease is challenging and according to Adler et al [19] there is only 26% accuracy for clinical diagnosis of PD in untreated patients and 53% accuracy in early PD patients responded to medication. So, multiple studies investigated the implementation of algorithms and

digital technology for the early diagnosis of PD. Most studies focus on the discrimination between patients with PD and healthy controls. However a lot of effort has been put for the implementation of digital technologies for diagnosis of prodromal PD. Both ***Prince and de Vos***[20] using algorithms for alternate finger tapping test data collected on smartphones and ***Mehrang et al*** [21] analysing 20-step walking by built in sensors of smartphones reported feasibility to discriminate PD from non PD subjects. ***Lipsmeier et al*** [22] evaluated phonation, rest tremor, finger tapping, balance and gait with a smartphone during active tasks and passive monitoring at home for 6 months. They found that the wearable devices can accurately discriminate patients with PD and healthy controls. In the study of ***Di Lazzaro et al*** [23] PD patients and healthy controls performed the MDS-UPDRS part III wearing inertial sensors. They distinguished patients from controls with an accuracy of 97%. ***Adams et al*** [24] in the WATCH-PD study evaluated patients and controls wearing smartwatch and smartphone in the clinic performing standard assessment and at home wearing the smartwatch for seven days after each clinic visit. Also at home patients completed motor, speech and cognitive tasks on the smartphone every other week. Parameters that differ between early PD patients and healthy subjects were arm swing, the proportion of time with tremor and finger tapping. ***Del Din et al*** [25]studied 14 gait characteristics with a wearable sensor placed on the lower back in healthy controls longitudinally four times at 2-year intervals. They found that gait variability and asymmetry of all gait characteristics were the best predictors for prodromal PD approximately 4 years before clinical diagnosis.

Touch technology with keystrokes dynamics during typing a computer offers another opportunity for studying motor symptoms in PD. Subjects type their computer at home and data collection from key strokes events as the participants press and release the keys (hold time, release latency, interkey latencies, flight time, alternating finger tapping)were stored in a platform and analyzed by a computational algorithm. All studies found that computer keyboard interaction discriminate patients with early PD from controls[26-29].

Subtle motor signs in the prodromal phase of PD are reduced facial expression (hypomimia) and voice changes (hypophonia).Different speech tasks have been tried for detection of speech abnormalities such as vowel phonation(«aaa»),syllable and sentence repetition and reading[30]. Smartphones used for capturing speech abnormalities (frequency variability, duration of pause intervals and rate of speech timing) succeeded to separate early PD patients from controls [30,31]. ***Singh and Xu*** after analysing 1000 voice samples (the subject said «aaah» for 10-s audio using a smartphone) propose a method that reaches 99% accuracy for predicting PD[32]. Furthermore ***Arora et al*** [33] found that voice was a discriminator factor for separating participants with idiopathic REM sleep behavior disorder from PD participants. For evaluation of facial expression computer vision and machine learning were used to measure the variance of facial movements (key eye and mouth related features) when the participants perform six basic emotions or when reading[30,34,35]. Especially ***Pegolo et al***[35] implemented a face tracking algorithm based on the Facial Action Coding System (56 landmarks describing the eyes ,the nose, the mouth, the cheeks).The studies concluded that quantitative evaluation of facial expression can assist in quantifying the degree of impairment in PD, identifying early PD patients from normal controls and classified emotions.

The iPROGNOSIS project supported by a European Horizon 2020 grant (coordinated by Aristotle University of Thessaloniki-Department of Electrical and Computer Engineering with the collaboration of the 3rd University Department of Neurology and the participation of different countries-U.K., Germany, Portugal, Sweden and Belgium) aimed to recognize patterns of motor and non-motor symptoms of PD for the early PD detection. Participants interact with their smartphones during all day activities. The parameters that recorded were: speech, movements by analysing the typing patterns on smartphone keyboards, facial expression in selfies and emotional content in text messages. Furthermore a smartwatch analysed sleep pattern and a smart belt was used for the assessment of real life eating difficulties. ***Iakovakis et al*** [36] evaluating PD patients and controls (interacting with touchscreen smartphones during natural typing) explored the combined discriminative potential of enriched keystroke variables (both timing and pressure) and achieved an AUC =0.92 and 0.82/0.81 sensitivity/specificity. Moreover, ***Iakovakis et al*** [37] in an analysis of validation dataset of 36.000 typing sessions (PD patients and controls) achieved AUC 0.89 with sensitivity/specificity:0.90/0.83. The estimations correlated significantly with the items 22/23/24 of the UPDRS. Further validation analysis on de novo PD patients resulted in AUC of 0.97 0.93/0.90 sensitivity/specificity. ***Papadopoulos et al*** [38] used a deep learning framework that analyses data captured during natural user-smart phone interaction to predict tremor and fine motor movements and achieved 0.86/0.93 sensitivity/specificity. In order to evaluate speech voice features from running speech signals were extracted from passively-captured recordings over voice calls [39]. ***Laganas et al*** [39] reported an AUC 0.68 for the classification of PD patients versus controls. Using a smartwatch triaxial accelerometry computed sleep metrics (sleep

efficiency index, total time sleep, sleep fragmentation index, sleep onset latency) used to discriminate between PD patients and controls [40].The univariate analysis achieved up to 0.77 AUC in early PD patients versus controls and a statistically significant association with the PD SleepScale 2 counterpart items. The iPROGNOSIS hypomimia (selfies) analysis module attempts to detect and quantify the decrease of variability of facial expressions in early PD patients[41]. Promising results (early PD patients versus controls) emerged from the study of ***Grammaticopoulou et al*** (sensitivity/specificity 0.79/0.82 for Hypomimia Severity Index)[41].For the assessment of real life eating difficulties ***Kyritsis et al*** [42] introduced the Plate -to-Mouth, an indicator that relates with the time spent by the hand operating the utensil to transfer food from the plate into the mouth. Wearable inertial measurement unit sensor data were collected in the clinics and in free living. The results reveal an AUC of 0.748 for the clinical dataset and 0.775/1.000 for the in-the-wild datasets towards the classification of in-meal eating behavior profiles to the PD and healthy control groups[42].The non-invasive evaluation of gastric motility –electrogastrography - in patients and controls was recorded by a special device(a smart belt).Analysis of electrogastrography signals captured after a 30-minute long electroga-strography (after 6 hours fasting) found differences between patients and controls primary for the post-prandial period[43].

Conclusions

The implementation of digital health technology will revolutionize PD diagnosis and treatment .Wearable devices will improve early diagnosis and identification of prodromal PD. Furthermore monitoring motor and non-motor symptoms in real life as well as response to treatment and motor fluctuations will drive us to a better precision medicine. Although the results of the studies are promising, there are several limitations on the use of wearable sensors such as small sample sizes of subjects in most studies, different number of sensors used, lack of consensus on the type and scope measures and the more appropriate approach for data captures, technical issued should be tackled and users should become familiar with technology [9, 44-46]. Future studies will help to adopt a widespread use of digital health technology.

References

[1] Dorsey ER, Sherer T, Okun M, Bloem B. The emerging evidence of the Parkinson pandemic. J Parkinsons Dis 2018;8(s1):S3-S8
[2] Titova N, Padmakumar C, Lewis S, Chaudhuri R. Parkinson's: a syndrome rather than a disease? J Neural Transm 2017;24(8):907-914
[3] Tolosa E, Garrido A, Scholz S, Poewe W. Challenges in the diagnosis of Parkinson's disease. Lancet Neurol 2021;20:385-397
[4] Heinzel S, Berg D, Gasser T, Chen H, Yao C, Postuma R et al. Update of the MDS research criteria for prodromal Parkinson's disease. Mov Disord 2019;34(10):1464-1470
[5] Berg D, Borghammer P, Fereshtehnejad S-M, Heinzel S, Horsager J, Schaeffer E et al. Prodromal Parkinson disease subtypes-key understanding heterogeneity. Nat Rev Neurol 2021;17(6) : 349-361
[6] Oung QW, Muthusamy H, Lee HL, Basah SN, Yaacob S ,Sarillee M et al. Technologies for assessment of motor disorders in Parkinson's disease: a review. Sensors 2015;15:21710-21745
[7] Luis-Martinez R, Monje M, Antonini A, Sanchez-Ferro A, Mestre T. Technology-enabled care: integrating multidisciplinary care in Parkinson's disease through digital technology. Front Neurol 2020;11:575995
[8] Adams J, Lizarraga K ,Waddell E, Myers T, Jensen-Roberts S, Modica J et al. Digital technology in movement disorders, applications and challenges. Curr Neurol Neurosci Rep 2021;21(4):16
[9] Chandrabhatla A, Pomeraniec J, Ksendzovsky A. Co-evolution of machine learning and digital technologies to improve monitoring of Parkinon's disease motor symptoms. NPG Digital Medicine 2022;5:32
[10] Moreau C, Rouaud T, Grabli D, Benatru I, Remy P, Marques AR et al. Overview on wearable sensors for the management of Parkinson's disease. NPJ Parkinson Dis 2023;9:153
[11] Monje M, Foffani G, Obeso J, Sanchez-Ferro A. New sensor and wearable technologies to aid in the diagnosis and treatment monitoring of Parkinson's disease. Annu Rev Biomed Eng 2019;21:111-143
[12] Sica M ,Tedesco S, Crowe C, Kenny L, Moore K, Timmons S et al. Continuous home monitoring of Parkinson's disease using inertial sensors: a systematic review. PLoS ONE 2021;16:e0246528
[13] Li P ,van Wezel R, He F, Zhao Y, Wang Y. The role of wrist-worn technology in the management of Parkinson's disease in daily life: a review. Front Neuroinform 2023;17:1135300
[14] Belic M, Bobic V, Bazda M, Solaja N, Duric-Jovicic M, Kostic V. Artificial intelligence for assisting diagnosis and assessment of Parkinson's disease. Clin Neurol Neurosurg 2019; 184:105442.
[15] Reichmann H, Klingelhoefer L, Bendig J. The use of wearables for the diagnosis and treatment of Parkinson's disease. J Neural Transm 2023;130:783-791

[16] NICE. NICE recommends NHS collects real-world evidence on devices that monitor people with Parkinson's disease. https://www.nice.org.uk/guidance/dg51-2023
[17] Bloem B, Post E, Hall D. An apple a day to keep the Parkinson's disease doctor away? Ann Neurol 2023;93:681-685
[18] [18] van Wamelen D, Sringean J, Trivedi D, Carroll C, Schrag A, Odin P et al. Digital health technology for non-motor symptoms in people with Parkinson's disease: futile or future? Parkinsonism Relat Disord 2021;89:186-194.
[19] Adler C, Beach T, Hentz J, Shill H, Caviness J, Driver-Dunckley E et al. Low clinical diagnostic accuracy of early vs advanced Parkinson disease. Neurology 2014;83:406-412
[20] Prince J, de Vos M.A deep learning framework for the remote detection of Parkinson's disease using smart-phone sensor data. Conf Proc IEEE Eng Biol Soc 2018;2018:2144-7
[21] Mehrang S, Jauhiainen M, Pietil J, Puustinen J ,Ruokolainen J, Nieminen H. Identification of Parkinson's disease utilizing a single self recorded 20-step walking test acquired by smartphone's inertial measurement unit. Conf Proc IEEE Eng Biol Soc 2018;2018:2913-6
[22] Lipsmeier F, Taylor K, Kilchenmann T, Wolf D, Scotland A, Schjodt-Eriksen J et al. Evaluation of smartphone -based testing to generate exploratory outcome measures in phase I Parkinson's disease clinical trial. Mov Disord 2018;33:1287-1297.
[23] Di Lazzaro G, Ricci M, Al-Wardat M, Schirinzi T, Scalise S ,Giannini F et al. Technology-based objective measures detect subclinical axial signs in untreated, de novo Parkinson's disease. J Parkinsons Dis 2020;10:113-122.
[24] Adams J, Kangarloo T, Tracey B, O'Donnell P, Volfson D ,Latzman R et al. Using a smartwatch and smartphone to assess early Parkinson's disease in the WATCH-PD study. NPJ Parkinson disease 2023;9:64
[25] Del Din S, Elshehabi M ,Galna B, Hobert M, Warmerdam E, Suenkel U et al. Gait analysis with wearable predicts conversion to Parkinson disease. Ann Neurol 2019;86:357-367
[26] Giancardo L, Sanchez -Ferro A, Gallego-Arryo T, Butterworth I, Mendoza C, Montero P et al. Computer keyboard interaction as an indicator of early Parkinson's disease. Sci Rep 2016;6:34468
[27] Adams W. High -accuracy detection of early Parkinson's disease using multiple characteristics of finger movement while typing. PLoS One 2017;12:e0188226
[28] Arroyo-Gallego T, Ledesma-Carbayo M, Butterworth I, Matarazzo M, Montero-Escribano P, Puertas-Martin V et al. Detecting motor impairment in early Parkinson's disease via natural typing interaction with keyboards: validation of the neuroQWERTY approach in an uncontrolled at-home setting. J Med Internet Res 2018;20:e89
[29] Liu WM ,Yeh CL, Chen PW, Lin CW ,Liu AB. Keystroke biometrics as a tool for the early diagnosis and clinical assessment of Parkinson disease. Diagnostics 2023;13:3061
[30] Lim WS, Chiu SI, Wu MC, Tsai SF, Wang PH, Lin KP et al. An integrated biometric voice and facial features for early detection of Parkinson's disease. NPJ Parkinson Disease 2022;8:145
[31] Rusz J, Hlavnicka J, Tykalova T, Novotny M, Dusek P, Sonka K et al. Smartphone allows capture of speech abnormalities associated with high risk of developing Parkinson's disease. IEEE Trans Neural Syst Rehabil Eng 2018;26:1495-1507
[32] Singh S, Xu W. Robust detection of Parkinson's disease using harvested smartphone voice data: a telemedicine approach. Telemed J E Health 2020;26:327-334
[33] Arora S, Baig F, Lo C, Barber T, Lawton M, Zhan A et al. Smartphone motor testing to distinguish idiopathic REM sleep behavior disorder, controls and PD. Neurology 2018;91:e1528-e1538
[34] Ali M, Myers T, Wagner E, Ratnu H, Dorsey R, Hoque E. Facial expressions can detect Parkinson's disease from videos collected on line. NPJ Digit Med 2021;4:129
[35] Pegolo E, Volpe D, Cucca A, Ricciardi L, Sawacha Z. Quantitative evaluation of hypomimia in Parkinson's disease: a face tracking approach. Sensors 2022;22:1358
[36] Iakovakis D, Hadjidimitriou S, Charisis V, Bostantjopoulou S, Katsarou Z, Hadjileontiadis L. Touchscreen typing-pattern analysis fro detecting fine motor skills decline in early-stage Parkinson's disease. Sci Rep 2018;8:7663
[37] Iakovakis D ,Chaudhuri R, Klingelhoefer L, Bostantjopoulou S, Katsarou Z, Trivedi D et al. Screening of Parkinsonian subtle fine-motor impairment from touchscreen typing via deep learning. Sci Rep 2020;10:12623
[38] Papadopoulos A, Iakovakis D, Klingelhoefer L, Bostantjopoulou S, Chaudhuri R, Kyritsis K et al. Unobtrusive detection of Parkinson's disease from multi-modal and in-the-wild sensor data using deep learning techniques. Sci Rep 2020;10:21370
[39] Laganas C, Iakovakis D, Hadjidimitriou S, Charisis V, Dias S, Bostantjopoulou S et al. Parkinson's disease detection based on running speech data from phone calls IEEE Trans Biomed

Eng 2022;69:1573-1584
[40] Iakovakis D, Mastoras R, Hadjidimitriou S, Charisis V, Bostantjopoulou S, Katsarou Z et al .Smartwatch-based activity analysis during sleep for early Parkinson's disease detection. Annu Int Conf IEEE Eng Med Biol Soc 2020;2020:4326-4329.
[41] Grammatikopoulou A, Grammalidis N, Bostantjopoulou S, Katsarou Z. Detecting hypomimia symptoms by selfie photo analysis. PETRA'19; Proceedings of the 12th ACM International Conference on PErvasive Technologies Related to Assistive Environments 2019:517-522
[42] Kyritsis K ,Fagerberg P, Ioakimidis I, Chaudhuri R, Reichmann H, Klingelhoefer L et al. Assessment of real life eating difficulties in Parkinson's disease patients by measuring plate to mouth movement elongation with inertial sensors. Sci Rep 2021;11:1632
[43] Charisis V, Hadjidimitriou S, Iakovakis D, DaSilva HP, Bostantjopoulou S, Katsarou Z et al. Evaluation of gastric motility of Parkinson's disease patients based on a novel wearable device and time-frequency analysis. AAN Annual Meeting 2020
[44] Espay A, Hausdorff J, Sanchez-Ferro A, Klucken J, Merola A, Bonato P et al. A roadmap for implementation of patient-centered digital outcome measures in Parkinson's disease obtained using mobile health technology. Mov Disord 2019;34:657-663
[45] Del Din S, Kirk C, Yarnall A, Rochester L, Hausdorff J. Body-worn sensors for remote monitoring of Parkinson's disease motor symptoms: vision, state of the art, and challenges. J Parkinsons Dis 2021;11(s1):S35-S47
[46] Laar A, de Lima A ,Maas B, Bloem B, de Vries N. Successful implementation of technology in the management of Parkinson's disease: barriers and facilitators. Clin Park Relat Disord 2023;8:100188

Main points:

The implementation of wearable devices and smartphones in Parkinson's disease is promising for:

a) early Parkinson's disease detection, even in the prodromal phase

b) objectively monitoring motor and non-motor symptoms and response to treatment

Useable points:

The implementation of wearable devices and smartphones in Parkinson's disease will improve:

a) medication adjustments

b) precision in treatment

c) clinical trial data

UTILIZING DIGITAL BIOMARKERS FOR THE MONITORING AND MANAGEMENT OF PARKINSON DISEASE

Foivos S. Kanellos[1,2], Konstantinos I. Tsamis[2], Dimitrios Chatzistefanidis[1], Nicholas Kostikis[1], Spyridon Konitsiotis[1]

[1] Department of Neurology, University Hospital of Ioannina
[2] Department of Physiology, Faculty of Medicine, School of Health Sciences, University of Ioannina

Abstract
Technological advancement has led to a notable surge of interest in the integration of digital technologies into medical care, particularly within the realm of chronic diseases. Quantitative metrics derived from digital health technology (DHT) have the potential to serve as Digital Biomarkers (DBs), facilitating the continuous and quantitative monitoring of disease symptoms, even outside clinical settings. This capacity extends to the ongoing and precise assessment of treatment responses, presenting an opportunity for swift adaptations in medication pathways. Moreover, the integration of DBs generated by wearable devices into innovative decision support systems holds promise for enhancing longitudinal disease management, complementing existing standard practices. Furthermore, these novel biomarkers not only advance diagnostic capabilities but also contribute to predicting clinical outcomes. As a result, the emergence of DBs holds considerable promise, representing a transformative force in precision neurology.

Keywords: Digital Biomarkers, Parkinson's Disease, Wearable Devices

Η ΧΡΗΣΗ ΨΗΦΙΑΚΩΝ ΒΙΟΔΕΙΚΤΩΝ ΓΙΑ ΤΗΝ ΠΑΡΑΚΟΛΟΥΘΗΣΗ ΚΑΙ ΤΗΝ ΔΙΑΧΕΙΡΗΣΗ ΤΗΣ ΝΟΣΟΥ ΠΑΡΚΙΝΣΟΝ

Φοίβος Σ. Κανέλλος[1,2], Κωνσταντίνος Ι. Τσάμης[2], Δημήτριος Χατζηστεφανίδης[1], Νικόλαος Κωστίκης[1], Σπυρίδων Κονιτσιώτης[1]
[1] Τμήμα Νευρολογίας, Πανεπιστημιακό Νοσοκομείο Ιωαννίνων
[2] Τομέας Φυσιολογίας, Τμήμα Ιατρικής, Σχολή Επιστημών Υγείας, Πανεπιστήμιο Ιωαννίνων

Περίληψη
Η αλματώδης εξέλιξη της τεχνολογίας έχει οδηγήσει σε μια αξιοσημείωτη αύξηση του ενδιαφέροντος για την ενσωμάτωση των ψηφιακών τεχνολογιών στην ιατρική, ιδίως στον τομέα των χρόνιων παθήσεων. Οι ποσοτικές μετρήσεις που προέρχονται από την χρήση ψηφιακών τεχνολογιών και χρησιμεύσουν ως ψηφιακοί βιοδείκτες (ΨΒ), διευκολύνουν τη συνεχή και ποσοτική παρακολούθηση των συμπτωμάτων της νόσου, ακόμη και εκτός κλινικών πλαισίων. Η ικανότητα αυτή επεκτείνεται στη συνεχή και ακριβή αξιολόγηση των αποκρίσεων στη θεραπεία, παρουσιάζοντας μια ευκαιρία για ταχείες προσαρμογές στο φαρμακευτικό σχήμα. Επιπλέον, η ενσωμάτωση των ΨΒ που παράγονται από φορέσιμες συσκευές σε καινοτόμα συστήματα υποστήριξης αποφάσεων, υπόσχεται την ενίσχυση της διαχρονικής διαχείρισης της νόσου, συμπληρώνοντας τις υπάρχουσες πρακτικές. Επιπλέον, αυτοί οι νέοι βιοδείκτες όχι μόνο προάγουν τις διαγνωστικές δυνατότητες αλλά συμβάλλουν και στην πρόβλεψη των κλινικών αποτελεσμάτων. Κατά συνέπεια, η εμφάνιση των ΨΒ υπόσχεται πολλά, αποτελώντας μια δύναμη μετασχηματισμού στη νευρολογία της ακριβείας.

Λέξεις κλειδιά: Ψηφιακοί Βιοδείκτες, Νόσος Πάρκινσον, Φορέσιμες Συσκευές

Introduction

Parkinson's disease (PD) currently stands as the second most prevalent neurodegenerative disorder following Alzheimer's disease. Global evidence underscores the escalating prevalence of PD, notably beyond the sixth decade, exhibiting an approximately tenfold surge in disease incidence between the sixth and ninth decades of life[1,2]. Anticipating a substantial rise in PD cases by 2030, the imperative to enhance healthcare systems and the escalating burden on healthcare providers globally may precipitate system overload and compromise patient care[3]. A crucial aspect of the pathological progression involves the degeneration of dopaminergic neurons within the pars compacta of the substantia nigra, leading to a significant reduction in dopamine levels

within the striatum. Replacement therapy utilizing the dopamine precursor levodopa typically yields a remarkable amelioration of fundamental motor symptoms, encompassing bradykinesia, rigidity, and resting tremor[4]. Regrettably, as the disease advances and treatment persists, the initially seamless and continuous therapeutic response tends to exhibit erratic behavior. This is marked by the gradual emergence of fluctuations, gait freezing, postural instability, and additional abnormal involuntary movements, often manifesting at the zenith of the therapeutic effect. Once these motor response complications manifest, they persist, intensifying in severity and unpredictability, thereby significantly diminishing the overall quality of life for both the patient and the carer[5,6].

Expert neurologists endeavor to mitigate these issues through adjustments to the timing and intensity of individual levodopa doses, incorporation of supplementary medications, or transitioning to treatment modalities tailored for advanced Parkinson's disease[7]. Nevertheless, symptoms tend to progressively deteriorate over spans of months or years, displaying fluctuating patterns from one day to the next or even within the same day, rendering treatment adaptations arduous[8]. Consequently, there arises a compelling need for precise information regarding the clinical manifestations of the disease to be promptly conveyed to physicians. This facilitates informed decision-making regarding treatment interventions at optimal junctures. Presently, patients typically engage with their treating physicians once annually or every 3-6 months, with minimal communication in between. However, this standard practice fails to accommodate the diverse needs of all patients, as some experience a more accelerated disease progression necessitating evaluations every one or two months, while others maintain a comparatively stable condition.

Digital health technologies (DHTs), such as smart monitoring systems and wearable solutions, have emerged over the past two decades as supplementary tools to traditional face-to-face clinical assessments[9]. Notably, individuals affected by Parkinson's disease, along with their caregivers and healthcare professionals, have increasingly adopted these healthcare practices to address accessibility challenges related to healthcare facilities. Besides the imperative for objective symptom detection, which is crucial for informing treatment decisions, clinicians may exhibit hesitancy in embracing the paradigm shift toward the digitalization of their practice, often adhering to traditional methods[10]. Clinical evaluations are inherently subjective, reliant on the experience and expertise of clinicians, mostly relying on widely employed rating scales which may exhibit rating variability [11,12]. Advances in monitoring systems have facilitated precise recording of motor symptoms associated with parkinsonism using handheld devices, thereby supporting an objective assessment of patients[13].

Taking a significant stride toward "personalized care" in Parkinson's disease, wearable technology enables continuous monitoring with data collection within the home environment. This approach affords a detailed analysis of the patient's clinical status throughout the day, encompassing routine daily activities. Furthermore, it allows for a quantitative assessment of the patient's progression over extended periods spanning months and years. These technological advancements align with the established standard of care, enhancing it significantly and heralding a paradigm shift compared to prevailing practices.

Digital Health Technologies in PD and the Digital Biomarkers (DB's)

In the past decade, substantial financial resources have been directed towards the identification of biomarkers to elucidate the progression of Parkinson's disease (PD), primarily utilizing molecular, fluid, or imaging modalities. These endeavors have yielded valuable insights into PD, encompassing mechanistic targets, disease subtypes, and imaging biomarkers. While significant knowledge has been gained, the practical implementation of robust biomarkers for disease progression, serving as tools to quantify changes in disease status or severity, remains a challenging pursuit.

Biomarkers, as demonstrated in other fields such as oncology, have proven instrumental in improving health outcomes and expediting drug approvals, particularly in areas with critical unmet needs. However, in the context of PD, the development of progression markers is imperative across all stages of the disease. This not only acts as a catalyst for advancing drug development by enabling interventions aimed at halting or slowing disease progression but also facilitates the development of symptomatic treatments tailored to moderate stages of the disease.

The diffusion of wearable digital technologies in healthcare, yielding substantial volumes of big data, has given rise to a paradigm shift in medical information. DBs, derived from patient-generated data regarding their disease state or health management through digital health technologies, represent a pivotal development in the modern healthcare landscape. This evolution is particularly germane to Parkinson's disease (PD), where DBs play a pivotal role in enhancing diagnostic and therapeutic precision[14]. DBs, within the context of PD, encompass meticulous quantification of motor symptoms (bradykinesia, rest tremor, rigidity, postural instability and gait disturbances) and the concurrent treatment

related complications, activities of daily living, and nuanced information on non-motor symptoms and treatment elements. This comprehensive dataset facilitates remote and continuous monitoring, providing actionable insights into the nuanced biological state of individuals[15].

The integration of these technologies into routine medical practice signifies a transformative approach. It heralds a multi-level strategy aimed at not only refining patient management and enhancing quality of life but also reshaping the structural dynamics and resource allocation within health systems. Furthermore, the establishment of a unified framework for research application fosters a new landscape for investigating innovative treatments and meticulously evaluating existing therapeutic modalities. This augurs well for advancing medical science and improving patient outcomes in the field of neurodegenerative diseases[16].

Remote symptom monitoring: Is it trustworthy and feasible at the same time?

A demand for a more objective and continuous monitoring of Parkinson's disease (PD) features arises due to the challenges associated with accurately assessing the presence and severity of symptoms through solely subjective means and the lapses in care continuity due to infrequent in-person visits. Telemedicine services, including camera-based consultations, have emerged as viable solutions. However, in the context of Parkinson's disease, these modules do not always provide physicians with a complete assessment of patients. This limitation is attributed to the absence of a comprehensive view, coupled with time constraints reminiscent of traditional office visits. Quantitative parameters evaluating motor condition, derived from wearable technologies, are becoming increasingly recognized in the movement disorder community as the most credible option that has come to fill the void. Especially for advanced patents, who often do not easy to access to their treating physician, telemedicine empowered by wearable devices has turned out to be very helpful. Among the array of technologies, inertial measurement units (IMUs) emerge as the predominant choice. In the domain of PD remote monitoring, IMUs have been seamlessly integrated into patient-worn devices, encompassing wearable sensors and systems. Over time, wearable monitoring systems have consistently improved their efficacy in discerning Parkinsonian symptoms. Despite promising outcomes, the incorporation of wearables into routine clinical practice remains limited, and a dearth of "practical recommendations" persists, hindering the optimization of outcomes for PD patients, their caregivers, and healthcare professionals.

Most devices currently available on the market and approved for medical use, are considered reliable in detecting several cardinal motor symptoms, as well as treatment-related complications such as the OFF state and dyskinesias. Each monitoring system has undergone clinical validation to confirm that it provides relevant and correct information.

Familiarity with the existence of these devices does not represent a recent attainment. Their evolution has traversed multiple stages, spanning a duration of at least three decades. Tremor was one of the first symptoms recorded by wearable systems[17–19]. Moreover, ambulatory monitoring has proven effective in quantifying bradykinesia, dyskinesia, and overall activity in patients with Parkinson's disease[20–22]. Conversely, concerning gait analysis, although sensors were early applied to measure gait parameters and general activity, it took more time for their application in the detection of gait disturbance in PD[23,24]. Particularly for freezing of gait and postural instability —symptoms prevalent in the more advanced stages of the disease and crucial indicators for the risk of falling—machine learning techniques have advanced to discern and identify these symptoms[25–27].

However, while most systems exhibit good accuracy in measuring bradykinesia, tremor, gait, and detecting ON/OFF fluctuations and dyskinesias, only one has been identified as having the capability to simultaneously capture the entire spectrum[28–30].Whilst the first sensors and algorithms developed focused on detecting specific symptoms without being able to visualize the wide range of motor impairments and their variation over the course of each day, advanced systems possess the capability to continuously detect symptoms over time and subsequently present them to the physician, thereby generating a comprehensive digital file of disease history.

Although wearables have gained acceptance from both the medical community and patients, several factors may impede their widespread use. The complexity of operation and technological unfamiliarity among patients emerge as the primary barriers, contributing to low adherence despite reported high acceptance levels.

Longitudinal Management of PD patients using digital monitoring systems

Parkinson's disease, classified as a neurodegenerative disorder, unfolds along an extended and gradually advancing trajectory for the majority of afflicted individuals. Within this temporal progression, patients traverse successive stages marked by a gradual escalation of both motor and nonmotor symptoms over protracted intervals spanning months and years[31,32]. Noteworthy fluctuations in symptomatology also manifest within daily and hourly contexts, necessitating vigilant monitoring by medical practitioners. This diligence is imperative for fa-

cilitating judicious interventions and the expeditious recalibration of pharmacotherapeutic regimens to align with dynamic patient requirements[33].

Clinical evaluations and Parkinson's disease (PD) scales have exhibited suboptimal reliability in capturing nuanced changes throughout the continuum of disease progression. In contrast, the application of machine learning algorithms to data derived from wearable sensors demonstrates a notable capacity to discriminate between discrete stages of PD. This technological approach proffers a robust and objective means for systematically monitoring the dynamic evolution of the disease[12,34].

Wearable systems, specifically designed for at-home utilization, facilitate continuous and precise monitoring. This enables proactive and preventive monitoring of diseases, as well as optimization of treatment protocols. This paradigm shift is poised to enhance medical care significantly, surpassing the efficacy of analogous devices functioning solely as Holter monitors. While Holter deployment remains a viable option, the unparalleled advantage of continuous usage provides a more comprehensive understanding of the patient's condition. This continuous, objective monitoring yields invaluable insights into the patient's status and symptom fluctuations over time, thus offering an enhanced foundation for the judicious adjustment of Parkinson's therapy[35]. The wealth of information obtained through this continuous monitoring approach is pivotal for optimizing treatment strategies.

The implementation of continuous, objective monitoring holds the potential for early detection of symptoms and fluctuations in patients who may not yet be cognizant of their presence or unable to articulate a precise understanding of their manifestations. The early identification and prompt treatment of motor fluctuations are anticipated to significantly enhance the prospects of leading a normal life or sustaining occupational effectiveness over extended periods. This bears a substantive impact on both the quality of life for patients and the health economics of the healthcare system[36,37].

Of particular significance is the identification of gait-related symptoms, including freezing of gait and postural instability, as pivotal components in the optimization of pharmacological and nonpharmacological interventions for Parkinson's disease[38]. These symptoms exert a profound influence on the overall quality of life. Consequently, wearable systems designed to monitor gait impairment, among other symptoms, address an unmet need in the comprehensive evaluation and treatment of Parkinson's disease patients. Furthermore, even in the advanced stages of Parkinson's disease, patients persist in experiencing both motor and nonmotor fluctuations, albeit potentially of reduced amplitude compared to earlier stages. A monitoring system remains highly pertinent even in this late disease stage, as ongoing treatment optimization continues to be imperative, representing the closest approximation to a sustainable cure for the disease[39].

Understanding how symptoms change throughout the day could help make treatments better, especially for managing levodopa-induced dyskinesia. Some patients aren't happy with even mild dyskinesia, while others can handle more severe symptoms. Most people get dyskinesia when their medication is at its highest level, but some get it when the medication is wearing off or in a different pattern. So, knowing how symptoms vary during the day is crucial for improving Parkinson's disease management[40]. This diversity in symptomatology unfolds across different temporal phases, characterized by varying intensity and duration. However, during conventional clinical encounters, physicians are afforded a mere snapshot of the patient's condition, thereby missing the comprehensive panorama. Consequently, the availability of data that methodically depicts, in a clinically meaningful manner, the dynamic conditions of the patient throughout the day—encompassing both symptom fluctuations and dyskinesias—becomes paramount. Such visual representations hold the promise of refining the current management paradigms for Parkinson's disease as they urge the physicians to decide based on objective outcomes and not on their inner ranking.

At present, there exists a pronounced underutilization of advanced therapies in the realm of Parkinson's disease, primarily attributed to the challenges encountered by physicians in accurately identifying suitable candidates. A number of Parkinson's disease centers have incorporated objective monitoring into the patient screening process for advanced therapy, a trend likely to gain prominence in the future[41,42]. This approach provides a more precise foundation for decisions regarding the necessity and type of invasive therapy, concurrently furnishing valuable support to decision-makers within both state and private insurance sectors.

Simultaneously, the management of patients undergoing advanced therapy stands to benefit significantly from objective monitoring, facilitating informed decisions pertaining to treatment adjustments to optimize efficacy. Conversely, should optimal results remain elusive, such monitoring aids in the deliberation to transition care delivery from secondary to primary levels. Additionally, extant ambiguities surrounding therapeutic choices are anticipated to prompt eventual regulatory or insurance imperatives, mandating healthcare providers to substantiate their decisions through objective validation concerning patient stratification for these invasive and resource-intensive interventions[43,44].

Despite the significant role played by DBs in the early detection of symptoms, the mitigation of motor fluctuations, and the objective referral for second-line therapies, reliance on single biomarker proves insufficient for the reliable prognosis of Parkinson's disease (PD). This inadequacy extends to predicting responses to specific drugs or identifying distinct patient subgroups. The complexity and heterogeneity inherent in PD, influencing a multitude of biological mechanisms, preclude the efficacy of isolated biomarkers. Consequently, the imperative arises for comprehensive, multifactorial biomarker signatures to enhance diagnostic precision and prognostic capabilities in the context of Parkinson's disease progression and therapeutic responses.

Digital Biomarkers' prognostic potential

The identification and validation of such marker signatures present formidable challenges demanding state-of-the-art methodologies. In recent significant developments in Parkinson's disease research, there have been instances of predicting an individual patient's risk of receiving a clinical diagnosis of PD. This prediction is made using routinely collected data from electronic health records, with a foresight of about 5 years in advance[45]. Another notable example involves using a machine learning approach to predict the progression of Parkinson's disease. This approach utilizes a signature composed of a mix of inflammatory cytokines measured in blood serum[46]. Additionally, there's a study where data from mobile phone gyroscopes and accelerometers, combined with demographic and clinical information, have been used to predict various measures of Parkinson's disease symptom severity[47].

Altogether, an escalating cognizance underscores the imperative to transition towards precision neurology, demanding a holistic conceptualization of the disease. This entails a synergistic integration of aging processes, genetic and epigenetic variants, environmental determinants, lifestyle factors, comorbidities, and clinical assessments. Within the existing paradigm, the emergence of technologies geared towards furnishing a comprehensive and easily interpretable portrait of patient status, bolstering physician decision-making through Clinical Decision Support Systems (CDSS), epitomizes the most innovative domain in the evolution of precision neurology [48].

However the extensive use of big data AI technologies to strengthen the prognostic, progression and the overall delivery of care in PD entail multiple ethical, legal and social implications[49]. In this context mechanisms are launched in order to determine the framework of use with respect to ensure ethical and legal data processing and AI engagement and human accountability[50,51].

Conclusion

Based on the findings of this narrative, we posit that in the forthcoming years, DB's will assume a heightened significance in this context. Consequently, we anticipate that DMs could be synergistically integrated with other data modalities, encompassing genetic variants, to enable earlier, more resilient, and precise diagnosis of the disease and prediction of its progression.

References

[1] Pringsheim T, Jette N, Frolkis A, Steeves TDL. The prevalence of Parkinson's disease: a systematic review and meta-analysis. ***Mov Disord Off J Mov Disord Soc***. 2014;29(13):1583-1590. doi:10.1002/mds.25945

[2] Poewe W, Mahlknecht P. Pharmacologic Treatment of Motor Symptoms Associated with Parkinson Disease. ***Neurol Clin***. 2020;38(2):255-267. doi:10.1016/j.ncl.2019.12.002

[3] Moore H, Shpiner DS, Luca CC. Management of Motor Features in Advanced Parkinson Disease. ***Clin Geriatr Med***. 2020;36(1):43-52. doi:10.1016/j.cger.2019.09.010

[4] Xia R, Mao ZH. Progression of motor symptoms in Parkinson's disease. ***Neurosci Bull***. 2012;28(1):39-48. doi:10.1007/s12264-012-1050-z

[5] Mosley PE, Moodie R, Dissanayaka N. Caregiver Burden in Parkinson Disease: A Critical Review of Recent Literature. ***J Geriatr Psychiatry Neurol***. 2017;30(5):235-252. doi:10.1177/0891988717720302

[6] Zhao N, Yang Y, Zhang L, et al. Quality of life in Parkinson's disease: A systematic review and meta-analysis of comparative studies. ***CNS Neurosci Ther***. 2021;27(3):270-279. doi:10.1111/cns.13549

[7] Armstrong MJ, Okun MS. Diagnosis and Treatment of Parkinson Disease: A Review. ***JAMA***. 2020;323(6):548-560. doi:10.1001/jama.2019.22360

[8] Vűradi C. Clinical Features of Parkinson's Disease: The Evolution of Critical Symptoms. ***Biology***. 2020;9(5):103. doi:10.3390/biology9050103

[9] Ancona S, Faraci FD, Khatab E, et al. Wearables in the home-based assessment of abnormal movements in Parkinson's disease: a systematic review of the literature. ***J Neurol***. 2022;269(1):100-110. doi:10.1007/s00415-020-10350-3

[10] Pedrosa DJ, Timmermann L. Review: management of Parkinson's disease. ***Neuropsychiatr Dis Treat***. 2013;9:321-340. doi:10.2147/NDT.S32302

[11] Shulman LM, Armstrong M, Ellis T, et al. Disability Rating Scales in Parkinson's Disease: Critique and Recommendations. ***Mov Disord Off J Mov Disord Soc***. 2016;31(10):1455-1465. doi:10.1002/mds.26649

[12] Regnault A, Boroojerdi B, Meunier J, Bani M, Morel T, Cano S. Does the MDS-UPDRS provide the precision to assess progression in early Parkinson's disease? Learnings from the Parkinson's progression marker initiative cohort. ***J Neurol***. 2019;266(8):1927-1936. doi:10.1007/s00415-019-09348-3

[13] Adams JL, Lizarraga KJ, Waddell EM, et al. Digital Technology in Movement Disorders: Updates, Applications, and Challenges. ***Curr Neurol Neurosci Rep***. 2021;21(4):16. doi:10.1007/s11910-021-01101-6

[14] Motahari-Nezhad H, Fgaier M, Mahdi Abid M, Péntek M, Gulácsi L, Zrubka Z. Digital Biomarker-Based Studies: Scoping Review of Systematic Reviews. ***JMIR MHealth UHealth***. 2022;10(10):e35722. doi:10.2196/35722

[15] Dorsey ER, Papapetropoulos S, Xiong M, Kieburtz K. The First Frontier: Digital Biomarkers for Neurodegenerative Disorders. ***Digit Biomark***. 2017;1(1):6-13. doi:10.1159/000477383

[16] Verification, analytical validation, and clinical validation (V3): the foundation of determining fit-for-purpose for Biometric Monitoring Technologies (BioMeTs) | npj Digital Medicine. Accessed December 21, 2023. https://www.nature.com/articles/s41746-020-0260-4

[17] Cole BT, Roy SH, De Luca CJ, Nawab S. Dynamic neural network detection of tremor and dyskinesia from wearable sensor data. ***Annu Int Conf IEEE Eng Med Biol Soc IEEE Eng Med Biol Soc Annu Int Conf***. 2010;2010:6062-6065. doi:10.1109/IEMBS.2010.5627618

[18] Rigas G, Tzallas AT, Tsipouras MG, et al. Assessment of tremor activity in the Parkinson's disease using a set of wearable sensors. ***IEEE Trans Inf Technol Biomed Publ IEEE Eng Med Biol Soc***. 2012;16(3):478-487. doi:10.1109/TITB.2011.2182616

[19] Hoff JI, Wagemans EA, van Hilten BJ. Ambulatory objective assessment of tremor in Parkinson's disease. ***Clin Neuropharmacol***. 2001;24(5):280-283. doi:10.1097/00002826-200109000-00004

[20] Dunnewold RJ, Hoff JI, van Pelt HC, Fredrikze PQ, Wagemans EA, van Hilten BJ. Ambulatory quantitative assessment of body position, bradykinesia, and hypokinesia in Parkinson's disease. ***J Clin Neurophysiol Off Publ Am Electroencephalogr Soc***. 1998;15(3):235-242. doi:10.1097/00004691-199805000-00007

[21] Klapper DA, Weaver J, Fernandez H, Ohno-Machado L. Classification of movement states in Parkinson's disease using a wearable ambulatory monitor. ***AMIA Annu Symp Proc AMIA Symp***. 2003;2003:896.

[22] Keijsers NL, Horstink MW, Gielen SC. Online monitoring of dyskinesia in patients with Parkinson's disease. ***IEEE Eng Med Biol Mag Q Mag Eng Med Biol Soc***. 2003;22(3):96-103. doi:10.1109/memb.2003.1213632

[23] Bouten CV, Koekkoek KT, Verduin M, Kodde R, Janssen JD. A triaxial accelerometer and portable data processing unit for the assessment of daily physical activity. ***IEEE Trans Biomed Eng***. 1997;44(3):136-147. doi:10.1109/10.554760

[24] alarian A, Russmann H, Vingerhoets FJG, et al. Gait assessment in Parkinson's disease: toward an ambulatory system for long-term monitoring. ***IEEE Trans Biomed Eng***. 2004;51(8):1434-1443. doi:10.1109/TBME.2004.827933

[25] Tripoliti EE, Tzallas AT, Tsipouras MG, et al. Automatic detection of freezing of gait events in patients with Parkinson's disease. ***Comput Methods Programs Biomed***. 2013;110(1):12-26. doi:10.1016/j.cmpb.2012.10.016

[26] Schlachetzki JCM, Barth J, Marxreiter F, et al. Wearable sensors objectively measure gait parameters in Parkinson's disease. ***PloS One***. 2017;12(10):e0183989. doi:10.1371/journal.pone.0183989

[27] BorzúL, Mazzetta I, Zampogna A, Suppa A, Olmo G, Irrera F. Prediction of Freezing of Gait in Parkinson's Disease Using Wearables and Machine Learning. ***Sensors***. 2021;21(2):614. doi:10.3390/s21020614

[28] Moreau C, Rouaud T, Grabli D, et al. Overview on wearable sensors for the management of Parkinson's disease. ***NPJ Park Dis***. 2023;9(1):153. doi:10.1038/s41531-023-00585-y

[29] Angelo Antonini, , Michela Garon, ,Giovanni Gentile, et al. Towards Objective Monitoring of Parkinson's Disease Motor Symptoms Using a Wearable Device: Wearability and Performance Evaluation of PDMonitor®. ***Front Neurol***. in press.

[30] Kanellos FS, Tsamis KI, Rigas G, et al. Clinical Evaluation in Parkinson's Disease: Is the Golden Standard Shiny Enough? ***Sensors***. 2023;23(8):3807. doi:10.3390/s23083807

[31] Kim HJ, Mason S, Foltynie T, Winder-Rhodes S, Barker RA, Williams-Gray CH. Motor Complications in Parkinson's Disease: 13-Year Follow-up of the CamPaIGN Cohort. ***Mov Disord***. 2020;35(1):185-190. doi:10.1002/mds.27882

[32] Chung SJ, Yoo HS, Lee YH, Lee HS, Lee PH, Sohn YH. Initial motor reserve and long-term prognosis in Parkinson's disease. ***Neurobiol Ag-***

ing. 2020;92:1-6. doi:10.1016/j.neurobiolaging.2020.02.028

[33] Kwok JYY, Huang TW, Tretriluxana J, et al. Symptom Burden and Unmet Support Needs of Patients With Parkinson's Disease: A Cross-Sectional Study in Asia-Pacific Regions. ***J Am Med Dir Assoc***. 2021;22(6):1255-1264. doi:10.1016/j.jamda.2020.09.012

[34] Evers LJW, Krijthe JH, Meinders MJ, Bloem BR, Heskes TM. Measuring Parkinson's disease over time: The real-world within-subject reliability of the MDS-UPDRS. ***Mov Disord Off J Mov Disord Soc***. 2019;34(10):1480-1487. doi:10.1002/mds.27790

[35] Tsamis KI, Odin P, Antonini A, Reichmann H, Konitsiotis S. A Paradigm Shift in the Management of Patients with Parkinson's Disease. ***Neurodegener Dis***. 2023;23(1-2):13-19. doi:10.1159/000533798

[36] Dodel R, Tinelli M, Deuschl G, Petersen G, Oertel W, Ahmerkamp-Böhme J. The economic benefit of timely, adequate, and adherence to Parkinson's disease treatment: the Value of Treatment Project 2. ***Eur J Neurol***. 2021;28(2):707-716. doi:10.1111/ene.14584

[37] Antonini A, Pahwa R, Odin P, et al. Comparative Effectiveness of Device-Aided Therapies on Quality of Life and Off-Time in Advanced Parkinson's Disease: A Systematic Review and Bayesian Network Meta-analysis. ***CNS Drugs***. 2022;36(12):1269-1283. doi:10.1007/s40263-022-00963-9

[38] Scherbaum R, Moewius A, Oppermann J, et al. Parkinson's disease multimodal complex treatment improves gait performance: an exploratory wearable digital device-supported study. ***J Neurol***. 2022;269(11):6067-6085. doi:10.1007/s00415-022-11257-x

[39] Rosqvist K, Horne M, Hagell P, Iwarsson S, Nilsson MH, Odin P. Levodopa Effect and Motor Function in Late Stage Parkinson's Disease. ***J Park Dis***. 2018;8(1):59-70. doi:10.3233/JPD-171181

[40] Thanvi B, Lo N, Robinson T. Levodopa-induced dyskinesia in Parkinson's disease: clinical features, pathogenesis, prevention and treatment. ***Postgrad Med J***. 2007;83(980):384-388. doi:10.1136/pgmj.2006.054759

[41] Moro E, Schüpbach M, Wächter T, et al. Referring Parkinson's disease patients for deep brain stimulation: a RAND/UCLA appropriateness study. ***J Neurol***. 2016;263:112-119. doi:10.1007/s00415-015-7942-x

[42] Heldman DA, Giuffrida JP, Cubo E. Wearable Sensors for Advanced Therapy Referral in Parkinson's Disease. ***J Park Dis***. 2016;6(3):631-638. doi:10.3233/JPD-160830

[43] Chaudhuri KR, Pickard AS, Alobaidi A, et al. The Cost Effectiveness of Levodopa-Carbidopa Intestinal Gel in the Treatment of Advanced Parkinson's Disease in England. ***PharmacoEconomics***. 2022;40(5):559-574. doi:10.1007/s40273-022-01132-y

[44] Afentou N, Jarl J, Gerdtham UG, Saha S. Economic Evaluation of Interventions in Parkinson's Disease: A Systematic Literature Review. ***Mov Disord Clin Pract***. 2019;6(4):282-290. doi:10.1002/mdc3.12755

[45] Yuan W, Beaulieu-Jones B, Krolewski R, et al. Accelerating diagnosis of Parkinson's disease through risk prediction. ***BMC Neurol***. 2021;21(1):201. doi:10.1186/s12883-021-02226-4

[46] Ahmadi Rastegar D, Ho N, Halliday GM, Dzamko N. Parkinson's progression prediction using machine learning and serum cytokines. ***Npj Park Dis***. 2019;5(1):1-8. doi:10.1038/s41531-019-0086-4

[47] Sieberts SK, Schaff J, Duda M, et al. Crowdsourcing digital health measures to predict Parkinson's disease severity: the Parkinson's Disease Digital Biomarker DREAM Challenge. ***Npj Digit Med***. 2021;4(1):1-12. doi:10.1038/s41746-021-00414-7

[48] Timotijevic L, Hodgkins CE, Banks A, et al. Designing a mHealth clinical decision support system for Parkinson's disease: a theoretically grounded user needs approach. ***BMC Med Inform Decis Mak***. 2020;20(1):34. doi:10.1186/s12911-020-1027-1

[49] Ethics and governance of artificial intelligence for health: WHO guidance. Published online 2021.

[50] Radley-Gardner O, Beale H, Zimmermann R, eds. ***Fundamental Texts On European Private Law***. Hart Publishing; 2016. doi:10.5040/9781782258674

[51] Ethics guidelines for trustworthy AI | Shaping Europe's digital future. Published April 8, 2019. Accessed December 24, 2023. https://digital-strategy.ec.europa.eu/en/library/ethics-guidelines-trustworthy-ai

ΚΛΙΝΙΚΟΙ ΒΙΟΔΕΙΚΤΕΣ ΣΤΗ ΝΟΣΟ ΠΑΡΚΙΝΣΟΝ ΚΑΙ ΣΤΟΝ ΑΤΥΠΟ ΠΑΡΚΙΝΣΟΝΙΣΜΟ

Μαρία Χονδρογιώργη [1], Σπυρίδων Κονιτσιώτης [1,2]

[1] Νευρολογική Κλινική, Πανεπιστημιακό Γενικό Νοσοκομείο Ιωαννίνων
[2] Τμήμα Νευρολογίας, Σχολή Επιστημών Υγείας, Ιατρική Σχολή Πανεπιστημίου Ιωαννίνων

Περίληψη

Η χρήση κλινικών βιοδεικτών στη διαφορική διάγνωση και πρόγνωση των παρκινσονικών συνδρόμων παραμένει πολύτιμη, παρά την εξέλιξη στον εντοπισμό εργαστηριακών, απεικονιστικών και γενετικών δεικτών. Στην παρούσα ανασκόπηση γίνεται αναφορά στα κριτήρια της Movement Disorders Society για την πρόδρομη νόσο Πάρκινσον (ΝΠ) τα οποία αποσκοπούν στην αξιολόγηση της πιθανότητας παρουσίας πρόδρομης ΝΠ σε άτομα χωρίς ή με πολύ ήπια κινητικά συμπτώματα. Περιγράφεται επίσης η προγνωστική αξία μεμονωμένων βιοδεικτών για την ιδιοπαθή ΝΠ, όπως η ηλικία έναρξης και ο κινητικός υπότυπος, καθώς και η προγνωστική αλλά και η διαφοροδιαγνωστική ικανότητα στο σύνολο των παρκινσονικών συνδρόμων της διαταραχής συμπεριφοράς ύπνου REM, της υποσμίας, των διαταραχών οφθαλμοκινητικότητας, της ορθοστατικής υπότασης, της στοματοφαρυγγικής δυσλειτουργίας και ποικίλων νευροψυχιατρικών εκδηλώσεων. Όσον αφορά τη διαταραχή συμπεριφοράς ύπνου REM, πέραν της υψηλότατης προγνωστικής της αξίας ως πρόδρομης κατάστασης α-συνουκλεϊνοπάθειας, σημαντική είναι και η χρήση της στην ταξινόμηση των ασθενών σύμφωνα με το προσφάτως προτεινόμενο μοντέλο κεντρικής ή περιφερικής έναρξης της παθολογίας α-συνουκλεΐνης στην ιδιοπαθή ΝΠ. Η χρήση του μοντέλου αυτού επιφυλάσσει χρήσιμες παθοφυσιολογικές και προγνωστικές προεκτάσεις όπως αυτές περιγράφονται συνοπτικά στο παρόν κείμενο. Τέλος, επισημαίνονται βασικές παράμετροι οι οποίοι καθοδηγούν τη θεραπευτική στρατηγική στην ιδιοπαθή ΝΠ, όπως η ηλικία, πιθανές συννοσηρότητες και ο τρόπος ζωής των ασθενών.

Λέξεις ευρετηρίου: νόσος Πάρκινσον; Άτυπα Παρκινσονικά σύνδρομα; Διαταραχή συμπεριφοράς ύπνου REM; κινητικός υπότυπος; Υποσμία

CLINICAL BIOMARKERS IN PARKINSON'S DISEASE AND ATYPICAL PARKINSONISM)

Maria Chondrogiorgi[1], Spyridon Konitsiotis[1,2]
[1] Department of Neurology, University General Hospital of Ioannina
[2] Department of Neurology, School of Health Sciences, Medical School, University of Ioannina

Abstract

The use of clinical biomarkers in the differential diagnosis and prognosis of parkinsonian syndromes remains valuable, despite the progress made in the identification of laboratory, imaging and genetic markers. The present review includes a reference on Movement Disorders Society criteria for prodromal Parkinson's disease (PD) which were designed to estimate the probability of prodromal PD in individuals with any or very mild motor symptoms. The prognostic value of individual biomarkers for idiopathic PD is also discussed, such as age of onset and motor subtype, as well as the role of REM sleep behavior disorder, hyposmia, oculomotor disorders, postural hypotension, oropharyngeal dysfunction and various neuropsychiatric manifestations in the differential diagnosis among parkinsonian syndromes. Regarding REM sleep behavior disorder, in addition to its high prognostic value as a state of an emerging α-synucleinopathy, its use in the classification of patients according to the recently proposed brain first and body first model for idiopathic PD is also important. The use of this model holds useful pathophysiological and prognostic implications which are also summarized in this review. Finally, key parameters that guide the therapeutic strategy in idiopathic PD are highlighted, such as patients' age and lifestyle and possible comorbidities.

Keywords: Parkinson's disease; Atypical Parkinsonian syndromes; REM sleep behaviour disorder; motor subtype; Hyposmia

Κύρια σημεία της ανασκόπησης

- *Ο εντοπισμός και η χρήση κλινικών βιοδεικτών παραμένει ουσιώδης παρά την εξέλιξη στον εντοπισμό εργαστηριακών βιοδεικτών*
- *Η εφαρμογή των κριτηρίων της Movement Disorders Society για την πρόδρομη ΝΠ επιτρέπουν τον πολύ πρώιμο εντοπισμό επίνοσων ατόμων*
- *Το μοντέλο διαχωρισμού των ασθενών με ΝΠ σε κεντρικής και περιφερικής έναρξης νόσο (brain first/ body first) παρέχει ενδιαφέροντα παθοφυσιολογικά και προγνωστικά στοιχεία*
- *Η διαταραχή συμπεριφοράς ύπνου REM, η υποσμία, η ορθοστατική υπόταση, η διαταραχή της οφθαλμοκινητικότητας και της στοματοφαρυγγικής λειτουργίας και το πρότυπο πιθανών νευροψυχιατρικών διαταραχών συμβάλλουν σημαντικά στη διαφοροδιάγνωση και πρόγνωση των παρκινσονικών συνδρόμων*

Αξιοποιήσιμα σημεία στην κλινική **πράξη**

- Η πρώιμης έναρξης ΝΠ σχετίζεται με βραδύτερη εξέλιξη της νόσου αλλά ταυτόχρονα σημαντική επιβάρυνση της ποιότητας ζωής
- Ο κινητικός υπότυπος παρέχει χρήσιμα προγνωστικά στοιχεία αλλά πιθανώς όχι μακροπρόθεσμα λόγω της δυνητικής μεταβλητότητάς του στην πορεία του χρόνου
- Η διαταραχή συμπεριφοράς ύπνου REM αποτελεί σε ένα εξαιρετικά υψηλό ποσοστό πρόδρομη εκδήλωση α-συνουκλεϊνοπάθειας
- Η παρουσία διαταραχής συμπεριφοράς ύπνου REM ως προκινητικό σύμπτωμα στη ΝΠ σχετίζεται με βαρύτερη διαταραχή του αυτόνομου νευρικού συστήματος και ταχύτερη γνωστική εξασθένιση
- Η υποσμία είναι συχνότερη στην ιδιοπαθή ΝΠ παρά στον άτυπο παρκινσονισμό
- Η ορθοστατική υπόταση όταν προηγείται του παρκινσονισμού και της γνωστικής έκπτωσης υποστηρίζει τη διάγνωση της ατροφίας πολλαπλών συστημάτων
- Το πρότυπο της γνωστικής έκπτωσης συμβάλλει στη διαφορική διάγνωση μεταξύ των άτυπων παρκινσονικών συνδρόμων

Review highlights

- The identification and use of clinical biomarkers remains essential despite the advances in the identification of laboratory and imaging biomarkers
- Application of the Movement Disorders Society criteria for prodromal PD allows the very early identification of individuals about to develop clinical PD
- Brain first and body first model provides valuable pathophysiological and prognostic clues
- REM sleep behavior disorders, hyposmia, orthostatic hypotension, oculomotor and oropharyngeal dysfunction, and the pattern of neurophychiatric disorders contribute significantly to the differential diagnosis and prognosis of parkinsonian syndromees

Useful points to clinical practice

- Early-onset PD is associated with a slower disease progression but at the same time with a significant burden on the quality of life
- Motor subtypes provide useful prognostic evidence in the short term but probably not in the long term due to their potential variability over time
- REM sleep behavior disorder represents at a high percentage an early stage of α-synucleinopathy
- The presence of REM sleep behavior disorder as a promotor symptom in PD is associated with more severe autonomic dysfunction and faster cognitive decline
- Hyposmia is more common in idiopathic PD than in atypical parkinsonism
- Orthostatic hypotension preceding parkinsonism and cognitive decline supports the diagnosis of multiple system atrophy
- The pattern of cognitive decline contributes to the differential diagnosis among atypical parkinsonian syndrom

Introduction

Despite the significant advances in the identification of laboratory, imaging and genetic biomarkers, the diagnosis of Parkinson's disease (PD) and atypical parkinsonism is still heavily relied on clinical examination. However, the diagnostic procedure can be proved demanding due to a substantial overlap among parkinsonian syndromes, with misdiagnosis rates even by movement disorders experts approaching the percent of 20% [1]. The neurodegenerative process that underlies these diseases is known to start many years before the emergence of motor symptoms [2] and specific non-motor symptoms that arise from this long-term procedure serve either as supportive criteria of the diagnosis of PD or as red flags for atypical parkinsonism. The identification of subtle motor symptoms is also crucial for the early and precise diagnosis. In the pursuit of precision medicine and personalized therapeutic approaches, clinical biomarkers have emerged as promising candidates for unraveling the intricate pathophysiology of PD and atypical parkinsonism.

This paper aims to depict the evolving landscape of clinical biomarkers in the context of PD and atypical parkinsonism. By delving into the current state of

knowledge surrounding these biomarkers, we seek to elucidate their potential role in not only diagnostic precision but also in prognostication, disease monitoring, and targeted therapeutic interventions.

Movement Disorders Society research criteria for prodromal PD

In 2015 the Movement Disorders Society published the first research criteria for prodromal PD [3]. Using a Bayesian classifier approach, the study provided likelihood ratios (LRs) for specific markers supposed to increase the probability for prodromal PD. The marker with the highest positive likelihood ratio was found to be polysomnography-proven REM sleep behavior disorder (LR 130), followed by a clearly abnormal dopaminergic PET/SPECT (LR 40), and possible subthreshold parkinsonism (LR 10). Other factors that were reported to increase the probability of prodromal PD included the presence of a sibling with PD with age at onset <50, olfactory loss, constipation, excessive daytime somnolence, symptomatic hypotension, severe erective dysfunction, depression, pesticide or solvent exposure, nonuse of caffeine, and male sex. In 2019 the criteria were updated, providing updated predictive values of the markers referred in the original criteria, while four new markers were also introduced, which included diabetes mellitus, cognitive deficits, physical inactivity, and low plasma urate levels in men [4].

Age of onset in PD

Age of onset was among the first clinical characteristics that were hypothesized to associate with different disease phenotypes and prognosis. The age of 40 was initially set as the cut-off to define young-onset PD (YOPD) but most recent studies have used a cut-off of 50 or even 55 years of age [5]. YOPD represents a maximum percentage of 10% of the entire PD population and a considerable variability exists within this group due to different genetic substrate etc. Despite this rather small sample size and the existing heterogeneity, YOPD has been associated in general with a favorable prognosis regarding both the motor and the non-motor part of the disease. Specifically, the large body of literature suggests that YOPD patients exhibit slower disease progression with less postural instability and autonomic dysfunction, more preserved cognitive function and less severe hyposmia [5,6]. In the study of Pagano et al [7] that used a dataset of Parkinson Progression Markers Initiative (PPMI), greater dopaminergic dysfunction on DaTSCAN and greater reduction of CSF α-syn and t-tau levels were observed in patients with PD of later onset. In the same study, the Hoehn and Yahr stage and the unified Parkinson's disease rating scale (UPDRS)-part III score was higher at the time of diagnosis in older patients. On the other hand, YOPD patients tend to develop earlier motor complications and dyskinesias and the impact of the disease on quality of life is more severe due to possible implications on employment, higher rates of depression and anxiety and more affected emotional well-being [5,6]. The frequency of RBD and other sleep problems was found to be similar among PD patients regardless of the age of disease onset [7]. At any case, comparisons of clinical characteristics according to age at disease onset should always be made under the light of changes that accompany the normal aging. To end with, the study of Kempster et al [8] found that YOPD patients delay to reach the disability milestones that define the advanced stage PD, but once this stage is reached the progression accelerates to match that of the older onset patients.

Motor subtype

The predominance of specific motor symptoms was also very early acknowledged as a valuable criterion to categorize patients into groups with different prognosis. The akinetic-rigid and tremor-dominant subtypes were the first motor subtypes described. The development of UPDRS allowed a more thorough classification, that included the tremor-dominant, postural instability and gait difficulty (PIGD), the axial-dominant, appendicular-dominant and rigidity dominant subtypes. However, the appendicular-dominant and rigidity dominant subtypes were largely replaced in clinical practice by the intermediate type. Patients with tremor-dominant phenotype are generally considered to present a more benign course with slower disease progression and lower rates of hyposmia, dementia, depression and other non-motor symptoms than those of PIGD subtype [6]. The term "benign tremulous parkinsonism" has been also used to describe a type of the disease with tremor predominance, absence of gait disorder and a relatively mild progression over many years [9]. Nevertheless, the predictive value of a motor subtype is compromised by its possible instability in the long term, as some longitudinal studies have provided related evidence. Specifically, the study of Simuni et al [10] showed that after one year of the diagnosis 39% of the patients with PIGD subtype and 18% of the tremor-dominant subtype shifted to another subtype, while another more recent study with a follow-duration of 3 years reported a conversion rate of 50% and 38% for PIGD and tremor-dominant patients respectively [11]. As additional factors are involved in the general prognosis of PD, in 2017 Fereshtehnejad et al [12] using data from PPMI proposed a new subtyping method encompassing both motor and non-motor features. A motor summary score and three non-motor characteristics (cognitive impairment, RBD, and dysautonomia) were used for the classification to "mild motor-prodominant",

"diffuse malignant" and "intermediate" subtype. During the follow-up period, patients classified under "diffuse malignant" and "intermediate" subtypes showed a significantly greater and more rapid progression of motor symptoms, as assessed by UPDRS-Part II. A similar progression of non-motor symptoms assessed with UPDRS – Part I was also observed. Particularly within the "diffuse malignant" subtype, the steeper decline was observed in cognition and in the activities of daily living.

REM Sleep Behavior Disorder (RBD)

RBD is a parasomnia characterized by dream-enacting behaviors. It is attributed to impairment of locus subcoeruleus and other pontine structures including magnocellular reticular formation as these nuclei are involved in the regulation of sleep-wake cycle and the maintenance of muscle atonia during REM sleep. Post-mortem studies on patients with idiopathic RBD have identified α-synouclein pathology in these areas [13] indicating RBD as a prodromal manifestation of α-synoucleinopathies. Multiple cohort studies have also reported a percentage of 73.5-92.5% of idiopathic RBD converting to PD, DLB, or MSA within 10-14 years [14,15]. Under this prism RBD has been proposed as the strongest predictor of α-synoucleinopathies and contributes significantly to their differentiation from tauopathies. The percentage of idiopathic RBD cases that convert to PD or DLB is similar, while phenoconversion to MSA is more unusual (about 5% of total RBD cases). RBD is such a rare phenomenon in PSP and CBD that the occurrence of RBD in patients with other clinical characteristics suggestive of CBS has been attributed to diffuse Lewy body disease manifesting as CBS [16]. Moreover, significantly reduced cardiac MIBG uptake, consistent with sympathetic denervation of the same magnitude as that in patients with diagnosed PD has been observed in subjects with idiopathic RBD [17]. Notably, a history of RBD preceding parkinsonism is also used to distinguish body-first PD from brain-first PD as discussed more extensively below.

Olfactory dysfunction

Hyposmia presenting as impairment of odor detection, odor identification, odor discrimination and odor-recognition memory has been recognized as a common feature of PD and may antedate clinical diagnosis by even more than 20 years [18]. Borghammer and Van Den Berge [19] recently hypothesized that the olfactory bulb might be an entry point of pathogens and toxins that initiate the α-synoulein pathology and then swallowed nasal secretions expose gastrointestinal lining to the same pathogens. Odor identification threshold corresponds to the level at which subjects are able not only to detect but also to recognize a stimulus. Odor identification testing has been found to provide high diagnostic accuracy in distinguishing PD patients from healthy individuals. Marked deficits in odor identification have been also associated with impaired visuospatial and executive function, revealing a potential of hyposmia for its use as biomarker of PD-related cognitive decline [20]. Cholinergic denervation in temporolimbic areas is strongly associated with both of hyposmia and cognitive impairment and appears to play and an important role in the correlation of these conditions along with degenerative changes in the orbitofrontal cortex [21]. Moreover, post-mortem studies have shown that the degree of olfactory impairment does not reflect the degree of α-synouclein pathology in the olfactory bulb but is mostly associated with a more widespread cortical and subcortical α-synouclein pathology [22,23]. Hyposmia is also a significant predictor of phenoconversion in patients with idiopathic RBD [15]. Furthermore, in the study of Kim et al [24] MIBG uptake was independently related to hyposmia in de novo PD patients. Olfactory deficits are less prominent in MSA than PD and affect less than 25% of the patients [25]. Similarly, hyposmia is infrequent in PSP and CBD. In the study of Shill et al [26] a sensitivity of 93.4% and specificity of 64.7% was suggested for PSP among patients presenting with parkinsonism and normosmia. The use of standardized olfactory tests and consideration of confounding factors including age increase the prognostic accuracy of hyposmia.

Oculomotor dysfunction

Oculomotor disturbances are mostly prominent in PSP with the typical vertical supranuclear gaze palsy combined with astonished facial expression. Macro square wave jerks, curved vertical saccades and slow velocity of vertical saccades are also strongly suggestive of PSP. Eyelid opening apraxia is possibly indicative for either PSP or CBS. Apraxia of saccades and slow velocity of vertical saccades can also be present in CBS. Hypermetric saccades, reduced VOR-suppression and saccadic eye sequences are probably indicative for MSA. Additional features of MSA, despite more infrequent can be the presence of downbeat or rebound nystagmus [27]. DLB has not been linked with any specific oculomotor dysfunction.

Orthostatic hypotension

Individuals with orthostatic hypotension have been found to run an about 2-fold higher risk of PD than healthy population. In a longitudinal study performed at an autonomic disorders clinic, it was found that 19% of patients with orthostatic hypotension and 25% of patients with delayed orthostatic hypotension converted to an α-synoucleinopathy within 10 years of diagnosis [28]. In a study that compared patients with PD and atypical parkinsonian syndromes,

the prevalence of orthostatic hypotension was 81% among MSA patients and lower in the other patient groups (PD 18%, DLB 31%, PSP 26%, CBD 7%) [29]. Despite the occurrence of orthostatic hypotension at a considerable percentage in almost all types of degenerative parkinsonism, its early manifestation is mostly supportive of MSA since in it typically antedates motor symptoms in PD and cognitive impairment in DLB [30].

Oropharyngeal dysfunction

Inspiratory stridor, usually nocturnal, is a symptom highly specific for MSA. Due to its high positive predictive value, it has been included in the diagnostic criteria as additional feature of possible MSA. Moreover, it has been proposed that the early occurrence of laryngeal stridor contributes to reduced survival. On the other hand, dysphagia can present in either PD, MSA-P or PSP patients with the proposed shared mechanism being the degeneration of the cholinergic neurons of the pedunculopontine nucleus [31]. Early dysphagia argues against PD and indicates additional dysfunction of brainstem related to atypical parkinsonism syndromes.

Neuropsychiatric symptoms

Obvious cognitive dysfunction should not be an early symptom in PD but can be a key feature in the diagnosis and differential diagnosis of atypical parkinsonian syndromes. Regarding PSP, executive deficits are typically prominent at the time of diagnosis and frequently precede it up to three years [32]. In the study of Rittman et al [33] impairment in verbal fluency was found to strikingly distinguish PSP from PD patients. Progressive non-fluent aphasia can be a presentation of either PSP or CBD underlying pathology [34]. Apraxia of speech, agraphia and social cognition impairment are particularly supportive of CBD [35]. The cognitive pattern of the prodromal phase of DLB on the other hand typically includes attention, executive and visual processing deficits and relatively preserved memory and object naming. Depression is an independent predictor of quality of life in all parkinsonian syndromes and is associated with a higher frequency of other non-motor symptoms such as sleep disturbances, anxiety, and cognitive decline [36]. The prevalence of depression has been found to be significantly higher in MSA and PSP compared to PD and other parkinsonian syndromes [35]. However, in a study that investigated non-motor symptoms preceding cognitive impairment in DLB, about one third of the patients had depressive symptoms with a mean duration of 4.5 to 10.7 years [30]. Apathy is also most frequent in PSP and an apathetic profile has been associated with increased mortality among these patients [37]. Among PSP subtypes, the PSP-RS subtype was found in a comparative study to comprise in total more neuropsychological and neurobehavioural deficits than the PSP-P subtype [38]. Delirium and visual hallucinations or even depression and anxiety can occur during the predementia stage of DLB and correspond to the delirium-onset and psychiatric-onset types of prodromal DLB [39].

Brain-first and body-first model

The brain-first and body-first hypothesis arose from the observation that Braak's theory was found to be not valid for all PD cases at post-mortem [40]. Specifically, some cases of PD and DLB were found to lack Lewy pathology in dorsal motor nucleus of the vagus nerve despite presenting pathology in substantia nigra and other structures corresponding to higher Braak stages [41]. Moreover, in some patients RBD is not prominent at the time of diagnosis but appears during the course of the disease. Third, severe autonomic dysfunction is observed in individuals with idiopathic RBD but a significant percentage of early PD patients show normal cardiac sympathetic innervation with deterioration at more advanced stages of the disease [17, 42,43]. Considering the above findings, it was hypothesized that PD comprises two subtypes according to the initial origin of α-synouclein pathology [40]. The two subtypes are supposed to differ at the early stages of the disease in some clinical and imaging markers but in later stages the two subtypes converge due to the spreading of α-synouclein pathology. The presence of RBD was proposed as the key clinical symptom to differentiate the two subtypes. The body-first subtype is defined by an established history of RBD preceding the symptoms of parkinsonism. On the other side, brain-first subtype is characterized by nigrostriatal dopaminergic dysfunction prior to involvement of the autonomic peripheral nervous system and patients under this subtype should not present RBD at the time of PD diagnosis. The early involvement of the autonomic system and lower brainstem structures in the body-first patients manifests in general with a higher burden of autonomic symptoms including orthostatic hypotension, constipation, urinary and sexual dysfunction. Moreover, body-first patients present higher rates of cognitive decline and a more rapid progression to dementia than patients of the brain-first subtype. A higher frequency of depression has been also found by some studies in the body-first subtype but the link between antidepressant use and RBD demasking should be taken into account in the interpretation of such findings. A tremor-dominant subtype has been reported to be more pronounced in brain-first patients. However literature is still inconclusive on the ability of brain-first and body-first model to predict accurately the PD motor subtype.

"One size does not fit all" in PD treatment

Despite the concept of precision medicine is relatively new in PD, a personalized medicine strategy according to basic patient characteristics is already followed by the majority of neurologists. The spectrum of these clinical characteristics that define clinical decisions is constantly growing. Age is usually the first characteristic that attending physician considers in order to decide a treatment plan. To begin with, the use of dopamine agonists is typically limited in older patients due to the high frequency of side-effects. Deep-brain stimulation is also usually avoided in patients of more than 70 years of age. At any case we suggest that differences between "chronological" and "biological" aging should be considered at every single patient, as well as possible comorbidities (e.g. diabetes mellitus with secondary autonomic dysfunction, osteoporosis with increased risk of fracture in case of fall, hyperomocysteinaemia possibly worsened by high dose levodopa therapy). Personality traits that predispose to impulse control disorders, dopamine dysregulation and punding should also be taken into account, with such including high alcohol consumption, novelty seeking behavior and history of substance or drug addiction [44]. Certain lifestyle and daily routine should also be considered particularly for younger patients for which side effects such as sedation during working hours can be incapacitating, while the opportunity of rescue therapy should also be offered [45].

Conclusion

The incorporation of biomarkers in Parkinson's disease research and clinical practice serves several crucial purposes. It facilitates a deeper comprehension of mechanisms, aids in the design of optimal treatment strategies, helps in avoiding medications with a high likelihood of side effects, and enables a more precise guidance to patients and caregivers. The Movement disorders criteria for prodromal PD provide a framework for the identification of individuals with early signs of PD, even during the crucial for research premotor phase. The brain-first and body-first model, by categorizing the PD patients into two different categories according to their initial symptoms provides prognostic clues since the body-first subtype has been associated with a higher burden of autonomic symptoms and more rapid cognitive decline.RBD is a biomarker with high prognostic value that tends to be considered as a stage of an emerging α-synoucleinopathy. The use of additional biomarkers such as olfactory disruption, orthostatic hypotension and cognitive decline can assist the differential diagnosis among α-synoucleinopathies. Oculomotor disturbance and specific patterns of neuropsychiatric manifestations are particularly helpful in the early diagnosis of PSP and CBD. PD motor subtypes provide insights into disease prognosis, but their long-term stability may be limited. Finally, age, comorbidities, personality traits, and lifestyle are recognized as critical factors influencing treatment decisions in PD. Research on several promising candidate markers may improve the accuracy in the early diagnosis and prognosis of parkinsonian syndromes.

References

[1] Rizzo G, Copetti M, Arcuti S, Martino D, Fontana A, Logroscino G. Accuracy of clinical diagnosis of Parkinson disease: A systematic review and meta-analysis. ***Neurology***. 2016;86(6):566-576

[2] Kalia LV, Lang AE. Parkinson's disease. ***Lancet***. 2015;386(9996):896-912.

[3] Berg D, Postuma RB, Adler CH, Bloem BR, Chan P, Dubois B, et al. MDS research criteria for prodromal Parkinson's disease. Mov Disord. 2015;30(12):1600-1611

[4] Heinzel S, Berg D, Gasser T, Chen H, Yao C, Postuma RB; MDS Task Force on the Definition of Parkinson's Disease. Update of the MDS research criteria for prodromal Parkinson's disease. Mov Disord. 2019;34(10):1464-1470

[5] Schrag A, Schott JM. Epidemiological, clinical, and genetic characteristics of early-onset parkinsonism. ***Lancet Neurol***. 2006;5(4):355-363

[6] Fereshtehnejad SM, Postuma RB. Subtypes of Parkinson's Disease: What Do They Tell Us About Disease Progression?. ***Curr Neurol Neurosci Rep***. 2017;17(4):34

[7] Pagano G, Ferrara N, Brooks DJ, Pavese N. Age at onset and Parkinson disease phenotype. ***Neurology***. 2016;86(15):1400-1407

[8] Kempster PA, O'Sullivan SS, Holton JL, Revesz T, Lees AJ. Relationships between age and late progression of Parkinson's disease: a clinico-pathological study. ***Brain***. 2010;133(Pt 6):1755-1762

[9] Josephs KA, Matsumoto JY, Ahlskog JE. Benign tremulous parkin- sonism. Arch Neurol. 2006;63(3):354–357

[10] Simuni T, Caspell-Garcia C, Coffey C, Lasch S, Tanner C, Marek K, et al. How stable are Parkinson's disease subtypes in de novo patients: analysis of the PPMI cohort? Parkinsonism Relat Disord. 2016;28:62–67

[11] Kohat AK, Ng SYE, Wong ASY, Chia NSY, Choi X, Heng DL, et al. Stability of MDS-UPDRS Motor Subtypes Over Three Years in Early Parkinson's Disease. ***Front Neurol***. 2021;12:704906

[12] Fereshtehnejad SM, Zeighami Y, Dagher A, Postuma RB. Clinical criteria for subtyping Parkinson's disease: biomarkers and longitudinal

progression. ***Brain***. 2017;140(7):1959-1976
[13] Turner RS, D'Amato CJ, Chervin RD, Blaivas M. The pathology of REM sleep behavior disorder with comorbid Lewy body dementia. ***Neurology***. 2000;55(11):1730-1732
[14] Iranzo A, Fernúndez-Arcos A, Tolosa E, Serradell M, Molinuevo JL, Valldeoriola F, et al. Neurodegenerative disorder risk in idiopathic REM sleep behavior disorder: study in 174 patients. ***PLoS One***. 2014;9(2):e89741
[15] Postuma RB, Iranzo A, Hu M, Högl B, Boeve BF, Manni R, et al. Risk and predictors of dementia and parkinsonism in idiopathic REM sleep behaviour disorder: a multicentre study. Brain. 2019;142(3):744-759
[16] Kasanuki K, Josephs KA, Ferman TJ, Murray ME, Koga S, Konno T, et al. Diffuse Lewy body disease manifesting as corticobasal syndrome: A rare form of Lewy body disease [published correction appears in Neurology. 2018 Nov 20;91(21):986]. ***Neurology***. 2018;91(3):e268-e279
[17] Miyamoto T, Miyamoto M, Inoue Y, Usui Y, Suzuki K, Hirata K. Reduced cardiac 123I-MIBG scintigraphy in idiopathic REM sleep behavior disorder. ***Neurology***. 2006;67(12):2236-2238
[18] Fereshtehnejad SM, Yao C, Pelletier A, Montplaisir JY, Gagnon JF, Postuma RB. Evolution of prodromal Parkinson's disease and dementia with Lewy bodies: a prospective study. ***Brain***. 2019;142(7):2051-2067
[19] Borghammer P, Van Den Berge N. Brain-First versus Gut-First Parkinson's Disease: A Hypothesis. ***J Parkinsons Dis***. 2019;9(s2):S281-S295
[20] Mao CJ, Wang F, Chen JP, Yang YP, Chen J, Huang JY, et al. Odor selectivity of hyposmia and cognitive impairment in patients with Parkinson's disease. ***Clin Interv Aging***. 2017;12:1637-1644
[21] Lee JE, Cho KH, Ham JH, Song SK, Sohn YH, Lee PH. Olfactory performance acts as a cognitive reserve in non-demented patients with Parkinson's disease. ***Parkinsonism Relat Disord***. 2014;20(2):186-191
[22] Nag S, Yu L, VanderHorst VG, Schneider JA, Bennett DA, Buchman AS, et al. Neocortical Lewy bodies are associated with impaired odor identification in community-dwelling elders without clinical PD. ***J Neurol***. 2019;266(12):3108-3118.
[23] Tremblay C, Serrano GE, Intorcia AJ, Sue LI, Wilson JR, Adler CH, et al. Effect of olfactory bulb pathology on olfactory function in normal aging. ***Brain Pathol***. 2022;32(5):e13075
[24] Kim JS, Park HE, Park IS, Oh YS, Ryu DW, Song IU, et al. Normal 'heart' in Parkinson's disease: is this a distinct clinical phenotype?. ***Eur J Neurol***. 2017;24(2):349-356
[25] Zhang L, Cao B, Ou R, Wei QQ, Zhao B, Yang J, et al. Non-motor symptoms and the quality of life in multiple system atrophy with different subtypes. ***Parkinsonism Relat Disord.*** 2017;35:63-68
[26] Shill HA, Zhang N, Driver-Dunckley E, Mehta S, Adler CH, Beach TG. Olfaction in neuropathologically defined progressive supranuclear palsy. ***Mov Disord***. 2021;36(7):1700–1704
[27] Schröter N, van Eimeren T, Classen J, Levin J, Redecker C, Wolz M, et al. Significance of clinical symptoms and red flags in early differential diagnosis of Parkinson's disease and atypical Parkinsonian syndromes. ***J Neural Transm (Vienna)***. 2023;130(6):839-846
[28] Gibbons CH, Freeman R. Clinical implications of delayed orthostatic hypotension: A 10-year follow-up study. ***Neurology***. 2015;85(16):1362-1367
[29] Ha AD, Brown CH, York MK, Jankovic J. The prevalence of symptomatic orthostatic hypotension in patients with Parkinson's disease and atypical parkinsonism. ***Parkinsonism Relat Disord***. 2011;17(8):625-628
[30] Chiba Y, Fujishiro H, Iseki E, Ota K, Kasanuki K, Hirayasu Y, Satoa K. Retrospective survey of prodromal symptoms in dementia with Lewy bodies: comparison with Alzheimer's disease. Dement Geriatr Cogn Disord. 2012;33(4):273-81.
[31] Alfonsi E, Versino M, Merlo IM, Pacchetti C, Martignoni E, Bertino G, et al. Electrophysiologic patterns of oral-pharyngeal swallowing in parkinsonian syndromes. ***Neurology***. 2007;68(8):583-589
[32] Street D, Whiteside D, Rittman T, Rowe JB. Prediagnostic Progressive Supranuclear Palsy - Insights from the UK Biobank. ***Parkinsonism Relat Disord***. 2022;95:59-64
[33] Rittman T, Ghosh BC, McColgan P, Breen DP, Evans J, Williams-Gray CH, et al. The Addenbrooke's Cognitive Examination for the differential diagnosis and longitudinal assessment of patients with parkinsonian disorders. ***J Neurol Neurosurg Psychiatry***. 2013;84(5):544-551
[34] Santos-Santos MA, Mandelli ML, Binney RJ, Ogar J, Wilson SM, Henry ML, et al. Features of Patients With Nonfluent/Agrammatic Primary Progressive Aphasia With Underlying Progressive Supranuclear Palsy Pathology or Corticobasal Degeneration. JAMA Neurol. 2016;73(6):733-742
[35] Ananthavarathan P, Patel B, Peeros S, Obrocki R, Malek N. Neurological update: non-motor symptoms in atypical parkinsonian syndromes. J Neurol. 2023;270(9):4558-4578
[36] Dissanayaka NN, Sellbach A, Silburn PA,

O'Sullivan JD, Marsh R, Mellick GD. Factors associated with depression in Parkinson's disease. J Affect Disord 2011;132(1–2):82–88.

[37] Lansdall CJ, Coyle-Gilchrist ITS, Vázquez Rodríguez P, Wilcox A, Wehmann E, Robbins TW, et al. Prognostic importance of apathy in syndromes associated with frontotemporal lobar degeneration. Neurology. 2019;92(14):e1547-e1557

[38] Srulijes K, Mallien G, Bauer S, Dietzel E, Gröger A, Ebersbach G, et al. In vivo comparison of Richardson's syndrome and progressive supranuclear palsy-parkinsonism. J Neural Transm (Vienna). 2011;118(8):1191-1197

[39] McKeith IG, Ferman TJ, Thomas AJ, Blanc F, Boeve BF, Fujishiro H, et al. Research criteria for the diagnosis of prodromal dementia with Lewy bodies. Neurology. 2020 Apr 28;94(17):743-755

[40] Horsager J, Knudsen K, Sommerauer M. Clinical and imaging evidence of brain-first and body-first Parkinson's disease. ***Neurobiol Dis***. 2022;164:105626.

[41] Jellinger KA. Is Braak staging valid for all types of Parkinson's disease?. ***J Neural Transm (Vienna)***. 2019;126(4):423-431.

[42] Kashihara K, Imamura T, Shinya T. Cardiac 123I-MIBG uptake is reduced more markedly in patients with REM sleep behavior disorder than in those with early stage Parkinson's disease. ***Parkinsonism Relat Disord***. 2010;16(4):252-255

[43] Hamada K , Hirayama M , Watanabe H , Kobayashi R , Ito H , Ieda T, et al. Onset age and severity of motor impairment are associated with reduction of myocardial 123I-MIBG uptake in Parkinson's disease***. J Neurol Neurosurg Psychiatry*** 2003;74:423–426

[44] Katzenschlagger R, Evans A. Impulse dyscontrol and dopamine dysregulation syndrome. In: Chaudhuri KR, Tolosa E, Schapira AHV, Poewe W, eds. Non-motor Symptoms of Parkinson's Disease. 2nd ed. Oxford, UK: Oxford University Press 2014;420- 435

[45] Titova N, Chaudhuri KR. Personalized medicine in Parkinson's disease: Time to be precise. ***Mov Disord***. 2017;32(8):1147-1154

Ενημερωτικές Σελίδες...

Συνέδρια - Ημερίδες - Συμπόσια - Επιστημονικές εκδηλώσεις

2024

- **8-9 Μαρτίου 2024: Επετειακή Επιστημονική Εκδήλωση «Παρελθόν, παρόν και μέλλον στη Νευρολογία»,** *Θεσσαλονίκη*
- **28-30 Μαρτίου 2024: 15ο Πανελλήνιο Συνέδριο Ελληνικής Εταιρείας Κεφαλαλγίας,** *Αθήνα*
- **15-17 Μαΐου 2024: 10th European Stroke Organization Conference, Basel,** *Switzerland*
- **30 Μαΐου-2 Ιουνίου 2024: 35ο Πανελλήνιο Συνέδριο Νευρολογίας,** *Ρόδος*
- **29 Ιουνίου – 2 Ιουλίου 2024: 10th EAN Congress 2024,** *Elsinki*
- **21-24 Νοεμβρίου 2024: 12ο Πανελλήνιο Συνέδριο Αγγειακών Εγκεφαλικών Νόσων,** *Αθήνα*
- **12-15 Δεκεμβρίου 2024: 11ο Πανελλήνιο Συνέδριο Ελληνικής Ακαδημίας Νευροανοσολογίας,** *Θεσσαλονίκη*

Αρχεία Κλινικής Νευρολογίας

Για λόγους ενημέρωσης αρχείου, παρακαλούμε συμπληρώστε τα στοιχεία αλληλογραφίας σας και στείλτε το απόκομμα με fax στο: **210 7247556** ή αποστείλετε τα στοιχεία στο e-mail: **info@jneurology.gr**

ΟΝΟΜΑΤΕΠΩΝΥΜΟ:

.....

.....

ΤΟΠΟΣ ΑΠΟΣΤΟΛΗΣ:

❑ ΔΙΕΥΘΥΝΣΗ ΟΙΚΙΑΣ:

Τ.Κ. ..... ΠΕΡΙΟΧΗ .....

ΤΗΛ.: .....

❑ ΔΙΕΥΘΥΝΣΗ ΙΑΤΡΕΙΟΥ:

Τ.Κ. ..... ΠΕΡΙΟΧΗ .....

ΤΗΛ.: ..... FAX: .....

ΚΙΝΗΤΟ: .....

- *Εάν επιθυμείτε να λαμβάνετε το περιοδικό «Αρχεία Κλινικής Νευρολογίας» και σε ηλεκτρονική έκδοση συμπληρώστε την ηλεκτρονική σας διεύθυνση:*

e-mail: .....

Σημειώσεις

Σημειώσεις

Σημειώσεις

Οδηγίες προς τους συγγραφείς

Το περιοδικό *ΑΡΧΕΙΑ ΚΛΛΙΝΙΚΗΣ ΝΕΥΡΟΛΟΓΙΑΣ* κυκλοφορεί κάθε δύο μήνες και αποτελεί το επίσημο όργανο της Ελληνικής Νευρολογικής Εταιρείας. Με την Υπουργική Απόφαση ΔΥ2α/Γ.Π.οικ. 66198/1/6/2006, που δημοσιεύθηκε στο Φ.Ε.Κ. 1034/Β/1-08-2006, προστέθηκε στον κατάλογο των περιοδικών με Εθνική Αναγνώριση.

Ύλη του Περιοδικού

1. Ανασκοπικά Άρθρα: Η έκτασή τους δεν πρέπει να υπερβαίνει τις 6.000 λέξεις.
2. Εργασίες: Κλινικές ή εργαστηριακές μελέτες. Δεν πρέπει να υπερβαίνουν τις 4.000 λέξεις (συμπεριλαμβανομένων έως 6 πινάκων και εικόνων). Δεν πρέπει να έχει προηγηθεί δημοσίευσή τους σε άλλο έντυπο. Περιλαμβάνουν σελίδα τίτλου, δομημένη περίληψη, εισαγωγή, μέθοδο, αποτελέσματα, συζήτηση και βιβλιογραφία.
3. Σύντομες ανακοινώσεις και Γράμματα προς τη σύνταξη: Σχόλια για εργασίες που έχουν δημοσιευθεί ή σύντομες αναφορές σε ένα θέμα. Δεν πρέπει να υπερβαίνουν τις 1.500 λέξεις και περιλαμβάνουν έως 2 πίνακες ή εικόνες.
4. Ενδιαφέροντα περιστατικά: Όριο λέξεων 1.500, με τη σελίδα τίτλου, περίληψη και τις βιβλιογραφικές αναφορές. Επιτρέπονται μέχρι 2 εικόνες ή πίνακες.
5. Νευρολογικές Εικόνες με εκπαιδευτικό ενδιαφέρον: Όριο 4 εικόνες για το ίδιο θέμα και 200 λέξεις.
6. Επιλογές και σχολιασμός της βιβλιογραφίας.
7. Νευρολογικά Νέα - Ειδήσεις - Ενημερωτικές Σελίδες, όπως νέα της Ελληνικής Νευρολογικής Εταιρείας και συγγενών εταιρειών, ανακοινώσει συνεδρίων και άλλων εκπαιδευτικών δραστηριοτήτων.

Δομή της ύλης

Γίνονται δεκτές εργασίες στα ελληνικά ή αγγλικά.
Υποβάλλεται πάντοτε ο τίτλος, τα ονόματα των συγγραφέων και η περίληψη και στα αγγλικά.
Τα κείμενα θα πρέπει να αποστέλλονται σε μορφή Microsoft Word document.
Σελίδα τίτλου: Περιέχει τον τίτλο, τα πλήρη ονόματα των συγγραφέων, το ίδρυμα προέλευσης, τη διεύθυνση και το τηλέφωνο του υπευθύνου για την αλληλογραφία και τον καταμετρημένο αριθμό λέξεων.
Περίληψη: Παρουσιάζει τα κυριότερα σημεία της εργασίας. Δεν πρέπει να υπερβαίνει τις 250 λέξεις. Στο τέλος της παρατίθενται 3-10 λέξεις ευρετηρίου.
Αγγλική περίληψη: Παρουσιάζει σε συντομία την εργασία. Η έκτασή της είναι ως 400 λέξεις. Στην αρχή της γράφονται τα ονόματα των συγγραφέων και ο τίτλος της εργασίας στα αγγλικά.
Λέξεις-κλειδιά: έως 6 λέξεις κλειδιά.
Βιβλιογραφία: Οι βιβλιογραφικές παραπομπές αριθμούνται με αύξοντα αριθμό ανάλογα με τη σειρά εμφάνισής τους στο κείμενο (Vancouver). Όλες οι βιβλιογραφικές παραπομπές να αναφέρονται μέσα σε αγκύλες. Π.χ. Ο Smith [1] ανέφερε ότι ... και τα ευρήματα αυτά επιβεβαιώθηκαν από τον Adams και συν [2]. Αναγράφονται έως και οι 6 πρώτοι συγγραφείς. Στον πίνακα της βιβλιογραφίας περιλαμβάνονται μόνο εκείνες οι βιβλιογραφικές παραπομπές που αναφέρονται στο κείμενο και ο πίνακας συντάσσεται με αύξοντα αριθμό που αντιστοιχεί στη σειρά εμφάνισης των βιβλιογραφικών παραπομπών στο κείμενο π.χ.
Πίνακες: Γράφονται σε ξεχωριστή σελίδα, μετά το τέλος των βιβλιογραφικών αναφορών. Αριθμούνται με τη σειρά εμφάνισής τους στο κείμενο και συνοδεύονται από σύντομη επεξήγηση.
Εικόνες: Αποστέλλονται τα πρωτότυπα σχέδια ή φωτογραφίες καλής ποιότητας. Να υποβάλλονται σαν αρχεία εικόνας ξεχωριστά από το κείμενο του MS Word. Αριθμούνται με τη σειρά εμφάνισης στο κείμενο. Στο κείμενο θα πρέπει να υπάρχει σαφής παραπομπή στον τίτλο των ηλεκτρονικών αρχείων. Σε ξεχωριστή σελίδα αναγράφονται οι τίτλοι των εικόνων και οι τυχόν επεξηγήσεις.
Ιατρική Δεοντολογία: Σε περιπτώσεις ερευνών που αφορούν ανθρώπους, η έρευνα πρέπει να έχει γίνει με βάση τη διακήρυξη του Ελσίνκι (1975). Σε περιπτώσεις φωτογραφιών ασθενών, θα πρέπει να υπάρχει έγγραφη συγκατάθεση.

Συνοδευτικό έντυπο υποβαλλόμενης εργασίας

Θα πρέπει να συμπληρωθούν ΟΛΑ τα σημεία του εντύπου. Άλλη συνοδευτική επιστολή δεν είναι απαραίτητη.

Είδος άρθρου (σημειώστε μόνο ένα)

❑ Ερευνητική εργασία ❑ Βραχεία εργασία - ενδιαφέρον περιστατικό ❑ Ανασκόπηση

❑ Βραχεία ανασκόπηση ❑ Ειδικό άρθρο ❑ Γράμμα στη σύνταξη ❑ Νευρο-εικόνες

Τίτλος:

Υπεύθυνος για την αλληλογραφία συγγραφέας:

Διεύθυνση:

Τηλέφωνο: FAX: e-mail:

Επιβεβαιώστε την πληρότητα της υποβολής του χειρογράφου σας, σημειώνοντας ΟΛΑ τα παρακάτω σημεία

- ❑ Τίτλος του άρθρου στα Ελληνικά και στα Αγγλικά με μικρά γράμματα
- ❑ Ονόματα συγγραφέων στα Ελληνικά και στα Αγγλικά *(πλήρη ονόματα π.χ. Νικόλαος Παπαδόπουλος)*
- ❑ Κέντρο προέλευσης της εργασίας στα Ελληνικά και στα Αγγλικά
- ❑ Δομημένη περίληψη στα Ελληνικά και στα Αγγλικά
- ❑ Έως πέντε λέξεις ευρετηριασμού *(κατά προτίμηση από το MeSH Hellas-Βιοϊατρική Ορολογία)* στα Ελληνικά και στα Αγγλικά
- ❑ Όλα τα ονόματα των συγγραφέων στις βιβλιογραφικές παραπομπές (*μέχρι 6 και στη συνέχεια* «*και συν.*» *ή* «*et al*»)
- ❑ Η βιβλιογραφία στις τελευταίες σελίδες των άρθρων

Δήλωση

Δηλώνω υπεύθυνα ότι:

1. Όλοι οι συγγραφείς της εργασίας συμφωνούν με το περιεχόμενό της και με την υποβολή της στο περιοδικό: *Αρχεία Κλινικής Νευρολογίας.*
2. Το ίδιο κείμενο ή τα αποτελέσματα της εργασίας δεν έχουν υποβληθεί για δημοσίευση σε άλλο Ελληνικό ή ξένο περιοδικό.
3. Δηλώνω υπεύθυνα ότι δεν υπάρχει θέμα υποκλοπής πνευματικής ιδιοκτησίας (σε περίπτωση εικόνων, πινάκων ή υλικού από άλλες δημοσιεύσει έχει ζητηθεί και ληφθεί η νόμιμη άδεια η οποία και συνυποβάλλεται).
4. Δεν υπάρχουν θέματα σύγκρουσης συμφερόντων – σε περίπτωση εξωτερικής χρηματοδότησης αυτό θα πρέπει να αναφέρεται στο τέλος της εργασίας.

Ο υπεύθυνος για την αλληλογραφία συγγραφέας

(υπογραφή)