FLUID BIOMARKERS IN PARKINSON'S DISEASE

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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

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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.

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