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Modern Directions in Early Biomarker Diagnostics of Parkinson's Disease – From Molecular Information Carriers to Non-Invasive Tissue Tests

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Abstract

Background: Parkinson's disease (PD) is the fastest-growing neurological disorder globally. Traditional diagnosis, which relies on motor symptoms, is associated with a 15–24% error rate and occurs after significant loss of dopaminergic neurons. Early biomarker identification is essential for biological redefinition of PD and timely intervention. **Objective**: To synthesize evidence from the past five years on minimally invasive biomarkers and assess whether multimodal panels offer greater diagnostic precision than single tests. **Methods**: A systematic review of PubMed and Scopus (January 2021–January 2026) was conducted per PRISMA guidelines, including meta-analyses and original studies on α-synuclein seed amplification assays (SAA), neuronally derived extracellular vesicles (LIEVs), neurofilament light chain (NfL), and advanced imaging (OCT-A, MRI). **Results**: SAA exhibited 96% sensitivity in GBA1 mutation carriers and 98.6% in hyposmic individuals with dopaminergic deficits, but only 67.5% in LRRK2 carriers. Serum LIEV-associated α-synuclein distinguished prodromal cases with high precision (AUC=0.91). NfL predicted functional decline (OR=2.54) up to five years before diagnosis. Advanced neuroimaging and skin RT-QuIC tests showed diagnostic accuracies from 82% to 100%. **Conclusion**: PD diagnosis is shifting from clinical observation to biological profiling. Multimodal biomarker panels integrating molecular, microvascular, and imaging data provide the most precise risk stratification, enabling timely, targeted neuroprotective interventions by rehabilitation specialists.

Keywords: Parkinson's disease, Early biomarkers, Alpha-synuclein, Neurodegeneration, Dopaminergic imaging

INTRODUCTION

Parkinson's disease (PD) is currently the fastest-growing neurological disorder globally and the second most prevalent neurodegenerative disease (Dorsey & Bloem, 2024; Tolosa et al., 2021). Projections indicate that its worldwide prevalence will double within the next generation (Dorsey & Bloem, 2024). The pathological hallmark of PD is the progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta, accompanied by intracellular protein aggregates known as Lewy bodies, whose principal component is misfolded α-synuclein (Bloem et al., 2021; Tolosa et al., 2021). According to Braak's hypothesis, these pathological processes originate in peripheral sites - such as the olfactory bulb and the enteric nervous system - and then propagate in a caudo-rostral manner to the brainstem and cortex (Braak & Del Tredici, 2024; Tolosa et al., 2021). This model accounts for a prolonged prodromal phase during which non-motor symptoms - including hyposmia, constipation, or depression - may precede the onset of motor manifestations by decades (Heinzel et al., 2024; Tolosa et al., 2021).

Despite advances in medicine, the routine diagnosis of PD continues to rely primarily on identifying motor symptoms (bradykinesia, resting tremor, rigidity), a method associated with a diagnostic error rate of 15–24% (Tolosa et al., 2021). This challenge stems from the clinical overlap between early PD and atypical Parkinsonian syndromes - such as multiple system atrophy (MSA), progressive supranuclear palsy (PSP), and corticobasal syndrome (CBS - despite their distinct pathophysiologies and prognoses (Höglinger et al., 2024; Tolosa et al., 2021). Crucially, by the time clinical diagnosis is reached, 50–70% of dopaminergic neurons have typically been lost, severely restricting the efficacy of potential disease-modifying therapies (Mitchell et al., 2021; Tolosa et al., 2021). At present, the most robust clinical predictor of progression to overt synucleinopathy is isolated REM sleep behavior disorder (iRBD), with a greater than 80% risk of developing PD, MSA, or dementia with Lewy bodies within 12 years (Iranzo et al., 2024; Tolosa et al., 2021).

Modern neurology seeks to reconceptualize PD as a biological entity defined by objective biomarkers rather than purely clinical features (Siderowf et al., 2023). However, this transformation is complicated by the profound molecular heterogeneity of the disease (Montero-Calle et al., 2025; Siderowf et al., 2023). Evidence from the PPMI cohort shows that α-synuclein seed amplification assays (SAA) achieve nearly 96% sensitivity in GBA mutation carriers but only 67.5% in those with LRRK2 mutations, highlighting distinct biotypes within

the same clinical syndrome (Siderowf et al., 2023). Other promising strategies include studying neuronally derived extracellular vesicles (LIEVs) and assessing protein seeding in accessible tissues such as skin and saliva (Chahine et al., 2024; Wang et al., 2024). Additionally, neuroinflammatory processes - particularly activation of the NLRP3 inflammasome - lead to elevated cerebrospinal cytokine levels (IL-1β, TNF-α) and are increasingly recognized as diagnostic adjuncts (Gao et al., 2025). Non-invasive retinal microvascular assessment using optical coherence tomography angiography (OCT-A) further expands the repertoire of biomarkers (Kashani et al., 2023).

The aim of this study is to synthesize scientific literature from the past five years on early PD biomarkers, with a particular focus on minimally invasive methods, which are critical for early patient stratification and optimizing physiotherapeutic interventions.

MATERIALS AND METHODS

2.1. Search Strategy and Qualification Criteria

A systematic literature review was conducted in accordance with PRISMA guidelines (Page et al., 2021) to synthesize current knowledge on early biomarkers of Parkinson's disease (PD). Electronic searches were performed in PubMed and Scopus, focusing on publications from January 2021 to January 2026. The search strategy incorporated keywords such as Parkinson’s disease, genetic markers, imaging markers, and prodromal markers. Search syntax was optimized using Boolean operators tailored to each database, with specific strings including: ("Parkinson's disease" or "Parkinson disease") and ("biomarkers" or "markers") and ("genetics" or "GBA" or "LRRK2") and ("imaging" or "MRI" or "SPECT" or "PET" or "OCT-A") AND ("prodromal" or "preclinical" or "premotor" or "iRBD" or "REM sleep behavior disorder"). The specified timeframe (2021–2026) was strictly applied during digital filtering to capture the most recent advances in fluid, tissue, and neuroimaging diagnostics. Reference lists of all relevant reviews and meta-analyses were manually screened using a secondary snowballing approach to identify additional high-impact studies that may have been missed in the primary search.

2.2. Inclusion and Exclusion Criteria

Meta-analyses and original research articles published in English or Polish, addressing the diagnosis of the prodromal phase of PD, were included. Exclusion criteria encompassed editorial articles, case reports, conference abstracts, studies published before January 2021, and animal studies lacking direct translational relevance to human diagnostics. To maintain high clinical evidence and statistical power, original articles were limited to prospective cohort studies, multicenter cross-sectional investigations, and rigorously characterized diagnostic accuracy studies involving human subjects with documented prodromal states (e.g., idiopathic REM sleep behavior disorder, severe hyposmia, or pathogenic mutations such as GBA1 or LRRK2). Studies were excluded if they lacked clearly defined control cohorts, omitted standardized reference standards for clinical validation (e.g., DaT-SPECT or quantitative clinical assessment scales), or presented only composite biomarker results without isolated statistical indices (e.g., sensitivity, specificity, area under the curve, odds ratios). In vitro studies focused solely on biochemical kinetics, without clinical biofluid or tissue specimen validation, were also excluded to ensure translational relevance.

2.3. Data Selection and Quality Analysis

The review process comprised two stages: initial screening of titles and abstracts, followed by full-text evaluation for relevance to study objectives. Identified biomarkers were then categorized according to methodological criteria outlined in the Results section. During preliminary screening, two independent reviewers assessed records against predefined eligibility criteria, achieving high inter-rater reliability. Disagreements were resolved by consensus or, if necessary, consultation with a senior third reviewer. Data extraction employed standardized electronic templates to collect key variables, including author, year, cohort characteristics (e.g., PPMI, Oxford Discovery), biomarker measurement platforms (e.g., Simoa for NfL, RT-QuIC for SAA), and detailed performance metrics. The methodological quality and risk of bias of included diagnostic accuracy studies were rigorously appraised using criteria based on the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool (Whiting et al., 2011), with focused attention on patient selection, index test execution, reference standard reliability, and the flow and timing of patient assessments to ensure robust overall validity.

RESULTS – REVIEW OF DIAGNOSTIC METHODS

3.1. α-Synuclein Seed Amplification Assays (SAA)

Innovative α-synuclein seed amplification assays (SAA) demonstrate nearly 96% sensitivity in individuals with GBA mutations, while sensitivity drops to 67.5% in LRRK2 mutation carriers (Siderowf et al., 2023). In subgroups presenting with a hyposmic phenotype and documented dopamine transporter (DaT) deficit, these assays achieve the highest diagnostic efficacy (Siderowf et al., 2023). SAA, which includes techniques such as real-time quaking-induced conversion (RT-QuIC) and protein misfolding cyclic amplification (PMCA), represents a fundamental shift toward molecular quantification of synucleinopathy (Siderowf et al., 2023; Wang et al., 2024). In clinical populations with objective olfactory loss (UPSIT score ≤ 25), SAA achieves a remarkable sensitivity of 98.6%, establishing a direct molecular link between peripheral autonomic dysfunction and central protein aggregation kinetics (Siderowf et al., 2023). Conversely, reduced sensitivity in asymptomatic or motor-manifest LRRK2 (specifically G2019S) mutation carriers suggests a distinct pathological mechanism, indicating that dopaminergic cell loss may proceed through pathways less reliant on widespread fibrillar macro-aggregates of α-synuclein, thereby demonstrating the biological heterogeneity of the Parkinsonian phenotype (Siderowf et al., 2023).

3.2. Neuronally Derived Extracellular Vesicles (LIEVs)

Analysis of neuronally derived extracellular vesicles (LIEVs) isolated from serum enables identification of prodromal cases (iRBD) with high precision (AUC = 0.91) (Yan et al., 2023). The concentration of α-synuclein in LIEVs correlates with time to phenoconversion (Chahine et al., 2024; Iranzo et al., 2024). Systematic stratification of CNS-enriched extracellular vesicles from peripheral blood reveals that vesicle cargo concentrations provide a highly reproducible diagnostic platform (Chahine et al., 2024). Quantitative immunoassays targeting α-synuclein within immunocaptured L1CAM- or NCAM-positive neural vesicles show statistically significant elevations in prodromal cohorts compared to age-matched controls (95% CI: 0.86–0.96) (Yan et al., 2023). Longitudinal data indicate that higher concentrations of oligomeric α-synuclein within these vesicles predict shorter intervals before progression from isolated sleep

disturbances to overt motor synucleinopathies, providing a reliable systemic proxy for central pathogenic progression (Chahine et al., 2024; Iranzo et al., 2024).

3.3. Neurofilament Light Chain (NfL)

Serum NfL is an important biomarker of axonal neurodegeneration (Halloway et al., 2022). A doubling of NfL concentration is associated with a 2.54-fold increase in PD risk (OR = 2.54; 95% CI: 1.70–3.81), and this marker is detectable more than five years before clinical diagnosis (Halloway et al., 2022). Statistical modeling in large prospective cohorts establishes serum NfL as a critical index of neurodegenerative velocity (Halloway et al., 2022). Beyond predicting incidence, incremental rises in circulating NfL exhibit a dose-dependent relationship with accelerated motor and functional decline (Halloway et al., 2022). Individuals in the highest NfL quartiles (>37.3 pg/mL) experience a significantly faster annual deterioration score (0.15 units) across standardized physical assessments, including timed gait, tandem coordination, and chair-stand protocols, underscoring NfL's value as a direct macromolecular measure bridging subclinical neurodegeneration and functional motor impairment (Halloway et al., 2022).

3.4. Imaging Biomarkers

3.4.1. Magnetic Resonance Imaging (MRI)

Free-water imaging: Increased free water in the posterior substantia nigra (SN) is detectable as early as the iRBD stage and correlates with early PD progression (Mitchell et al., 2021).

Neuromelanin-sensitive MRI: Reduced signal in the SN and locus coeruleus identifies the prodromal phase with 82.5% sensitivity (Mitchell et al., 2021).

Nigrosome-1 (SWI/QSM): Loss of the nigrosome-1 signal differentiates PD from controls with an accuracy of 94–98% (Mitchell et al., 2021). Advanced diffusion imaging sequences mapping the extracellular fluid compartment show that free-water elevation within the ventral and posterior substantia nigra tracks early tissue changes (Mitchell et al., 2021). This shift reflects active microglial activation and breakdown of the surrounding cellular matrix (Mitchell et al., 2021). Neuromelanin-sensitive sequences quantify the progressive depigmentation of catecholaminergic nuclei, revealing significant reductions in SN and locus coeruleus signal

during preclinical phases (sensitivity 82.5%, specificity 81.0%) (Mitchell et al., 2021). When combined with susceptibility-weighted imaging (SWI) or quantitative susceptibility mapping (QSM), the loss of hyperintense nigrosome-1 structure yields diagnostic accuracy of up to 98% for distinguishing early idiopathic Parkinsonism from normal aging (Mitchell et al., 2021).

3.4.2. Nuclear Medicine (PET/SPECT)

Dopamine transporter deficit (DAT-SPECT) is present in approximately 50% of iRBD patients before motor symptoms emerge (Iranzo et al., 2024; Mitchell et al., 2021). Metabolic imaging (FDG-PET) identifies the Parkinson's Disease-Related Pattern (PDRP) (Mitchell et al., 2021). Molecular neuroimaging with single-photon emission computed tomography (SPECT) that tracks the presynaptic dopamine transporter (DAT) confirms that spatial asymmetry and a quantitative reduction in striatal ligand binding are prominent in the late prodromal state (Mitchell et al., 2021). Roughly half of iRBD individuals demonstrate putaminal DAT deficits years before clinical motor criteria are met (Iranzo et al., 2024; Mitchell et al., 2021). FDG-PET spatial covariance analysis consistently identifies PDRP - a network defined by hypermetabolism in the globus pallidus, putamen, pons, and motor cortex, and hypometabolism in the posterior parietal and occipital areas - serving as a validated metabolic marker for systemic disease progression (Mitchell et al., 2021).

Table 1. Biomarkers of the Central Nervous System (Imaging and Fluid Analysis)

Biomarker	**Sample Source**	**Disease Stage**	**Sensitivity/ Specificity**	**Key Advantage**
DAT-SPECT	Brain Imaging	Early Clinical	High / High	Validated, detects dopaminergic deficit
Dopaminergic PET	Brain Imaging	Early Clinical	Very High / High	Quantitative accuracy

Biomarker	Sample Source	Disease Stage	Sensitivity/ Specificity	Key Advantage
Neuromelanin MRI	Brain Imaging	Prodromal/ Early	Mod / Mod	Non-invasive, SN/LC changes
QSM/Free-water MRI	Brain Imaging	Prodromal/Early	Mod / Mod	Detects iron/structural collapse
Total α-synuclein	CSF	Early Clinical	Variable	Ease of measurement
Oligomeric alpha-synuclein	CSF	Early Clinical	Mod / High	Disease-specific

3.5. Non-Invasive Tissue Markers (Skin and Saliva)

RT-QuIC skin tests exhibit 94% sensitivity and 98% specificity for PD diagnosis (Wang et al., 2024). In saliva, total α-synuclein is significantly decreased, while oligomeric forms are elevated (Chahine et al., 2024). Ultrastructural tissue profiling by cutaneous biopsy reveals that misfolded α-synuclein (αSynP) seeding activity can be detected with very high diagnostic accuracy in peripheral autonomic neural networks (Wang et al., 2024). Dermal punch biopsies from cervical and lower extremities, analyzed with optimized RT-QuIC amplification, yield a sensitivity of 94% and specificity of 98%, reaching 100% accuracy at sites near the brainstem (e.g., upper neck, occipital region) (Wang et al., 2024). Salivary analysis reveals a distinct biochemical shift: physiological α-synuclein monomers are markedly reduced, whereas oligomeric and phosphorylated forms are significantly elevated relative to healthy controls (Chahine et al., 2024).

Table 2. Peripheral Tissue and Molecular Seeding Markers

Biomarker	Sample Source	Disease Stage	Sensitivity/ Specificity	Key Advantage
RT-QuIC / SAA	CSF, Skin, Saliva	Prodromal/ Early	Very High ($>90\%$)	Gold standard for protein seeding
Phospho-alpha-syn	Skin Biopsy	Prodromal/ Early	High / High	Detects peripheral pathology
Salivary Gland Biopsy	Salivary Tissue	Prodromal/ Early	Mod / High	High periphery fidelity
Genetic Variants	Blood (DNA)	Pre/Prodromal	Variable	Stratifies high-risk groups

3.6. Systemic and Functional Clinical Indicators

To achieve comprehensive early detection, non-specific neurodegenerative biomarkers, clinical sleep-olfactory phenotypes, and emerging exploratory omics platforms must be integrated into diagnostic frameworks (Gao et al., 2025; Halloway et al., 2022; Kashani et al., 2023). The operational characteristics of systemic and functional markers are summarized in Table 3. Clinical surveillance during the prodromal phase demonstrates that early identification cannot rely solely on brain-derived structural markers; systematic functional measurements must also be included to achieve optimal accuracy (Mitchell et al., 2021; Tolosa et al., 2021).

Serum neurofilament light chain (NfL) is a sensitive biomarker of advanced axonal degeneration in long-projecting motor tracts (Halloway et al., 2022). Longitudinal prospective

studies indicate that dynamic tracking of systemic NfL changes provides a real-time proxy for ongoing structural damage and impending loss of physical autonomy (Halloway et al., 2022). At the same time, peripheral biofluid approaches - such as blood-based metabolomics and exploratory proteomics - are emerging as highly sensitive, non-invasive tools for constructing cross-disciplinary multi-marker panels (Siderowf et al., 2023).

While isolated inflammatory proteins such as interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and high-sensitivity C-reactive protein (hs-CRP) lack sufficient diagnostic specificity due to systemic confounders, their aggregation into composite inflammatory indices shows a significant predictive correlation with accelerated motor decline and early structural deterioration (Gao et al., 2025).

Additionally, validated clinical and behavioral phenotypes offer powerful, non-invasive predictors of imminent neurodegeneration (Iranzo et al., 2024). Isolated REM sleep behavior disorder (iRBD), verified by comprehensive multi-channel video-polysomnography, remains the strongest individual clinical predictor of phenoconversion, conferring a risk exceeding 80% over twelve years of follow-up (Iranzo et al., 2024). When combined with quantitative olfactory testing - such as the University of Pennsylvania Smell Identification Test (UPSIT) or Sniffin' Sticks protocols - the resulting clinical-functional matrix significantly enhances diagnostic confidence (Siderowf et al., 2023).

These accessible, cost-effective operational tools capture early disturbances in CNS homeostasis, providing a critical, multi-tiered screening strategy for patient stratification and the timely initiation of targeted prehabilitative interventions (Heinzel et al., 2024; Siderowf et al., 2023).

Table 3. Systemic, Functional, and Emerging Markers

Biomarker	**Sample Source**	**Disease Stage**	**Sensitivity/ Specificity**	**Key Advantage**
Neurofilament (NfL)	Blood / CSF	Early Clinical	Mod / Mod	Indices of axonal decay
Inflammatory Profile	Blood	Prodromal/ Early	Low / Low	Registers systemic inflammation
Hyposmia (UPSIT)	Clinical Test	Prodromal	High / Low	Simple, inexpensive
RBD (olysomnography	Sleep/ Clinical	Prodromal	Very High / Mod	Strong predictor
Omics Profiles	Blood / CSF	Experimental	Emerging	Biomarker panel potential

DISCUSSION

4.1. Interpretation of Main Results and Biological Heterogeneity

This review demonstrates that the field of Parkinson's disease (PD) diagnostics is rapidly advancing toward a multidimensional biological redefinition (Siderowf et al., 2023). A key finding is the exceptional sensitivity of α-synuclein seed amplification assays (SAA) in the prodromal phase, achieving nearly 98.6% in patients with hyposmia and confirmed dopaminergic deficits (Siderowf et al., 2023) - a substantial improvement over traditional

clinical criteria, which carry a diagnostic error rate of up to 24% (Tolosa et al., 2021). This evidence suggests that SAA testing may emerge as a new "gold standard" for early patient stratification, especially in GBA1 mutation subgroups, where sensitivity approaches 96% (Siderowf et al., 2023).

Mechanistically, the outstanding accuracy of SAA in hyposmic individuals highlights the strong spatiotemporal link between olfactory dysfunction and early-stage Lewy body pathology (Braak & Del Tredici, 2024; Chahine et al., 2024). Misfolded protein seeds propagate from the olfactory bulb along olfactory tracts, accumulating in these tissues before widespread cortical involvement (Braak & Del Tredici, 2024). This anatomical alignment explains the near-perfect correlation between severe sensory deficits and positive seeding activity (Siderowf et al., 2023). The use of real-time quaking-induced conversion (RT-QuIC) techniques now enables clinicians to bypass reliance on overt motor symptoms, shifting diagnosis from a reactive to a proactive, biology-driven approach (Wang et al., 2024).

However, comparison with the literature reveals marked biological heterogeneity in PD (Montero-Calle et al., 2025; Siderowf et al., 2023). For example, SAA sensitivity in LRRK2 mutation carriers drops to 67.5% (Siderowf et al., 2023), suggesting that protein aggregation pathways differ by genetic background and necessitate a personalized diagnostic strategy (Montero-Calle et al., 2025). A lower rate of positive SAA results may reflect a reduced pathological burden of α-synuclein in certain biotypes (Siderowf et al., 2023). LRRK2-associated Parkinsonism may follow distinct pathogenic routes, such as LRRK2 kinase hyperactivation, disrupted endolysosomal trafficking, and direct mitochondrial impairment (Siderowf et al., 2023; Montero-Calle et al., 2025). Thus, a negative SAA result in a pathogenic LRRK2 carrier does not rule out prodromal PD, highlighting the need for non-synuclein biomarkers to avoid false negatives in genetically distinct groups (Montero-Calle et al., 2025).

4.2. Innovation of Tissue and Fluid Diagnostics

Emerging diagnostics based on peripheral tissues and extracellular vesicles are highly promising. The 100% sensitivity of RT-QuIC in skin biopsies (occipital region) confirms that α-synuclein pathology is systemic and detectable peripherally well before brainstem involvement (Wang et al., 2024). Combining this with neuronally derived extracellular vesicle

(LIEV) analysis, which identifies iRBD cases with high precision (AUC = 0.91), enables the concept of a comprehensive "fluid biological profile" (Chahine et al., 2024).

Dermal biopsies offer unique structural value, as small autonomic nerve fibers in the skin serve as accessible reservoirs of misfolded α-synuclein (Wang et al., 2024). These tissues mirror central pathology, providing a minimally invasive window into the brain's disease state (Wang et al., 2024). LIEV isolation represents a leap in "liquid biopsy" technology, with CNS-specific vesicles carrying protected molecular cargo (Chahine et al., 2024). The quantitative relationship between vesicular protein levels and phenoconversion enables dynamic, real-time disease tracking, long before neuroimaging reveals brain damage (Iranzo et al., 2024; Mitchell et al., 2021). Evaluation of dermal nerve architecture also tracks small-fiber denervation rates, paralleling central dopamine decline (Wang et al., 2024).

4.3. NfL in Physical Performance Prediction and Prehabilitation

Incorporating neurofilament light chain (NfL) into diagnostic panels provides new opportunities for early patient stratification in rehabilitation (Halloway et al., 2022). NfL offers unique insight into the pace of axonal degeneration and associated physical decline. Notably, increased NfL can predict functional loss five years prior to overt motor symptoms (Halloway et al., 2022), and levels above 25.4 pg/mL may serve as objective triggers for early prehabilitation (Halloway et al., 2022).

Unlike α-synuclein, which indexes a specific proteinopathy, serum NfL is a highly sensitive, non-specific indicator of ongoing axonal injury in long-projecting motor and cortical tracts (Halloway et al., 2022). As neurodegeneration accelerates, neuronal structural integrity is lost, releasing neurofilament proteins into the bloodstream (Halloway et al., 2022). Tracking the trajectory of NfL elevation provides a valuable timeline for intervention. Biomechanically, degradation of corticospinal tracts and basal ganglia loops impairs motor recruitment and force development (Halloway et al., 2022), manifesting as early bradykinesia and gait variability. Identifying a biological threshold for intervention enables the initiation of neuro-prehabilitation - combining high-intensity aerobic training, sensorimotor dual-tasking, and coordination exercises - to build functional reserve and delay disability (Halloway et al., 2022).

4.4. Multimodal Approach and the Role of Neuroinflammation

The data underscore the necessity for a multimodal diagnostic approach. While PET/SPECT is critical for confirming dopaminergic deficit, advanced MRI (free water, QSM) provides detailed insight into neurodegenerative dynamics (Mitchell et al., 2021). Analysis of the NLRP3 inflammasome reveals that neurodegeneration is closely tied to immune activation (IL-1β, TNF-α), which can precede classical synuclein pathology (Gao et al., 2025).

This early neuroinflammatory cascade, marked by NLRP3 activation in microglia, creates a toxic microenvironment that disrupts the blood-brain barrier and promotes α-synuclein misfolding and spread (Gao et al., 2025). Microvascular changes can be non-invasively tracked using optical coherence tomography angiography (OCT-A), where reduced retinal capillary density in prodromal states mirrors central microangiopathy (Kashani et al., 2023). By integrating inflammatory markers, microvascular imaging, and advanced neuroimaging, a robust diagnostic matrix is achieved, capturing the full spectrum of proteinopathy, microvascular collapse, and immune dysregulation in early PD (Gao et al., 2025; Kashani et al., 2023; Mitchell et al., 2021).

4.5. Limitations and Future Research Directions

Despite the high value of the studies analyzed, important limitations remain (Siderowf et al., 2023). Most cohorts are composed of White individuals, limiting generalizability (Siderowf et al., 2023). Additionally, widespread adoption of technologies such as SAA and LIEV isolation will require standardized protocols and cost reduction (Siderowf et al., 2023).

To address these challenges, future research should prioritize inclusion of multi-ethnic cohorts to validate biomarkers across diverse populations (Siderowf et al., 2023). The absence of universally accepted technical guidelines hinders reproducibility for sensitive assays like RT-QuIC and vesicle isolation (Wang et al., 2024). Standardizing biospecimen collection, optimizing tissue sampling, and automating workflows are critical next steps. Overcoming these operational barriers will facilitate the transition of molecular diagnostics from specialized laboratories to routine clinical use (Siderowf et al., 2023).

SUMMARY AND CONCLUSIONS

Contemporary Parkinson's disease (PD) diagnostics are moving decisively toward biological patient profiling (SAA, LIEVs, OCT-A, NfL), rather than relying solely on clinical symptom assessment (Siderowf et al., 2023). This paradigm shift is critical, as motor scales only detect disease after extensive, irreversible dopaminergic loss has occurred (Mitchell et al., 2021; Tolosa et al., 2021). Adopting molecular-driven frameworks enables clinicians to identify the onset of synucleinopathy at a subclinical stage, allowing for proactive rather than reactive intervention (Bloem et al., 2021; Siderowf et al., 2023).

α-Synuclein seed amplification assays (SAA) are essential for early stratification, demonstrating the highest efficacy in individuals with hyposmia (Chahine et al., 2024; Siderowf et al., 2023). Amplification of misfolded protein seeds from biofluids or peripheral tissues offers an objective measure that reliably distinguishes prodromal synucleinopathies from atypical Parkinsonian syndromes and non-degenerative mimics (Höglinger et al., 2024; Siderowf et al., 2023). This biological specificity renders SAA indispensable for forming mutation-specific cohorts for targeted clinical trials (Montero-Calle et al., 2025; Siderowf et al., 2023).

Serum NfL is a sensitive biomarker that predicts the rate of functional decline years before diagnosis (Halloway et al., 2022). Whereas protein aggregation assays index disease presence, NfL quantifies the velocity of axonal destruction and structural decay (Halloway et al., 2022). Monitoring NfL levels provides clinicians with a dynamic, real-time proxy for predicting structural damage and loss of physical autonomy (Halloway et al., 2022).

Integrating neuroinflammatory markers (NLRP3 pathway) with microvascular imaging (OCT-A) enables a comprehensive assessment of early disturbances in CNS homeostasis (Gao et al., 2025; Kashani et al., 2023). By combining fluid indicators of microglial activation (e.g., IL-1β, TNF-α) with high-resolution retinal capillary density metrics, clinicians can map the full prodromal pathophysiological landscape, capturing proteinopathy, immune dysregulation, and microvascular collapse simultaneously (Gao et al., 2025; Kashani et al., 2023; Mitchell et al., 2021).

Practical Applications: Early identification of biological markers (e.g., NfL above 25.4 pg/mL) allows physiotherapists and sports medicine specialists to implement targeted physical activity

programs - such as aerobic training and dual-tasking - when neuronal reserve remains, significantly delaying motor disability onset (Halloway et al., 2022). Detecting elevated axonal or microvascular markers during the prodromal phase creates a therapeutic window for neuro-prehabilitation (Halloway et al., 2022; Kashani et al., 2023), harnessing preserved neuroplasticity before irreversible motor symptoms appear (Bloem et al., 2021; Halloway et al., 2022).

Physiotherapy teams can act on these biological alerts by shifting from generic exercises to highly specialized, neuroprotective regimens (Bloem et al., 2021). High-intensity aerobic training (e.g., treadmill or cycling at 70–80% HRmax) should be deployed, as this intensity upregulates brain-derived neurotrophic factor (BDNF), boosts mitochondrial efficiency, and may suppress the NLRP3 inflammasome cascade (Gao et al., 2025). Early integration of complex sensorimotor dual-tasking - combining challenging motor patterns with cognitive demands - strengthens cognitive and movement reserves (Halloway et al., 2022), reinforcing neural networks and buffering the motor system against future neurodegenerative decline (Halloway et al., 2022; Kashani et al., 2023).

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