IMAGING BIOMARKERS IN PARKINSON'S DISEASE

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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

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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

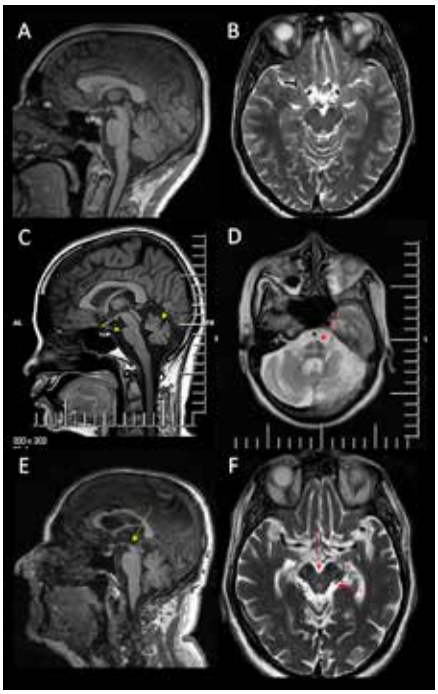

Figure 1. Classic MRI.in the differential diagnosis of idiopathic Parkinson's Disease A, B: Normal brain. C, D: Patient with Multiple System Atrophy. Cerebellar and pontine atrophy are visible in image C (yellow arrows). The "hot cross bun sign" can bre seen in image D. This sign is produced by a selective 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)

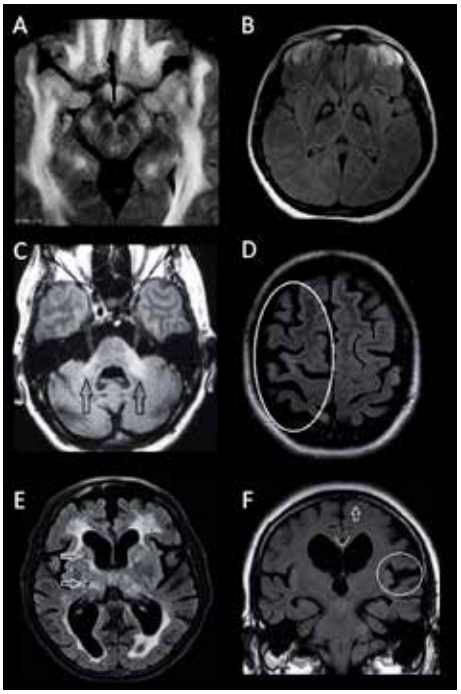

Figure 2. Typical images in various syndromes that share clinical features with Parkinson's disease.
Image A: The "face of the giant Panda" in Wilson's disease. This image, in T2 sequence, is a result of high signal intensity of the midbrain tegmentum with normal hypointense red nuclei forming the eyes, 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.

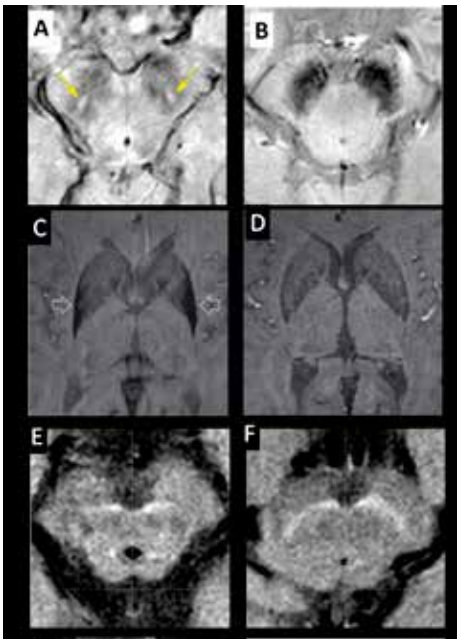

Figure 3. Advanced MRI techniques used in the diagnosis and follow up of parkinsonism.
Images A and B Iron sensitive SWI. The Dorsal Nigral Hyperintensity (DNH) is visible bilaterally in the brain of a healthy subject (Image A, arrows) but not in a patient with parkinsonism, due to accumulation of iron.
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)

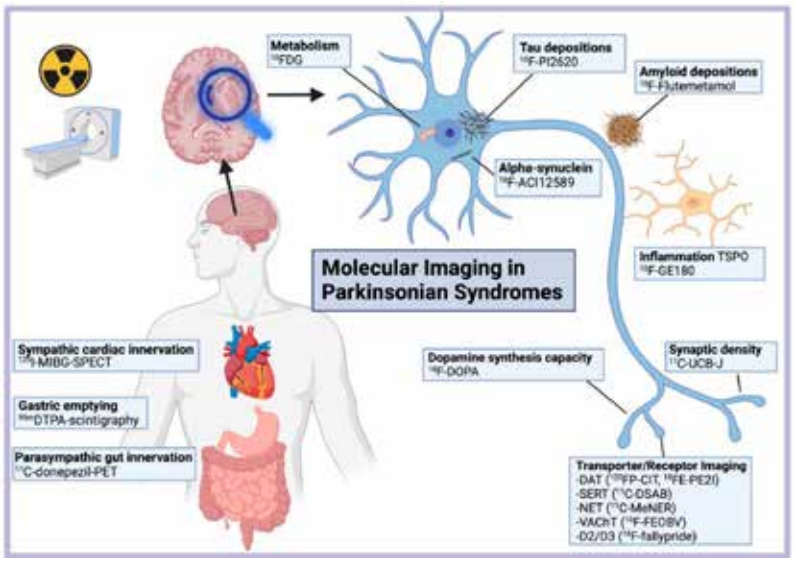

Figure 4. Overview of the targets and tracers for molecular imaging in movement disorders. Source: Prange et al. (2022) [12].

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.

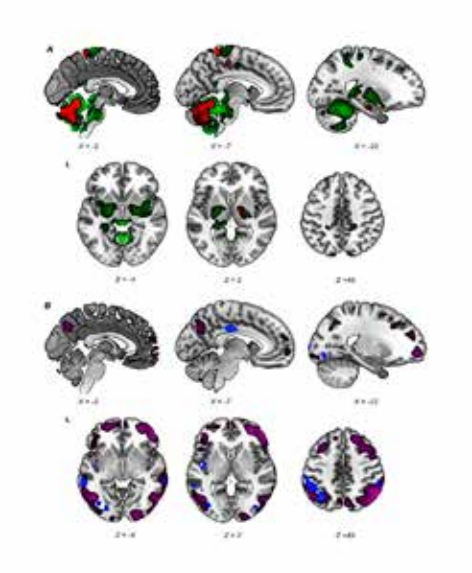

Figure 5. FDG-PET in PD, depicting brain metabolism. A specific Parkinson's Disease Related Pattern (PDRP) can be identified, with increased metabolic activity in the globus pallidus, putamen, thalamus, pons, cerebellum and motor cortex (green areas) along with decreased activity in the lateral cortex 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)

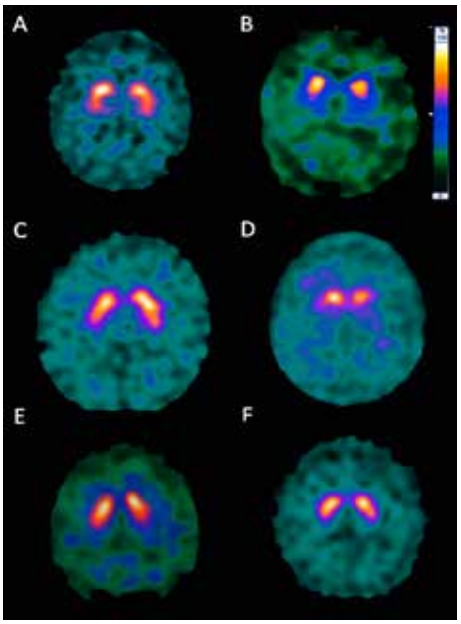

Figure 6. DaT scan for the diagnosis of degenerative parkinsonism
Image A. Man 56 years old, 15-y history of bilateral action tremor. Normal DaT scan. Diagnosis: Essential tremor (ET)
Image B. Man 72y, 1 year history of bradykinesia. Frequent falls. Poor response to levodopa. DaT scan: Bilateral degenerative 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)

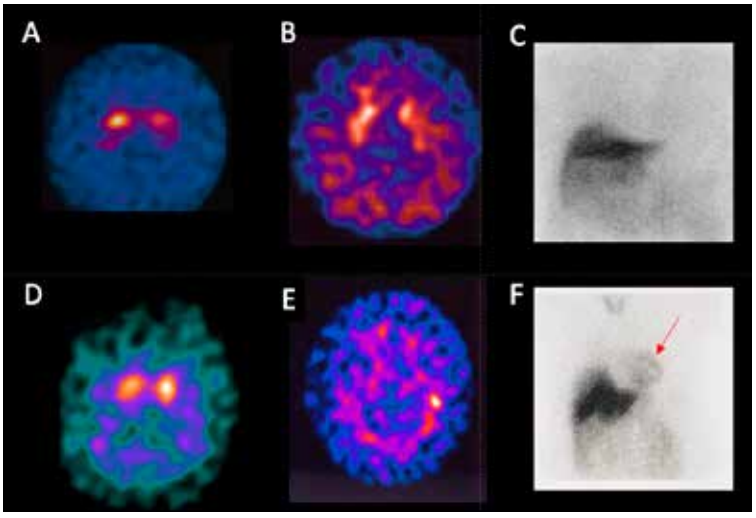

Figure 7. Differential diagnosis of idiopathic Parkinson's Disease from Parkinson Plus using D2 imaging (IBZM) and cardiac scintigraphy (MIBG) that identifies sympathetic aponeurosis of the heart.
Upper row: Patient with Parkinson's Disease. Lower row: Patient with Multiple System Atrophy (MSA). Images 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

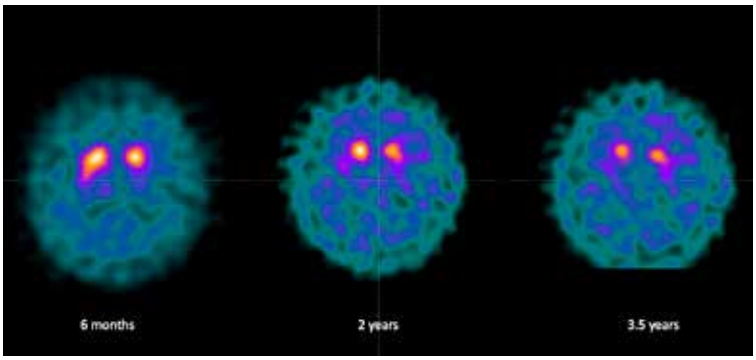

Figure 8. Serial DaT scans in a patient with Parkinson's Disease. Images are taken 6 months, 2 years and 3,5 years after the appearance of the symptoms. In has been observed that striatal dopaminergic markers follow an exponential decline during the first two years followed by a slower decline in the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

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Figure 1.

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

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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

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