Thesis

The Role of Neurofilament Light Chain (NfL) as a Biomarker in Parkinson ‘s Disease

submitted by

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Graz, 08.09.2025 Timur Günes m.p.

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Zusammenfassung

Ziel

Ziel dieser Studie war es, den Zusammenhang zwischen Serum-Neurofilament light chain (NfL)-Werten und klinischen Parametern zu untersuchen sowie den diagnostischen und prognostischen Wert zu ermitteln, um zwischen Parkinson-Krankheit (PK) und gesunden Probandinnen und Probanden differenzieren zu können. Ergänzend wurde eine systematische Literaturrecherche zum aktuellen Forschungsstand zu NfL und der PK durchgeführt. Für die Analyse wurden Daten aus der Prospektive Datenbank für Bewegungsstörungen (PROMOVE, Graz, 2010), einer prospektiven Langzeitstudie, verwendet und mit den Ergebnissen der systematischen Literaturrecherche verglichen.

Methoden

Unsere Studie umfasste 93 Teilnehmende, darunter 46 PK erkrankte Patientinnen/Patienten (PK-Gruppe) aus der PROMOVE-Datenbank und 47 gesunde Personen (HC-Gruppe) aus der Austrian Stroke Prevention Family Study (ASPS-Family). Die Serum-NfL-Werte wurden mit dem Simoa HD-1 Analyzer (Quanterix, NF-Light™ Advantage Assay-Kit) gemessen. Zu Studienbeginn erfolgten klinische und bildgebende Untersuchungen des Gehirns. Im Verlauf der Studie erhielten Personen mit PK bis zu sechs Jahre lang jährliche klinische Untersuchungen.

Ergebnisse

Die NfL-Werte waren zu Studienbeginn und im Verlauf signifikant höher in der PK-Gruppe im Vergleich zur HC-Gruppe. Im longitudinalen Verlauf stiegen die NfL-Werte in der PD-Gruppe stärker an als in der HC-Gruppe, insbesondere bei männlichen Teilnehmenden unserer Kohorte. In der PK-Gruppe zeigte sich keine signifikante Korrelation zwischen Mini-Mental State Examination (MMSE)- und NfL-Werten. In der HC-Gruppe hingegen waren höhere NfL-Ausgangswerte prädiktiv für niedrigere MMSE-Werte bei der Follow-up-Untersuchung. Zudem zeigte sich bei der Follow-up-Untersuchung in der HC-Gruppe eine negative Korrelation zwischen den NfL-Werten und den MMSE-Werten. Wiederum zeigte die neuropsychologische Testbatterie Consortium to Establish a Registry for

Alzheimer's Disease (CERAD), dass in der PK-Gruppe höhere NfL-Ausgangswerte prädiktiv für niedrigere globale kognitive Leistungen bei der Follow-up-Untersuchung waren. Im Unterschied dazu zeigten die NfL-Werte zu Studienbeginn und im Verlauf keinen Zusammenhang mit motorischer Beeinträchtigung oder der Progression motorischer Symptome und hatten keinen prädiktiven Wert für den motorischen Verlauf.

Schlussfolgerungen

Wie bereits vorbeschrieben waren auch in unserer Kohorte die NfL-Werte in der PK-Gruppe höher als in der HC-Gruppe und zeigten im Zeitverlauf einen stärkeren Anstieg. Bei Personen mit PK waren erhöhte NfL-Werte mit kognitiven Beeinträchtigungen assoziiert und prädiktiv für eine zukünftige kognitive Verschlechterung. Im Gegensatz zu anderen Studien war eine Korrelation zwischen NfL und motorischer Beeinträchtigung nicht vorhanden. Zusammenfassend zeigt sich in unserer Studie, dass NfL eher ein Biomarker für kognitive als für motorische Beeinträchtigungen ist.

Abstract

Objective

This study aimed to assess the relationship between serum neurofilament light chain (NfL) levels and clinical markers of disease severity, as well as their diagnostic and prognostic value in patients with Parkinson's disease (PD) compared to healthy controls (HC). In addition, a systematic literature review was conducted to provide an overview of the current evidence on NfL levels in PD. Data from the Prospective Registry on Movement Disorders (PROMOVE, 2010, Graz), was used to support and compare with the findings from the systematic literature review.

Methods

In total, 93 participants were enrolled in the study, including of 46 patients with PD (PD group) from the PROMOVE registry, and 47 HC (HC group) from the Austrian Stroke Prevention Family Study (ASPS-Family), who served as the control group. Serum NfL samples were analyzed using Simoa HD-1 Analyzer (Quanterix, NF-Light™ Advantage Assay-Kit). All participants underwent clinical examinations and neuroimaging at baseline. Over the course of the study, PD patients received annual clinical assessments for up to six years.

Results

NfL levels were significantly higher in PD patients compared to HC at baseline and follow-up, with a greater longitudinal increase observed in the PD group, particularly among males in our cohort. No significant correlation was found between NfL and Mini-Mental State Examination (MMSE) scores in the PD group. In contrast, in the HC group, higher baseline NfL predicted lower follow-up MMSE scores. At follow-up, NfL levels were also negatively associated with MMSE scores in the HC group.

In PD patients, higher baseline NfL levels predicted lower follow-up global cognition scores, as measured by the neuropsychological test battery Consortium to Establish a Registry for Alzheimer’s Disease (CERAD). In contrast, NfL levels showed no association with motor severity or progression at either baseline or follow-up and had no predictive value for motor symptom development.

Conclusion

Consistent with previous research, NfL levels were elevated in PD patients compared to HC and increased more rapidly over time in the PD group. In PD, higher NfL levels were associated cognitive impairment and predicted future cognitive decline. In contrast to other studies, we found no significant association between NfL levels and motor severity. Overall, our results support NfL as a promising biomarker of cognitive, but not motor impairment, in PD.

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List of Abbreviations

AD ..... Alzheimer's disease
ADL ..... activities of daily living
ALS ..... amyotrophic lateral sclerosis
APD ..... atypical Parkinsonian disorder
AUC ..... area under the curve
Aβ ..... amyloid beta
Aβ40 ..... amyloid beta 1–40
Aβ42 ..... amyloid beta 1–42
BDI-II ..... Beck Depression Inventory-II
BJLO ..... Benton Judgment of Line Orientation test
BMI ..... body mass index
CBD ..... corticobasal degeneration
CBS ..... corticobasal syndrome
CDSS ..... Cumulative Dysautonomia Symptoms Scale
CERAD ..... Consortium to Establish a Registry for Alzheimer's Disease
CI ..... confidence interval
CJD ..... Creutzfeldt–Jakob disease
CNS ..... central nervous system
CRP ..... c-reactive protein
CSF ..... cerebrospinal fluid
CTh ..... cortical thickness
CX3CL1 ..... chemokine (C-X3-C motif) ligand 1
CXCL12 ..... chemokine (C-X-C motif) ligand 12
DAT ..... dopamine transporter
DBS ..... deep brain stimulation
DLB ..... dementia with Lewy bodies
DMV ..... dorsal motor nucleus of the vagus
DRS-2 ..... Dementia Rating Scale-2
DTI ..... diffusion tensor imaging
ECL ..... electrochemiluminescence
ELISA ..... enzyme-linked immunosorbent assay
EV ..... extracellular vesicle
FOG ..... freezing of gait
FTD ..... frontotemporal Dementia
GABA ..... gamma-aminobutyric acid
GBA1 ..... glucocerebrosidase gene
GFAP ..... glial fibrillary acidic protein
GPe ..... globus pallidus externus
GPi ..... globus pallidus internus
H&Y ..... Hoehn and Yahr scale
HC ..... healthy control

HFABP ..... heart-type fatty acid–binding protein
HVLT ..... Hopkins Verbal Learning Test
IF ..... intermediate filament
IFN-γ ..... interferon gamma
IL-1β ..... interleukin-1 beta
IL-4 ..... interleukin-4
IL-6 ..... interleukin-6
iLBD ..... incidental Lewy body disease
INF ..... cerebral infarctions
IPS ..... idiopathic Parkinson's syndrome
LB ..... Lewy bodies
LC ..... locus coeruleus
LNS ..... Letter–Number Sequencing test
log NfL ..... log-transformed neurofilament light chain levels
LRRK2 ..... leucine-rich repeat kinase 2 gene
MCI ..... mild cognitive impairment
MD ..... mean diffusivity
MDS-UPDRS ..... Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale
MDS-UPDRS total ..... sum of parts I–IV of the MDS-UPDRS (overall motor and non-motor disability)
MDS-UPDRS-II ..... part II of the MDS-UPDRS (motor aspects of daily living)
MDS-UPDRS-III ..... part III of the MDS-UPDRS (motor examination)
MHPG 3-methoxy-4-hydroxyphenylethyleneglycol
miRNA ..... microRNA
MMSE ..... Mini-Mental State Examination
MoCA ..... Montreal Cognitive Assessment
MS ..... multiple sclerosis
MSA ..... multiple system atrophy
MT ..... microtubule
NBM ..... nucleus basalis of Meynert
NF ..... neurofilament
NF-H ..... neurofilament heavy chain
NF-M ..... neurofilament medium chain
NfL ..... neurofilament light chain
NMS ..... non-motor symptoms
NMSS ..... Non-Motor Symptoms Scale
p-tau ..... phosphorylated tau
PD ..... Parkinson's disease
PD-CI ..... Parkinson's disease with cognitive impairment
PD-MCI ..... Parkinson's disease with mild cognitive impairment
PD-NC ..... Parkinson's disease with normal cognition
PDD ..... Parkinson's disease dementia

PIGD ..... postural instability and gait disorder
PINK1 ..... PTEN-induced kinase 1 gene
pNF-H ..... phosphorylated neurofilament heavy chain
PRKN ..... parkin gene
pro-IL-1β ..... precursor interleukin-1 beta
PROMOVE ..... prospective, longitudinal registry on movement disorders
PSP ..... progressive supranuclear palsy
Rab ..... ras-related in brain proteins
RAB10 ..... ras-related in brain protein 10
RAB12 ..... ras-related in brain protein 12
RAB29 ..... ras-related in brain protein 29
RBD ..... rapid eye movement behavior disorder
sAPP-α ..... soluble amyloid precursor protein alpha
SD ..... standard deviation
SDMT ..... Symbol Digit Modalities Test
Simoa ..... single molecule array
SN ..... substantia nigra
SNCA ..... alpha-synuclein gene
sncRNA ..... small non-coding RNA
SNpc ..... substantia nigra pars compacta
SNpr ..... substantia nigra pars reticularis
sPD ..... sporadic Parkinson's disease
TBI ..... traumatic brain injury
TD ..... tremor-dominant
TNF-α ..... tumor necrosis factor alpha
TS1 ..... Total Score 1 of the CERAD battery
TS2 ..... Total Score 2 of the CERAD battery
TUG ..... Timed Up and Go test
UPDRS ..... Unified Parkinson's Disease Rating Scale
UPDRS total ..... sum of parts I–IV of the UPDRS (overall motor and non-motor disability)
UPDRS-II ..... part II of the UPDRS (motor aspects of daily living)
UPDRS-III ..... part III of the UPDRS (motor examination)
USSLB ..... Unified Staging System for Lewy Body Disorders
VaD ..... vascular dementia
VPS35 ..... vacuolar protein sorting 35 gene
YKL-40 ..... chitinase-3-like protein 1
α-syn ..... α-synuclein

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1 Introduction

1.1 Parkinson's Disease

1.1.1 Definition

Parkinson’s disease (PD) can be defined as a neurodegenerative disorder of the central nervous system (CNS), characterized by a progressive loss of dopaminergic neurons in the nigrostriatal system. PD symptoms gradually worsen over time, and non-motor symptoms (NMS) may precede the more noticeable onset of motor symptoms. Motor signs in PD comprise bradykinesia, rest tremor and rigidity [1].

Movement disorders in general can be classified into hypokinetic disorders and hyperkinetic disorders. Parkinsonism is a hypokinetic movement disorder. PD is the most frequent cause of Parkinsonism. Other diseases that share similar symptoms with PD are for example vascular Parkinsonism and atypical Parkinsonian syndromes. The differentiation between different types of parkinsonian disorders can be difficult [2].

1.1.2 Epidemiology

After Alzheimer’s disease (AD), PD ranks as the second most common neurodegenerative disorder [3]. Over the period from 1990 to 2015, PD cases have increased by nearly 100%, and its prevalence continues to grow each year [4]. In 2019, more than 8.5 million people worldwide were living with PD [5], and estimates suggest this number could rise to 12 million by 2040 [4]. Between 2000 and 2019, the disability-adjusted life years associated with PD increased by around 81%, reaching 5.8 million in 2019. During this time, PD-related deaths more than doubled, with 329,000 deaths reported in 2019 [5]. In 2017, there were 1.02 million newly diagnosed cases of PD. With ongoing improvements in medical care, years lived with disability among PD patients rose by 8.9% from 1990 to 2007, but the increase slowed to 1.0% between 2007 and 2017, suggesting that medical advances may have contributed to a slowing of the severity of disability associated with PD [6].

The global incidence of PD is approximately 17 cases per 100,000 person-year [7]. The risk of developing PD increases with age. Among those aged 65 and

older, the incidence is 160 cases per 100,000 person-years, peaking around age 80. Geographically, PD prevalence seems lower in Africa compared to Europe and America, whereas incidence rates are relatively consistent across Asia, Europe, and the Americas. Racial comparisons remain inconsistent. One study found a higher incidence of PD in Black individuals compared to White individuals, while a larger study reported higher rates in Hispanic populations than in non-Hispanic, Asian, and Black populations. Sex-specific data reveal that men are more likely to develop PD than women, with a male-to-female ratio ranging from 1.3:1 to 2.0:1. However, ratios as low as 0.95:1 have been reported in Asia, possibly due to variations in smoking habits, which have been found to reduce the risk of PD [3].

1.1.3 Etiology

Parkinson's syndrome can be classified into four main categories. These are idiopathic Parkinson's syndrome (IPS), also known as PD, familial PD, atypical Parkinsonian disorders (APDs), and secondary parkinsonism [8,9]. Table 1 provides an overview of these classifications with their etiologies.

Parkinson's syndrome	Etiology
Idiopathic Parkinson's syndrome (IPS)	Degeneration of dopaminergic neurons occurs in the substantia nigra (SN) and locus coeruleus (LC). Multifactorial (including genetic predisposition)
Genetic forms of Parkinson's syndrome	Single-gene mutations that result in the loss of dopaminergic neurons, with gene loci identified as PARK 1-23
Atypical Parkinsonian disorder	Multiple system atrophy (MSA)
	Progressive supranuclear palsy (PSP)
	Corticobasal degeneration (CBD)
	Dementia with Lewy bodies (DLB)
Secondary parkinsonism	Drug-induced parkinsonism (Most common): Typical antipsychotics, Lithium, Antiemetics (metoclopramide), Anticonvulsive agent (Valproat)
	Trauma (Traumatic brain injury (TBI)
	Tumor
	Toxic (carbon monoxide, manganese exposure)
	Metabolic (Morbus Wilson)
	Vascular diseases (Binswanger's disease)

	Inflammatory: HIV encephalopathy and Encephalitis lethargica

Table 1. Classification and etiology of parkinsonian syndromes [8–10]

Parkinsonian disorders are multifactorial diseases with several pathophysiological mechanisms contributing to their clinical presentation. In the case of multifactorial IPS, also known as sporadic PD (sPD), the etiology of the degenerative process remains unknown and is believed to have a polygenic background [11]. Compared to sporadic PD, genetic forms of PD have been linked to several identified gene loci (PARK 1-23) associated with the degenerative process [12].

Heritability estimates for PD range from 22% to 40%, pointing to the importance of genetic predisposition [11]. At least 19 monogenic mutations have been identified as pathogenic, with 10 following an autosomal dominant inheritance pattern and 9 following an autosomal recessive pattern [8]. Furthermore, four risk loci (PARK3, PARK10, PARK12, and PARK16) have been associated with this condition, though they have not yet demonstrated direct pathogenicity [12]. Approximately 10% of all PD cases are believed to result from causative mutations (familial PD), with an earlier onset of disease. Mutations in the PARK2 locus represents nearly 50% of early-onset PD cases, while alterations in the alpha-synuclein gene (SNCA) are relatively rare [10]. Mutations in the SNCA gene, lead to the overproduction of α-synuclein (α-syn), which aggregates into toxic oligomers and fibrils, a key feature of PD pathology. Additionally, mutations in the glucocerebrosidase gene (GBA1), associated with Gaucher disease, are significant risk factors for PD. These mutations impair lysosomal function, resulting in decreased degradation of α-syn and leading to its pathological accumulation and cellular damage [11].

The most common mutation observed in PD is the Gly2019Ser variant in the leucine-rich repeat kinase 2 (LRRK2) gene. LRRK2 plays a key role in regulating immune responses and mitochondrial function, with its dysfunction contributing to PD pathogenesis. Beyond PD, LRRK2 is also associated with Crohn’s disease, leprosy, and mycobacterial infections, suggesting that its variants may influence a range of conditions through shared pathogenic mechanisms [11].

1.1.4 Pathophysiology

PD is primarily characterized by dopamine deficiency at striatal receptors, caused by the degeneration of dopaminergic neurons in the SN. This results in a loss of the disinhibitory effect normally mediated by dopamine, leading to overactivity of basal ganglia structures, which suppress signals in the pyramidal motor pathways. The resulting imbalance between inhibition and excitation within basal ganglia circuits gives rise to bradykinesia, resting tremor, and muscular rigidity [9]. While dopaminergic depletion is a key feature of PD, several underlying pathophysiological processes contribute to disease onset and progression. These include molecular mechanisms involving mitochondrial and lysosomal dysfunction, immune and inflammatory responses, α-syn aggregation [11], and gut–brain interactions [8]. The following sections outline these mechanisms in detail, providing a comprehensive understanding of PD pathogenesis.

1.1.4.1 Molecular mechanisms

Mitochondria play a crucial role in PD by regulating energy production and signaling, which influences cell survival or degeneration. Early stages of PD are characterized by synaptic damage and mitochondrial dysfunction, leading to structural changes that result in increased production of reactive oxygen species, pathological calcium levels, and reduced adenosine triphosphate synthesis, all contributing to neurodegeneration. Mitochondrial dysfunction in PD has been linked to both environmental toxins, such as pesticides, and a set of 11 genetic mutations, including SNCA, parkin gene (PRKN), PTEN-induced kinase 1 gene (PINK1), and LRRK2. Due to their high energy demands, dopaminergic neurons are especially vulnerable, with PINK1 and PRKN playing essential roles in mitophagy, the selective removal of damaged mitochondria. Accumulated α-syn further damages mitochondrial membranes, increases oxidative stress, both of which promote the formation of α-syn species, including fibrils and oligomers, thereby accelerating disease progression [11].

Lysosomes are vital for breaking down misfolded proteins, including α-syn. The progression of PD is strongly linked to impairments in protein degradation mechanisms, particularly the autophagy-lysosomal and ubiquitin-proteasome pathways. When these systems become less effective, proteins like α-syn

accumulate, forming toxic aggregates. The role of lysosomes in clearing such proteins through autophagy and mitophagy is crucial, but this function can be compromised by GBA1 gene mutations. These mutations reduce the activity of an enzyme called acid β-glucocerebrosidase, resulting in the buildup of glucosylceramide and toxic glucosylsphingosine, which in turn promote neuroinflammation through activation of astrocytes and microglia [11].

Furthermore, vacuolar protein sorting (VPS35) [13] and LRRK2 genes play vital roles in PD, particularly in endocytosis and intracellular transport [11]. Endocytosis facilitates the formation of endosomes, which eventually fuse with lysosomes for breakdown [11]. Mutations in LRRK2 enhance the phosphorylation[11] of ras-related in brain (Rab) proteins [14], such as RAB10, RAB12, and RAB29, which are crucial for both endocytosis and lysosomal transport [11]. Similarly, mutations in VPS35 can activate LRRK2. The overactivation of mutated LRRK2 impairs lysosomal function and interferes with the cell's ability to respond to membrane damage, leading to disturbances in endosomal transport. These disruptions compromise lysosomal autophagy and contribute to the accumulation of α-syn [11].

1.1.4.2 Immune and Inflammatory Mechanisms

Immune and inflammatory processes play a key role in PD progression. Inflammation occurs both in the CNS and in peripheral blood, where elevated cytokine levels are associated with accelerated disease progression. PD is characterized by enhanced pro-inflammatory activity in monocytes and T cells, particularly T helper 1 and T helper 17. Immune cell infiltration and microglial activation promote chronic neuroinflammation. Although immune activity may aid in clearing misfolded proteins in early-stage PD, prolonged activation can worsen neuronal damage. Genetic factors such as human leukocyte antigen polymorphisms and LRRK2 mutations influence immune responses. Abnormal α-syn activates microglia and monocytes through Toll-like receptors, while mitochondrial dysfunction further increase inflammatory response [11].

Environmental factors, particularly pathogens entering through the gut and nasal pathways, may worsen inflammation in PD by triggering intestinal inflammation and α-syn aggregation. In PD, gut microbiome changes favor pro-inflammatory

bacteria, raising endotoxin levels and lowering anti-inflammatory short-chain fatty acids. This gut inflammation may contribute to brain pathology, either alone or through a combination of mechanisms, including increased gut permeability, α-syn spread along the vagus nerve, and migration of α-syn-specific T-cells to affected brain regions [11].

1.1.4.3 Prodromal Stage

In the prodromal phase of PD, neurodegeneration is thought to begin at the molecular level. A key player in this process is α-syn, a 140-amino-acid protein highly concentrated at neuronal synapses, where it regulates vesicle trafficking and neurotransmitter release. In PD and related disorders such as Parkinson's disease dementia (PDD) and dementia with Lewy bodies (DLB), it aggregates in the cytoplasm of neurons, forming filaments that give rise to Lewy bodies (LB) and Lewy neurites, sequestering organelles like mitochondria and lysosomes. The aggregation process begins with toxic monomers and oligomers in the neuronal cytoplasm, which impair cellular function early in the disease and later form filaments that combine into LBs. While LBs have long been considered key pathological markers, current evidence suggests that smaller oligomers are the more neurotoxic aggregates driving neurodegeneration [11].

To explain the early extranigral involvement in PD, Braak et al. proposed a pathophysiological mechanism known as the dual-hit hypothesis, which is linked to the pathology described in Braak stage 1 (see Section 1.1.6). According to this hypothesis, a pathogen, for example a virus may enter the nervous system through two distinct routes. In the first route, it enters via the nasal mucosa, spreads to the olfactory bulb, and then progresses to the temporal lobe. In the second route, it travels through the gastrointestinal tract to the Meissner's plexus and then reaches the vagus nerve. Animal studies support this gut–brain pathway, demonstrating LB pathology in parasympathetic and sympathetic nerve fibers. However, autopsy studies have found that approximately 10% of PD patients do not show α-syn pathology in the vagus nerve [8].

In line with this Braak et al.'s model, numerous studies link the gut microbiome to PD. Animal models support this connection, demonstrating that intestinal microbes can worsen PD symptoms, while on the other hand, antibiotic treatment may

alleviate them. In humans, PD patients exhibit distinct gut microbiota profiles, characterized by reduced levels of bacteria such as Prevotella, Blautia, Roseburia, and Faecalibacterium, and increased levels of Lactobacillaceae, Akkermansia, and Bifidobacterium. However, it remains unclear whether these alterations contribute to the pathogenesis of the disease or rise as a consequence of PD [15].

Additional evidence for a gastrointestinal origin of PD comes from clinical and epidemiological data. Constipation, a common early symptom of PD, has been associated with faster cognitive decline and an increased risk of mild cognitive impairment (MCI) and dementia. Inflammatory bowel disease, particularly Crohn's disease, is also linked to PD, likely due to shared genetic factors such as LRRK2 variants [16]. Increased intestinal permeability, often referred to as "leaky gut" may allow toxins, such as bacterial lipopolysaccharides, to enter the enteric nervous system, promoting inflammatory responses and initiating α-syn aggregation [15]. Interestingly, nicotine and coffee consumption may reduce PD risk by positively influencing the gut microbiota, underscoring the gut–brain axis's role in disease pathogenesis [8].

1.1.4.4 Symptomatic stage

The nervous system can compensate for neural damage by using its internal reserves, allowing surviving neurons to maintain function by increasing their activity to compensate for the damage. In PD, up to 60–80% of dopamine-producing neurons in the nigrostriatal pathway may be lost before motor symptoms appear [17]. Symptoms emerge once this compensatory mechanism reaches its limit[17], mainly due to degeneration of the substantia nigra pars compacta (SNpc) [18].

Deterioration of the SNpc leads to various changes in the basal ganglia neurotransmission. In healthy individuals, the SNpc primarily activates inhibitory neurons in striatum that use gamma-aminobutyric acid (GABA) as their neurotransmitter. In PD, two distinct pathways are affected by SNpc degeneration. In one pathway, degeneration of the SNpc reduces dopaminergic stimulation of striatal neurons projecting to the globus pallidus externus (GPe), resulting in decreased GPe activity. This disinhibits the subthalamic nucleus, which becomes overactive and increases excitatory input to the globus pallidus internus (GPi) and

the substantia nigra pars reticulata (SNpr), which in turn enhances their inhibitory effect on the thalamus. In the second pathway, degeneration of the nigrostriatal projection from the SNpc reduces activation of GABAergic striatal neurons that normally inhibit the GPi and SNpr. This loss of inhibition further increases their inhibitory effect on the thalamus. Both pathways lead to excessive thalamic inhibition, disrupting communication between the thalamus and motor cortex and resulting in the characteristic bradykinesia observed in PD [18].

1.1.5 Clinical Presentation

1.1.5.1 Clinical Symptoms

The first signs of PD are usually non-motor symptoms that coincides with the onset of motor symptoms in the later stages of the disease [1]. PD is characterized by three main motor symptoms, including resting tremor, bradykinesia, and rigidity (stiffness). These symptoms generally start on one side of the body and gradually spread to both sides, often remaining more pronounced on the initially affected side. The progression and presentation of PD vary widely among individuals. A subset of patients experience a tremor-dominant (TD) type, marked by significant tremor with less rigidity or gait impairment. Others have an akinetic-rigid form, dominated by bradykinesia and stiffness [19]. However, NMS often cause greater disability than motor symptoms and significantly reduce quality of life [10].

1.1.5.1.1 Motor symptoms

In PD, the initial motor symptoms commonly begin in one arm, often leading to reduced dexterity for precise tasks and occasionally a resting tremor. Some individuals experience a subtle drag in one leg or develop a shuffling gait. A general slowing of movement, reduced facial expression, or diminished arm swing while walking may also occur. Additionally, patients often report pain in the affected limb, with symptoms sometimes accompanied by frozen shoulder or back pain [20].

PD tremors typically appear at rest (resting tremor), occur at a frequency of 4–5 Hz [10], often affect one or both hands [19], and may be accompanied by reduced arm swing and shoulder pain [10]. Stress or cognitive tasks can increase tremor intensity [21]. These rhythmic, involuntary movements tend to diminish with voluntary action but may reappear during sustained postures, such as holding the

arms outstretched [19]. The tremor is characterized by alternating bursts in opposing muscle groups (agonist and antagonist), known as an “alternating tremor” [22]. Approximately half of PD patients exhibit the characteristic “pill-rolling” tremor [22], involving the thumb and index finger moving in a supination and pronation pattern [19], or it may present as flexion and extension movements in the fingers, hand, or foot [2]. It can also spread to other limbs and commonly affects one or both extremities on one side of the body before becoming bilateral [2], and may also involve the jaw, tongue, or eyelids [22]. Head tremors are uncommon in PD but frequently occur in essential tremor (ET) [19].

Less common tremor types, such as re-emergent and postural tremors, represent subtypes of action tremors. Action tremors typically manifest at a frequency of 7–8 Hz, most prominent in the extended fingers and hands. Unlike the slower, rhythmic resting tremor characteristic of PD, these action tremors appear during voluntary movement and generally diminish when the patient is relaxed. Notably, they may precede the classic resting tremor and can coexist with it [22]. Re-emergent tremors are marked by a delayed onset (a few seconds) when holding a position, such as with outstretched arms, while postural tremors arise when a person maintains a position against gravity [10].

Bradykinesia and rigidity are additional symptoms that frequently occur alongside other signs of PD. These symptoms typically present on the symptomatic side in PD but may also manifest as subtle signs on the contralateral side or as midline features, such as hypomimia (reduced facial expression). Symptom onset may be subtle or postponed when bradykinesia is the initial sign, especially if it begins on the nondominant side [10]. Bradykinesia, a core PD feature, involves slowed voluntary movements and reduced movement amplitude (hypokinesia), with speed and range diminishing during repetitive tasks [19]. It can also affect involuntary actions like arm swing [2].

Rigidity is characterized by increased muscle tone, resulting in constant resistance to passive limb movement. It affects both agonist and antagonist muscles and often leading to a flexed posture. Unlike spasticity, which is often strongest at the start of passive movement (clasp-knife phenomenon) and varies between muscles, rigidity shows persistent resistance. When combined with tremor, rigidity

may produce cogwheel rigidity, which is a ratchet-like interruption in passive movement [2].

Gait disturbances in PD typically involve slow walking with short, shuffling steps (marche à petit pas), increasing the risk of falls. Arm swing is often reduced, especially on the side where tremor or rigidity is more pronounced. Festination may occur in some patients, characterized by a slow walking start that progressively accelerates with shorter steps [19]. In advanced stages of PD, freezing of gait (FOG) can occur, causing a temporary inability to initiate movement. This makes it especially difficult to start walking in narrow spaces or while turning, often resulting in falls due to combined FOG and postural instability (reduced balance) [10].

Facial expressivity is also affected. Hypomimia, is characterised by palpebral fissures, low blinking frequencies and fixed fascial expressions [2]. While a typical blink rate is 12–20 times per minute, PD patients often blink only 5–10 times per minute, resulting in a widening of the space between the eyelids and a characteristic staring expression [22]. Micrographia, where handwriting becomes progressively smaller, slower, and eventually illegible, may also occur. Fine motor skills required for everyday tasks, such as handling utensils, often deteriorate. Some patients develop striatal deformities, which gradually lead to joint abnormalities in the hands and feet [10]. Painful dystonia (involuntary muscle contractions) may also arise, including toe extension and jaw clenching. A particularly notable symptom is camptocormia, a form of axial dystonia involving severe forward spinal flexion that typically improves when lying down or when pushing against a walker [22].

Another prominent symptom is hypokinetic dysarthria, which impacts both speech and swallowing [22]. This condition results in hypophonia (a soft voice) [2] along with rushed, monotonous, and unclear speech that can sometimes diminish to a whisper [22]. Swallowing is similarly affected, making eating a slow and effortful task [22]. With disease progression, some patients may also develop pseudobulbar palsy, which contributes to dysarthria (impaired speech articulation), dysphagia (difficulty swallowing), glossplegia (tongue paralysis) and facial paralysis [23]. Together, these impairments disrupt both communication and nutritional intake, further complicating daily activities for those affected [22].

Additional motor symptoms may include blepharoclonus, characterized by involuntary twitching of closed eyelids, and blepharospasm, where the eyelid close involuntary. Change in reflexes are uncommon, though hyperreflexia can occur on the symptomatic side in some PD patients [2]. Some patients also exhibit the Myerson sign (also known as the glabellar reflex), which is characterized by an inability to suppress blinking when tapped repeatedly on the forehead [2], whereas, in healthy individuals, the response fades after a few blinks [19].

1.1.5.1.2 Non-motor symptoms (NMS)

NMS are now recognized as a core feature of PD, often emerging in the early or prodromal phase, by which point about one-third of dopamine neurons are already lost [24]. They affect diverse domains, including fatigue, personality changes [2], pain (30–85%), depression (35%), psychosis (40%), anxiety (60%), apathy (loss of motivation (60%), visual dysfunction (22–78%), and hyposmia (weakened sense of smell (>90%) [25]. Cognitive issues range from mild impairment to dementia [2], affecting 30% of patients initially [10] and up to 83% after 20 years [25]. These cognitive deficits often involve executive functions such as working memory, planning, and organization [10].

Other frequent NMS include olfactory deficits, such as anosmia (complete loss of smell) [2], and a variety of sleep disturbances. These may present as insomnia, excessive daytime sleepiness, vivid dreams, fragmented sleep, REM sleep behavior disorder (RBD) [2] and sleep apnea [10]. In RBD, individuals may physically perform dream-related movement such as kicking, hitting, or running [19]. Autonomic dysfunctions are also common, with symptoms including constipation, muscle cramps, erectile dysfunction, orthostatic hypotension, seborrhea (oily skin), and excessive sweating [22]. Additional features include bladder issues [2] and sialorrhea (drooling), which is likely caused by reduced swallowing frequency [22].

1.1.5.2 Disease Progression

PD typically progresses through five stages, as outlined by the Hoehn and Yahr (H&Y) scale. In stage 1, symptoms are limited to one side of the body but become bilateral in stage 2, which often lasts 5 to 10 years [20]. As the disease advances, patients experience postural instability in stage 3, severe disability in stage 4, and,

on average, reach a chairbound state in stage 5 [20] about 7.5 years after onset [22]. Approximately 10% of patients have a slower course, with mild symptoms and clinical stability lasting over 10 years. Advances in treatment have also impacted these trajectories, helping some patients retain mobility and function longer [22].

PD progression also includes a prodromal phase, marked by NMS such as RBD, constipation, depression and hyposmia. These NMSs often appear years before the onset of motor signs and become more frequent as the disease advances. In the early stage, classic motor symptoms like tremor, rigidity, and bradykinesia emerge, often alongside MCI. The mid-stage is marked by additional complications, including orthostatic hypotension, urinary symptoms, and axial deformities. In the late stage, patients frequently experience postural instability and significant NMS such as hallucinations and dementia [24].

1.1.6 Pathology

Macroscopically, PD involves neurodegeneration in key brain regions like the SNpc and LC, with structural changes such as frontal cortex atrophy and ventricular enlargement. Other affected areas include the nucleus basalis of Meynert (NBM), dorsal motor nucleus of the vagus (DMV), pedunculopontine nucleus, raphe nuclei, hypothalamus, and olfactory bulb [26].

Microscopically, PD is marked by extracellular pigmentation, neuronal degeneration, gliosis, and LBs, which are cytoplasmic inclusions. These are primarily found in neurons of the SNpc, LC, DMV and spinal cord (substantia innominata and intermediolateral cell column), but can also appear in peripheral tissues such as the myenteric and cardiac plexuses, highlighting PD as a multisystem disorder [10]. Brainstem LBs have an eosinophilic core with a pale halo, while cortical LBs lack a halo and have irregular borders [26]. They mainly consist of hyperphosphorylated neurofilaments, lipids, iron, ubiquitin, and α-syn [10]. While normal aging reduces SN dopaminergic neurons to approximately 200,000 by age 80, PD causes a more severe decline to 30% or less of that found in healthy individuals of the same age [22].

Braak's staging system (2003) outlines six neuropathological stages of PD, reflecting a caudo-rostral progression of LB pathology [26] (see Figure 1).

According to this model, PD may originate in peripheral tissues such as the gastrointestinal tract or olfactory system and gradually progress to brain regions, including subcortical and cortical structures [11]. Stages 1–3 represent the prodromal phase, mainly affecting the spinal cord, brainstem and forebrain [8]. This presymptomatic phase may involve autonomic and olfactory symptoms but lack motor signs. In early PD (Stages 1–2), LBs first appear in the DMV, preganglionic vagal axons and anterior olfactory nucleus, a pattern consistent with the “dual-hit hypothesis”. The pathology then extends to the raphe nuclei, gigantocellular reticular nucleus, and LC [27]. Additionally, in Stage 2, efferent pathways to the enteric nervous system begin to show involvement [8].

Stages 3–6 mark the symptomatic phase, beginning with SNpc involvement and progressing from brainstem to limbic and cortical regions, corresponding with the emergence of motor symptoms [8]. In Stage 3, neuronal loss affects the LC, amygdala, pedunculopontine nucleus, and basal forebrain (including the NBM), while LBs appear in the SNpc and hypothalamus without associated cell loss. By Stage 4, SNpc degeneration and limbic involvement in the entorhinal cortex, mesocortex, and amygdala [27] contribute to cognitive decline [8]. In Stage 5, pathology spreads to the temporal and prefrontal neocortex [27], with prefrontal involvement contributing to apathy and reduced motivation [8]. In Stage 6, LBs reach the primary sensory and motor cortices. Stages 5 and 6 represent advanced PD [27] and are characterized by severe cognitive decline or dementia, as well as gait disturbances [26].

Although Braak staging links LB distribution to disease progression, this pattern isn’t consistent across all patients [27]. Some patients may show a brainstem dominant form of the disease without peripheral involvement, while others may present with olfactory pathology first, followed by either brainstem- or limbic-predominant patterns [11]. Additionally, incidental Lewy body disease (iLBD), found in individuals without parkinsonism or cognitive impairment, may represent a presymptomatic phase of PD [27]. However, not all with iLBD develop PD, and some show early signs of DLB. Autopsies studies have identified iLBD in 8–17% of neurologically normal individuals over the age of 60 [28]. The presence of LB pathology in both central and peripheral regions in iLBD challenges Braak’s caudo-rostral model and suggests PD is a dynamic process that may manifest

once α-syn accumulation crosses a critical threshold. Variations from the caudo-rostral progression have been reported in 6–43% of cases. Some patients show advanced αSyn pathology in regions such as the SN or cortex with minimal clinical symptoms, and 7–8.3% lack DMV involvement altogether. These findings challenge the DMV as the initial site of pathology and support a more variable or simultaneous subcortical–cortical involvement. Furthermore, autopsy studies reveal weak correlation between αSyn pathology and symptoms such as dementia or extrapyramidal symptoms in advanced Braak stages, while the presence of motor symptoms in early stages further underscores the mismatch between pathology and clinical manifestation [27].

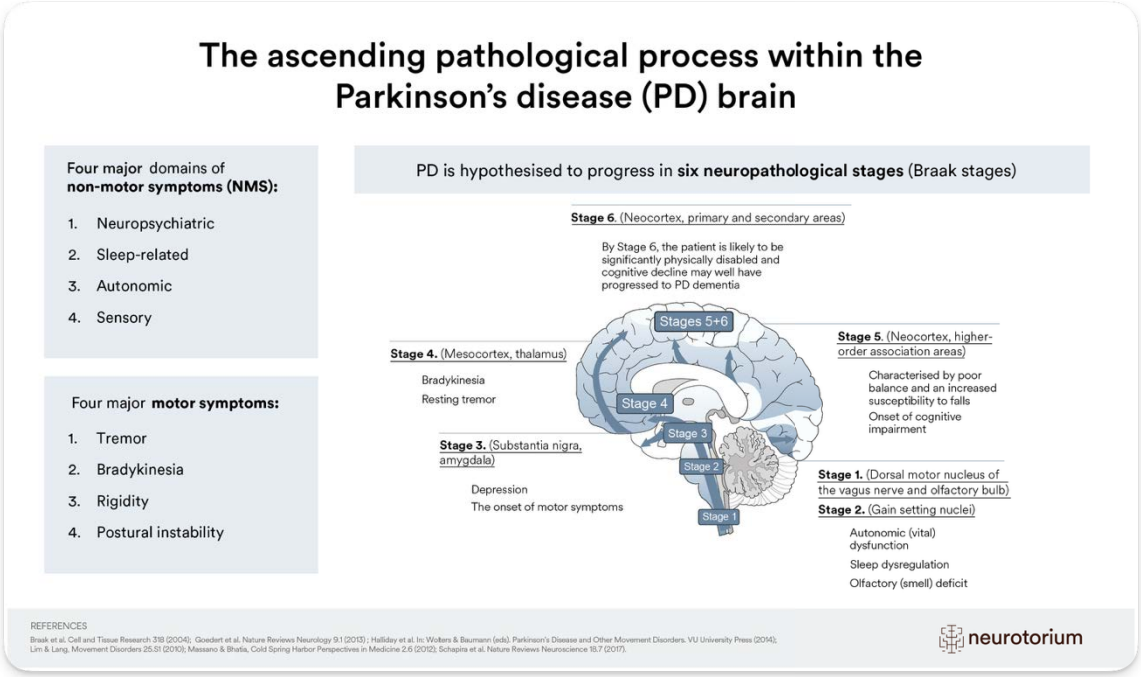

Figure 1. Braak staging: Ascending neuropathological progression and symptom development in PD [29]. Image © Neurotorium.org. Reproduced with permission. Available from: https://neurotorium.org/image/parkinsons-disease-and-braak-stages/.

Cognitive symptoms in PD, often seen in Braak stages 5–6, are frequently linked to tau pathology in the limbic system rather than α-syn alone. Comorbid AD pathology complicates the clinical picture, especially in PDD and DLB, where both α-syn and tau protein contribute to symptoms. Tau and α-syn are often found in the same vulnerable regions such as LC, NBM, and amygdala, indicating shared pathogenic mechanisms that may be further intensified by amyloid-β (Aβ). This challenges clear distinctions between PD, DLB, and AD. Additionally, disease

progression patterns vary, as early-onset cases with long disease duration in PD tend to aligning with Braak staging, whereas late-onset or rapidly progressing cases often exhibit widespread LB pathology that is either already present at onset or rapidly spreads early in the disease course [27].

While Braak staging provides a framework for understanding the progression of LB pathology, it primarily focuses on LB distribution and neglects other factors, such as neuronal loss. Evidence suggests neuron death may depend on regional or cell-specific factors, rather than LB accumulation. As a result, advanced LB pathology doesn't always match symptom severity, highlighting the need to refine staging systems [27]. This heterogeneity in LB distribution and disease manifestation led to the development of the Unified Staging System for Lewy Body Disorders (USSLB) in 2009, which incorporates alternative progression patterns and therefore enables a more precise allocation of pathological changes [11].

1.1.7 Diagnostics

The diagnosis of PD mainly relies on a detailed patient history and clinical examination. Although various diagnostic tools, including biomarkers and neuroimaging techniques, have been developed, they lack the sensitivity and specificity needed to detect PD in its early stages. As a result, diagnosis continues to be based on clinical criteria, which reflect the typical progression of the disease and describe common symptoms that emerge throughout its course. This often delays diagnosis until more advanced stages, when symptoms become more apparent. Heterogeneous presentations, overlapping signs with other conditions or aging, and reliance on expert neurologists makes early detection difficult. NMS often precede motor symptoms by years but are typically mild and may be overlooked, contributing to delayed diagnosis [30]. Nevertheless, early identification is crucial for improving treatment outcomes and quality of life, emphasizing the need for a comprehensive diagnostic approach combining clinical, laboratory, and imaging tools.

1.1.7.1 Laboratory diagnostics

The current clinical diagnosis of PD is based primarily on symptom observation, with an accuracy of about 80% when verified through post-mortem analysis, as confirmation of diagnosis is only possible after death [31]. Biomarkers offer great

potential to improve PD diagnosis and treatment by enabling earlier detection, tracking disease progression, distinguishing PD from APD, and guiding the development of targeted therapies, and assessing responses to treatment. Early diagnosis is crucial as most PD cases are diagnosed late in the disease course, limiting the effectiveness of available therapies [32].

1.1.7.1.1 Cerebrospinal fluid (CSF) biomarkers

Cerebrospinal fluid (CSF) analysis offers direct access to the CNS through its close interaction with the CNS extracellular space, making it a valuable tool for diagnosing various neurological diseases. As an ultrafiltrate of serum, CSF contains proteins, which are mainly brain-specific (20%), making it a promising medium for identifying individuals at risk for neurological conditions. It is particularly useful for differentiating between infectious, autoimmune, and degenerative diseases. In PD, the disruption of the blood-brain barrier highlights CSF's potential for identifying disease-specific biomarkers [32].

Recent research on α-syn has shown that CSF levels of total α-syn are significantly lower in PD patients compared to controls, likely due to intracellular aggregation that reduces its presence in the CSF. These findings show high sensitivity (95.3%) and specificity (98%) for identifying LB-synucleopathies and have proven effective in distinguishing PD from healthy control (HC). However, this biomarker alone is insufficient to reliably differentiate PD from other neurodegenerative diseases, including DLB and AD [32].

Glial fibrillary acidic protein (GFAP), a brain-specific protein released from damaged astrocytes, is emerging as a promising biomarker. Elevated GFAP in CSF or serum indicates intracranial injury but lacks disease specificity, as levels are increased in PD, DLB, AD, frontotemporal dementia (FTD), and Creutzfeldt–Jakob disease (CJD). Despite this, GFAP may help monitor disease progression in PD and neurological damage overall [32].

Tau, phosphorylated tau (p-tau), and amyloid beta 1–42 (Aβ42), known AD biomarkers, are also being investigated in PD. These markers are typically normal or slightly reduced in the CSF of PD patients compared to HC. Notably, low Aβ42 have been associated to cognitive decline. Furthermore, studies have also shown that higher tau and p-tau levels in PDD compared to those with normal cognition

may aid in distinguishing cognitive subtypes. In DLB, AD-like CSF profiles are more frequent than in PD, supporting their utility in differential diagnosis [32].

Another candidate biomarker currently under investigation is chitinase-3-like protein 1 (YKL-40) [33], a neuroinflammatory protein secreted by activated astrocytes, which has been found to be elevated in AD, FTD, amyotrophic lateral sclerosis (ALS), and PD. While animal models show increased YKL-40 in PD brain tissue and CSF compared to HC, human studies report poor accuracy in distinguishing PDD from other dementias such as AD and vascular dementia (VaD) [32].

In summary, while biomarkers such as α-syn, GFAP, tau, Aβ42, and YKL-40 show promise in PD research, their limited specificity and inconsistent findings highlight the need for further studies to clarify their diagnostic utility.

1.1.7.1.2 Serum biomarkers

A reliable serum biomarker for PD could provide a non-invasive, accessible diagnostic tool, eliminating the need for costly imaging or invasive lumbar punctures required for CSF analysis. Such a biomarker could aid in PD diagnosis, to differentiate APDs, and predict symptom severity or cognitive impairment [32].

α-syn is a promising serum biomarker for PD due to its strong pathological relevance. Although initially detected in plasma in 2003, studies on total serum α-syn have yielded inconsistent results, largely due to methodological differences. A meta-analysis reported elevated levels in PD patients compared to controls, but high heterogeneity limited its reliability. Consequently, research now focuses on specific α-syn forms to improve diagnostic accuracy [32].

Erythrocyte (red blood cell) α-syn shows potential as a PD biomarker, as majority of blood α-syn is found in red blood cells. One study reported that elevated phosphorylated cytosolic erythrocyte α-syn distinguishes PD from HC with approximately 70% sensitivity and specificity. Another study found that higher total and oligomeric α-syn levels also correlated with motor decline in early PD [32].

α-syn in neuron-derived extracellular vesicles (EVs) also shows promise as a blood-based PD biomarker. Elevated EV α-syn levels have been found in PD patients compared to HC. One study reported 1.57-fold higher levels in PD than in HC and showed that α-syn effectively distinguishing it from DLB and progressive

supranuclear palsy (PSP). Another study detected a β-sheet–structured form of α-syn in all PD cases but not in HC. However, clinical use requires standardized methods and deeper understanding of α-syn isoforms [32].

Recent studies identify GFAP as a strong predictor of progression from PD to PDD, outperforming neurofilament light chain (NfL) in some cases. One study found that higher GFAP levels were strongly associated with the development of PDD, with the area under the curve (AUC) reaching approximately 0.9. In contrast, NfL was not significantly associated with PDD development. Moreover, the study found that GFAP was also associated with cognitive impairment. Other studies showed that combining GFAP and NfL further improved differentiation between PDD and PD, as well as between PD and RBD. These findings highlight GFAP as a promising biomarker for cognitive decline in PD. In addition to GFAP, p-tau and amyloid have emerged as potential PD biomarkers [32].

Beyond protein-based biomarkers, inflammatory markers are gaining attention in PD due to the central role of neuroinflammation. A recent meta-analysis found that PD patients had higher blood levels of inflammatory markers such as interleukin-6 (IL-6), interleukin 1-beta (IL-1β), and tumor necrosis factor alpha (TNF-α), while interleukin-4 (IL-4) and interferon gamma (IFN-γ) levels were reduced compared to controls [10,32]. Additionally, other markers such as c-reactive protein (CRP), chemokine (C-C motif) ligand 2 (CCL2) [34], chemokine (C-X3-C motif) ligand 1 (CX3CL1) [35], and chemokine (C-X-C motif) ligand 12 (CXCL12) [36] were also elevated in PD [32].

Importantly, certain blood markers including soluble vascular cell adhesion molecule-1 (sVCAM-1) [37], NOD-like receptor thermal protein domain associated protein 3 (NLRP3), IL-1β, CXCL12 [32], and interleukin-8 (IL-8) [10,32] showed strong diagnostic performance with AUC values above 0.80 in distinguishing PD from HC [32]. In contrast, markers like pentraxin 3 (PTX3) [38], serum amyloid A (SAA), CX3CL1, amyloid precursor protein-alpha (sAPP-α), TNF-α, and IL-6 exhibited lower diagnostic accuracy, with AUC values ranging from 0.60 to 0.80. Several studies also link inflammatory markers to disease severity and cognitive decline. One study found that elevated TNF-α and IL-6 levels were associated with severe PD. Another study reported increased precursor interleukin-1 beta (pro-IL-1β) [32,39] and TNF-α in neuron-derived EVs, with pro-IL-1β, IL-6, IL-10 [32,40],

and TNF-α correlating with cognitive impairment in PD [32]. A third study found that a combination of three biomarkers, CRP, albumin, melanoma inhibitory activity protein (MIA), accurately predicted rapid cognitive decline (AUC = 0.809) [32].

MicroRNA (miRNA) and non-coding RNA (sncRNA) in exosomes also show promise as PD biomarkers. One study linked reduced α-syn–related miRNA to motor severity and reported high diagnostic accuracy (AUC = 0.86) in differentiating PD patients from controls. Another study identified downregulated of a miRNA called hsa-miR-144-3p in early-stage, untreated PD patients compared to controls. A meta-analysis reported a pooled AUC of 0.87 for miRNA panels in differentiating PD from controls. Despite these advances, methodological inconsistencies and lack of standardization across studies remain obstacles to clinical application [32].

Overall, while blood-based biomarkers such as α-syn, GFAP, inflammatory cytokines, sncRNAs, and microRNAs show strong potential for PD diagnosis and prognosis, inconsistent findings, methodological variability, and lack of standardization still limit their clinical utility, underscoring the need for further research.

1.2 Neurofilaments (NFs)

NfL are intermediate filaments (IF) exclusively expressed in the cytoplasm of neurones. Together with Peripherin, α-internexin, neurofilament medium chain (NF-M) and neurofilament heavy chain (NF-H) they form cylindrical proteins called neurofilaments (NFs; see Figure 2). The diameter of these proteins are ~10 nm and their main function is to provide stability and structural integrity within neurons [41]. NFs are primarily located in the axons, dendrites, and soma of neurons. Their concentration is highest in axons, and therefore, damage to larger diameter axons results in elevated NFs levels. Following neuronal damage, NFs are released to the interstitial fluid, CSF, and peripheral blood [42].

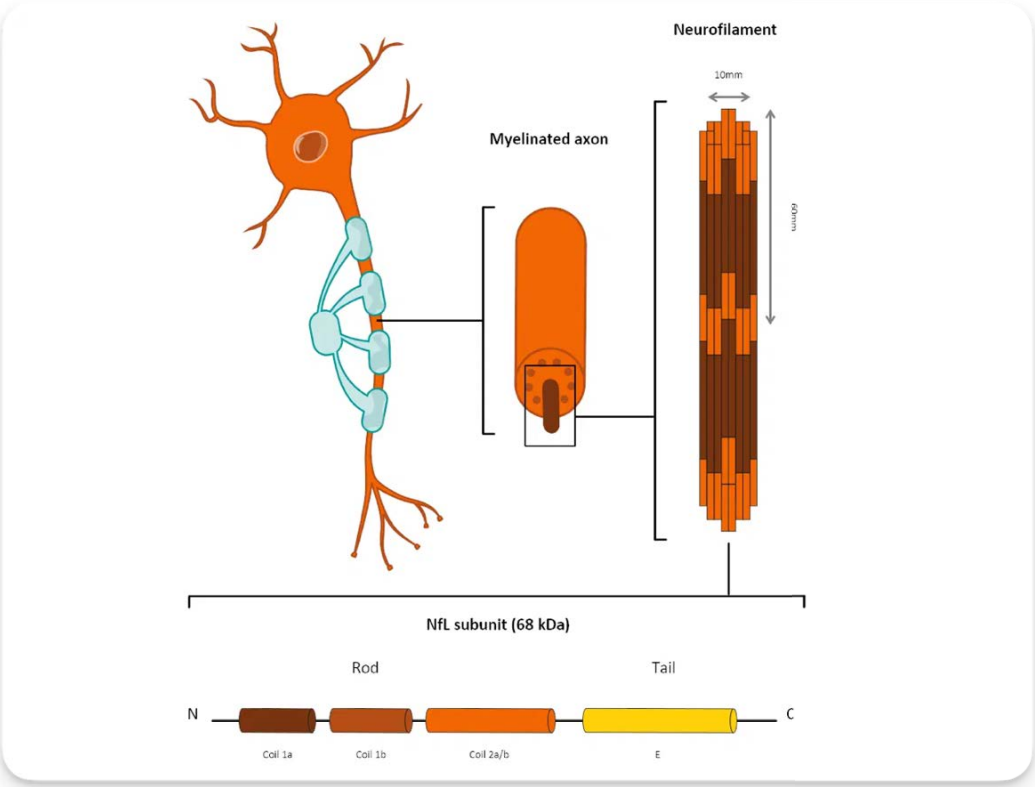

Figure 2. Structural organization and axonal localization of NfL [43]. Image © Siemens Healthineers AG, 2025. Reproduced with permission. Available from: https://www.siemens-healthineers.com/de/laboratory-diagnostics/assays-by-diseases-conditions/neurologie/neurofilament-leichtketten-assay.

1.2.1 Neurofilament Structure and Assembly

NFs are built from subunits with a consistent three-part structure: a head, a rod-shaped body, and a tail. The head, rich in the amino acids serine and threonine, can be modified in various ways (phosphorylation and glycosylation). The rod domain maintains a uniform α-helical structure across all subunits, while the tail is primarily composed of glutamic acid, lysine and serine. Assembly begins with the formation of a dimer, in which two subunits (monomers) interact via their rod domains. Dimers then align in opposite orientations to form tetramers. Eight tetramers then link together to generate a short filament, known as a unit-length filament (UFL). These short filaments then align end-to-end and condense to form the final NF structure. The assembly process is illustrated in Figure 3. After synthesis, NF subunits undergo post-translational modifications that protect them from proteolytic degradation [41].

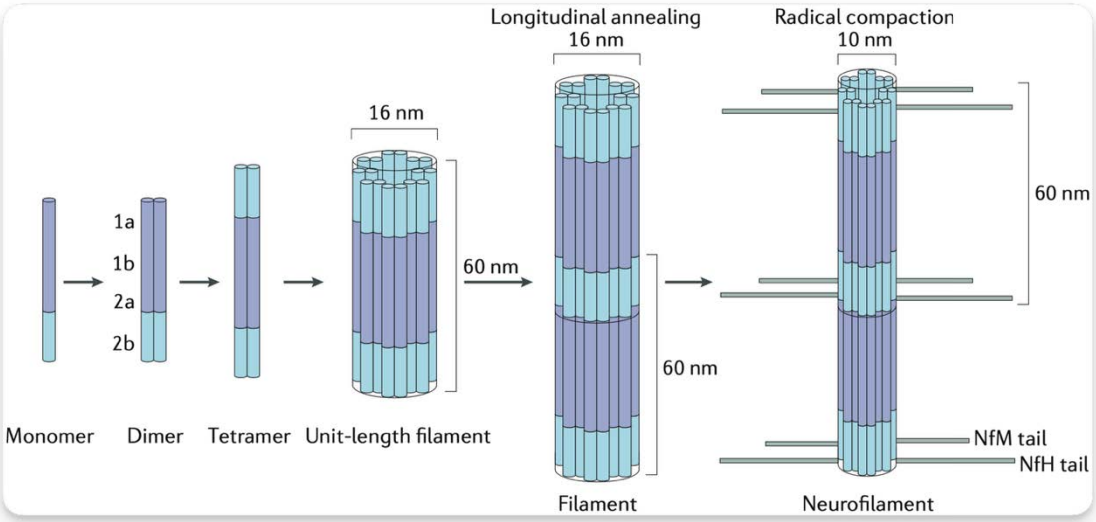

Figure 3. Overview of neurofilament subunits and their hierarchical assembly [41]. Licensed for use under Copyright Clearance Center License Number [6071910043763].

1.2.2 Neurofilament Function

Despite ongoing investigation, NFs are believed to support axonal growth and stability, enabling rapid nerve conduction [41]. Several studies have also identified interactions between NFs and various cellular components, including mitochondria, actin, tubulin, brain spectrin, kinases, phosphatases, molecular motors, receptors and proteases. Moreover, NFs are thought to regulate microtubule (MT) organization in axons through tubulin-binding sites located in their head domains (shared by all subunits), that suppress MT polymerization. The same binding sites may also exist in the tail domains of NF-H and NF-M, potentially contributing to MT regulation. One animal study also showed that NfL-derived peptides inhibit MT polymerization, reduce cell proliferation, and suppress tumor growth. These findings highlight the importance of NF and MT interactions in maintaining axonal structure and intracellular transport. In addition, NFs help organize organelles such as synaptic vesicles, endosomes, and the endoplasmic reticulum (ER). They also guide the positioning of these structures, including lysosomes. Notably, NfL interacts with mitochondria and may play a role in regulating their function [44].

1.2.3 Instrumental diagnostics

Detecting NFs in blood and CSF was once difficult, but advances in diagnostic tools have improved sensitivity and specificity. The first method used was immunoblots (1st-generation immunoassays), which could detect but not precisely measure NF levels in blood and CSF [41]. In 1971 Engvall and Perlmann invented a new method of performing immunoassays, called enzyme-linked immunosorbent assay (ELISA) [45].

1.2.3.1 Enzyme-Linked Immunosorbent Assay (ELISA): Second-Generation Immunoassay

ELISA, a second-generation immunoassay, enabled the first quantitative measurement of NF-H and NfL in CSF [41]. The first study to use ELISA for detecting NfL in CSF was published in 1996, reporting reliable quantification with a detection limit of 125 ng/L. However, NfL levels in most HC were undetectable in CSF and remained below the detection limit in blood in all subjects. Increases in CSF NfL levels were associated with age and were significantly higher in patients with ALS, AD, VaD, olivopontocerebellar atrophy (OPCA), normal pressure hydrocephalus (NPH), cerebral infarctions (INF), and relapsing-remitting multiple sclerosis (MS). ALS patients, especially with upper motor neuron (UMN) signs, had the highest levels. While not reaching statistical significance due to the small sample size, CSF NfL showed potential as a diagnosic and prognosic biomarker, particularly for ALS and other neurodegenerative diseases [46].

The first commercially available immunoassay for detecting NfL, introduced in 2003 [42], achieved a sensitivity of 60 ng/L [47]. The first study to use this ELISA revealed significant CSF NfL elevations across various neurological disorders compared to HC. The highest mean concentrations were observed in patients with INF, followed by those with ALS and relapsing-remitting MS. Among extrapyramidal disorders, PD had the lowest levels, while multiple system atrophy (MSA) and PSP averaged higher NfL levels. Moderate increases were also seen in dementias, with VaD showing higher levels than late-onset AD. In contrast, most HC had NfL levels below the assay's detection threshold [47].

This newer, commercially available ELISA improved upon previous methods, offering enhanced sensitivity, defined epitope specificity, no cross-reactivity with

NF proteins (NF-M and NF-H), and an readily available antibody supply, which made it valuable for diagnosis, prognosis, and disease monitoring [47]. However, interlaboratory variability remains a challenge. An international multicenter study involving 35 laboratories reported high consistency within individual labs when testing samples, but large differences between labs when comparing NfL levels from the same samples, largely due to errors in sample preparation. With standardized protocols, variability was reduced from 59% to 14%, highlighting the importance of accurate standard preparation and precise pipetting for reliable NfL measurement. As a result, this technique demands expert laboratories to ensure high accuracy and reproducibility [48].

1.2.3.2 Electrochemiluminescence (ECL) Assay: Third-Generation Immunoassay

The first electrochemiluminescence (ECL)-based immunoassay, developed in 2013 [49], marked the third generation of NfL detection tools [41]. Compared to ELISA, ECL offers higher sensitivity (15.6 pg/mL), detects NfL in both CSF and blood [49], and requires less sample volume [41]. ECL was first applied in a 2013 study that measured NfL levels in blood across several neurodegenerative diseases. In that study, the assay was validated for serum NfL, showing significantly elevated NfL levels in ALS, Guillain-Barré Syndrome (GBS), and AD compared to control patients. Serum NfL levels correlated strongly with CSF concentrations, except in control subjects, and the assay showed high reproducibility, supporting its clinical potential [49].

1.2.3.3 Single Molecule Array (Simoa) Assay: Fourth-Generation Immunoassay

In 2014, Quanterix introduced the single molecule array (Simoa) [50], a 4th-generation immunoassay that revolutionized diagnostics [41]. It is 126 times more sensitive than ELISA and 25 times more sensitive than ECL for detecting NfL in CSF [41]. The Simoa assay also demonstrates the strongest correlation between CSF and serum NfL levels compared to ELISA and ECL, confirming its superiority as the most sensitive and reliable method for quantifying NfL [51]. Simoa uses antibody-coated paramagnetic particles [52] in beads (2.7 μm in diameter) [41] that bind target proteins in sample volume containing biotinylated detection antibodies

and a streptavidin-conjugated reporter enzyme [52]. This forms sandwich immunocomplexes, consisting of two antibodies and one antigen [41]. The beads are then distributed into a disc containing approximately 240,000 microwells, each capturing a single bead that can carry only one analyte. After exposure to enzyme substrates, beads with bound analytes emit fluorescent signals, which are quantified by Simoa's analysis software [52].

Simoa's key advantage is its ultra-high sensitivity, which is up to 1,000 times greater than that of ELISA, allowing detection of NfL at femtogram (fg/ml) levels [53]. This is particularly important given that blood NfL concentrations are significantly lower than those in CSF. Compared to CSF analysis, blood-based NfL testing is less invasive and more practical, avoiding lumbar punctures and enabling safer, repeated sampling for longitudinal analyses of NFs [41]. Serum NfL has been shown to be stable under storage conditions, including room temperature and repeated freeze-thaw cycles from −80 °C, making it a reliable marker for tracking longitudinal changes in disease progression. It is valuable for early diagnosis, monitoring disease progression, and assessing treatment response [49], even in conditions where CSF sampling is uncommon [41]. In research settings, blood NfL can be used to evaluate drug efficacy and neuroaxonal damage, particularly in early-phase trials such as proof-of-concept and dose-finding studies [42]. Identifying disease at an early stage may enable earlier therapeutic intervention.

While promising, NfL still requires further validation to confirm its diagnostic and prognostic utility across neurological disorders. To ensure consistency and comparability across studies and clinical settings, standardized measurement protocols and age- and sex-specific reference values are essential. Moreover, factors such as age, comorbidities, and peripheral nerve involvement must be accounted for to enable accurate interpretation of NfL levels. Despite these limitations, blood NfL provides a cost-effective, non-invasive, and real-time indicator of axonal injury, making it a valuable biomarker in both research and clinical applications [41].

1.2.4 Physiological Neurofilament levels

With increasing age, neurodegeneration is reflected by brain atrophy and increasing NfL levels in both blood and CSF. In healthy individuals, CSF NfL levels rise approximately 2.5-fold between ages 20 and 50, and double again by age 70, possibly due to reduced CSF turnover or slow axonal damage, though the underlying cause remains unclear [41]. Blood NfL increases from 27.9 pg/mL at age 30 to 65.1 pg/mL by age 70, while CSF levels range from 387 to 2,417 pg/mL between ages 20 and 80 [42]. Data from fourth-generation assays indicate a 2.2% annual increase in blood NfL between ages 18 and 70, possibly reflecting aging processes in both the central and peripheral nervous systems. As a results, standardized age-related reference values in HC are essential for interpreting NfL as a biomarker in clinical and research settings [41].

1.2.5 Pathological Neurofilament levels

In neurological disorders such as ALS, DLB, and PD, NF structures form abnormal “liquid crystal gel networks”, a process influenced by subunit stoichiometry and phosphorylation [41]. Although NfL levels naturally increase with age in healthy individuals, neurodegeneration leads to further elevations [42]. Genetic mutations in NF-related genes such as PRPH (which encodes peripherin), NEFH (encoding the NF-H), and NEFM (encoding the NF-M) may contribute to NF accumulation and have been linked to ALS and familial PD. Additionally, mutations in genes encoding heat-shock proteins (linked to Charcot–Marie–Tooth disease), gigaxonin (linked to giant axonal neuropathy), and superoxide dismutase (associated with ALS) can indirectly impair NF function [41].

NFs have been extensively researched in neurological disorders like MS, dementias (e.g., AD, FTD), stroke, TBI, ALS, PD, and Huntington’s disease, where they have proven useful for diagnosis, prognosis, or treatment monitoring. In other conditions such as epilepsy, encephalitis, meningitis, hypoxia, optic neuropathies, increased intracranial pressure, neurotoxicity, and peripheral neuropathies, their usefulness remains uncertain due to limited systematic research [41].

Among the subunits, NfL reliably indicates axonal damage but lacks disease specificity. Although it cannot clearly distinguish between disorders with overlapping NfL levels, it can aid in identifying differences in disease progression

and severity, as well as in distinguishing disorders that involve neurodegeneration from those that do not. To enhance its diagnostic utility, NfL should be evaluated in combination with clinical assessments, other biomarkers, and neuroimaging data [42].

NfL has received increased attention compared to other NF subunits due to its abundance and higher solubility, which allow for more consistent plasma distribution and reliable measurement. Nonetheless, phosphorylated NF-H may still have diagnostic value in diseases such as ALS [42]. As a sensitive biomarker, NfL has the potential to detect and monitor neurodegeneration, thereby enabling improved clinical decision-making and patient care.

1.2.6 Neurofilaments in Parkinson's Disease

The diagnosis of PD remains a significant challenge. Although it's a widespread neurodegenerative disease, there are no established fluid biomarkers available for clinical use [41]. Diagnosis is primarily based on clinical criteria, often at a point when the disease has already progressed significantly, highlighting the critical need for early and accurate diagnostic tools [30]. A summary of the systematic literature review is provided below. Unless otherwise specified, reported associations refer to cross-sectional correlations between NfL levels and clinical measures. For details on the individual studies, see Section 3.1.

1.2.6.1 Neurofilament Light Chain in Parkinson's Disease and Atypical Parkinsonian Disorders

The first study to investigate NfL levels in bodily fluids as a potential biomarker for PD was conducted by Holmberg et al. in 1998 [41]. It showed elevated CSF NfL levels in APDs such as PSP and MSA compared to PD [54]. These findings were further supported by a subsequent study focused on CSF NF-H [55]. Since then, numerous studies have shown that NfL concentrations are significantly higher in APDs, including MSA, PSP, and corticobasal syndrome (CBS), compared to PD [33,54,56–67]. Specifically, CSF NfL was elevated in PSP [33,54,56,59,61,62,65,67], MSA [33,54,56,57,61,62,64–66], and CBS [33,56,59,61,63] relative to PD. DLB patients also showed higher CSF NfL levels than those with PD [56]. Similarly, plasma NfL were elevated in MSA, PSP, and CBS compared to PD [57,58,68–70], with MSA exceeding those in PSP [69].

Serum findings support this overall pattern of elevated NfL in APDs relative to PD [70]. Meta-analyses further confirmed elevated CSF NfL in MSA versus PD [71] and across APDs compared to PD [72].

NfL levels in CSF, plasma, and serum have also been shown to reliably distinguish PD from APDs and DLB. CSF NfL levels were significantly higher in APDs (MSA, PSP, CBS) than in PD, with high diagnostic accuracy reported across studies [56–58,62,67,70,72,73]. CSF NfL also differentiated PD from MSA [62,72,73], PSP [67,72], and DLB [73]. Peripheral NfL measurements (plasma and serum) showed similarly high accuracy in distinguishing PD from APDs [57,58,68,70,74,75]. Notably, a serum NfL cutoff of 14.8 ng/L was associated with a 36-fold increased risk of APD (MSA and PSP) diagnosis [58], and plasma NfL was also shown to differentiate PD from MSA [68]. Additionally, serum NfL has been reported to distinguished PD from ET [76].

Beyond single-analyte comparisons, NfL also differentiated PD from APDs, and diagnostic accuracy was further improved by combining NfL with other biomarkers. For example, combining β-amyloid 42, total tau and NfL improved differentiation between PD and APDs (MSA and PSP) compared to NfL alone [69]. Similarly, studies found that combining NfL with α-syn outperformed either marker alone in differentiating PD from PSP, CBS, and MSA [57]. More specifically, for PD versus MSA, combining CSF NfL with total tau improved accuracy [62,64], while for PD versus PSP, the NfL/Aβ42 ratio performed better than NfL alone [67]. Nevertheless, one study suggests NfL alone performs as well as or better than other individual markers, including α-syn, Aβ42, total tau, and p-tau, and that adding additional biomarkers does not substantially improve overall diagnostic accuracy [56]. Supporting this, one study found that CSF NfL alone outperformed tau, phosphorylated neurofilament heavy chain (pNF-H), and noradrenergic metabolite 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG) in distinguishing PD from MSA [64].

Longitudinal studies have demonstrated that CSF NfL levels significantly increase over time in individuals with PD, reflecting ongoing neurodegeneration [33,77,78]. Within PD subgroups, research has shown that GBA mutation carriers generally exhibited lower CSF NfL levels than IPD, but levels were higher in those with mild or severe risk GBA mutations compared to low-risk carriers [79]. Plasma NfL

further distinguished genetic forms of PD, with symptomatic GBA, LRRK2, and SNCA mutation carriers showing higher levels than individuals with IPD, and both groups exhibiting higher levels than asymptomatic genetic mutation carriers [80]. These findings underscore the value of NfL as a clinically relevant biomarker for differentiating PD from APDs and other movement disorders, as well as for tracking disease progression and enabling genetic risk assessment across biological fluids.

1.2.6.2 Neurofilament Light Chain in Parkinson's Disease and Healthy Controls

Several studies have shown that NfL levels are significantly elevated in individuals with PD compared to HC across CSF, serum, and plasma [59,61,67–70,75,76,78–93]. At baseline, CSF NfL levels were higher in PD patients than in HC [59,61,67,78–81,83,86,88], and this elevation was mirrored in serum [61,70,75,76,80,83,89,93] and plasma [68,69,82,84,85,87,91,92]. Longitudinal studies confirm that serum NfL increases more in PD than in HC over time [80,92]. One study reported higher plasma NfL in females with PD compared to HC, a difference not seen in males [89]. Additionally, individuals who later converted to PD showed higher serum NfL during follow-up, despite similar baseline levels to HC [90].

NfL has also been shown to reliably differentiate PD from HC across biofluids, including plasma [68,87,91], serum [61,76,83], and CSF [61,83,86]. Combining NfL with other biomarkers, such as α-syn, Aß1-42 and amyloid beta 1–40 (Aß1-40), further improves discrimination between PD and HC [69,83]. However, one study reported that α-syn alone outperformed NfL in distinguishing PD from HC [87]. Overall, NfL appears to be a reliable marker for differentiating PD from HC, especially when used in combination with other biomarkers.

1.2.6.3 Association of Neurofilament Light Chain with Demographics, Disease Duration, and Non-Motor Symptoms in Parkinson's Disease

Extensive research has shown that NfL levels increase with age in PD, with strong age-dependent elevations observed in CSF [56,57,60,67,70,77–79,83,86,94], serum [58,70,76,83,90,95–99] and plasma [57,68,82,84,90,100,101], highlighting

the need to adjust for age when interpreting NfL values. Additionally, serum NfL has been found to be negatively correlated with body mass index (BMI), suggesting higher BMI in PD is associated with lower NfL levels [96]. NfL levels also tend to increase with disease duration in plasma [70,102], CSF [77,79], and serum [99].

The relationship between NfL levels and sex in PD has been inconsistent across studies and biofluids. Some studies report higher NfL concentrations in females than in males, observed in both plasma [70] (63 females vs. 108 males) and serum [80] (140 females vs. 257 males), while other studies found the opposite, with higher NfL levels in males, particularly in plasma [101] (13 females vs. 13 males) and CSF [79] (135 females vs. 236 males). These conflicting findings suggest that the influence of sex on NfL levels may be affected by cohort characteristics (e.g., sample size, age distribution, disease stage), the biological fluid analyzed, and whether key confounding variables such as age and BMI are controlled.

Elevated plasma NfL levels in PD have also been associated with a greater NMS burden. Higher baseline NfL levels have been linked to more severe depressive symptoms at baseline, and predicted a higher likelihood of progression to dementia at follow-up [82]. Patients with RBD showed higher baseline NfL than those without [100]. Additionally, higher baseline NfL levels correlated with worse scores on various NMS measures, such as the Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS) part I, the Non-Motor Symptoms Scale (NMSS), and the Cumulative Dysautonomia Symptoms Scale (CDSS). Elevated NfL also predicted an increased risk of institutionalization and dependency in activities of daily living (ADL) at follow-up [82]. Elevated NfL in both plasma and CSF was also associated with the presence of orthostatic hypotension [57]. In CSF, longitudinal correlations have been observed between NfL and hyposmia severity at both baseline and follow-up [78].

Taken together, these findings suggest that NfL levels in PD are influenced by demographic and clinical factors, are associated with NMS burden, and underscore their value as markers of disease progression and ongoing neurodegeneration.

1.2.6.4 Association of Neurofilament Light Chain with Motor Dysfunction in Parkinson's Disease

Multiple studies have shown that NfL levels in serum, CSF, and plasma are positively correlated with motor severity and progression in PD. Baseline NfL concentrations in serum [76,93,95–97,99], CSF [57,78,79,81,94], and plasma [57,68,70,81,82,84,100] showed significant positive correlations with baseline scores on Part III of the MDS-UPDRS (MDS-UPDRS-III; motor examination) and the original Unified Parkinson's Disease Rating Scale (UPDRS-III), indicating that higher NfL levels were associated with greater motor impairment. Similar correlations have been reported between scores on Part II of the MDS-UPDRS (MDS-UPDRS-II;motor aspects of daily living) [82] or UPDRS (UPDRS-II) [98] and NfL levels in both plasma [82] and serum [98]. Furthermore, studies have shown that baseline serum [97] and plasma [82] NfL levels predict motor decline, as reflected by follow-up MDS-UPDRS-III scores [97]. Additionally, elevated baseline CSF and plasma NfL levels were positively correlated with the rate of change in MDS-UPDRS-III scores, suggesting a faster rate of motor decline, especially in older individuals with higher NfL concentrations [81].

Further associations between NfL and specific motor symptoms have also been reported. Elevated baseline serum NfL levels were linked to an increased risk of FOG [103], as well as higher akinetic-rigid symptoms at both baseline [102] and follow-up [97]. In CSF, longitudinal correlations have been observed between NfL and worsening bradykinesia and axial motor impairment (based on items from UPDRS-III), at both baseline and follow-up. Follow-up CSF NfL also correlated with greater severity of postural instability and freezing of gait, while baseline levels were associated with poorer timed up-and-go (TUG) test performance, indicating impaired mobility and postural control [78]. Collectively, these findings support NfL as a promising biomarker for tracking motor symptom severity and progression in PD across multiple domains and biological fluids.

1.2.6.5 Association of Neurofilament Light Chain with Disease Severity in Parkinson's Disease

Several studies have demonstrated that NfL levels are strongly associated with disease severity in PD, as measured by H&Y stage and MDS-UPDRS total scores. NfL concentrations in plasma [68,84,100] and serum [76] increased progressively

across H&Y stages 1 to 5, with significantly higher levels observed in more advanced stages [84]. Multiple studies reported that baseline NfL levels correlated positively with H&Y stage in serum [61,76,96,98,99], plasma [57,70], and CSF [33,56,57,78,79]. One study found that patients with higher baseline plasma NfL levels reached more advanced H&Y stages at both baseline and follow-up [82]. Notably, elevated plasma NfL levels were associated to a threefold increased risk [84], and elevated serum NfL to a sixfold increased risk [98], of reaching H&Y stage ≥ 3. In addition, baseline NfL also correlated with baseline UPDRS or MDS-UPDRS total scores in serum [80,97] and CSF [78]. Follow-up serum NfL were similary correlated with follow-up UPDRS total scores [98]. Collectively, these findings support the utility of NfL as a robust biomarker for tracking disease severity and progression in PD.

1.2.6.6 Association of Neurofilament Light Chain with Cognitive Impairment in Parkinson's Disease

NfL has been identified in multiple studies as a strong biomarker of cognitive impairment and decline in PD across plasma, serum, and CSF. Higher baseline NfL levels were linked to more sever cognitive deterioration, as evidenced by negative correlations with baseline global cognitive measures, including the Mini-Mental State Examination (MMSE) in plasma [68,84,100,101], serum [61,76,83] and CSF [61], the Montreal Cognitive Assessment (MoCA) in serum [92,93,95,96,99] and CSF [59,79,104], and the Dementia Rating Scale-2 (DRS-2) in plasma [81] and CSF [59,81]. In addition, one study found that baseline CSF NfL levels were negatively correlated with the annual rate of change in DRS-2 scores, indicating that higher NfL were associated with faster cognitive decline [59].

Several studies also demonstrated that elevated baseline plasma NfL levels were strong predictors of cognitive decline in PD. Higher baseline concentrations were associated with over a fivefold increased risk of cognitive conversion (PD with normal cognition (PD-NC) to PD with MCI (PD-MCI)/PDD or PD-MCI to PDD) [81] and a threefold risk of direct progression from PD to PDD [84]. Another study reported that patients with high serum NfL had more than a threefold greater risk of cognitive deterioration compared to those with lower NfL levels [96]. Furthermore, NfL levels were shown to increase in with the severity of cognitive

impairment, rising progressively from PD-NC to PD-MCI and further to PDD, as observed in both plasma [68,84,100,101] and serum [76]. Similarly, CSF NfL were higher in cognitively impaired PD patients than PD-NC [79,104], with the highest levels observed in patients with PDD [56,57,67]. One study found that CSF NfL levels were also elevated in PD patients with baseline cognitive impairment compared to those who developed it later or maintained normal cognition [79]. A CSF NfL threshold above 1100 ng/L was identified as predictive of future PDD risk [67] and high serum NfL levels in PD and PD-MCI were similarly associated with an increased risk of progression to PDD [92]. Moreover, combining CSF NfL with additional markers such as heart-type fatty acid–binding protein (HFABP) and Aβ42 improved predictive accuracy compared to NfL alone [67].

Higher baseline and longitudinal serum NfL levels were further associated with greater decline in cognitive performance across multiple studies. Affected domains include global cognition, measured by MoCA, processing speed and attention, assessed using the Symbol Digit Modalities Test (SDMT), and episodic memory, evaluated with the Hopkins Verbal Learning Test (HVLT). These associations were seen both at baseline [92] and during follow-up [92,93]. These associations were particularly pronounced in individuals with PD-MCI and in amyloid-positive PD patients [92]. At baseline, serum NfL also showed negative correlations with clinical scores in visuospatial ability (assessed by Benton Judgment of Line Orientation [BJLO] test), processing speed/attention (evaluated using [SDMT]), and language (measured by Semantic Fluency) [93]. Furthermore, baseline serum NfL negatively correlated with follow-up performance in specific cognitive domains, including executive function and working memory (measured by Letter-Number Sequencing [LNS] test), visuospatial ability (BJLO) and language (semantic fluency). Higher longitudinal NfL levels were also linked to poorer follow-up outcomes in visuospatial function and language, as measured by BJLO and Semantic Fluency tests, respectively [92].

Finally, plasma NfL has been shown to effectively differentiate PD patients with cognitive impairment (PD-CI) from those with PD-NC. Studies reported that higher plasma NfL levels reliably distinguished PD-CI from PD-NC and also separate individuals with PD-NC or PD-MCI from those with PDD. Meta-analyses further confirm that NfL levels were higher in PD-CI than PD-NC [100]. Collectively, these

findings highlight NfL as a valuable biomarker for identifying and monitoring cognitive decline in PD, including differentiation between PD-NC, PD-MCI, and PDD, as well as predicting progression between these stages.

1.2.6.7 Association of Neurofilament Light Chain with Clinical Subtypes in Parkinson's Disease

Studies have shown that NfL levels differed among PD subtypes, particularly between the postural instability and gait disorder (PIGD) and TD phenotypes. Serum NfL was significantly higher at baseline in PIGD patients compared to non-PIGD patients. Baseline PIGD scores (derived from the MDS-UPDRS scores) correlated positively with baseline NfL levels [95,98], and elevated serum NfL predicting faster PIGD progression, even in those with mixed PIGD and tremor subtypes [97]. Plasma NfL was also higher in PIGD than in TD patients and was associated with worse motor function (derived from the MDS-UPDRS scores) and poorer cognitive performance (assessed by MoCA and MMSE) in the PIGD group [91]. In CSF, both PIGD and TD subtypes showed higher NfL levels than HC [78]. These findings supported the use of NfL as a biomarker for distinguishing PD subtypes and tracking severity and progression, particularly in the PIGD phenotype.

1.2.6.8 Associations Between Neurofilament Light Chain with Prognostic Indicators in Parkinson's Disease

Elevated NfL concentrations in CSF have been associated with increased mortality risk and shorter survival in individuals with PD [57,78], as well as in cohorts including both PD and MSA patients [60]. Longitudinal studies showed that high serum NfL was negatively correlated with ADL scores, indicating greater functional impairment in patients with elevated NfL. Moreover, a serum NfL threshold of 18.4 pg/mL was predictive of reaching key clinical milestones, including the need for a walking aid, nursing home admission, and death [98]. These findings suggested that higher CSF NfL levels could serve as a prognostic biomarker for mortality in parkinsonian disorders and underscored the value of NfL as a marker not only of neurodegeneration but also of key clinical milestones in PD.

1.2.6.9 Association of Neurofilament Light Chain with Neuroimaging Markers in Parkinson's Disease

NfL levels were closely associated with neuroimaging markers of neurodegeneration in PD, reflecting structural brain changes linked to disease progression. One study showed that higher plasma NfL concentrations were negatively correlated with global and regional cortical thickness (CTh), including the frontal, temporal, parietal, and insular cortices [84]. Similarly, another study found that baseline serum NfL was negatively associated with baseline CTh in the temporal and fusiform regions, while higher follow-up serum NfL correlated with greater cortical thinning, reflected by lower follow-up CTh in the temporo-occipital, posterior cingulate/precuneus, superior parietal, and orbitofrontal areas. Cortical thinning in these regions was linked to poorer cognitive performance on related cognitive tasks. Orbitofrontal thinning was associated with worse HVLT scores, lower CTh in the superior temporal sulcus correlated with reduced semantic fluency, and cortical thinning in the precentral and lateral occipital cortices was related to lower SDMT performance [93]. In a separate study, higher plasma NfL levels were also associated with reduced hippocampal volume [84].

Another study reported that baseline serum NfL correlated with increased baseline mean diffusivity (MD) on diffusion tensor imaging (DTI) in the parahippocampal/entorhinal and temporo-occipital regions at baseline, while follow-up NfL was linked to elevated follow-up MD in the temporal and superior frontal cortices. These MD increases, reflecting microstructural damage, were associated with poorer baseline performance on cognitive tasks such as the BJLO and the LNS tests. At follow-up, increased MD was also associated with lower scores on the SDMT. The affected brain regions supported the specific cognitive functions assessed, suggesting that region-specific microstructural damage contributed directly to the observed deficits. Specifically, executive dysfunction, associated with fronto-striatal damage, is reflected in poorer performance on LNS and SDMT. In contrast, damage to posterior cortical regions is linked to impairments in visuospatial ability, language, and memory, as demonstrated by lower BJLO scores [93].

In addition, one study showed that higher baseline serum and CSF NfL predicted greater longitudinal loss of dopamine transporter (DAT) binding in the putamen

[78,97] and caudate [78], as measured by single-photon emission computed tomography (SPECT) [10], indicating faster dopaminergic neurodegeneration [78,97]. CSF NfL was also associated with increased axonal damage on DTI, initially in the internal capsule at baseline, progressing to frontal tracts at 1 year, and extending to the pons and limbic lobe by 3 years [78]. Together, these findings establish NfL as an imaging correlate of structural and microstructural brain changes in PD, supporting its potential as a biomarker of neurodegenerative progression and cognitive decline.

1.2.6.10 Association of Neurofilament Light Chain with Deep Brain Stimulation in Parkinson's Disease

One study investigated the effects of deep brain stimulation (DBS), a neurosurgical treatment for motor symptoms in advanced PD, on NfL levels and found a transient postoperative increase, likely reflecting the impact of the surgical procedure itself rather than stimulation or disease progression. The type of anesthesia, number of electrodes implanted, and baseline NfL levels did not significantly influence this NfL increase. Importantly, NfL concentrations returned to presurgical levels within 2 to 8 months after surgery, and no significant increase was observed following the initiation of DBS stimulation at either 2 or 8 months postsurgery [99]. These findings suggest that NfL elevations after DBS are procedural rather than a result of ongoing neurodegeneration.

In conclusion, NfL is a reliable marker of axonal damage with potential for diagnosing and monitoring various neurological diseases. While NfL shows diagnostic potential in PD, its greater value may lie in supporting clinicians in distinguishing PD from APDs, especially in cases with overlapping features. Nevertheless, strong and longitudinal correlations between NfL and clinical outcomes are still required to support its routine implementation in clinical practice [42].

2 Methods

2.1 Systematic literature review

2.1.1 Search strategy

A systematic literature review was conducted to provide an overview over the current state on NfL levels in PD. To evaluate the current knowledge on this topic, we constructed a search strategy to provide information on relevant scientific theories, methods and potential research gaps. The database used for the systematic literature review was PubMed. To increase our chance in finding all articles related to our topic, we applied Boolean operators with multiple search terms, as shown in Figure 4. Additionally, we reviewed the reference lists of selected articles and included further relevant studies. To be eligible for inclusion in our analysis, studies published in other languages were required to have detailed English abstracts.

```
("Parkinson Disease"[MeSH Terms] OR "Parkinson disease*"[Title/Abstract] OR
"Parkinson's Disease*"[Title/Abstract] OR ("Parkinsonian Disorders"[MeSH
Terms] OR "Multiple System Atrophy"[MeSH Terms] OR "Supranuclear Palsy,
Progressive"[MeSH Terms] OR "Parkinsonian*"[Title/Abstract] OR
"Parkinsonism*"[Title/Abstract] OR "Multiple system atrophy*"[Title/
Abstract] OR "Multisystem* Atroph*"[Title/Abstract] OR "Progressive
Supranuclear Pals*"[Title/Abstract])) AND ("Neurofilament Proteins"[MeSH
Terms] OR "Neurofilament*"[Title/Abstract]) AND ("Cerebrospinal Fluid"[MeSH
Terms] OR "Blood"[MeSH Terms] OR "Biomarkers/Blood"[MeSH Terms] OR
"Biomarkers/Cerebrospinal fluid"[MeSH Terms] OR "Neurofilament proteins/
Blood"[MeSH Terms] OR "Neurofilament proteins/Cerebrospinal fluid"[MeSH
Terms] OR "Cerebrospinal fluid*"[Title/Abstract] OR "Blood*"[Title/
Abstract] OR "Serum*"[Title/Abstract])
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Figure 4. Boolean search strategy for identifying studies on neurofilament biomarkers in parkinsonian syndromes. The search yielded 190 results, with the most recent publication dated January 2022.

The selection criteria used for this literature review included articles that reported data on NfL-levels measured using Simoa in PD and APDs and examined their potential correlation or association with clinical outcomes. We also included studies examining the utility of NfL in differentiating PD from APDs. Exclusion criteria for the literature review were articles that compared primarily other Biomarkers or did not provide any clinical data related to NfL levels in PD. Studies

investigating multiple biomarkers in relation to clinical outcomes were included only if NfL levels were analyzed separately. Additionally, studies involving participants with genetic forms of PD were only included when NfL level comparisons were made with sporadic forms of PD. To ensure all studies meet the exclusion criteria, all articles were title and abstract screened. In total, 27 studies were included in the final analysis, including cross-sectional studies and longitudinal studies.

2.2 Study design, ethical approval and patient consent

To gain a better understanding of the role NfL levels play in PD, data from the prospective, longitudinal registry on movement disorders (PROMOVE) in Graz were used to supplement and compare with the findings from the systematic literature review. In total 93 participants (47 HC and 46 PD) were included in our study. We examined data of 46 patients with PD from PROMOVE registry. In addition, 47 healthy individuals from the Austrian Stroke Prevention Family Study (ASPS Family) served as controls [105]. These participants were age and sex matched and did not have any first-degree relatives with movement disorders.

The PROMOVE registry is a longitudinal observational study on movement disorders conducted at the University Clinic for Neurology, Graz, Austria, with approval from the Ethics Committee of the Medical University of Graz. Since its initiation in January 2010, the registry has enrolled over 380 participants, all of whom provided informed consent prior to inclusion. All participants underwent a comprehensive baseline evaluation, which included physical and laboratory assessments, cognitive testing, blood sampling for biomarker analysis, and neuroimaging. Longitudinal follow-up assessments were conducted at four predefined intervals, enabling repeated collection of clinical, laboratory, and imaging data to assess disease progression over time. Detailed inclusion and exclusion criteria are provided in Table 2.

Inclusion criteria	Exclusion criteria
Confirmed diagnosis of a movement disorder	Patients without a solicitor and considered by the study doctor to be unable to sign the consent form
Age requirement: > 18 years	Age exclusion: < 18 years
MMSE score > 24 was required to authorize signing of the consent form. Otherwise, a solicitor's signature was needed.	No confirmed decision by patient or legal representative to sign the consent form
	Patient enrolled in a clinical trial for experimental medical treatment of a movement disorder

Table 2. Study inclusion and exclusion criteria.

2.2.1 Clinical Assessment

On the day of clinical assessment, the dopaminergic medications of PD patients were temporarily paused, and the evaluations were carried out in the off-medication state. To reliably assess PD severity, the levodopa equivalent dose (LED) was calculated based on the prescribed medication at the time of evaluation [106]. The H-Y scale [107] and the MDS-UPDRS [108] were utilized to determine the disease stage and severity of patients.

NMS were evaluated using the NMS questionnaire (30 items), which covers various categories, such as attention/memory, cardiovascular, mood/cognition, sleep/fatigue, perceptual problems, gastrointestinal, urinary, sexual function, and miscellaneous symptoms [109,110]. Depression in older adults was evaluated using the Geriatric Depression Scale (GDS), a 30-item assessment tool that evaluates mood, motivation, and overall sense of well-being. The severity of depressive symptoms is indicated by higher scores on the GDS [111–113].

All PD patients underwent cognitive assessment using the neuropsychological test battery from the Consortium to Establish a Registry for Alzheimer's Disease (CERAD), specifically the extended version known as CERAD-Plus, as detailed in Table 3 [114]. The total scores for the CERAD utilized in this trial were referred to as Total Score 1 (TS1) and Total Score 2 (TS2). The TS1 was calculated using verbal fluency, modified Boston Naming Test, constructional praxis, word list memory, word list recall and word list recognition. Similarly, TS2 included all the tests in TS1 and a constructional recall test. A separate CERAD memory score

was also calculated based on word list recall, word list recognition, and constructional praxis recall [115,116].

CERAD	CERAD-Plus
Tests for verbal fluency	Tests for verbal fluency
Word list memory test	Word list memory test
Modified Boston Naming Test	Modified Boston Naming Test
MMSE	MMSE
Test for constructional praxis	Test for constructional praxis
Tests for word list recall and recognition	Tests for word list recall and recognition
	Trail Making Test (TMT) parts A and B
	Phonematic fluency test (“s-words”)

Table 3. Overview of neuropsychological tests included in CERAD and CERAD-Plus.

2.2.2 Laboratory analysis

The laboratory conducted measurements using blood samples collected at baseline, with serum samples stored at -80 ◦ C until biomarker analyses. The serum NfL levels were measured in duplicate using Simoa NF-Light assay (Quanterix) on the HD-1 platform, following standard procedures.

2.2.3 Statistical analysis

Continuous variables were presented as mean ± standard deviation (SD) if normally distributed, or as median if not. The categorical variables were expressed as numbers or percentage. To test for normal distribution, the Kolmogorov–Smirnov and Shapiro–Wilk tests were used, confirming that all variables were normally distributed. Pearson correlation was used to examine the relationship between NfL and continuous variables. Independent t-tests were used to compare the two groups: PD and HC. Statistical significance was defined as a p-value less than 0.05. All statistical analysis were performed using the software IBM SPSS Statistics version 27 (IBM Corporation, Armonk, New York, USA).

Log-transformation of NfL levels (log NfL) was applied to address uneven distribution and the presence of extreme values (outliers) in the raw data. This adjustment helped to normalize the distribution and reduce variability, thereby increasing the robustness and reliability of statistical analyses. Similar approaches using log NfL levels have been employed in other studies [33,58,80,83,84,91,99].

In our dataset, the log-transformed NfL values also demonstrated stronger statistical significance than the raw NfL values (pg/ml), supporting the validity of this approach.

3 Results

3.1 Systematic Literature Review

A summary of the systematic literature review on NfL levels in PD is presented in in Sections 3.1.1, 3.1.2 and 3.1.3, including details on study design, sample characteristics, biofluid analyzed, and key findings.

3.1.1 Cross-Sectional Studies

Source	Study Population + Assay (CSF/Serum)	Key findings	Remarks
[76]	• Total (n = 288): 146 IPD, 82 ET, 60 HC • Assay: Simoa • Body fluids: Serum • Biomarkers: NfL	• NfL positively correlated with age in HC (r = 0.30, p = 0.02), PD (r = 0.25, p = 0.002), and ET (r = 0.30, p = 0.006). • Serum NfL levels in PD (16.6 ± 3.5 pg/ml) higher than ET (12.2 ± 2.4 pg/ml) and HC (11.8 ± 2.4 pg/ml, all p < 0.01). • NfL cutoff of 13.75 pg/mL distinguished PD from HC with 76% sensitivity, 85% specificity, and AUC of 0.869. • NfL cutoff of 13.65 pg/mL distinguished PD from ET with 76.7% sensitivity, 84.1% specificity, and AUC of 0.854. • PD: NfL levels increased with H-Y stage: 12.2, 15.1, 18.0, 20.6, and 25.1 pg/mL for stages I–V (F = 104.1, p < 0.001). • PD: ANOVA: NfL increased with cognitive impairment: HC (11.8	• Disease stage: Early-stage PD (H&Y stage 2.5). • *Adjusted for age, sex, education, BMI, age of onset, and disease duration.

		pg/mL), PD-NC (15.0 pg/mL), PD-MCI (19.6 pg/mL), and PDD (22.2 pg/mL; F = 114.8, p < 0.001). • PD: Positive correlation between NfL level and UPDRS-III score (r = 0.79, p < 0.001) + H-Y stage (r = 0.86, p < 0.001). • PD: Negative correlation between NfL level and MMSE scores (r = − 0.70, p < 0.001). • PD: Multivariate regression analysis showed that NfL is an independent predictor of motor symptom severity (H-Y stage: t = 8.75, VIF = 2.93, p < 0.001; UPDRS-III scores: t = 3.17, VIF = 2.92, p = 0.002) and cognition impairment (MMSE score: t = − 4.81, VIF = 1.73, p < 0.001) *.	
[103]	• Total (n = 361): de novo PD • Assay: Simoa • Body fluids: Serum • Biomarkers: NfL	• High NfL levels increased the risk of FOG* (continuous variable: HR = 1.03, p = 0.007, AUC = 0.71; dichotomous variable: HR = 1.57, p = 0.003, AUC = 0.68).	• Disease stage: de novo PD. • 189 Patients developed FOG over 5 years (median follow-up). • *Adjusted for Male sex, PIGD score, MoCA score, GDS score, and caudate DAT uptake.
[100]	• Total (n = 168): 38 HC, 130 PD [57 PD-NC, 34 PD-MCI, and 39 PDD] • Assay: Simoa • Body fluids: Plasma	• Mean NfL levels were higher in PDD (35.7 ± 21.7 pg/ml, p < 0.001) and PD-MCI (21.9 ± 10.3 pg/ml, p = 0.028) than HC (16.5 ± 7.3 pg/ml). • Mean NfL PD-NC (17.9 ± 8.9 pg/ml) and HC (16.5 ± 7.3 pg/ml) showed no significant difference.	• Disease stage: Early-stage PD (H&Y stage 2.1) • * Adjusted for age, sex, age at PD onset, disease duration, MDS-UPDRS-III score, H&Y

	• Biomarkers: NfL	• NfL: PD-NC < PD-MCI (p = 0.039). • NfL: PD-NC < PDD (p < 0.001). • NfL: PD-MCI < PDD (p = 0.004). • NfL showed positive correlation with age in controls (r = 0.69, p < 0.0001) and PD (r = 0.24, p = 0.005). • PD: NfL level positively correlate with MDS-UPDRS-III score (r = 0.29, p = 0.001). • NfL was higher in H&Y stage (p < 0.001) stage 5 (53.22 ± 31.2 pg/ml) than 1 (16.97 ± 8.8 pg/ml). • NfL in PD + RBD (28.67 ± 16 pg/ml) higher than without RBD alone (22.14 ± 15.8 pg/ml, p = 0.002). • PD: Negative correlation between NfL level and MMSE score (r = − 0.49, p < 0.0001). • ROC analysis: NfL distinguished PD from PD with cognitive impairment (AUC = 0.708, sensitivity 65.75%, specificity 71.93%; cutoff = 19.58 pg/mL). • ROC analysis: NfL distinguished PD-NC/PD-MCI from PDD (AUC = 0.7461, sensitivity 58.97%, specificity 86.81%; cutoff = 30.78 pg/mL). • Multiple linear regression: NfL levels (β = –0.11, p < 0.001) and H–Y stage (β = –1.332, p = 0.013) were both independent predictors of cognitive impairment *.	stage, and presence of RBD. • Subject with PD that developed Dementia after 1 Year were excluded. • Meta-analysis: Egger's and Begg's tests, along with the symmetrical funnel plot, indicated a low likelihood of publication bias.

		• Meta-analysis (this study + others): NfL levels association with cognitive impairment (pooled SMD: 1.286, $p < 0.001$; heterogeneity: I^2 = 78.8%). PD with cognitive impairment had substantially higher plasma NfL levels than those without.	
[73]	• Total (n = 113): 35 HC, 23 DLB, 26 MSA, 29 PD • Assay: Simoa • Body fluids: CSF • Biomarkers: NfL	• Mean NfL were higher in MSA (3,839.32 ± 615.85 pg/mL) than in PD (960.55 ± 108.82 pg/mL) and HC (810.00 ± 104.81 pg/mL; $p < 0.001$). • Mean NfL were higher in DLB (2,190.76 ± 421.46 pg/mL) than PD and HC (all $p < 0.001$). • ROC analysis: NfL could distinguish MSA + DLB from PD (AUC = 0.87, $p < 0.0001$); NfL could distinguish MSA + DLB from HC (AUC = 0.92, $p < 0.0001$). • ROC analysis: NfL could distinguish MSA from PD (AUC = 0.90, $p < 0.0001$); NfL could distinguish MSA from HC (AUC = 0.96, $p < 0.0001$). • ROC analysis: NfL could distinguish DLB from PD (AUC = 0.78, $p = 0.0008$); NfL could distinguish DLB from HC (AUC = 0.88, $p = < 0.0001$).	• Disease stage: Not available. • No sensitivity or specificity for ROC analysis given.
[57]	• Total (n = 355): 72 controls, 116 PD, 37 PDD, 64 DLB, 80 MSA, 58 PSP/CBS, 30 iAF, 19 iRBD • Assay: Simoa (plasma NfL),	• Age positively correlated with plasma NfL in PD ($\rho = 0.49$, $p < 0.0001$), PSP/CBS ($\rho = 0.44$, $p = 0.005$), and controls ($\rho = 0.71$, $p < 0.0001$). Similarly, age correlated with CSF NfL in PD ($\rho = 0.50$, $p < 0.0001$) and	• Disease stage: Early-stage PD (H&Y = 1.5). • PD vs. APD (ROC-analysis): No AUC for α-syn alone. • PD vs. PSP/CBS (ROC-analysis):

	ELISA (CSF NfL), RT-QuIC (CSF: α-syn), CLEIA (CSF: t-tau, p-tau, Aβ 42, and Aβ40) • Body fluids: CSF and plasma • Biomarkers: NfL, α-syn, t-tau, p-tau, Aβ42, and Aβ40	controls (ρ = 0.63, p = 0.005). • Median plasma NfL was higher in MSA (34.0 pg/ml) and PSP+CBS (26.6 pg/ml) than in controls (8.9 pg/ml) and PD (10.2 pg/ml; all p < 0.0001) *; MSA was slightly higher than PSP+CBS (p = 0.03) *. • Median CSF NfL was higher in MSA (3098.0 pg/ml) and PSP+CBS (1569.0 pg/ml) than in PD (566.5 pg/ml) and higher in MSA than PSP+CBS (all p < 0.0001) *. • ROC analysis: CSF NfL and plasma NfL distinguished PD from MSA (CSF: AUC = 0.991, 95.7% sensitivity, 100% specificity; cutoff = 1196 pg/mL; plasma: AUC = 0.972, 90.3% sensitivity, 96.4% specificity; cutoff = 17.2 pg/mL) and PSP/CBS (CSF: AUC = 0.940, 97.4% sensitivity, 80.8% specificity; cutoff = 1057 pg/mL; plasma: AUC = 0.936, 88.7% sensitivity, 87.8% specificity; cutoff = 16.6 pg/mL). • ROC analysis: PD vs. APD: CSF NfL (93.9% sensitivity, 90.8% specificity, AUC = 0.97) and plasma NfL (90.3% sensitivity, 91.7% specificity, AUC = 0.96). • ROC analysis: PD vs. APD, combined test (NfL + α-syn) higher sensitivity (91.4% α-syn; 93.9% CSF NfL; 98.3% combined test. α-syn alone had higher specificity (97.5% α-syn; 90.8% CSF NfL; 95.8%	No AUC for α-syn alone. • PD vs. MSA (ROC-analysis): No AUC for α-syn alone. • Biomarker values (pg/ml) are all ln-transformed. • *Adjusted for age. • PD vs. APD: combined test outperformed NfL or α-syn alone.

		combined). Combined test had highest diagnostic accuracy for PD vs. APD (AUC = 0.971). • ROC analysis: PD vs. PSP/CBS: combined test had highest diagnostic accuracy (AUC = 0.99) than CSF/plasma NfL or α-syn alone. • ROC analysis: PD vs. MSA combined test had higher AUC (0.97) than α-syn alone, but not CSF NfL alone (AUC = 0.99). • PDD (15.9 pg/ml) patients had lower median plasma NfL than MSA and PSP/CBS (all $p < 0.0001$). • PDD (807.5 pg/ml) had Higher CSF NfL than PD ($p = 0.039$) but lower than MSA and PSP/CBS ($p < 0.0001$). • PD+PDD: CSF and plasma NfL correlated negatively with MMSE ($\beta = -0.037$, $p = 0.001$; $\beta = -0.400$, $p = 0.001$), positively with H&Y stage ($\beta = 0.243$, $p < 0.0001$; $\beta = 0.200$, $p = 0.001$), UPDRS-III ($\beta = 0.014$, $p = 0.001$; $\beta = 0.009$, $p = 0.022$), and orthostatic hypotension ($\beta = 0.217$, $p = 0.030$; $\beta = 0.362$, $p = 0.005$). CSF NfL positively correlated with disease duration ($p = 0.007$). • PD/PDD: CSF NfL correlated positively with H&Y stage ($\beta = 0.143$, $p = 0.009$) and UPDRS-III ($\beta = 0.022$, $p = 0.019$) *. • CSF NfL levels were linked to survival in PD/PDD (HR = 12.6, $p = 0.01$). High CSF NfL	

		levels were strongly linked to shorter survival.	
[81]	• Plasma cohort (total n = 596): 198 HC, 79 MCI, 65 AD, 30 PPA, 35 bvFTD, 30 Tauopathy [PSP/ CBS], 20 DLB, 139 PD • CSF cohort (total n = 354): 51 HC, 20 MCI, 26 AD, 28 PPA, 31 bvFTD, 30 Tauopathy [PSP/ CBS], 19 DLB, 149 PD • Assay: Simoa • Body fluids: CSF and plasma • Biomarkers: NfL and other biomarkers	• PD: Plasma (r = 0.32, p = 0.00026) and CSF (r = 0.32, p = 0.00006) NfL levels in PD positively correlate with baseline MDS-UPDRS-III. Only plasma remained significant after adjustment* (p = 0.005, no coefficient mentioned). • PD: Plasma (p = 0.005) and CSF (p < 0.001) NfL levels predict rate of change in baseline MDS-UPDRS-III** (coefficient not mentioned). “Greater age and higher NfL measures associated with faster rate of motor decline.”. • PD: Plasma (p = 0.028) and CSF (p = 0.001) NfL levels predict rate of change in DRS-2 scores*** (coefficient not mentioned). “Higher NfL measures associated with faster rate of cognitive decline.”. • PD: plasma NfL levels in highest tertial had 5.34x higher chance of converting to MCI or dementia/MCI to Dementia (HR 5.34, p = 0.005) ***. • Median plasma NfL levels were higher in MCI, AD, PPA, bvFTD and PSP/ CBS than HC. • Median CSF NfL levels were higher in AD, PPA, bvFTD, PSP/CBS and PD (1024 pg/mL, p = 0.036) than HC (899.7 pg/mL). • PD: ROC analysis identified 14.6 pg/mL as the optimal plasma NfL	• Disease stage: Not available. • Median disease duration = 6 years. • *Adjusted for age, sex, disease duration, and education. • ** Adjusted for age, sex, disease duration, LEDD, and baseline MDS-UPDRS-III score. • *** Adjusted for age, sex, disease duration, education, and baseline DRS-2 score.

		cutoff for predicting MCI/dementia (AUC = 0.60, sensitivity 74.2%, specificity 54.0%).	
[82]	• Total (n = 137): 45 HC, 106 PD • Assay: Simoa • Body fluids: Plasma • Biomarkers: NfL	• NfL levels higher in PD (70.3 pg/ml) than HC (64.9 pg/ml, p < 0.001) *+sex. • PD: NfL levels positively correlated with baseline total MDS-UPDRS-I (r = 0.370, p = 0.002), -II (r = 0.336; p = 0.009) and -III (r = 0.232; p = 0.030) scores, CDSS (r = 0.394, p = 0.002) and NMSS total score (r = 0.280, p = 0.025). • PD: High NfL levels showed higher baseline H&Y stage (p = 0.004), MDS-UPDRS-II (p = 0.025)/III (p = 0.036), NMSS (p = 0.001), CDSS (p = 0.029), BDI-II scores (p = 0.035), and malignant phenotype prevalence (p = 0.019) than normal NfL levels. • High NfL levels showed higher MDS-UPDRS-II (p = 0.034) and NMSS scores (p = 0.015) than normal NfL levels *. • Baseline NfL levels correlated with age in PD (r = 0.546; p < 0.001). • High baseline NfL levels in PD correlated with follow-up: higher H&Y stage (p = 0.546), increased cases with progression to dementia (p = 0.003), institutionalization (p = 0.029) and dependency in ADL (p = 0.008) than in PD with normal NfL. • High baseline NfL levels in PD showed worsening in follow-up MDS-UPDRS-III	• Disease stage: Early-stage PD (H&Y = 2.0). • 14 PD Subjects were excluded due to conversion to APD, dementia, death and vascular chronic encephalopathy. • Subtyping: PD: High (H-NfL) or normal (N-NfL) based on the 80th percentile of age-matched HC; Malignant phenotype: required ≥2 of MCI, OH, or RBD. • Follow-up: 2 years (only for Clinical assessment). • *Adjusted for age. • ** Adjusted for age, sex, disease duration, baseline MDS-UPDRS-III, ΔLEDD and MMSE.

		than in PD with normal NfL (p = 0.004) **.	
[96]	• Total (n = 289): PD advanced stage • Assay: Simoa • Body fluids: Serum • Biomarkers: NfL, NT-proBNP, hsTnI	• NfL levels correlate with Age (r = 0.587, p < 0.001), BMI (r = -0.300, p < 0.001), MoCA scores (r = -0.372, p < 0.001). • NfL positively correlated with MDS-UPDRS-III (β = 1.84, p = 0.042) *. • NfL positively correlated with H&Y stages (β = 0.17, p = 0.001) *+FDR-adjustment. • High NfL levels were associated with a >3-fold increased risk of cognitive decline (HR = 3.23, p = 0.025) **. • NfL levels correlated with cardiac biomarkers (hsTnI: β = 1.20, p = 0.002, NT-proBNP (β = 1.49, p < 0.001) *. • NfL levels correlated with NT-proBNP (β = 1.43, p < 0.001) ***.	• Disease stage: Advanced stage PD. • Exclusion of 3 subjects due to sample collection after DBS. • DBS: 33% of subjects. • *Adjusted for age, sex, disease duration, LEDD and BMI. • **Adjusted for age, sex, disease duration and baseline MoCA. • ***Adjusted for age, sex, disease duration, LEDD, BMI, dysautonomia, hypertension, hypercholesterolemia, diabetes, prior myocardial infarction, prior stroke, atrial fibrillation, heart failure, eGFR<60 mL/min.
[83]	• Total (n = 191): 139 PD and 52 HC • Assay: Simoa (Serum) and ELISA (CSF) • Body fluids: Serum and CSF	• Median CSF (p < 0.001) and serum NfL (p = 0.005) levels were higher in PD (CSF: 866 pg/mL; serum: 18.7 pg/mL) than HC (CSF: 612 pg/mL; serum: 13.7 pg/mL). • CSF NfL was higher in PD than controls (p < 0.01) *. • Median CSF and serum NfL levels correlate with	• Disease stage: 76 PD early stage (disease duration ≤5 years and H&Y stage <3). • *Adjusted for age. • Combination of biomarkers outperformed

	• Biomarkers: NfL, o-/t-α-syn and p-/t-α-syn.	age in PD (CSF: r = 0.43, p = 0.005; serum: r = 0.49, p < 0.001) and HC (CSF: r = 0.57, p < 0.001; serum: r = 0.45, p = 0.002). • Binary logistic regression (PD vs. HC): CSF NfL could better differentiate PD from HC (AUC = 0.73, 68% sensitivity, 71% specificity; cutoff = 738 pg/mL) compared to serum NfL (AUC = 0.64, 61% sensitivity, 68% specificity; cutoff = 15.6 pg/mL). • Binary logistic regression (PD vs. HC): CSF NfL + CSF p-/t-α-syn + CSF o-/t-α-syn could differentiate PD from HC (AUC = 0.92, 85% sensitivity, 86% specificity). • Binary logistic regression (PD vs. HC): Serum NfL + CSF p-/t-α-syn + CSF o-/t-α-syn could differentiate PD from HC (AUC = 0.90, 91% sensitivity, 81% specificity). • Serum NfL levels negatively correlate with MMSE scores (β = -0.193; p = 0.02) *.	NfL alone: Serum NfL + CSF p-/t-α-syn + CSF o-/t-α-syn (91% sensitivity).
[84]	• Total (n = 170): 158 PD [42 PD-NC, 66 PD-MCI, 50 PDD], 12 HC • Assay: Simoa (NfL) and IMR (α-syn) • Body fluids: Plasma • Biomarkers: NfL and α-syn	• Log NfL levels were higher: in PD (2.76 ± 0.64) than HC (2.09 ± 0.40, p < 0.0001); in PD with advanced H&Y stage (3.01 ± 0.69) than early H&Y stage (2.68 ± 0.57, p = 0.008); in PDD (3.07 ± 0.62) than PD-MCI (2.73 ± 0.54, p < 0.05) and PD-NC (2.47 ± 0.64, p < 0.05). • PD: Plasma NfL is negatively associated with Cth (global, frontal,	• Disease stage: 47 PD advanced stage (H&Y > 3). • NfL levels: Log transformed. • *Adjusted for age, sex, and disease duration. • ** Adjusted for age and sex. • *** Adjusted for age, sex, disease

		temporal, parietal, and insula) and hippocampal volume (ρ = –0.21 to –0.26, p < 0.05). • PD: Plasma NfL is negatively associated with Cth (temporal (β = –0.06, p = 0.02) and insular (β = -0.09, p = 0.01) ***. • NfL levels correlated with age in PD (ρ = 0.56, p < 0.001). • NfL levels correlated with MDS-UPDRS-III motor scores (β = 2.18, p = 0.01) *. • NfL levels correlated with MMSE scores (β = –0.79, p = 0.02) *. • Higher NfL levels were associated with higher odds of advanced H&Y stage (OR = 2.97, p = 0.03). • Higher NfL levels were linked to a higher risk of developing PDD (OR = 2.81, p = 0.03).	duration, and MMSE scores.
[68]	• Total (n = 178): 116 PD, 22 MSA, 40 HC • Assay: Simoa (NfL) and IMR (α-syn) • Body fluids: Plasma • Biomarkers: NfL	• NfL levels correlate with age in PD (r = 0.25, p = 0.001) and HC (r = 0.59, p < 0.0001). • NfL levels were higher in MSA (35.8 ± 6.2 pg/mL) than PD (17.6 ± 2.8 pg/mL) and HC (10.6 ± 2.3 pg/mL, all p < 0.01). • NfL levels positively correlated with H&Y stage in PD (I–V: 10.7, 15.3, 28.8, 28.9, 66.8 pg/mL; p < 0.001). • ROC analysis: NfL could distinguish MSA from PD (AUC = 0.802, cutoff = 24.06 pg/mL, 75.3% sensitivity, 80.4% specificity). A 12.34 pg/mL cutoff (AUC = 0.754) differentiated PD	• Disease stage (PD): Not mentioned. • *Adjusted for age, sex, and disease duration. • **Adjusted for age, sex, disease duration, and baseline UPDRS-III scores. • *** Adjusted for age, sex, disease duration, and baseline MMSE scores.

		from controls (53.2% sensitivity, 90.5% specificity). • NfL levels positively correlated with H&Y stage in PD (r = 0.51, p < 0.0001) and UPDRS-III scores (r = 0.46, p < 0.0001). • Multivariate regression analysis (PD): NfL levels correlated with motor severity (UPDRS-III (β = 0.418, p < 0.001) *. • NfL levels PDD (25.6 ± 19.6 pg/mL) > PD-MCI (18.7 ± 13.1 pg/mL) > PD (10.4 ± 7.5 pg/mL) > HC (7.3 ± 3.8 pg/mL, all p < 0.001). • Multivariate regression analysis: NfL levels negatively correlated with MMSE scores in PD (β = −0.069, p = 0.003) *. • Cox regression analysis: High NfL levels associated with higher HR for motor symptom progression in PD (HR 1.03, p = 0.029) **. • ROC analysis (PD): NfL >21.84 pg/mL was linked to a higher risk of ≥2-point UPDRS-III progression than NfL <21.84 pg/mL (p = 0.002). • Cox regression analysis: High NfL levels linked to higher HR for cognitive progression in PD (HR = 1.03, p = 0.0152) ***. • ROC analysis: NfL >18.34 pg/mL as a risk threshold for cognition decline (p = 0.009).	
[58]	• Total (n = 137): 55 PD, 53 HC, 22 MSA, 7 PSP	• Serum NfL levels were higher in APD (MSA: 22.2 ± 11 ng/L; PSP: 25.6 ± 8.4 ng/L) than PD (10.4 ± 4.9	• Disease stage (PD): Early onset PD.

	• Assay: Simoa • Body fluids: Serum and CSF • Biomarkers: NfL	ng/L, p < 0.0001) and HC (11.5 ± 6.5 ng/L, p < 0.0001) *. • CSF NfL was higher in APD (MSA + PSP: 5,544 ng/L [MSA: 65,487 ng/L; PSP: 4,809 ng/L]) than in PD (1,239 ng/L, p < 0.0001) and controls (1,348 ng/L, p < 0.0001). • Serum NfL levels positively correlated with age in PD (r = 0.78, p < 0.0001) and HC (r = 0.66, p < 0.0001). • ROC analysis of CSF NfL: APD vs. PD (AUC = 0.90, sensitivity 75%, specificity 98%); APD vs. controls (AUC = 0.89, sensitivity 75%, specificity 100%). • ROC analysis of serum NfL: Differentiation of APD and PD was possible (AUC: 0.91, sensitivity = 86% and specificity = 85%; cutoff = 14.8 ng/L). • ROC analysis of serum NfL: Differentiation of APD and HC was possible (AUC = 0.88, sensitivity = 93% and specificity = 71%; cutoff = 13.6 ng/L). • With a 14.8 ng/L cutoff, serum NfL predicted APD (PPV = 76%) and PD (NPV = 92%) over 12 years. • Binary logistic regression: With 14.8 ng/L cutoff serum NfL (OR = 36) confirmed a 36-fold higher APD risk, independent of age. • APD: Serum NfL level positively association in APD with baseline International Cooperative Ataxia Rating Scale (r = 0.60, p = 0.003) and	• CSF: Cutoff values are missing. • *Adjusted for age and sample storage time. • Positive predictive value (PPV). • Negative predictive value (NPV).

		tandem gait test (r = 0.54, p = 0.004). • APD: Serum NfL positively correlated with follow-up (3-year) tandem gait (r = 0.53, p = 0.03), H&Y score (r = 0.68, p < 0.001), MMSE (r = 0.60, p = 0.019), and H&Y progression (r = 0.69, p < 0.001).	
[101]	• Total (n = 283): 59 HC, 26 PD-NC, 23 PDD, 119 AD, 56 MCI • Assay: Simoa • Body fluids: Plasma • Biomarkers: NfL	• Mean NfL levels (pg/ml): Higher in males (p = 0.03) and positively increased with age (r = 0.427, p < 0.001) across all groups. • PDD (23.3 ± 10.8 pg/ml) had higher NfL than PD-NC (15.4 ± 9.9 pg/ml, p = 0.04) and HC (17.8 ± 6.4 pg/ml, p = 0.048). • Higher plasma NfL correlated with lower MMSE in all subjects (r = −0.491, p < 0.001) and in PD (r = −0.323, p = 0.023).	• Disease stage (PD): not mentioned.
[69]	• Total (n = 99): 45 PD, 13 MSA, 8 PSP, 33 HC • Assay: Simoa • Body fluids: Plasma • Biomarkers: NfL, α-syn, total tau, Aβ42, Aβ40	• Plasma NfL was elevated in APD (MSA: 86.53 ± 33.74 pg/mL; PSP: 53.90 ± 22.97 pg/mL) vs. PD (20.43 ± 14.09 pg/mL) and HC (16.00 ± 5.18 pg/mL, all p < 0.0001) *. • NfL levels MSA > PSP (p = 0.02). • Best diagnostic panels: For APD vs. HC is Aβ42 + Aβ40 + Aβ42/40 + NfL + α-syn (AUC = 0.983, 98.5% sensitivity, 93.9% specificity). Same panel for PD vs. HC (AUC = 0.977, sensitivity 97.8, specificity 93.9%). • PD vs. APD: NfL + Aβ42 + total tau (AUC = 1.000, sensitivity and specificity 100%).	• Disease stage: Early stage (mean H&Y stage < 3 (SD 1.31) and disease duration < 5 (SD 4.92). • *Adjusted for age, sex, and disease duration. • Comparison with other biomarkers: NfL outperformed all biomarkers in differentiating: APD vs. PD/HC.

		• NfL reliably differentiated: PD vs. APD (AUC = 0.963, sensitivity 88.9%, specificity 95.0%), PD vs. MSA (AUC = 0.983, sensitivity 100%, specificity 95.6%), and PD vs. PSP (AUC = 0.993, sensitivity 82.5%, specificity 100%).	
[61]	• Total (n = 313): α-syn-related disorders [151 PD, 17 MSA, 45 DLB], tau protein-related disorders [38 PSP, 16 CBS, 11 AD, 15 FTD/ALS], 20 HC • Assay: Simoa • Body fluids: Serum and CSF • Biomarkers: NfL and pNF-H	• NfL levels were higher in CSF and serum across all groups vs. HC, with the highest CSF levels in CBS, MSA, PSP and DLB ($p < 0.001$) *. • NfL: MSA > DLB ($p < 0.001$) and PSP > DLB ($p < 0.002$). • CSF pNF-H levels were higher in all groups vs. HC ($p < 0.05$), except DLB. • NfL showed an AUC of 0.769 in CSF and 0.773 in serum for distinguishing PD from HC (for CSF pNF-H AUC was 0.668) **. • PD: serum NfL positively correlated with H&Y ($r = 0.27$, $p = 0.003$). • CSF NfL: MSA > PD/DLB ($p < 0.05$). • CSF NfL: MSA, PSP, and CBS > PD ($p < 0.05$). • In PD subjects, CSF and serum NfL correlated negatively with MMSE ($\tau = -0.22$ and -0.24; all $p < 0.001$).	• Disease stage (PD): Advanced stage (> 3 median H&Y stage). • * NfL levels not provided, just a box plot. • ** No specificity and sensitivity and cutoff values. • NfL outperforms pNF-H.
[74]	• KCL cohort (Group A, total n = 805): 158 CU, 86 MCI, 59 EOAD, 102 AD dementia, 54 FTD, 140 PD, 59 PDD/DLB, 19 CBS/PSP, 50	• Group A: NfL elevated in all vs. CU ($p < 0.0001$), except PD, DS, depression, and EOAD; NfL higher in all cognitively impaired and atypical parkinsonian groups than in PD ($p <$	• Disease stage: KCL and Lund (Early-stage PD (H&Y stage = 1.9). • NfL levels no provided, just a box plot.

	ALS, 41 DS, 37 Depression • Lund cohort (Group B, total: n = 1,464): 376 CU, 209 SCD, 280 MCI, 23 EOAD, 134 AD dementia, 150 FTD, 171 PD, 46 PDD/DLB, 24 CBS/PSP, 29 MSA, 22 VaD, 23 EOAD • Assay: Simoa • Body fluids: plasma • Biomarkers: NfL	0.0001). No NfL values available, only box plot. • Both cohorts: NfL levels showed large effect sizes compared to PD for APD (CBS/PSP: g = 2.0; MSA: g = 1.4) and cognitive impairment disorders (VaD: g = 1.88; FTD: g = 1.4; PDD/DLB: g = 1.1; AD dementia: g = 1.0). • NfL distinguished APD from PD with high specificity: Group A (AUC = 0.86, 56% sensitivity, 89% specificity); Group B for CBS/PSP (AUC = 0.95, 51% sensitivity, 100% specificity) and MSA (AUC = 0.88, 57% sensitivity, 90% specificity).	• No cutoff values for AUC. • Cognitively unimpaired (CU). • Early-onset Alzheimer's disease (EOAD): < 65 years. • Subjective cognitive decline (SCD). • Down Syndrome (DS). • Author's Conclusion: High plasma NfL levels in parkinsonism suggest APD due to greater axonal damage compared to PD.
[75]	• Total (n = 2061): 222 APD [101 PSP, 55 MSA, 40 CBS, 26 indeterminate], 76 HC, 1763 PD • Assay: Simoa • Body fluids: Serum • Biomarkers: NfL	• Mean NfL was higher in PD patients (26.5 pg/L) than HC (16.4 pg/L, p < 0.01). • Mean NfL in PD was lower than in PSP (47.4 pg/L, p < 0.05) and CBS (53.1 pg/L, p < 0.05). • Serum NfL differentiated PD from all PSP+CBS (AUC = 0.80, p < 0.05). No specificity or sensitivity available.	• Disease stage: not mentioned. • Death: 44 APD patients died (mean disease duration: 5.9 ± 2.3 years). • Autopsy: 17 of 44 deaths were confirmed postmortem, all matching antemortem diagnoses.
[70]	• Lund cohort (total n = 278): 171 PD, 30 MSA, 19 PSP, 5 CBS, 53 HC • London cohort (total n = 117): 20 PD, 30 MSA, 29 PSP, 12 CBS, 26 HC	• Lund cohort: Plasma NfL positively correlated with age in the whole cohort (ρ = 0.449), HC (ρ = 0.436) and PD (ρ = 0.577, all p < 0.001). • Lund cohort: female had higher NfL levels than male in the whole cohort (p = 0.041) and PD (p =	• Disease stage: Lund (Early-stage PD [mean H&Y stage 1.9, mean disease duration 5.3 years]); London (Early-stage PD [mean H&Y stage 2.5, mean disease duration

	• Early disease cohort (total n = 109): 53 PD, 28 MSA, 22 PSP, 6 CBS • Assay: ELISA (CSF), Simoa (serum and plasma) • Body fluids: Plasma (Lund), serum (London), serum/plasma (Early disease cohort) and CSF (Lund and London) • Biomarkers: NfL	0.040). No NfL values available. • Lund cohort: Plasma NfL positively correlated with CSF NfL in the whole cohort (ρ = 0.730, p < 0.001), HC (ρ = 0.572, p < 0.001), PD (ρ = 0.589, p < 0.001), and APD (ρ = 0.419, p = 0.003) *. • Lund cohort: Mean plasma NfL was higher in PSP (2,656 ng/L), MSA (3,435 ng/L), and CBS (2,498 ng/L) than PD (896 ng/L, all p < 0.001) **. Plasma NfL was higher in APD than HC (p < 0.001) *. • Lund cohort: In PD, plasma NfL positively correlated with disease duration (β = 0.278, p < 0.001), LED (β = 0.235, p = 0.003), H&Y stage (β = 0.187, p = 0.004), UPDRS-III (β = 0.227, p < 0.001), Timed Up and Go Test (β = 0.164, p = 0.036), and tandem gait test (β = 0.237, p = 0.045), reflecting worse motor symptoms. In APD, it correlated only with H&Y stage (β = 0.286, p = 0.040) and UPDRS-III (β = 0.449, p = 0.001). • London cohort: Serum NfL positively correlated with age in the whole cohort (ρ = 0.290, p = 0.001), HC (ρ = 0.411, p = 0.037) and PD (ρ = 0.483, p = 0.031), but not APD. • London cohort: In female NfL levels higher than male only in APD (p = 0.048). No NfL value available.	9.7 years]); Early disease cohort (mean H&Y stage 2.0, mean disease duration ≤3 years). • No cutoff value for AUC • *Adjusted for age and sex • ** Adjusted for age, sex and disease duration • Blood-based NfL performed as well as CSF NfL in discriminating PD from APD. • Author's conclusion: CSF and blood NfL are not elevated in PD, likely due to milder axonal degeneration compared to APD. NfL levels and disease duration in APD show no correlation, suggesting that myelinated axon degeneration likely occurs at a constant rate.

		• London cohort: Serum NfL positively correlated with CSF NfL in the whole cohort (β = 0.796, p < 0.001), HC (β = 0.022, p = 0.022) and APD (β = 0.677, p < 0.001) *. • London cohort: Mean serum NfL was higher in PSP (2,284 ng/L), MSA (3,004 ng/L), and CBS (2,845 ng/L) than in HC (638 ng/L) and PD (2,041 ng/L, all p < 0.001) *. PD also showed higher NfL than HC (p = 0.011) *. Higher NfL levels in APD (PSP, p = 0.011; MSA, p = 0.002; CBS, p < 0.001) than PD **. • Early disease cohort: Blood NfL was higher in PSP, MSA, and CBS than in early PD (all p < 0.001). No NfL values available, only box plot. • Diagnostic accuracy PD vs. APD (Lund cohort): Plasma NfL (AUC = 0.91, specificity 91%, sensitivity 82%); CSF NfL (AUC = 0.96, 93% specificity, 92% sensitivity). • Diagnostic accuracy PD vs. APD (London cohort): Serum NfL (AUC = 0.85, 90% specificity, 80% sensitivity). • Diagnostic accuracy PD vs. APD (Early disease cohort): AUC = 0.81, 80% specificity, 70% sensitivity. • Diagnostic accuracy PD vs. APD (all cohorts): Similar results with individual APD groups (PSP, MSA, and CBS).	
[97]	• Total (n = 376): de novo PD	• Baseline NfL levels correlate with Age (ρ = 0.60, p < 0.001).	• Subtypes: 264 TD, 43 indeterminate

	• Assay: Simoa • Body fluids: Serum • Biomarkers: NfL	• Higher baseline NfL levels positively correlate with MDS-UPDRS total scores (ρ = 0.17, p < 0.001) and MDS-UPDRS-III scores (ρ = 0.16, p = 0.001). • Higher baseline NfL levels negatively correlated MoCA scores (ρ = −0.21, p < 0.001). • A 10 pg/mL higher baseline serum NfL level was associated with annual increases of 0.52 ± 0.17 points in MDS-UPDRS-III (p = 0.003) and 0.96 ± 0.28 points in MDS-UPDRS total score (p < 0.001); males showed greater progression (+0.54 ± 0.24, p = 0.024; +1.10 ± 0.38, p = 0.005) *. • A 10 pg/mL higher baseline serum NfL level was linked to a 2.46 ± 0.43 point per decade increase in PIGD scores (p < 0.001). • Akinetic-rigid scores increased faster with higher baseline NfL (p = 0.006) *. No model coefficient available. • Higher baseline NfL predicted faster MDS-UPDRS-III and total score progression in PD+PIGD (p = 0.006) and worsening PIGD scores in both PIGD (p < 0.001) and tremor-dominant (p = 0.039) subtypes *. No model coefficient available. • Higher baseline serum NfL levels were associated with greater longitudinal loss of putamen DAT binding ratio (−0.14 ± 0.06 units per decade per 10 pg/mL	PD and 69 PIGD-dominant. • Disease stage: de novo PD. • * Adjusted for “sex, age at baseline, disease duration at baseline, their interactions with years in the study, and time varying LEDD”. • Progressive decline in DAT binding was associated with increasing MDS-UPDRS-III, MDS-UPDRS total scores, and akinetic-rigid scores (*p* < 0.001).

		increase; p = 0.013), though no association was seen at baseline *.	

Abbreviations	Explanation
AD	Alzheimer's disease
ADL	activities of daily living
ALS	amyotrophic lateral sclerosis
ANOVA	analysis of variance
APD	atypical Parkinsonian disorders
AUC	area under the curve
Aβ40	amyloid beta 1–40
Aβ42	amyloid beta 1–42
BDI-II	Beck Depression Inventory – II
BMI	body mass index
bvFTD	behavioral variant frontotemporal dementia
CBS	corticobasal syndrome
CDSS	Cumulative Dysautonomia Symptoms Scale
CLEIA	chemiluminescent enzyme-immunoassay
Cox regression analysis	proportional hazards regression model
CSF	cerebrospinal fluid
CTh	cortical thickness
DAT	dopamine Transporter
DBS	deep brain stimulation
DLB	dementia with Lewy bodies
DRS	Dementia Rating Scale
eGFR	estimated glomerular filtration rate
ELISA	enzyme-linked immunosorbent assay
ET	essential tremor
F	F-statistic (analysis of variance)
FDR	false discovery rate
FOG	freezing of gait
FTD	frontotemporal dementia
GDS	Geriatric Depression Scale
H&Y	Hoehn and Yahr scale
HC	health control
Hedges' g (g)	standardized mean difference effect size
HR	hazard ratio
hsTnI	high-sensitivity troponin I
I^2	Higgins' I-squared statistic for heterogeneity
iAF	isolated autonomic failure
IMR	immunomagnetic Reduction
IPD	idiopathic Parkinson's disease
iRBD	isolated REM sleep behavior disorder
KCL cohort	Multicenter participant group collected at the Maurice Wohl Clinical Neuroscience Institute, King's College London
Kendall's tau (τ)	Kendall's tau rank correlation coefficient
LED	levodopa equivalent dose
LEDD	levodopa equivalent daily dose

ln	natural logarithm
London cohort	participants recruited through clinics at the National Hospital for Neurology and Neurosurgery, Queen Square, London, United Kingdom
Lund cohort	participants enrolled at the Neurology and Memory Clinics, Skåne University Hospital, Lund, Sweden
MCI	mild cognitive impairment
MDS-UPDRS	Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale
MDS-UPDRS total	sum of parts I–IV of the MDS-UPDRS (overall motor and non-motor disability)
MDS-UPDRS-I	part I of the MDS-UPDRS (non-motor experiences of daily living)
MDS-UPDRS-II	part II of the MDS-UPDRS (motor aspects of daily living)
MDS-UPDRS-III	part III of the MDS-UPDRS (motor examination)
MMSE	Mini-Mental State Examination
MoCA	Montreal Cognitive Assessment
MSA	multiple system atrophy
NfL	neurofilament light chain
NMSS	Non-Motor Symptoms Scale
NT-proBNP	N-terminal prohormone of brain natriuretic peptide
o-/t-α-syn	oligomeric α-synuclein/total α-synuclein ratio
OH	orthostatic hypotension
OR	odds ratio
p	probability value
p-/t-α-syn	phosphorylated α-synuclein/total α-synuclein ratio
p-tau	phosphorylated tau
PD	Parkinson's disease
PD-MCI	Parkinson's disease with mild cognitive impairment
PD-NC	Parkinson's disease with normal cognition
PDD	Parkinson's disease dementia
PIGD	postural imbalance and gait difficulty
pNF-H	phosphorylated neurofilament heavy chain
PPA	primary progressive aphasia
PSP	progressive supranuclear palsy
r	Pearson's correlation coefficient
RBD	REM sleep behavior disorder
ROC	receiver operating characteristic
RT-QuIC	real-time quaking-induced conversion
Simoa	single-molecule array
SMD	standard mean difference
t	t statistic
t-tau	total tau protein

TD	tremor-dominant
UPDRS	Unified Parkinson's Disease Rating Scale
UPDRS-III	part III of the UPDRS (motor examination)
UPDRS-IV	part IV of the UPDRS (motor complications)
VaD	vascular dementia
VIF	variance inflation factor
α-syn	alpha-synuclein
β	standardized regression coefficient
ρ	Spearman's rank correlation coefficient

Table 4. Overview of cross-sectional studies investigating NfL concentrations in PD and APDs. The table includes details on assay method, biological fluid analyzed, biomarker measured, sample size, key findings, and reported associations between NfL levels and clinical features.

These findings are further explored in the context of longitudinal data, as summarized below.

3.1.2 Longitudinal Studies

Source	Study Population + Assay (CSF/Serum)	Key findings	Remarks
[98]	• Total (n = 85): IPD • Assay: Simoa • Body fluids: Serum • Biomarkers: NfL	• Baseline: High NfL levels positively correlated age ($p < 0.001$). • Baseline: High NfL levels positively correlated with UPDRS-II ($\beta = 4.44$, $p = 0.000$), H&Y stage ($\beta = 0.54$, $p = 0.003$) and PIGD-score ($\beta = 1.29$, $p = 0.050$). • Baseline: High NfL levels negatively correlated with ADL ($\beta = -18.93$, $p = 0.001$). • Cox regression analysis (cutoff = 18.4 pg/mL): Higher NfL levels predicted clinical milestone: Use of a walking aid (HR = 3.48, $p = 0.006$); Nursing home admission (HR = 5.08, $p < 0.001$); Progression to H&Y stage 5 (HR = 6.16, $p = 0.000$); Death (HR = 4.07, $p = 0.001$) *.	• Death: 32 PD. • Disease stage: not mentioned (no H&Y stage available). • No cutoff values, sensitivity and specificity available for AUC. • * Adjusted for age at onset, disease duration and sex.

		• Baseline NfL predicted progression milestones with AUCs of 0.68–0.71 (adjusted for age). Walking-aid (AUC = 0.705), Nursing home (AUC = 0.699), H&Y stage 5 (AUC = 0.680), Dementia (AUC = 0.669) and Death (AUC = 0.680). • Follow-up NfL levels positively correlated with follow-up UPDRS-II (β = 5.88, p = 0.020), UPDRS-total (β = 16.82, p = 0.025), H&Y stage (β = 0.79, p = 0.009) and PIGD-score (β = 3.73, p = 0.009). • Follow-up: High NfL levels negatively correlated with ADL (β = -20.24, p = 0.024).	
[117]	• Total (n = 578): 396 early-stage PD, 182 HC • Assay: Simoa • Body fluids: Serum • Biomarkers: NfL	• Rate of NfL increase in PD peaks at baseline 18.8 pg/mL (2.0 pg/mL/year, inverted-U curve). • Controls peak at 13.4 pg/mL (0.61 pg/mL/year). • NfL rises rapidly after symptom onset in PD, with the rate of increase slowing after 10 years. Over 30 years, serum NfL increases by 153%, a greater change than seen in CSF Aβ42, α-syn, t-tau, or p-tau. • Serum NfL rose rapidly then slowed in PD-NC and PD-CI. • NfL remained elevated in PD-NC and PD-CI compared to controls for 30 years after motor onset. The rise was greater in PD-CI (285%) than PD-NC (217%), with NfL showing the most notable z-score changes.	• Disease stage: Early-stage PD (H&Y stage < 3). • Subtypes: PD-NC and PD-CI. • No p value was provided because the modeled trajectory describes trends using mathematical modeling, not statistical hypothesis testing.

[90]	• Total (n = 72): 16 PD Converter (PDC), 20 PD Progressor (PDP), 16 RBD, 20 HC • Assay: Simoa • Body fluids: Serum • Biomarkers: NfL	• NfL levels positively correlated with age in all groups ($p < 0.001$). No r value given. • PDC showed higher annual NfL increase (7.7%) than controls (4.4%, $p = 0.009$). • PDC had higher NfL levels than before conversion ($p = 0.008$) *. No NfL value given (only paired line plot). • PDC NfL was higher than in controls ($p = 0.039$) *.	• Subtypes: Converters: Developed PD during follow-up; Progressors: Gained new, lasting prodromal markers (depression, hyposmia or probable RBD) but did not develop PD. • Disease stage: No H&Y stage available. • *Adjusted for age.
[91]	• Total (n = 199): 149 early-stage PD [70 TD, 55 PIGD, 24 Indeterminate], 50 HC • Assay: Simoa • Body fluids: Plasma • Biomarkers: NfL	• Baseline NfL levels positively correlate with age in PD ($\rho = 0.557$, $p < 0.001$) and HC ($\rho = 0.294$, $p = 0.038$). • Baseline (PD): NfL levels negatively associated with disease duration ($\rho = -0.291$, $p < 0.001$). • NfL levels at baseline were higher in PD (16.2 ± 7.6 pg/ml) than HC (8.8 ± 3.4 pg/ml, $p < 0.001$) *. • Follow-up: NfL levels were higher in PIGD (18.4 ± 14.5 pg/ml) than TD (12.6 ± 4.4 pg/ml, $p = 0.025$) **. • Follow-up (ROC analysis): NfL levels could differentiate TD from PIGD (AUC = 0.656; 90% sensitivity, 30.4% specificity). • Baseline: High NfL levels were associated with worse MoCA ($\beta = -3.168$, $p < 0.05$) and MDS-UPDRS-III ($\beta = 10.687$, $p < 0.01$) in PIGD ***. • ROC analysis: NfL levels could differentiate PD	• Disease stage: Early-stage PD (H&Y 1.8). • No cutoff values for AUC. • *Adjusted for age and sex. • **Adjusted for age, sex and disease duration. • *** Adjusted for age, sex, disease duration and LEDD.

		from HC (AUC = 0.833, 60% sensitivity, 90% specificity). • In PIGD, each 1-unit increase in ln-NfL (≈2.72 pg/ml) predicted a 9.73-point increase in MDS-UPDRS-III (p = 0.002) and a 2.09-point decrease in MMSE (p = 0.024) *.	
[80]	• DeNoPa cohort (total n = 176): 98 PD, 61 HC, 17; OND: 2 MSA-P, 3 PSP, 3 DLB, 1 VaP, 3 ET, 2 cerebellar tremor, 3 unclear diagnosis) • Bridging cohort (total n = 514): 150 PD, 344 OND, 20 HC • PPMI cohort (total n = 1190): 397 de novo PD, 187 HC, 63 prodromal (hyposmia, iRBD), 226 affected and 309 unaffected genetic cohort (GBA, LRRK2, SNCA mutation carriers), 8 OND (5 MSA, 2 DLB, 1 CBS) • Assays: DeNoPa: ELISA; Bridging & PPMI: Simoa • Body fluids: DeNoPa cohort: CSF; Bridge cohort: CSF and serum; PPMI cohort: serum	• DeNoPa: CSF NfL highest in OND (median: 839 pg/mL) > PD (median 562 pg/mL) > HCs (median 494 pg/mL; all p = 0.01). • PPMI: In HC Age and sex explained 51% of the variability in log2NfL levels (R^2 = 0.51). • PPMI: Mean NfL was higher in PD than HC at each follow-up and throughout the first 5 years. Serum NfL increased longitudinal in PD compared to HC (β = 0.0541, P < 0.0001) *. • PPMI: Median serum NfL levels were highest in OND (MSA > DLB and CBS (16.23 pg/mL), followed by genetic (symptomatic) PD (13.36 pg/mL), prodromal participants (12.20 pg/mL), PD patients (11.73 pg/mL), unaffected PD mutation carriers (asymptomatic (11.63 pg/mL), and HC (11.05 pg/mL; all p < 0.0001) *. • PPMI: Median NfL rose 3.35% per year of age (p < 0.0001) and was 6.79% higher in female (p = 0.0002) than male *. • PPMI: Doubling serum NfL increased median MDS-UPDRS total score by 3.45 (β = +3.45, p =	• Bridging cohort is a 514-participant subset of PPMI with paired CSF and serum samples. • Disease stage: No H&Y stage available. • Autopsy: 2 deaths confirmed MSA and DLB with immunohistochemistry. • Subgroups: PPMI: Genetic group (symptomatic and asymptomatic). • *Adjusted for age and sex. • **Adjusted for LEDD, age and sex. • Author's conclusion: Serum NfL was higher in prodromal than PD or HC but lower than ONDs, reflecting iRBD's high risk of conversion to PD, DLB, or MSA; Smaller NfL increase in

	• Biomarkers: NfL	0.0115), decreased median SDMT total by 1.39 (β = -1.39, p = 0.026), decrease in median HVLT with discrimination recognition by 0.3 (β = –0.30, p = 0.03), and decrease in median HVLT with retention by 0.029 (β = -0.029, p = 0.04) **.	PD may be due to slower progression than others (e.g., multiple sclerosis), less cell death than others (e.g., Alzheimer's or CJD), and α-syn pathology in PD mainly in less myelinated neurons. NfL reflects neuronal damage in large, myelinated axons and is not disease-specific, aligning with PD's mild and gradual pathology.

Abbreviations	**Explanation**
ADL	activities of daily living
AUC	area under the curve
Aβ42	amyloid beta 1–42
CBS	corticobasal syndrome
CJD	Creutzfeldt–Jakob disease
Cox regression analysis	proportional hazards regression model
CSF	cerebrospinal fluid
DeNoPa cohort	participants from the De Novo Parkinson's Disease (DeNoPa) study, a prospective, longitudinal, single-center study conducted at Paracelsus-Elena-Klinik in Kassel, Germany
DLB	dementia with Lewy bodies
ELISA	enzyme-linked immunosorbent assay
ET	essential tremor
GBA	glucocerebrosidase gene
H&Y	Hoehn and Yahr scale
HC	healthy control
HR	hazard ratio
HVLT	Hopkins Verbal Learning Test
IPD	idiopathic Parkinson's disease
iRBD	isolated REM sleep behavior disorder
LEDD	levodopa-equivalent daily doses
ln NfL	natural logarithm–transformed values of neurofilament light chain

Log2NfL	log-base-2-transformed neurofilament light chain
LRRK2	leucine-rich repeat kinase 2 gene
MDS-UPDRS	Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale
MDS-UPDRS total	sum of parts I–IV of the MDS-UPDRS (overall motor and non-motor disability)
MDS-UPDRS-III	part III of the MDS-UPDRS (motor examination)
MMSE	Mini-Mental State Examination
MoCA	Montreal Cognitive Assessment
MSA	multiple system atrophy
MSA-P	multiple system atrophy - parkinsonian type
NfL	neurofilament light chain
OND	other neurodegenerative disorders
p	probability value
p-tau	phosphorylated tau
PD	Parkinson's disease
PD-CI	Parkinson's disease with cognitively impairment
PD-NC	Parkinson's disease with normal cognition
PDC	Parkinson's disease converter
PDP	PD Progressor
PIGD	postural imbalance and gait disorder
PPMI cohort	individuals enrolled in the Parkinson's Progression Markers Initiative (PPMI), a global, multicenter, longitudinal study designed to identify biomarkers associated with Parkinson's disease
PSP	progressive supranuclear palsy
r	Pearson's correlation coefficient
R^2	coefficient of determination
RBD	rapid eye movement behavior disorder
ROC	receiver operating characteristic
SDMT	Symbol Digit Modality Test
Simoa	single molecule array
SNCA	alpha-synuclein gene
t-tau	Total tau protein
TD	tremor dominant
UPDRS	Unified Parkinson's Disease Rating Scale
UPDRS total	sum of parts I–IV of the UPDRS (overall motor and non-motor disability)
UPDRS-II	part II of the UPDRS (motor aspects of daily living)
VaP	vascular parkinsonism
z	z score (standard score)
α-syn	alpha-synuclein
β	standardized regression coefficient
ρ	Spearman's rank correlation coefficient

Table 5. Overview of longitudinal studies investigating NfL concentrations in PD and APDs. The table includes details on assay method, biomarkers measured,

biological fluid analyzed, sample size, key findings, and reported associations between NfL levels, clinical features, and clinical progression over time.

Findings from both cross-sectional and longitudinal studies are summarized below to provide an integrated view of NfL associations in PD.

3.1.3 Cross-sectional and Longitudinal studies

Source	Study Population + Assay (CSF/Serum)	Key findings	Remarks
[92]	• Total (n = 445): 144 HC, 301 de novo PD • Assays: Simoa • Body fluids: Serum • Biomarkers: NfL	• Baseline NfL levels were higher in PD (13.00 pg/mL) than HC (11.71 pg/mL, p = 0.031). • Baseline NfL was higher in PD-MCI (14.4 pg/mL) vs. HC (p < 0.001). • Baseline: NfL negatively correlated with MoCA in PD (β = –0.030, p = 0.033) *. • Baseline: Higher NfL in PD predicted more severe decline in global cognition (MoCA, β = –0.014, p < 0.001). • Baseline: Higher NfL in PD predicted more severe decline in episodic memory (HVLT, p < 0.05). HVLT Total Recall (β = –0.010, p = 0.024), HVLT Delayed Recall (β = –0.017, p = 0.001) and HVLT Retention (β = –0.019, p < 0.001). • Baseline (PD): Higher NfL in PD predicted more severe decline in visuospatial functioning (BJLO, β = –0.013, p = 0.025), executive function/working memory (LNS, β = –0.023, p = 0.002), language (Semantic Fluency, β = –0.015, p = 0.001) and	• Subtyping: PD-NC: MoCA > 26; PD-MCI: MoCA 22–26; PDD: MoCA < 22. • Disease stage: de novo PD. • * Adjusted for age, gender, educational level, APOE ε4 carrier status, and disease duration. • The study defined amyloid positivity in PD and HC using CSF Aβ42 levels, with a cutoff of < 250 pg/mL (amyloid positive).

		processing speed/attention (SDMT, β = –0.024, p < 0.001). Highest NfL tertile predicted decline in cognition and in most domains (MoCA, HVLT, LNS, Semantic Fluency and SDMT), except for BJLO. • Baseline: PD-NC or PD-MCI with highest tertile of NfL level had higher risk of progressing to PDD (HR 6.33, p = 0.001) compared to the lowest tertile *. • NfL increased faster in de novo PD (1.42 pg/mL/year), PD-NC (1.27 pg/mL/year), and PD-MCI (1.71 pg/mL/year) vs. controls (0.69 pg/mL/year, p < 0.001). • Follow-up (PD): A greater longitudinal increase in NfL predicted more decline in MOCA (β = –0.002, p < 0.001), HVLT Total Recall (β = –0.003, p < 0.001), HVLT Delayed Recall (β = –0.004, p < 0.001), HVLT Retention (β = –0.004, p < 0.001), HVLT Recognition Discrimination (β = –0.003, p = 0.008), BJLO (β = –0.002, p = 0.021), Semantic Fluency (β = –0.002, p = 0.002), and SDMT (β = –0.004, p < 0.001). • NfL (both baseline and longitudinal change) is a strong marker of cognitive decline in both PD-NC and PD-MCI, but the associations are broader (more domains affected)	

		and stronger in PD-MCI than PD-NC. • NfL (both baseline and longitudinal change) is associated with cognitive decline in both amyloid negative and amyloid positive PD, but the associations are broader and stronger in amyloid positive PD, affecting more cognitive domains.	
[93]	• Total (n = 189): 133 de novo PD, 56 HC • Assays: Simoa • Body fluids: Serum • Biomarkers: NfL	• Baseline: NfL was higher in PD (12.28 ± 5.6 pg/ml) than HC (10.47 ± 3.6 pg/ml, p = 0.010). • Follow-up: One-year increase in NfL was greater in PD than HC (2.90 ± 11.1 vs. 0.39 ± 2.2, p = 0.030). • Baseline: NfL levels in PD correlated with BJLO (r = –0.25, p = 0.004), SDMT (r = –0.35, p < 0.001), semantic fluency (r = –0.21, p = 0.013), MDS-UPDRS-III (r = 0.19, p = 0.032), and one-year MoCA decline (r = –0.21, p = 0.019). • Baseline: Higher NfL in PD predicted one-year MoCA decline (β = –0.23, p = 0.023) and HVLT decline (β = –0.22, p = 0.034) *. • Follow-up: In PD one-year NfL increase negatively correlated with one-year SDMT scores (β = –0.25, p = 0.019) *. • Baseline: Higher NfL in PD was linked to lower temporal and fusiform cortical thickness (Cth) (p < 0.05). • Follow-up of 1-year: Longitudinal NfL in PD	• Disease stage: De novo PD. • Cortical thickness from T1-MRI indicates macrostructural cortical atrophy, while intracortical mean diffusivity from DTI shows microstructural damage. • Bankssts: Banks of the superior temporal sulcus. • * Adjusted for age, sex and educational level.

		increases were linked to more cortical thinning: temporo-occipital, posterior-cingulate-cortex/precuneus, superior-parietal, and orbitofrontal regions ($p < 0.05$). • Baseline: Higher NfL levels in PD were linked to increased mean diffusivity (MD): Parahippocampal/entorhinal and temporo-occipital regions ($p < 0.05$). • Follow-up of 1-year: Longitudinal NfL in PD increase was associated with higher MD: Temporal and superior frontal regions ($p < 0.05$). • Baseline: Temporal Cth in PD correlated with LNS scores ($r = 0.18$, $p = 0.04$). • Baseline: In PD MD in temporo-occipital and parahippocampal-entorhinal regions was associated with BJLO ($r = -0.25$, $p = 0.007$; $r = -0.33$, $p < 0.001$), LNS ($r = -0.23$, $p = 0.016$; $r = -0.25$, $p = 0.007$), and SDMT scores ($r = -0.27$, $p = 0.004$; $r = -0.35$, $p < 0.001$). • Follow-up of 1-year: In PD increases in MD over time in superior temporal and frontal regions were linked to lower BJLO scores ($r = -0.27$, $p = 0.045$; $r = -0.31$, $p = 0.022$). • Follow-up of 1-year: In PD lower orbitofrontal Cth linked to lower HVLT ($r = 0.23$, $p = 0.029$); lower bankssts Cth linked to lower semantic fluency (r	

		= 0.23, p = 0.026); lower Cth in precentral (r = 0.24, p = 0.019) and lateral-occipital (r = 0.21, p = 0.041) regions was linked to lower SDMT. • Follow-up of 1-year: In PD higher MD in inferior-temporal region was linked to lower SDMT (r = -0.28, p = 0.041).	
[99]	• Best medical treatment (BMT)/Group A (total n = 57): 57 PD • Chronic DBS (Group B, total n = 92): 92 PD • Longitudinal DBS surgery cohort (Group C, total n = 21): 21 PD • Assay: Simoa • Body fluids: Serum • Biomarkers: NfL	• Baseline (Group A + B): NfL correlated with age (ρ = 0.5), disease duration (ρ = 0.41), H&Y stage (ρ = 0.44), UPDRS-III (ρ = 0.35) and lower MoCA scores (ρ = −0.43; all p < 0.0001). • Group A and B: Age, MOCA score, and disease duration were identified as key factors influencing serum NfL levels, together accounting for 46% of the variance in log NfL (R^2 = 0.46). • Group C: NfL increased from pre-surgery baseline (median 14.8 pg/ml) to 3–5 days post-surgery (median 33.5 pg/ml, p < 0.00001), likely due to surgery (authors' conclusion). Anesthesia type (awake vs. general anesthesia), number of implanted electrodes, and baseline NfL had no significant effect. • Group C: NfL levels increased further from time point 1 to 2 (median 56.1 pg/ml, p < 0.00001). From time point 3 to 4, NfL levels normalized to baseline (median 13.8 pg/ml, p < 0.00001).	• Disease stage: Group A (early-stage PD (H&Y stage = 2); Group B (advanced stage PD (H&Y stage = 3); Group C (early-stage PD (H&Y stage = 2). • Time point: 0 (baseline/ pre-surgery); 1 (3–5 days postoperative (no stimulation); 2 (6–8 weeks postoperative (no stimulation); 3 (3–5 days after testing/stimulation); 4 (8 months post-surgery (with stimulation). • Authors' conclusion: No NfL increase after stimulation, and no difference in NfL between group A and B, suggests electrical stimulation does not induce neuronal injury.

Abbreviations	Explanation
APOE ε4	apolipoprotein E epsilon 4
Aβ42	amyloid beta 1–42
BJLO	Benton Judgment of Line Orientation test
CSF	cerebrospinal fluid
Cth	cortical thickness
DAT	dopamine transporter
DBS	deep brain stimulation
DTI	diffusion tensor imaging
H&Y	Hoehn and Yahr scale
HC	health control
HR	hazard ratio
HVLT	Hopkins Verbal Learning Test
LNS	Letter-Number Sequencing test
log NfL	log-transformed neurofilament light chain levels
MD	mean diffusivity
MDS-UPDRS	Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale
MDS-UPDRS-III	part III of the MDS-UPDRS (motor examination)
MoCA	Montreal Cognitive Assessment
NfL	neurofilament light chain
p	probability value
PD	Parkinson's disease
PD-MCI	Parkinson's disease with mild cognitive impairment
PD-NC	Parkinson's disease with normal cognition
PDD	Parkinson's disease with dementia
r	Pearson's correlation coefficient
R^2	coefficient of determination
SDMT	Symbol Digit Modality Test
Simoa	single molecule array
UPDRS	Unified Parkinson's Disease Rating Scale
UPDRS-III	part III of the UPDRS (motor examination)
β	standardized regression coefficient
ρ	Spearman's rank correlation coefficient

Table 6. Summary of cross-sectional and longitudinal studies investigating NfL concentrations in PD and APDs. The table includes biological fluid analyzed, assay method, sample size, diagnostic groups, biomarkers measured, and key findings, and reported associations between NfL levels, clinical features, and disease progression.

3.2 Results of the Present Study

In our study we analyzed a total of 93 patients. The study population consists of 46 patients with PD and 47 sex- and age-matched HC.

3.3 Demographic characteristics

Of the 93 patients enrolled in this trial, 58 (60.3%) were male and 35 (39.7%) female. In patients with PD 16 were female (34.8%) and 30 were male (65.2%) and in HC 19 were female (40.4%) and 28 were male (59.6%). The mean age was 66.1 ± 6.7 years, with no significant difference between PD patients (mean 66.2, SD 6.6) and HC participants (mean 66.1, SD 6.8; p = 0.959). The age of participants ranged from 50 to 78 years.

3.4 Clinical Characteristics

There were no significant differences between the PD group and HC in terms of BMI or cognitive performance. The mean BMI was 27.3 kg/m^2 (SD 3.4) in the HC group and 26.5 kg/m^2 (SD 4.5) in the PD group (p = 0.323). Mean MMSE scores at baseline were slightly higher in HC participants (28.3, SD 1.3) compared to PD patients (27.9, SD 1.3), though the difference did not reach statistical significance (p = 0.094). At follow-up, MMSE scores remained similar between groups, with HC scoring 28.0 (SD 1.8) and PD 28.2 (SD 1.5; p = 0.455).

Baseline clinical motor assessment of PD patients showed a mean MDS-UPDRS-III score of 28 in the OFF-medication state, indicating moderate motor symptom severity. The mean Hoehn and Yahr (H&Y) stage in the OFF-medication state was 1.96 (SD 0.3), with a median of 2.0, and an interquartile range (IQR) of 0, indicating that at least 50% of patients were at the same disease stage. The average disease duration was 4.0 years (SD 3.5).

3.5 Cognition

3.5.1 Comparative Analyses of MMSE Scores

In a mixed cohort of 93 participants, MMSE scores remained stable over time, with mean values of 28.1 (SD = 1.3) at baseline and 28.1 (SD = 1.7) at follow-up. The score range slightly expanded from 6 to 7 points, reflecting minor increases in variability, suggesting preserved overall cognition despite individual differences.

Sex-specific analysis in mixed cohort revealed consistent trends over time. Among males, the mean MMSE scores did not change between baseline and follow-up, with a mean baseline score of 28.0 (SD = 1.3) and a follow-up score of 27.9 (SD =

1.7). The score range showed minimal variability, broadening from 6 to 7 points. Among females, the mean MMSE scores also showed no change, with a baseline of 28.3 (SD = 1.2) and a follow-up of 28.4 (SD = 1.6), while the score range widened from 5 to 7 points. Overall, these findings indicate relatively stable cognition.

Group-wise comparisons between HC and individuals with PD indicated subtle but consistent patterns over time. HC mean MMSE scores were 28.3 (SD = 1.3) at baseline and 28.0 (SD = 1.8) at follow-up, with the range shifting from 5 to 7 points. In PD participants, MMSE scores also remained stable, with 27.9 (SD 1.3) at baseline and 28.2 (SD 1.5) at follow-up, and a consistent range of 6 points. These changes were not clinically significant and indicate relatively stable cognitive performance in both groups over time.

Sex-specific analyses in HC and PD indicated minimal changes in cognitive performance over time without clinical significance. Among HC, males had mean MMSE score of 28.5 (SD = 1.2) at baseline and 28.0 (SD = 1.9) at follow-up, with the range shifting from 4 to 7 points. HC female scored 28.0 (SD = 1.3) at baseline and 27.9 (SD = 1.7) at follow-up, with a range shifting from 5 to 7 points. PD males had mean MMSE scores of 27.5 (SD = 1.3) at baseline and 27.8 (SD = 1.5) at follow-up, with a stable range of 6 points. PD females scored 28.6 (SD = 1.0) at baseline and 29.1 (SD = 1.2) at follow-up, with the range shifting from 4 to 3 points. These results suggest that cognitive performance remained generally stable across subgroups.

Independent samples t-tests revealed no significant differences in MMSE scores between HC and individuals with PD, neither at baseline ($t(91) = 1.692$, $p = 0.094$), nor at follow-up ($t(91) = –0.750$, $p = 0.455$).

Sex-stratified independent samples t-tests showed that HC males scored significantly higher than PD males at baseline, with a mean MMSE score of 28.5 (SD = 1.2) compared to 27.5 (SD = 1.3), $t(56) = 3.172$, $p = 0.002$. At follow-up, there was no significant difference between HC and PD males ($t(56) = 0.525$, $p = 0.602$). Among females, there was no significant difference at baseline between HC and PD ($t(33) = -1.375$, $p = 0.178$). At follow-up, however, PD females scored

significantly higher than HC females, with a mean MMSE score of 29.1 (SD = 1.2) compared to 27.9 (SD = 1.7), t(33) = -2.287, p = 0.029.

3.6 Neurofilament Light Chain (NfL)

This section presents the analysis of serum NfL levels in HC and patients with PD at baseline and follow-up. Group comparisons were performed using independent samples t-tests to evaluate differences in both raw and log-transformed NfL concentrations (see Table 7).

	HC (n = 47)	PD (n = 46)	P-Value (HC vs. PD)
NfL baseline (pg/ml)	12.60 (7.64)	17.35 (8.89)	0.007
NfL follow up (pg/ml)	15.27 (8.00)	24.86 (16.23)	< 0.001
NfL baseline (log pg/ml)	1.05 (0.21)	1.19 (0.19)	< 0.001
NfL follow up (log pg/ml)	1.14 (0.19)	1.33 (0.22)	< 0.001

Table 7. Comparison of serum Neurofilament light (NfL)-levels (raw) between healthy control (HC) and Parkinson´s disease (PD) patients at baseline and follow-up.

3.6.1 Comparative Analyses of NfL

3.6.1.1 Baseline and Follow-up NfL levels

To compare NfL levels between PD and HC groups at individual time points, independent samples t-tests were conducted, including sex-stratified analyses of log NfL values. Results showed significantly higher log NfL levels in PD group compared to HC at both baseline and follow-up (see Figure 5). At baseline, the mean log NfL level in the PD group was 1.19 (SD = 0.20), compared to 1.05 (SD = 0.21) in the HC group. PD participants had a mean log NfL level that was 0.15 units higher than HC participants (95% CI: −0.23 to −0.06; t(91) = −3.404, p < 0.001). At follow-up, PD patients had a mean log NfL level of 1.33 (SD = 0.22), while HC participants had 1.14 (SD = 0.19). This corresponded to a 0.19-unit higher log NfL level in the PD group (95% CI: −0.28 to −0.10; t(91) = −4.403, p < 0.001).

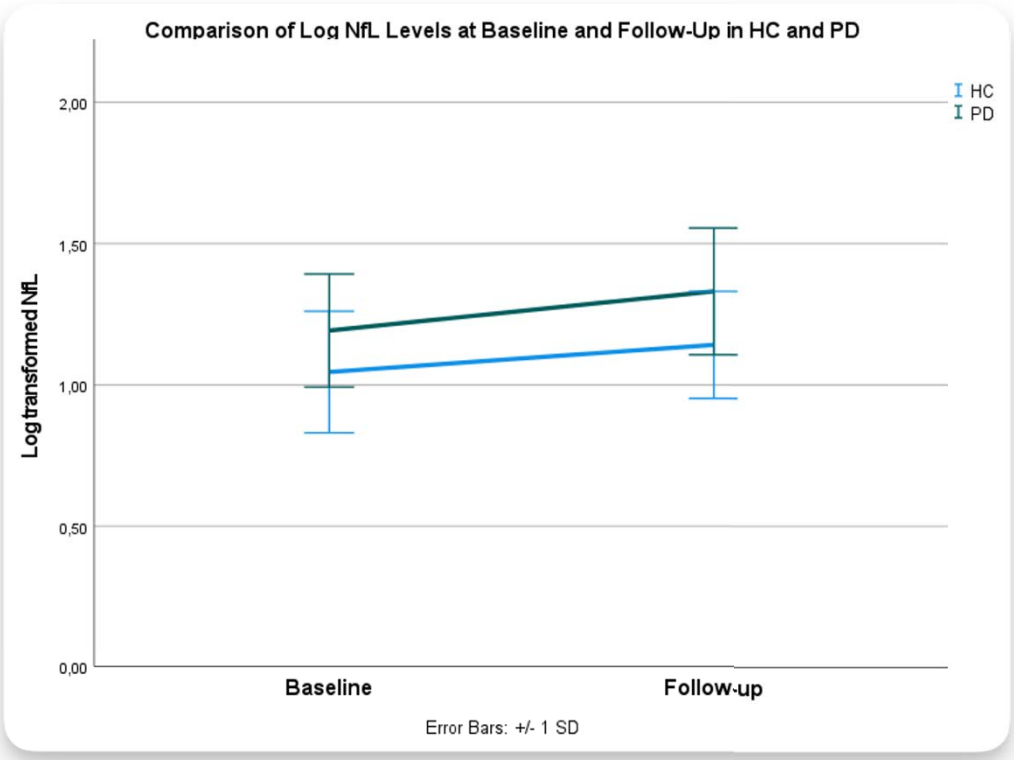

Figure 5. Comparison of log transformed Neurofilament light (NfL)-levels at baseline and follow-up in healthy control (HC) and Parkinson´s disease (PD).

In the sex-stratified analysis (see Figure 6), log NfL levels increased from baseline to follow-up in all groups. Among HC, males showed an increase from 1.05 (SD = 0.19) to 1.10 (SD = 0.18), and females from 1.04 (SD = 0.26) to 1.20 (SD = 0.19). In the PD group, males had higher log NfL levels overall, increasing from 1.23 (SD = 0.21) to 1.37 (SD = 0.22), while females showed an increase from 1.13 (SD = 0.18) to 1.26 (SD = 0.22).

Independent samples t-tests showed significantly higher log NfL levels in PD males compared to HC males at both baseline and follow-up. At baseline, PD males had a mean log NfL level that was 0.18 units higher than HC males (95% CI: –0.28 to –0.07; $t(56) = –3.391$, $p = 0.001$). At follow-up, this difference increased to 0.27 units (95% CI: –0.37 to –0.16; $t(56) = –5.002$, $p < 0.001$). In contrast, no significant differences were found between PD and HC females at either baseline ($t(33) = –1.190$, $p = 0.242$) or follow-up ($t(33) = –0.891$, $p = 0.379$).

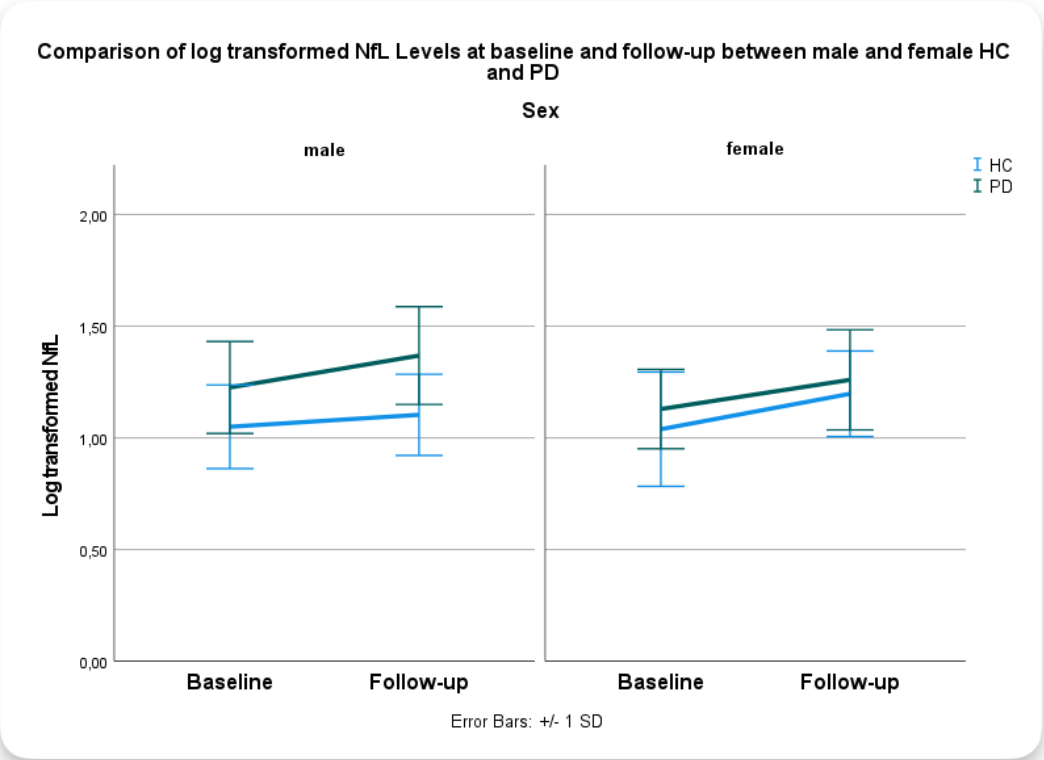

Figure 6. Comparison of log transformed Neurofilament light (NfL)-levels at baseline and follow-up between male and female healthy control (HC) and Parkinson´s disease (PD).

These results underscore that log NfL levels were significantly higher in PD patients compared to HC at both baseline and follow-up, with this difference reaching significance only in males. This indicates that differences in NfL levels between groups are observable at specific time points and may follow distinct sex-specific trajectories over time.

3.6.1.2 Longitudinal Changes in Serum NfL

To assess changes in NfL levels over time within each group, paired samples t-tests were performed separately for the HC and PD groups, including sex-stratified analyses of log NfL values. Overall, the results demonstrated that log NfL levels increased significantly from baseline to follow-up in both HC and PD groups. In the HC group (see Figure 7), log NfL levels increased significantly by an average of 0.10 units (95% CI: −0.14 to −0.06; $t(46) = -4.851$, $p < 0.001$). Similarly, the PD group (see Figure 8) demonstrated a larger increase of 0.14 units over time (95% CI: −0.20 to −0.08; $t(45) = -4.568$, $p < 0.001$).

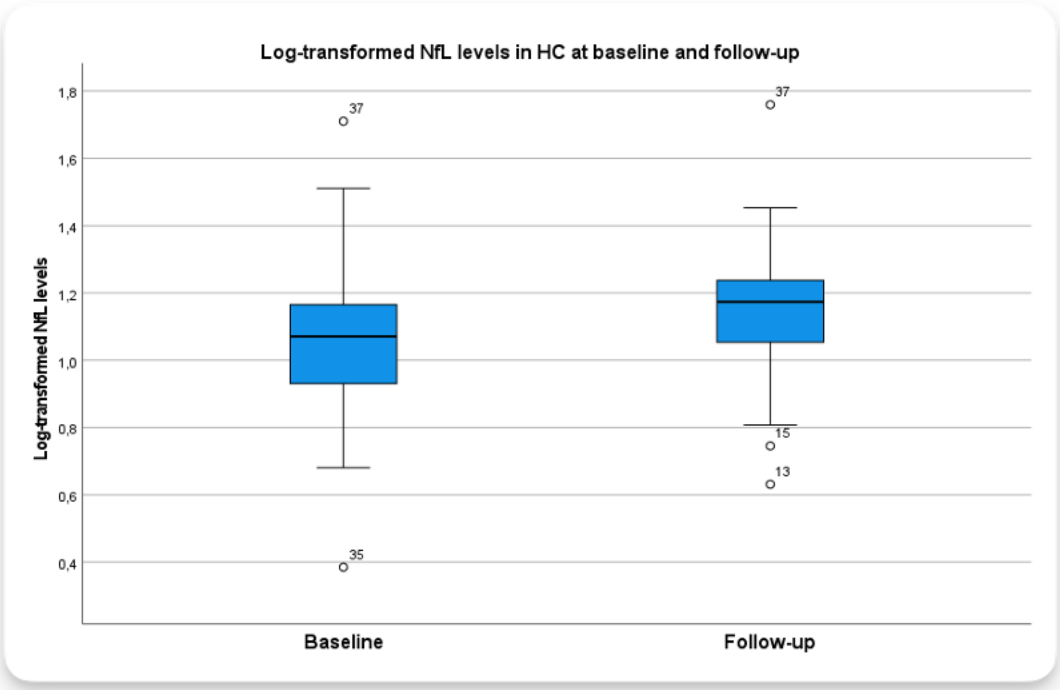

Figure 7. Log-transformed Neurofilament light (NfL)-levels in healthy control (HC) at baseline and follow-up.

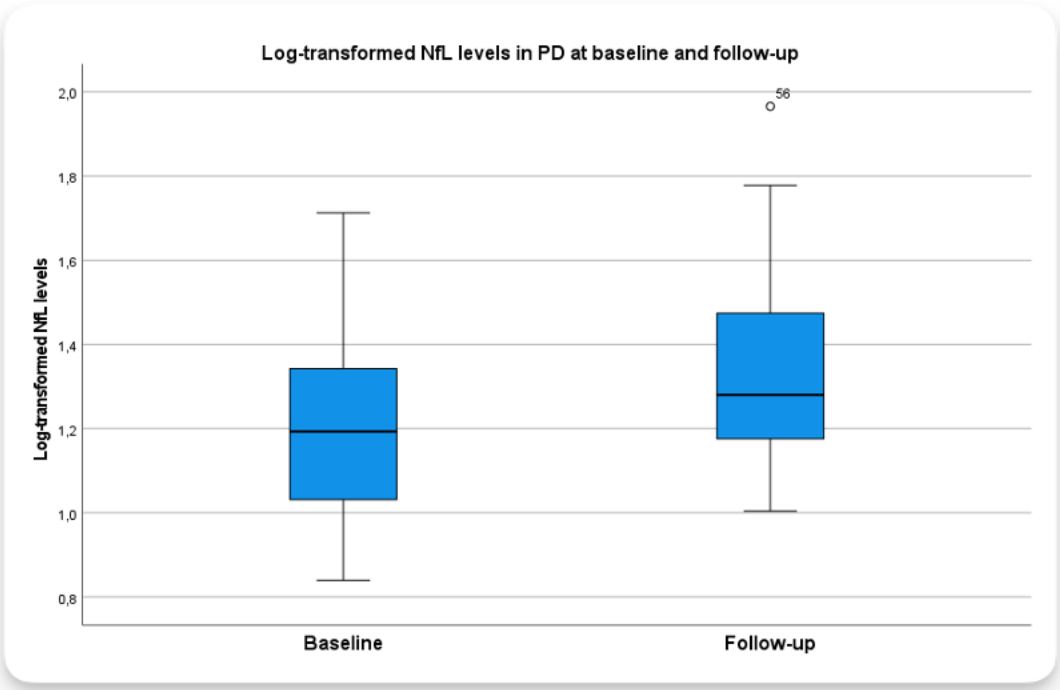

Figure 8. Log-transformed Neurofilament light (NfL)-levels in Parkinson´s disease (PD) at baseline and follow-up.

Sex-stratified analyses revealed significant increases in log NfL levels over time in both HC and PD groups (see Figure 9). Among males, HC participants showed a modest increase of 0.05 units (95% CI: –0.10 to –0.01; t(27) = –2.533, p = 0.017), while PD males exhibited a larger increase of 0.14 units (95% CI: –0.22 to –0.06; t(29) = –3.564, p = 0.001). In females, the increase was more pronounced in the HC group, with a 0.16-unit rise (95% CI: –0.23 to –0.09; t(18) = –4.732, p < 0.001), whereas PD females showed a smaller but still significant increase of 0.13 units (95% CI: –0.23 to –0.03; t(15) = –2.844, p = 0.012).

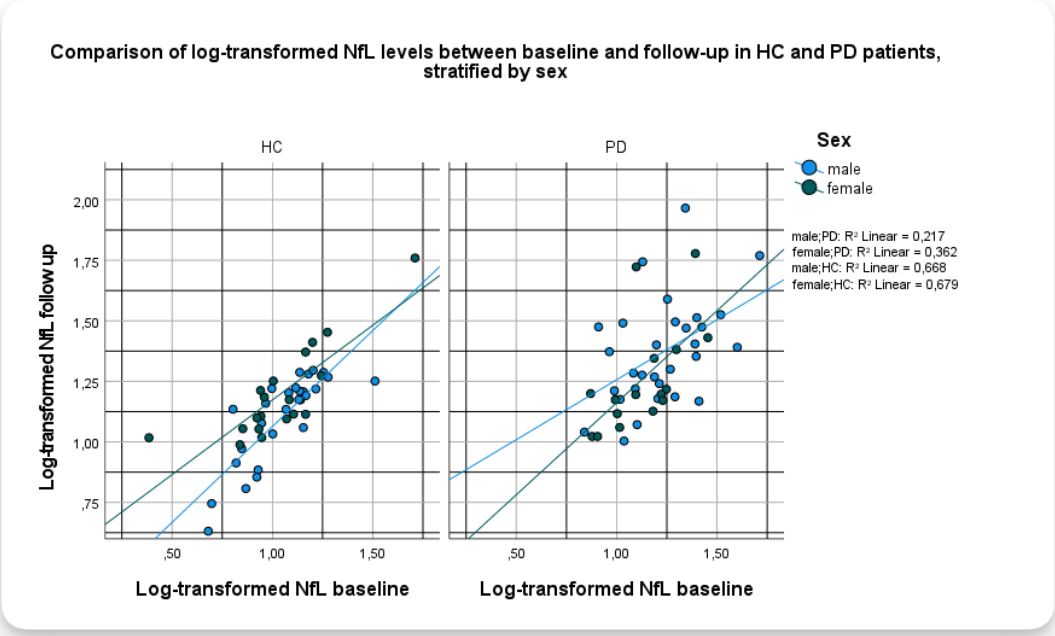

Figure 9. Comparison of log-transformed Neurofilament light (NfL)-levels between baseline and follow-up in healthy control (HC) and Parkinson´s disease (PD) patients, stratified by sex.

These findings underscore that log NfL levels increased over time within both HC and PD groups. The most pronounced changes were observed in PD males and HC females, suggesting potential sex-specific trajectories in NfL progression.

3.6.2 Correlation Analyses of NfL

3.6.2.1 Association Between NfL Levels and Age

Bivariate and partial correlation analyses were used to examine the relationship between age at baseline and NfL levels at both baseline and follow-up in the HC and PD groups, with partial correlations adjusting for relevant covariates.

3.6.2.1.1 Mixed Cohort of Participants

Across all participants (n = 93), age showed a moderate positive correlation with log NfL levels at both baseline ($r = 0.451$, $p < 0.001$) and follow-up ($r = 0.388$, $p < 0.001$). After adjusting for sex and BMI, partial correlations remained significant at both time points (baseline: $r = 0.460$, $p < 0.001$; follow-up: $r = 0.399$, $p < 0.001$).

When stratified by sex and adjusted for BMI, males showed a strong positive correlation between age and log NfL levels at both baseline ($r = 0.497$, $p < 0.001$) and follow-up ($r = 0.471$, $p < 0.001$). In females, a significant correlation was found only at baseline ($r = 0.393$, $p = 0.021$), with no significant association at follow-up.

In summary, age correlates moderately with log NfL levels, particularly in males, and these associations persist after adjustment for covariates.

3.6.2.1.2 Comparison of HC and PD Groups

In the HC group, age at baseline showed a moderate positive correlation with log NfL levels at both baseline ($r = 0.513$, $p < 0.001$) and follow-up ($r = 0.488$, $p < 0.001$). In the PD group, the correlations were weaker but still significant at baseline ($r = 0.437$, $p = 0.002$) and follow-up ($r = 0.372$, $p = 0.011$).

After adjusting for sex and BMI, age remained strongly correlated with log NfL levels in the HC group at both baseline ($r = 0.584$, $p < 0.001$) and follow-up ($r = 0.598$, $p < 0.001$). In the PD group, the correlations were weaker but still significant at baseline ($r = 0.397$, $p = 0.017$) and follow-up ($r = 0.318$, $p = 0.035$).

In conclusion, age-related increases in log NfL were observed in both groups, with stronger and more consistent associations in HC, even after adjusting for covariates.

3.6.2.2 Association of Cognitive Function and NfL

Bivariate and partial correlation analyses were used to examine the relationship between serum NfL levels and cognitive performance as measured by the MMSE at both baseline and follow-up in the HC and PD groups, with partial correlations adjusting for relevant covariates.

3.6.2.2.1 Mixed Cohort of HC and PD Participants

There were no significant correlations between baseline log NfL and MMSE scores at either baseline or follow-up, including in sex-stratified analyses. However, at follow-up, a weak negative correlation was observed between MMSE and log NfL ($r = –0.206$, $p = 0.047$).

Partial correlation analyses revealed no significant associations between log NfL levels and MMSE scores at baseline or follow-up, including models assessing baseline log NfL in relation to follow-up MMSE, regardless of adjustments for covariates. These findings remained consistent across all models, including sex-stratified analyses.

In summary, no significant correlations were found between MMSE and log NfL at baseline, nor between baseline log NfL and follow-up MMSE, while a weak association at follow-up suggests a potential but limited link between higher log NfL levels and lower cognitive performance.

3.6.2.2.2 Comparison of HC and PD Groups

In both the HC and PD groups, baseline NfL levels were not significantly correlated with MMSE scores at either baseline or follow-up. However, sex-stratified analyses revealed a significant negative correlation between baseline log NfL and follow-up MMSE in HC females ($r = –0.525$, $p = 0.021$). At follow-up, a significant negative correlation between log NfL and MMSE scores was observed in the HC group overall ($r = –0.385$, $p = 0.007$), with a particularly strong association in females ($r = –0.684$, $p = 0.001$). No significant associations were found at follow-up in HC males, or in the PD group or its sex-stratified subgroups.

After adjusting for covariates, partial correlation analyses showed no significant association between baseline MMSE and NfL in either group. However, in the overall HC group, baseline log NfL was borderline significantly negatively associated with MMSE at follow-up after adjustment for age and BMI ($r = -0.300$, $p = 0.045$). In sex-stratified analyses, a significant negative correlation was observed in HC females ($r = –0.503$, $p = 0.039$) after adjusting for age and BMI, but not in males. No significant correlations were found in the PD group or its sex-stratified subgroups.

At follow-up, partial correlations in the HC group revealed a strong negative association between MMSE and log NfL after adjustment for age, sex, and BMI ($r = –0.512$, $p < 0.001$). In sex-stratified models adjusted for age and BMI, significant associations were found in both HC females ($r = –0.673$, $p = 0.003$) and males ($r = –0.516$, $p = 0.007$). No such associations were observed in the PD group or its sex-stratified subgroups.

Overall, higher log NfL levels were linked to poorer cognitive performance at follow-up in the HC group, particularly among females and even after adjusting for covariates. Baseline log NfL also predicted lower follow-up cognition in the overall HC group and in female only, with the association remaining significant after covariate adjustment. No such associations were found in the PD group or within its sex-stratified subgroups.

3.6.3 PD Group

Bivariate and partial correlation analyses were conducted to assess the relationship between serum NfL levels and cognitive performance as measured by the CERAD scores (TS1, TS2), as well as motor symptom severity, as measured by MDS-UPDRS-III scores, in the HC and PD groups. Partial correlations were adjusted for relevant covariates.

3.6.3.1 Baseline NfL Levels

Baseline log NfL levels were significantly negatively correlated with follow-up TS2 z scores in the total group ($r = –0.404$, $p = 0.005$), as shown in Figure 10, where TS2 z represents the z-transformed TS2 of the CERAD neuropsychological test battery. When stratified by sex, this association remained significant in males ($r = –0.391$, $p = 0.033$) but was not significant in females.

After adjusting for disease duration and baseline MDS-UPDRS-III scores, baseline log NfL was significantly negatively associated with follow-up TS2 z score ($r = –0.419$, $p = 0.005$). In sex-stratified analyses adjusted for disease duration and baseline MDS-UPDRS-III scores, a significant negative association was observed in males ($r = –0.403$, $p = 0.033$), but not in females.

In contrast, baseline log NfL levels were not significantly correlated with MDS-UPDRS-III scores at either baseline ($r = 0.031$, $p = 0.838$) or follow-up ($r = 0.125$, p

= 0.441), even after adjusting for covariates. This lack of association also remained in sex-stratified analyses.

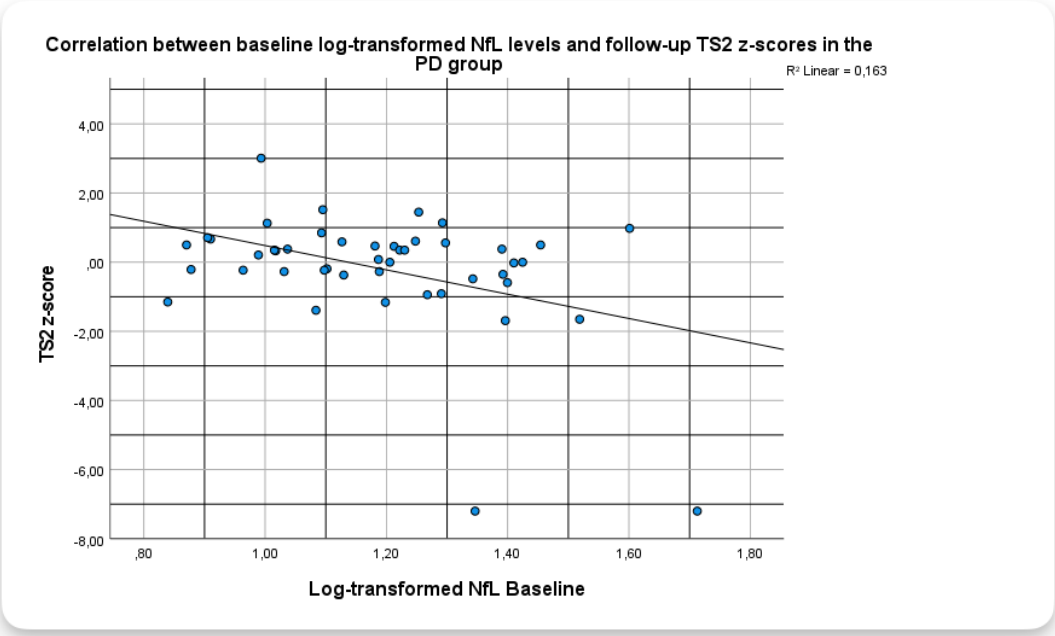

Figure 10. Correlation between baseline log-transformed Neurofilament light (NfL)-levels and follow-up Total Score 2 (TS2) z-scores in the Parkinson´s disease (PD) group.

In summary, higher baseline NfL levels were associated with lower follow-up TS2 z scores, reflecting greater cognitive decline, particularly in males. This association remained significant after adjusting for covariates. In contrast, NfL levels showed no significant correlation with motor scores at baseline or follow-up, even after covariate adjustment.

3.6.3.2 Follow-up NfL Levels

At follow-up, log NfL levels were negatively correlated with both CERAD TS1 (r = –0.355, p = 0.021) and CERAD TS2 (r = –0.341, p = 0.027), as shown in Figure 11 and Figure 12. However, these associations were not significant in sex-stratified analyses.

The negative associations between NfL levels and cognitive scores remained significant after adjusting for BMI, with correlations for CERAD TS2 (–0.401, p = 0.047) and CERAD TS1 (–0.399, p = 0.048). After separately adjusting for disease duration, these associations remained significant for CERAD TS2 (–0.313, p =

0.046) and CERAD TS1 (–0.329, p = 0.036). In sex-stratified analyses adjusted for age, disease duration, and BMI, a strong and significant negative correlation was found in females between NfL levels and CERAD TS2 (–0.849, p = 0.016), but remained non-significant in males. Similarly, for CERAD TS1, after adjustment for age and BMI, a strong and significant negative correlation was observed in females (r = –0.841, p = 0.009), whereas no association was found in males.

In contrast, log NfL levels at follow-up showed a positive but non-significant correlation with follow-up MDS-UPDRS-III scores (r = 0.282, p = 0.078), which remained non-significant after adjusting for covariates or stratifying by sex.

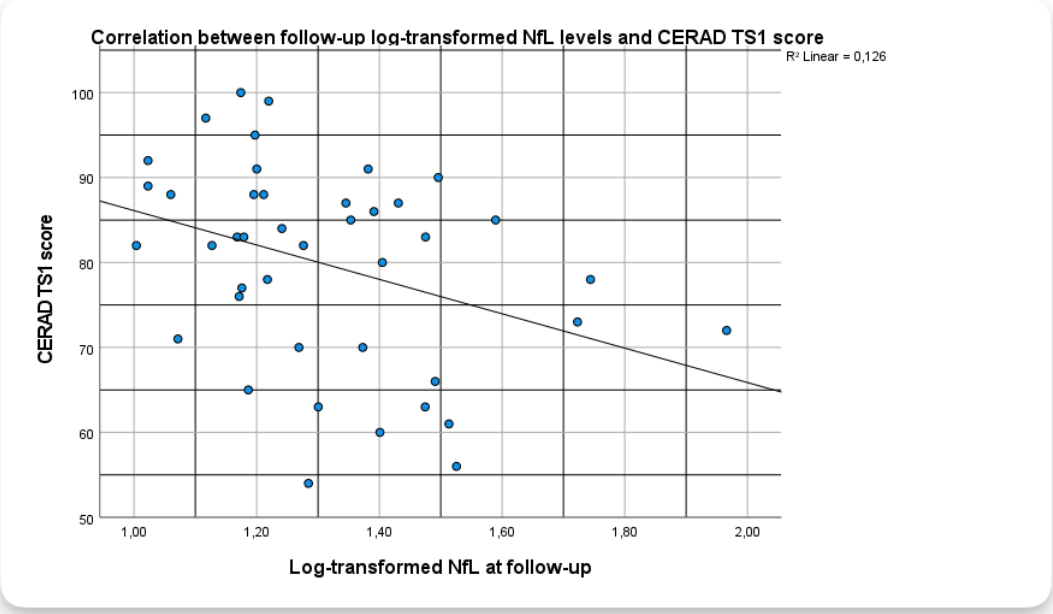

Figure 11. Correlation between follow-up log-transformed Neurofilament light (NfL)-levels and Consortium to Establish a Registry for Alzheimer's Disease Total Score 1 (CERAD TS1) scores.

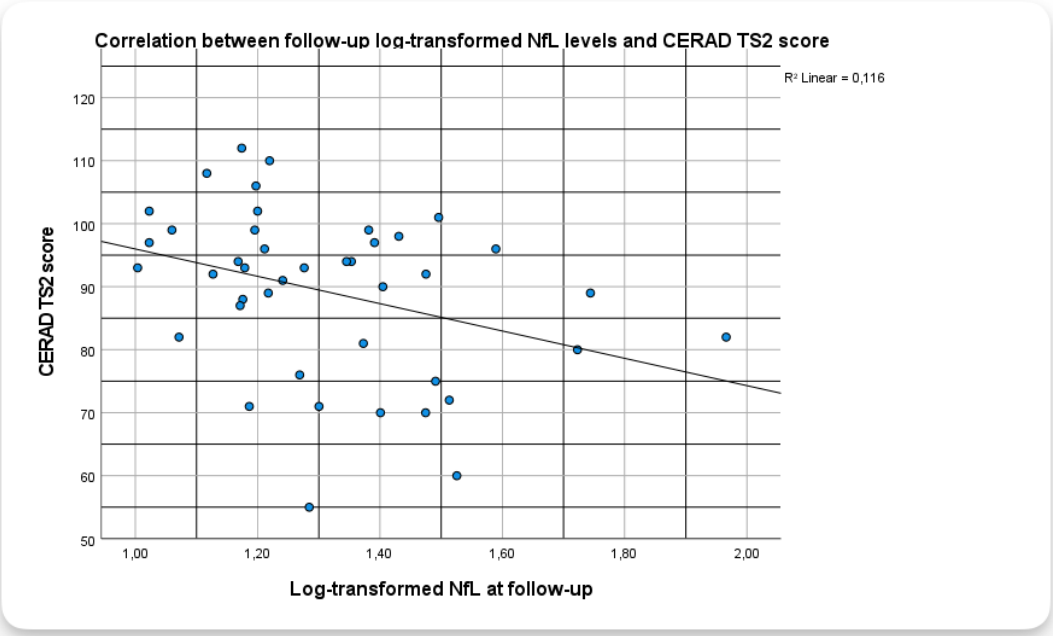

Figure 12. Correlation between follow-up log-transformed Neurofilament light (NfL)-levels and Consortium to Establish a Registry for Alzheimer's Disease Total Score 2 (CERAD TS2) score.

Overall, at follow-up higher NfL levels were significantly associated with poorer cognitive performance, as measured by CERAD TS2 and TS1, particularly for females, even after adjusting for covariates. In contrast, no significant correlations were found between follow-up NfL levels and motor severity (MDS-UPDRS-III scores). Together, these findings highlight the potential of NfL as a biomarker for cognitive impairment in PD, with possible sex-specific differences in its predictive value.

4 Discussion

4.1 Results

In this longitudinal study, we examined the association between serum NfL levels and motor and cognitive performance in an age- and sex-matched cohort of patients with PD and HC. Our aim was to evaluate serum NfL as a potential biomarker for disease progression in PD.

Serum NfL levels were consistently higher in PD patients compared to HC at both baseline and follow-up, both in the overall sample and in sex-stratified analyses for

males. The increase over time was more pronounced in the PD group, particularly among males, suggesting potential sex-specific trajectories of NfL progression. These findings support NfL as a marker of ongoing neuroaxonal injury and point to potential biological sex differences in PD progression [70,79,80,101]. NfL levels also showed stronger age dependence in the HC group than in the PD group. Even after adjusting for covariates, age remained a key predictor of NfL in HC but less so in PD, underscoring the importance of correcting for age and sex when interpreting NfL values.

The relationship between NfL and cognition differed by group. In PD, NfL was not associated with MMSE scores. In the HC group, however, higher follow-up NfL levels were linked to lower MMSE scores at follow-up, even after adjusting for covariates, and this association remained significant in both sexes when stratified. Baseline NfL were also significantly associated with follow-up MMSE decline in the overall HC cohort, particularly in females, after covariate adjustment.

In PD patients, higher baseline NfL were associated with lower global cognition scores (CERAD TS2 z score) at follow-up in the overall cohort and in males, even after adjusting for covariates, suggesting predictive value for cognitive decline. At follow-up, higher NfL levels also correlated with poorer CERAD TS1 and CERAD TS2 scores in the overall PD cohort and in females, independent of covariates, further reflecting greater cognitive impairment. However, serum NfL was not associated with MDS-UPDRS-III scores (motor function) at baseline or follow-up, nor did it predict changes over time in MDS-UPDRS-III scores.

Overall, these findings demonstrated that serum NfL levels were consistently higher in PD patients than in HC. They support the use of serum NfL as a marker of cognitive, but not motor symptoms in PD and underscore the importance of accounting for age and sex in its interpretation. The observed sex-specific differences in NfL and cognition may reflect distinct underlying pathomechanisms. Although NfL was elevated and increased over time in PD, its relationship to cognition appears complex and may offer predictive value when key covariates are considered.

4.2 Interpretation in the Context of Existing Literature

Our study largely confirms previous findings that serum NfL is elevated in PD compared to HC [61,70,75,76,80,83,89,93], consistent with findings in CSF [59,61,67,78–81,83,86,88] and plasma [68,69,82,84,85,87,91,92]. In our sex-stratified analysis, this elevation was evident in males, in contrast to one study that reported higher plasma NfL in females with PD compared to controls [89], indicating that sex-related differences in disease progression may exist but remain inconclusive. Although we did not assess diagnostic utility, other studies have shown that serum NfL can distinguish PD from HC [61,76,83], as well as NfL measured in other biofluids, including plasma [68,87,91] and CSF [61,83,86]. Moreover, other studies have also shown that both individual biomarkers and combinations with NfL can improve differentiation between PD and HC (see Section 1.2.6.2).

Consistent with other studies that examined CSF NfL, we also found that serum NfL levels increase over time [33,77,78], reflecting ongoing neuroaxonal degeneration. Moreover, in the PD group, we did not observe any differences in NfL levels between males and females at any individual time point, whereas other studies have reported such differences, though inconsistently [70,79,80,101]. For further details on associations between NfL levels and sex in PD, see Section 1.2.6.3. However, we found that serum NfL levels increased more rapidly over time in male PD patients compared to females, in contrast to a previous study that reported a steeper NfL increase in females, suggesting potential sex-specific trajectories of NfL or disease progression that may vary across cohorts [80]. The absence of sex differences at individual timepoints, such as baseline or follow-up, may be partly due to the small PD sample size (n = 46). Given the unequal sex distribution in our PD group (male-to-female ratio: 1.88:1), the observed faster NfL increase in males should be interpreted with caution.

In line with earlier findings, we found that age influences serum NfL levels in both PD [58,70,76,83,90,95–99] and HC [58,70,76,90]. NfL measured in other biofluids also correlates with age in PD, as shown for plasma [57,68,82,84,90,100,101] and CSF [56,57,60,67,70,77–79,83,86,94], and in HC, as reported for CSF [56,77–79] and plasma [68,70,100,101]. We found no correlation between serum NfL and BMI in either HC and PD Group, possibly due to the small sample sizes (PD: n = 46;

HC: n =47) despite moderate variability (HC: 27.3 (SD 3.4); PD: 26.5 (SD 4.5)). However, one study did report a negative association in PD [96].

We also did not find a correlation between NfL and disease duration. While most studies show NfL level increases with disease duration in plasma [70,102], CSF [77,79], and serum [99], one study reported an inverse correlation with plasma NfL [91], possibly due to cohort differences. The absence of a correlation with disease duration in our cohort may be explained by the relatively short mean disease duration (4.0 years, SD 3.5) and limited variability in H&Y stages (mean 1.96, SD 0.3).

In our study, we also did not observe any correlation between serum NfL levels and motor severity. However, previous studies have reported associations with the original UPDRS-III or MDS-UPDRS-III scores [76,93,95–97,99], indicating that higher NfL levels were associated with greater motor impairment. Similar associations have also been observed in other biofluids, including CSF [57,78,79,81,94], and plasma [57,68,70,81,82,84,100]. Additionally, correlations between blood NfL levels and UPDRS-II or MDS-UPDRS-II scores have been reported [82,98], suggesting that higher NfL levels may be associated with greater impairment in motor aspects of daily living. Several studies have also shown that higher baseline blood NfL levels predict faster motor decline, particularly in males, and older patients [81,82,97]. Elevated NfL levels have also been linked to FOG, akinetic-rigid symptoms, bradykinesia, axial impairment, PIGD severity, and poorer mobility [78,97,102,103]. These findings support NfL's potential as a biomarker for motor progression in PD. For a more detailed overview of NfL and motor dysfunction, see Section 1.2.6.4.

We also did not observe an association between NfL levels and disease severity as measured by H&Y stage. However, higher serum NfL has been linked to greater disease severity in PD in other studies [61,76,96,98,99]. Similar associations, have also been reported in plasma [57,70] and CSF [33,56,57,78,79]. For further details on NfL and disease severity, see Section 1.2.6.5.

The lack of significant associations of motor and disease severity in our cohort may be explained by the limited variability in clinical measures and the relatively

small sample size (n = 46 PD). Most patients had early to moderate disease stage, with a mean H&Y stage of 1.96 (SD 0.3, IQR 0) and a mean MDS-UPDRS-III score of 28. The mean disease duration was also relatively short at 4.0 years (SD 3.5). Together, these factors may have limited the ability to detect correlations between NfL levels and both motor impairment and disease severity.

In this study, no significant association was observed between NfL levels and MMSE scores in the PD group. In contrast, baseline NfL levels significantly predicted follow-up MMSE scores in the overall HC group and in females after adjustment for covariates. A negative correlation between NfL and MMSE was observed only in the HC group, both in the overall sample and in sex-stratified analyses at follow-up. This pattern likely reflects neurodegenerative changes associated with normal aging rather than disease-related neurodegeneration. These findings are not consistent with previous studies that have reported cross-sectional associations between serum NfL levels and MMSE scores in individuals with PD [61,76,83], as well as in other biofluids, including CSF [61] and plasma [68,84,100,101]. However, MMSE scores in both groups showed minimal variability and remained consistently above 27 at baseline and follow-up, reflecting the exclusion of participants with diagnosed dementia and the absence of cognitive decline. This limited range likely reduced the sensitivity of the MMSE, and the slight improvement in MMSE scores observed at follow-up may have further limited its ability to detect subtle cognitive changes, particularly in individuals with early to moderate stage PD.

Nonetheless, in our study, both cross-sectional and longitudinal associations were observed between NfL levels and cognitive performance assessed by the CERAD neuropsychological battery in PD. Consistent with other studies, higher blood and CSF NfL levels were associated with greater cognitive decline as measured by with MoCA [59,79,92,93,95,96,99,104], MMSE [61,68,76,83,84,100,101] and DRS-2 [59,81], and also predicted future cognitive deterioration [92,96]. However, in our study, when stratified by sex, the association between higher NfL levels and greater cognitive decline was significant only in female PD patients, whereas the prediction of future cognitive deterioration was significant only in males. According to our literature review, this sex-specific pattern has not been reported previously. The lack of association between NfL and MMSE in PD patients is likely explained

by the limited variability in MMSE scores, underscoring the value of more sensitive assessments such as the CERAD and CERAD-Plus for detecting subtle cognitive decline. For further details on NfL as a predictor and marker of cognitive impairment progression in PD, see Section 1.2.6.6.

Although these associations were not observed in our cohort, previous studies have demonstrated relationships between NfL and a wide range of clinical features, including diagnostic differentiation, NMS, disease progression, imaging markers, and treatment response in PD. These include NfL-based differentiation between PD and APD, as well as among PD genetic subtypes (see Section 1.2.6.1). NfL has also been associated with NMS burden, such as depression, RBD, autonomic dysfunction, and increased risk of institutionalization (see Section 1.2.6.3). Differences in NfL levels across PD subtypes and their clinical implications have been described (see Section 1.2.6.7). Furthermore, elevated NfL levels have been linked to mortality and key clinical milestones such as loss of independence (see Section 1.2.6.8). NfL levels have also been shown to correlate with imaging markers of structural and microstructural brain changes in PD, including cortical thinning, diffusion abnormalities, and reduced dopamine transporter availability (see Section 1.2.6.9). Finally, NfL has been studied in the context of neurosurgical interventions, with transient postoperative increases observed following DBS, likely reflecting procedural rather than disease-related effects (Section 1.2.6.10).

While many studies have reported sex differences in NfL levels among PD patients [70,79,80,101], our study extends these findings by demonstrating sex-specific differences across multiple clinical parameters, including age and cognitive performance. However, our findings regarding sex differences in NfL trajectories should be interpreted with caution, as the male-to-female ratio was unequal (HC: 1.47:1; PD: 1.88:1). This imbalance may have increased the likelihood of detecting effects in men while reducing detection sensitivity in women, potentially influencing the significance of the observed sex differences.

Our study both supports and extends previous findings regarding NfL as a biomarker in PD, while also highlighting several methodological factors that may have contributed to differences from earlier studies. Notably, our PD cohort consisted of individuals at H&Y stage 2, with a relatively short disease duration

(mean 4.0 years, SD 3.5) and high baseline cognitive functioning, as indicated by MMSE scores (27.9 in PD vs. 28.3 in HC). This suggests that PD patients were early-moderate disease stage and are less likely to show cognitive impairment, factors that may contribute to lower NfL levels and smaller group differences between PD and HC, due to less pronounced neurodegeneration. The limited variability in clinical characteristics likely narrowed the range of NfL values and cognitive performance, potentially limiting the detection of associations observed in more heterogeneous, older or advanced PD cohorts. Additionally, the sample size (47 HC and 46 PD) and single center recruitment (Graz) may have further contributed to the differences observed compared to larger, multicenter studies.

Beyond sample characteristics, differences in methods used to quantify NfL may also influence cross-study comparisons. Moreover, inter-laboratory and inter-assay variability, as well as the use of different platforms (e.g., ELISA vs. Simoa) or sample type (serum, plasma, or CSF), can contribute to variability. Collectively, these methodological differences, such as variation in disease stage, cognitive assessment tools, sample composition, and assay conditions, highlight the importance of cautious interpretation and careful comparison across NfL studies in PD.

4.3 Strength and Limitations

The strength of this study lies in the careful selection and matching of participants, with age- and sex-matched HC and PD groups and the exclusion of individuals with a family history of movement disorders. A homogeneous PD cohort (H&Y stage 2) reduces variability within the PD group, while detailed clinical and neuropsychological assessments (e.g., CERAD-Plus) allow for sensitive detection of cognitive changes. The longitudinal design allowed for tracking NfL changes over time and their association with clinical progression, including sex-stratified analyses. Lastly, biomarker measurements using the high-sensitivity Simoa platform ensured high reliability and reproducibility.

Despite these strengths, the study has some limitations. The overall sample-size was small, especially for the sex-specific analyses, and the higher proportion of males in the PD group may have made it harder to detect effects in females. Focusing on early-stage PD (H&Y stage 2) helped make the groups more

comparable but reduces applicability of the findings to patients with more advanced disease stages. Additionally, the relatively high baseline cognitive performance in the PD cohort may have made it harder to observe associations between NfL and overall cognitive decline. Another limitation is that we did not observe significant associations between NfL levels and motor scores, as reported in some previous studies. This may be due to the early disease stage of all PD patients (H&Y stage 2), where limited variability in motor impairment and short disease duration could reduce the likelihood of detecting such correlations. This suggests that relationships between NfL and motor function may be more pronounced in cohorts with a broader range of disease severity and larger sample sizes. Furthermore, NfL may reflect overall neuroaxonal damage rather than motor symptom severity, particularly in early-stage PD where clinical differences are low.

As a single-center study conducted in Austria, the findings may not fully represent more diverse populations or different healthcare systems. Although participants were carefully matched and grouped, detailed multivariable models could have controlled for additional confounding factors. Moreover, pre-analytic procedures can introduce bias due to differences in measurements, when samples are processed or analyzed in different groups, such as on different days, by different technicians, or using different reagent batches. Finally, technical issues such as inter-assay variability and differences between laboratories or test kits could have affected NfL measurements.

4.4 Implications

The findings of this study have several important implications for clinical application and future research. The demonstration of elevated serum NfL levels in early-stage PD, and their association with cognitive decline, supports the potential utility of NfL as a non-invasive biomarker for monitoring neurodegeneration and progression in PD. Prior to the introduction of highly sensitive laboratory diagnostic tools, such as Simoa, NfL levels could only be reliably measured in CSF using ELISA, which required invasive lumbar puncture. This was mainly because ELISA lacked the sensitive to detect NfL in blood. With Simoa, it is now possible to measure NfL levels in blood accurately [41]. This makes the process much less

invasive and allows NfL to be measured longitudinally to monitor changes over time.

Incorporating NfL into clinical assessments could enable better evaluation of disease severity [61,76,96,98,99] and progression risk [41], allowing clinicians to identify individuals with more aggressive disease courses and initiate earlier monitoring or intervention. Tracking NfL dynamics over time could also aid in detecting early signs of neurodegeneration before clinical symptoms emerge, as demonstrated by a study in which individuals who later converted from HC to PD showed similar serum NfL levels to HC at baseline, but elevated levels at follow-up [90]. Furthermore, it may support the evaluation of future disease-modifying treatments, help assess their efficiency, and aid in therapeutic decision-making by evaluating treatment response [41]. These insights could help guide the selection of initial medication and allow for dosage adjustments based on the activity of neurodegeneration, as reflected by NfL levels. The observed sex-specific differences in NfL levels also suggest that personalized approaches might be useful.

Despite the insights gained from our findings, several important questions remain unanswered. First, it remains unclear how serum NfL levels perform as a predictive biomarker across different stages of PD or in more diverse populations. The mechanisms underlying the observed sex differences in NfL progression are not fully understood and require further investigation. Additionally, it remains unclear how longitudinal changes in NfL levels are associated with clinical milestones, such as the onset of dementia or motor complications. There is also a need for larger, more diverse multicenter studies to establish standardized reference values across different populations, determine the prognostic value of NfL throughout the disease course, and evaluate its use in real-world clinical settings.

In summary, our study contributes to the growing evidence that serum NfL is a valuable biomarker for disease monitoring in early-stage PD. We found strong associations between NfL levels and cognitive decline, along with sex-specific differences. These results highlight the need for individualized assessment in PD. Although further research is needed to confirm these results in larger and more diverse cohorts, our findings suggest that NfL measurements may help in risk

assessment for cognitive decline and track neuroaxonal injury or disease progression by assessing longitudinal change in NfL. They may also support therapeutic decision-making, particularly in managing cognitive symptoms, as NfL predicted cognitive decline. Further investigation into the pathophysiological mechanisms and clinical applications of NfL will be critical for developing personalized treatments and improving clinical outcomes in PD.

5 Reference

1. Masuhr KF, Masuhr F, Neumann M. Duale Reihe Neurologie. 7. Auflage. Stuttgart: Thieme; 2016. 195–196 p. (Duale Reihe).

2. Simon RP. Clinical Neurology. 10th ed. New York, N.Y: McGraw-Hill Education LLC; 2018. 21, 312–314, 320–324 p. (McGraw-Hill's AccessMedicine).

3. Ascherio A, Schwarzschild MA. The epidemiology of Parkinson's disease: risk factors and prevention. Lancet Neurol. 2016 Nov;15(12):1257–72.

4. Dorsey ER, Sherer T, Okun MS, Bloem BR. The Emerging Evidence of the Parkinson Pandemic. J Park Dis. 2018;8(s1):S3–8.

5. Parkinson disease [Internet]. [cited 2025 June 19]. Available from: https://www.who.int/news-room/fact-sheets/detail/parkinson-disease

6. Ou Z, Pan J, Tang S, Duan D, Yu D, Nong H, et al. Global Trends in the Incidence, Prevalence, and Years Lived With Disability of Parkinson's Disease in 204 Countries/Territories From 1990 to 2019. Front Public Health. 2021;9:776847.

7. Hirsch L, Jette N, Frolkis A, Steeves T, Pringsheim T. The Incidence of Parkinson's Disease: A Systematic Review and Meta-Analysis. Neuroepidemiology. 2016;46(4):292–300.

8. Sturm D, Biesalski AS, Höffken O, editors. Neurologische Pathophysiologie: Ursachen und Mechanismen neurologischer Erkrankungen. Berlin: Springer; 2019. 144, 149–151, 155 p.

9. Parkinson-Syndrom und Parkinson-Krankheit - AMBOSS [Internet]. [cited 2025 June 19]. Available from: https://next.amboss.com/de/article/C30qkf?q=parkinson-syndrom%20und%20morbus%20parkinson#Zf3ee709a0b4756e72dde7bedd42ab8ef

10. Jankovic J, Mazziotta JC, Pomeroy SL, Newman NJ. Bradley and Daroff's neurology in clinical practice. 8. Edition. Edinburgh: Elsevier; 2022. 311–313, 710, 919, 1174, 1504–1507 p.

11. Morris HR, Spillantini MG, Sue CM, Williams-Gray CH. The pathogenesis of Parkinson's disease. Lancet Lond Engl. 2024 Jan 20;403(10423):293–304.

12. Deng H, Wang P, Jankovic J. The genetics of Parkinson disease. Ageing Res Rev. 2018 Mar;42:72–85.

13. PubChem. VPS35 - VPS35 retromer complex component (human) [Internet]. [cited 2025 June 28]. Available from: https://pubchem.ncbi.nlm.nih.gov/gene/VPS35/human

14. Stenmark H, Olkkonen VM. The Rab GTPase family. Genome Biol. 2001;2(5):REVIEWS3007.

15. Menozzi E, Macnaughtan J, Schapira AHV. The gut-brain axis and Parkinson disease: clinical and pathogenetic relevance. Ann Med. 2021 Dec;53(1):611–25.

16. Tan AH, Lim SY, Lang AE. The microbiome-gut-brain axis in Parkinson disease - from basic research to the clinic. Nat Rev Neurol. 2022 Aug;18(8):476–95.

17. Engelender S, Isacson O. The Threshold Theory for Parkinson's Disease. Trends Neurosci. 2017 Jan;40(1):4–14.

18. Silbernagl S, Lang F, Rüdiger Gay, Rothenburger A. Taschenatlas Pathophysiologie [Internet]. 6., vollständig überarbeitete Auflage. Stuttgart New York: Georg Thieme Verlag; 2020 [cited 2025 June 20]. 350–351 p. (Thieme eRef). Available from: https://eref-1thieme-1de-1pfo1q6y400c5.han.medunigraz.at/ebooks/pdf/cs_10313274/188240106_002_010_007.pdf

19. Berkowitz A, editor. Clinical neurology & neuroanatomy: a localization-based approach. Second edition. New York: McGraw-Hill LLC; 2022. 274–275 p. (McGraw-Hill's AccessMedicine).

20. Howard R, Kullmann D, Werring DJ, Zandi M, editors. Neurology: a queen square textbook. 3rd edition. Newark: John Wiley & Sons, Incorporated; 2024. 200–203 p.

21. Zach H, Dirkx MF, Pasman JW, Bloem BR, Helmich RC. Cognitive Stress Reduces the Effect of Levodopa on Parkinson's Resting Tremor. CNS Neurosci Ther. 2017 Mar;23(3):209–15.

22. Ropper AH, Samuels MA, Klein JP, Prasad S. Adams and Victor's principles of neurology. Twelfth edition. New York: McGraw-Hill LLC; 2023. 1073–1077 p. (McGraw-Hill's AccessMedicine).

23. Saleem F, Munakomi S. Pseudobulbar Palsy. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 [cited 2025 June 20]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK553160/

24. Váradi C. Clinical Features of Parkinson's Disease: The Evolution of Critical Symptoms. Biology. 2020 May 19;9(5):103.

25. Armstrong MJ, Okun MS. Diagnosis and Treatment of Parkinson Disease: A Review. JAMA. 2020 Feb 11;323(6):548–60.

26. Kouli A, Torsney KM, Kuan WL. Parkinson's Disease: Etiology, Neuropathology, and Pathogenesis. In: Stoker TB, Greenland JC, editors. Parkinson's Disease: Pathogenesis and Clinical Aspects [Internet]. Brisbane (AU): Codon Publications; 2018 [cited 2025 June 20]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK536722/

27. Jellinger KA. A critical evaluation of current staging of alpha-synuclein pathology in Lewy body disorders. Biochim Biophys Acta. 2009 July;1792(7):730–40.

28. Frigerio R, Fujishiro H, Ahn TB, Josephs KA, Maraganore DM, DelleDonne A, et al. Incidental Lewy body disease: do some cases represent a preclinical stage of dementia with Lewy bodies? Neurobiol Aging. 2011 May;32(5):857–63.

29. Parkinson's Disease and Braak stages [Internet]. Neurotorium. [cited 2025 June 20]. Available from: https://neurotorium.org/image/parkinsons-disease-and-braak-stages/

30. Tolosa E, Garrido A, Scholz SW, Poewe W. Challenges in the diagnosis of Parkinson's disease. Lancet Neurol. 2021 May;20(5):385–97.

31. Rizzo G, Copetti M, Arcuti S, Martino D, Fontana A, Logroscino G. Accuracy of clinical diagnosis of Parkinson disease: A systematic review and meta-analysis. Neurology. 2016 Feb 9;86(6):566–76.

32. Yamashita KY, Bhoopatiraju S, Silverglate BD, Grossberg GT. Biomarkers in Parkinson's disease: A state of the art review. Biomark Neuropsychiatry. 2023 Dec;9:100074.

33. Magdalinou NK, Paterson RW, Schott JM, Fox NC, Mummery C, Blennow K, et al. A panel of nine cerebrospinal fluid biomarkers may identify patients with atypical parkinsonian syndromes. J Neurol Neurosurg Psychiatry. 2015 Nov;86(11):1240–7.

34. Chemokine CCL2 - MeSH - NCBI [Internet]. [cited 2025 July 7]. Available from: https://www.ncbi.nlm.nih.gov/mesh?Db=mesh&Cmd=DetailsSearch&Term=%22Chemokine+CCL2%22%5BMeSH+Terms%5D

35. Chemokine CX3CL1 - MeSH - NCBI [Internet]. [cited 2025 July 7]. Available from: https://www.ncbi.nlm.nih.gov/mesh/68054428

36. Chemokine CXCL12 - MeSH - NCBI [Internet]. [cited 2025 July 7]. Available from: https://www.ncbi.nlm.nih.gov/mesh/68054377

37. Tchalla AE, Wellenius GA, Travison TG, Gagnon M, Iloputaife I, Dantoine T, et al. Circulating vascular cell adhesion molecule-1 is associated with cerebral blood flow dysregulation, mobility impairment, and falls in older adults. Hypertens Dallas Tex 1979. 2015 Aug;66(2):340–6.

38. PTX3 pentraxin 3 [Homo sapiens (human)] - Gene - NCBI [Internet]. [cited 2025 July 7]. Available from: https://www.ncbi.nlm.nih.gov/gene/5806

39. Interleukin-1beta - MeSH - NCBI [Internet]. [cited 2025 July 7]. Available from: https://www.ncbi.nlm.nih.gov/mesh?Db=mesh&Cmd=DetailsSearch&Term=%22Interleukin-1beta%22%5BMeSH+Terms%5D

40. Interleukin-10 - MeSH - NCBI [Internet]. [cited 2025 July 7]. Available from: https://www.ncbi.nlm.nih.gov/mesh/68016753

41. Khalil M, Teunissen CE, Otto M, Piehl F, Sormani MP, Gattringer T, et al. Neurofilaments as biomarkers in neurological disorders. Nat Rev Neurol. 2018 Oct;14(10):577–89.

42. Gaetani L, Blennow K, Calabresi P, Di Filippo M, Parnetti L, Zetterberg H. Neurofilament light chain as a biomarker in neurological disorders. J Neurol Neurosurg Psychiatry. 2019 Aug;90(8):870–81.

43. Neurofilament-Leichtketten (NfL)-Assay [Internet]. [cited 2025 June 21]. Available from: https://www.siemens-healthineers.com/de/laboratory-diagnostics/assays-by-diseases-conditions/neurologie/neurofilament-leichtketten-assay

44. Yuan A, Rao MV, Veeranna null, Nixon RA. Neurofilaments and Neurofilament Proteins in Health and Disease. Cold Spring Harb Perspect Biol. 2017 Apr 3;9(4):a018309.

45. Engvall E, Perlmann P. Enzyme-linked immunosorbent assay (ELISA). Quantitative assay of immunoglobulin G. Immunochemistry. 1971 Sept;8(9):871–4.

46. Rosengren LE, Karlsson JE, Karlsson JO, Persson LI, Wikkelsø C. Patients with amyotrophic lateral sclerosis and other neurodegenerative diseases have increased levels of neurofilament protein in CSF. J Neurochem. 1996 Nov;67(5):2013–8.

47. Norgren N, Rosengren L, Stigbrand T. Elevated neurofilament levels in neurological diseases. Brain Res. 2003 Oct 10;987(1):25–31.

48. Petzold A, Altintas A, Andreoni L, Bartos A, Berthele A, Blankenstein MA, et al. Neurofilament ELISA validation. J Immunol Methods. 2010 Jan 31;352(1–2):23–31.

49. Gaiottino J, Norgren N, Dobson R, Topping J, Nissim A, Malaspina A, et al. Increased neurofilament light chain blood levels in neurodegenerative neurological diseases. PloS One. 2013;8(9):e75091.

50. Simoa® Technology [Internet]. Quanterix. [cited 2025 June 21]. Available from: https://www.quanterix.com/simoa-technology/

51. Kuhle J, Barro C, Andreasson U, Derfuss T, Lindberg R, Sandelius Å, et al. Comparison of three analytical platforms for quantification of the neurofilament light chain in blood samples: ELISA, electrochemiluminescence immunoassay and Simoa. Clin Chem Lab Med. 2016 Oct 1;54(10):1655–61.

52. Evaluate the effect of autoantibodies by measuring baseline IFNα and cytokines with Simoa® technology [Internet]. Quanterix. 2025 [cited 2025 June 21]. Available from: https://www.quanterix.com/product-

brochures/evaluate-the-effect-of-autoantibodies-by-measuring-baseline-ifnα-and-cytokines-with-simoa-technology/

53. NfL Fact Sheet [Internet]. Quanterix. 2025 [cited 2025 June 21]. Available from: https://www.quanterix.com/product-brochures/nfl-assay-fact-sheet/

54. Holmberg B, Rosengren L, Karlsson JE, Johnels B. Increased cerebrospinal fluid levels of neurofilament protein in progressive supranuclear palsy and multiple-system atrophy compared with Parkinson's disease. Mov Disord Off J Mov Disord Soc. 1998 Jan;13(1):70–7.

55. Brettschneider J, Petzold A, Süssmuth SD, Landwehrmeyer GB, Ludolph AC, Kassubek J, et al. Neurofilament heavy-chain NfH(SMI35) in cerebrospinal fluid supports the differential diagnosis of Parkinsonian syndromes. Mov Disord Off J Mov Disord Soc. 2006 Dec;21(12):2224–7.

56. Hall S, Öhrfelt A, Constantinescu R, Andreasson U, Surova Y, Bostrom F, et al. Accuracy of a panel of 5 cerebrospinal fluid biomarkers in the differential diagnosis of patients with dementia and/or parkinsonian disorders. Arch Neurol. 2012 Nov;69(11):1445–52.

57. Quadalti C, Calandra-Buonaura G, Baiardi S, Mastrangelo A, Rossi M, Zenesini C, et al. Neurofilament light chain and α-synuclein RT-QuIC as differential diagnostic biomarkers in parkinsonisms and related syndromes. NPJ Park Dis. 2021 Oct 11;7(1):93.

58. Marques TM, van Rumund A, Oeckl P, Kuiperij HB, Esselink RAJ, Bloem BR, et al. Serum NFL discriminates Parkinson disease from atypical parkinsonisms. Neurology. 2019 Mar 26;92(13):e1479–86.

59. Olsson B, Portelius E, Cullen NC, Sandelius Å, Zetterberg H, Andreasson U, et al. Association of Cerebrospinal Fluid Neurofilament Light Protein Levels With Cognition in Patients With Dementia, Motor Neuron Disease, and Movement Disorders. JAMA Neurol. 2019 Mar 1;76(3):318–25.

60. Constantinescu R, Rosengren L, Eriksson B, Blennow K, Axelsson M. Cerebrospinal fluid neurofilament light and tau protein as mortality biomarkers in parkinsonism. Acta Neurol Scand. 2019 Aug;140(2):147–56.

61. Schulz I, Kruse N, Gera RG, Kremer T, Cedarbaum J, Barbour R, et al. Systematic Assessment of 10 Biomarker Candidates Focusing on α-Synuclein-Related Disorders. Mov Disord Off J Mov Disord Soc. 2021 Dec;36(12):2874–87.

62. Herbert MK, Aerts MB, Beenes M, Norgren N, Esselink RAJ, Bloem BR, et al. CSF Neurofilament Light Chain but not FLT3 Ligand Discriminates Parkinsonian Disorders. Front Neurol. 2015;6:91.

63. Constantinescu R, Rosengren L, Johnels B, Zetterberg H, Holmberg B. Consecutive analyses of cerebrospinal fluid axonal and glial markers in Parkinson's disease and atypical Parkinsonian disorders. Parkinsonism Relat Disord. 2010 Feb;16(2):142–5.

64. Abdo WF, Bloem BR, Van Geel WJ, Esselink RAJ, Verbeek MM. CSF neurofilament light chain and tau differentiate multiple system atrophy from Parkinson's disease. Neurobiol Aging. 2007 May;28(5):742–7.

65. Holmberg B, Johnels B, Ingvarsson P, Eriksson B, Rosengren L. CSF-neurofilament and levodopa tests combined with discriminant analysis may contribute to the differential diagnosis of Parkinsonian syndromes. Parkinsonism Relat Disord. 2001 Sept;8(1):23–31.

66. Singer W, Schmeichel AM, Shahnawaz M, Schmelzer JD, Boeve BF, Sletten DM, et al. Alpha-Synuclein Oligomers and Neurofilament Light Chain in Spinal Fluid Differentiate Multiple System Atrophy from Lewy Body Synucleinopathies. Ann Neurol. 2020 Sept;88(3):503–12.

67. Bäckström DC, Eriksson Domellöf M, Linder J, Olsson B, Öhrfelt A, Trupp M, et al. Cerebrospinal Fluid Patterns and the Risk of Future Dementia in Early, Incident Parkinson Disease. JAMA Neurol. 2015 Oct;72(10):1175–82.

68. Lin CH, Li CH, Yang KC, Lin FJ, Wu CC, Chieh JJ, et al. Blood NfL: A biomarker for disease severity and progression in Parkinson disease. Neurology. 2019 Sept 10;93(11):e1104–11.

69. Li Q, Li Z, Han X, Shen X, Wang F, Bai L, et al. A Panel of Plasma Biomarkers for Differential Diagnosis of Parkinsonian Syndromes. Front Neurosci. 2022;16:805953.

70. Hansson O, Janelidze S, Hall S, Magdalinou N, Lees AJ, Andreasson U, et al. Blood-based NfL: A biomarker for differential diagnosis of parkinsonian disorder. Neurology. 2017 Mar 7;88(10):930–7.

71. Hu X, Yang Y, Gong D. Cerebrospinal fluid levels of neurofilament light chain in multiple system atrophy relative to Parkinson's disease: a meta-analysis. Neurol Sci Off J Ital Neurol Soc Ital Soc Clin Neurophysiol. 2017 Mar;38(3):407–14.

72. Ge F, Ding J, Liu Y, Lin H, Chang T. Cerebrospinal fluid NFL in the differential diagnosis of parkinsonian disorders: A meta-analysis. Neurosci Lett. 2018 Oct 15;685:35–41.

73. Canaslan S, Schmitz M, Villar-Piqué A, Maass F, Gmitterová K, Varges D, et al. Detection of Cerebrospinal Fluid Neurofilament Light Chain as a Marker for Alpha-Synucleinopathies. Front Aging Neurosci. 2021;13:717930.

74. Ashton NJ, Janelidze S, Al Khleifat A, Leuzy A, van der Ende EL, Karikari TK, et al. A multicentre validation study of the diagnostic value of plasma neurofilament light. Nat Commun. 2021 June 7;12(1):3400.

75. Jabbari E, Holland N, Chelban V, Jones PS, Lamb R, Rawlinson C, et al. Diagnosis Across the Spectrum of Progressive Supranuclear Palsy and Corticobasal Syndrome. JAMA Neurol. 2020 Mar 1;77(3):377–87.

76. Huang Y, Huang C, Zhang Q, Shen T, Sun J. Serum NFL discriminates Parkinson disease from essential tremor and reflect motor and cognition severity. BMC Neurol. 2022 Jan 27;22(1):39.

77. Hall S, Surova Y, Öhrfelt A, Swedish BioFINDER Study, Blennow K, Zetterberg H, et al. Longitudinal Measurements of Cerebrospinal Fluid Biomarkers in Parkinson's Disease. Mov Disord Off J Mov Disord Soc. 2016 June;31(6):898–905.

78. Bäckström D, Linder J, Jakobson Mo S, Riklund K, Zetterberg H, Blennow K, et al. NfL as a biomarker for neurodegeneration and survival in Parkinson disease. Neurology. 2020 Aug 18;95(7):e827–38.

79. Lerche S, Wurster I, Röben B, Zimmermann M, Machetanz G, Wiethoff S, et al. CSF NFL in a Longitudinally Assessed PD Cohort: Age Effects and Cognitive Trajectories. Mov Disord Off J Mov Disord Soc. 2020 July;35(7):1138–44.

80. Mollenhauer B, Dakna M, Kruse N, Galasko D, Foroud T, Zetterberg H, et al. Validation of Serum Neurofilament Light Chain as a Biomarker of Parkinson's Disease Progression. Mov Disord Off J Mov Disord Soc. 2020 Nov;35(11):1999–2008.

81. Aamodt WW, Waligorska T, Shen J, Tropea TF, Siderowf A, Weintraub D, et al. Neurofilament Light Chain as a Biomarker for Cognitive Decline in Parkinson Disease. Mov Disord Off J Mov Disord Soc. 2021 Dec;36(12):2945–50.

82. Pilotto A, Imarisio A, Conforti F, Scalvini A, Masciocchi S, Nocivelli S, et al. Plasma NfL, clinical subtypes and motor progression in Parkinson's disease. Parkinsonism Relat Disord. 2021 June;87:41–7.

83. Oosterveld LP, Verberk IMW, Majbour NK, El-Agnaf OM, Weinstein HC, Berendse HW, et al. CSF or serum neurofilament light added to α-Synuclein panel discriminates Parkinson's from controls. Mov Disord Off J Mov Disord Soc. 2020 Feb;35(2):288–95.

84. Chen CH, Lee BC, Lin CH. Integrated Plasma and Neuroimaging Biomarkers Associated with Motor and Cognition Severity in Parkinson's Disease. J Park Dis. 2020;10(1):77–88.

85. Chang KH, Liu KC, Lai CS, Yang SY, Chen CM. Assessing Plasma Levels of α-Synuclein and Neurofilament Light Chain by Different Blood Preparation Methods. Front Aging Neurosci. 2021;13:759182.

86. Papuć E, Rejdak K. Increased CSF NFL in Non-demented Parkinson's Disease Subjects Reflects Early White Matter Damage. Front Aging Neurosci. 2020;12:128.

87. Lin WC, Lu CH, Chiu PY, Yang SY. Plasma Total α-Synuclein and Neurofilament Light Chain: Clinical Validation for Discriminating Parkinson's

Disease from Normal Control. Dement Geriatr Cogn Disord. 2020;49(4):401–9.

88. Gui Y, Liu H, Zhang L, Lv W, Hu X. Altered microRNA profiles in cerebrospinal fluid exosome in Parkinson disease and Alzheimer disease. Oncotarget. 2015 Nov 10;6(35):37043–53.

89. Chen JH, Chan L, Chung CC, Bamodu OA, Hong CT. Blood Neurofilament Light Chain in Parkinson's Disease: Comparability between Parkinson's Progression Markers Initiative (PPMI) and Asian Cohorts. J Clin Med. 2021 Oct 29;10(21):5085.

90. Wilke C, Dos Santos MCT, Schulte C, Deuschle C, Scheller D, Verbelen M, et al. Intraindividual Neurofilament Dynamics in Serum Mark the Conversion to Sporadic Parkinson's Disease. Mov Disord Off J Mov Disord Soc. 2020 July;35(7):1233–8.

91. Ng ASL, Tan YJ, Yong ACW, Saffari SE, Lu Z, Ng EY, et al. Utility of plasma Neurofilament light as a diagnostic and prognostic biomarker of the postural instability gait disorder motor subtype in early Parkinson's disease. Mol Neurodegener. 2020 June 5;15(1):33.

92. Ma LZ, Zhang C, Wang H, Ma YH, Shen XN, Wang J, et al. Serum Neurofilament Dynamics Predicts Cognitive Progression in de novo Parkinson's Disease. J Park Dis. 2021;11(3):1117–27.

93. Sampedro F, Pérez-González R, Martínez-Horta S, Marín-Lahoz J, Pagonabarraga J, Kulisevsky J. Serum neurofilament light chain levels reflect cortical neurodegeneration in de novo Parkinson's disease. Parkinsonism Relat Disord. 2020 May;74:43–9.

94. Gaetani L, Höglund K, Parnetti L, Pujol-Calderon F, Becker B, Eusebi P, et al. A new enzyme-linked immunosorbent assay for neurofilament light in cerebrospinal fluid: analytical validation and clinical evaluation. Alzheimers Res Ther. 2018 Jan 23;10(1):8.

95. Pötter-Nerger M, Dutke J, Lezius S, Buhmann C, Schulz R, Gerloff C, et al. Serum neurofilament light chain and postural instability/gait difficulty (PIGD) subtypes of Parkinson's disease in the MARK-PD study. J Neural Transm Vienna Austria 1996. 2022 Mar;129(3):295–300.

96. Niemann L, Lezius S, Maceski A, Leppert D, Englisch C, Schwedhelm E, et al. Serum neurofilament is associated with motor function, cognitive decline and subclinical cardiac damage in advanced Parkinson's disease (MARK-PD). Parkinsonism Relat Disord. 2021 Sept;90:44–8.

97. Ye R, Locascio JJ, Goodheart AE, Quan M, Zhang B, Gomperts SN. Serum NFL levels predict progression of motor impairment and reduction in putamen dopamine transporter binding ratios in de novo Parkinson's disease: An 8-year longitudinal study. Parkinsonism Relat Disord. 2021 Apr;85:11–6.

98. Ygland Rödström E, Mattsson-Carlgren N, Janelidze S, Hansson O, Puschmann A. Serum Neurofilament Light Chain as a Marker of Progression in Parkinson's Disease: Long-Term Observation and Implications of Clinical Subtypes. J Park Dis. 2022;12(2):571–84.

99. Frank A, Bendig J, Schniewind I, Polanski WH, Sobottka SB, Reichmann H, et al. Serum neurofilament indicates that DBS surgery can cause neuronal damage whereas stimulation itself does not. Sci Rep. 2022 Jan 27;12(1):1446.

100. Zhu Y, Yang B, Wang F, Liu B, Li K, Yin K, et al. Association between plasma neurofilament light chain levels and cognitive function in patients with Parkinson's disease. J Neuroimmunol. 2021 Sept 15;358:577662.

101. Lin YS, Lee WJ, Wang SJ, Fuh JL. Levels of plasma neurofilament light chain and cognitive function in patients with Alzheimer or Parkinson disease. Sci Rep. 2018 Nov 26;8(1):17368.

102. Chung CC, Chan L, Chen JH, Bamodu OA, Hong CT. Neurofilament light chain level in plasma extracellular vesicles and Parkinson's disease. Ther Adv Neurol Disord. 2020;13:1756286420975917.

103. Kim R, Jeon B. Serum neurofilament light chain predicts future freezing of gait in Parkinson's disease. Parkinsonism Relat Disord. 2021 Oct;91:102–4.

104. Bartl M, Dakna M, Galasko D, Hutten SJ, Foroud T, Quan M, et al. Biomarkers of neurodegeneration and glial activation validated in Alzheimer's disease assessed in longitudinal cerebrospinal fluid samples of Parkinson's disease. PloS One. 2021;16(10):e0257372.

105. Schmidt R, Enzinger C, Ropele S, Schmidt H, Fazekas F, Austrian Stroke Prevention Study. Progression of cerebral white matter lesions: 6-year results of the Austrian Stroke Prevention Study. Lancet Lond Engl. 2003 June 14;361(9374):2046–8.

106. Tomlinson CL, Stowe R, Patel S, Rick C, Gray R, Clarke CE. Systematic review of levodopa dose equivalency reporting in Parkinson's disease. Mov Disord Off J Mov Disord Soc. 2010 Nov 15;25(15):2649–53.

107. Hoehn MM, Yahr MD. Parkinsonism: onset, progression and mortality. Neurology. 1967 May;17(5):427–42.

108. Goetz CG, Tilley BC, Shaftman SR, Stebbins GT, Fahn S, Martinez-Martin P, et al. Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS): scale presentation and clinimetric testing results. Mov Disord Off J Mov Disord Soc. 2008 Nov 15;23(15):2129–70.

109. Chaudhuri KR, Martinez-Martin P, Schapira AHV, Stocchi F, Sethi K, Odin P, et al. International multicenter pilot study of the first comprehensive self-completed nonmotor symptoms questionnaire for Parkinson's disease: the

NMSQuest study. Mov Disord Off J Mov Disord Soc. 2006 July;21(7):916–23.

110. Chaudhuri KR, Martinez-Martin P, Brown RG, Sethi K, Stocchi F, Odin P, et al. The metric properties of a novel non-motor symptoms scale for Parkinson's disease: Results from an international pilot study. Mov Disord Off J Mov Disord Soc. 2007 Oct 15;22(13):1901–11.

111. Yesavage JA, Sheikh JI. 9/Geriatric Depression Scale (GDS): Recent Evidence and Development of a Shorter Version. Clin Gerontol. 1986 Nov 18;5(1–2):165–73.

112. Yesavage JA, Brink TL, Rose TL, Lum O, Huang V, Adey M, et al. Development and validation of a geriatric depression screening scale: a preliminary report. J Psychiatr Res. 1982 1983;17(1):37–49.

113. Yesavage JA. Geriatric Depression Scale. Psychopharmacol Bull. 1988;24(4):709–11.

114. Morris JC, Heyman A, Mohs RC, Hughes JP, van Belle G, Fillenbaum G, et al. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part I. Clinical and neuropsychological assessment of Alzheimer's disease. Neurology. 1989 Sept;39(9):1159–65.

115. Seo EH, Lee DY, Lee JH, Choo IH, Kim JW, Kim SG, et al. Total scores of the CERAD neuropsychological assessment battery: validation for mild cognitive impairment and dementia patients with diverse etiologies. Am J Geriatr Psychiatry Off J Am Assoc Geriatr Psychiatry. 2010 Sept;18(9):801–9.

116. Chandler MJ, Lacritz LH, Hynan LS, Barnard HD, Allen G, Deschner M, et al. A total score for the CERAD neuropsychological battery. Neurology. 2005 July 12;65(1):102–6.

117. Baek MS, Lee MJ, Kim HK, Lyoo CH. Temporal trajectory of biofluid markers in Parkinson's disease. Sci Rep. 2021 July 20;11(1):14820.