UNIVERSITY OF PADOVA

Department of General Psychology

Master Degree in Cognitive Neuroscience and Clinical Neuropsychology

Final dissertation

Neurofilament light chain protein in patients with Parkinson's disease and cognitive deficits

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Abstract

Neurofilaments are cytoskeletal proteins responsible for radial growth and length of long myelinated axons. In total there are 6 neurofilament subunits, yet the focus of this thesis lies on neurofilament light chain and its implication in cognition of patients with Parkinson's disease.

Neurofilament light protein has emerged as a promising biological marker across various neurodegenerative disorders, offering insights into axonal damage crucial for non-invasive neurological biomarker research. Initially investigated in cerebrospinal fluid (CSF), neurofilament light chains reveal distinctive patterns of cortical and subcortical damage. However, the invasive nature of CSF collection prompted scientists to devise alternative quantification methods. Despite advancements, the transition to blood-based biomarker research faces challenges, mainly the lack of universally standardized baseline measures for neurofilament light proteins in healthy subjects and affected patients.

Recently, the potential clinical implications of neurofilament light protein have gained traction, with researchers worldwide leveraging it for diagnostic, prognostic, and treatment monitoring purposes. While it shows promise across several neurodegenerative diseases like multiple sclerosis, Alzheimer's disease, and frontotemporal lobar degeneration, its role in Parkinson's disease remains contentious. Factors contributing to this discordance include the clinical heterogeneity of Parkinson patients, divergent quantification methodologies, conflicting study outcomes, and the absence of a universally established baseline for neurofilament light protein.

Nevertheless, this work underscores a growing body of evidence linking cognition in Parkinson's disease patients to blood-based and serum-based levels of neurofilament light chain protein, suggesting that further exploration and clinical application are warranted.

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Neurofilaments

Axons are prolonged segments of the neurons located in the center of the cell, in between the soma and the axon terminals. Their cytoskeleton is made of a scaffolding of proteins with 6 different types. Out of six of them, the most important are type IV proteins called *intermediate filaments* with a size of about 10nm diameter. They are called intermediate as their size is intermediate between the smaller microfilaments (around 7nm) and larger microfilaments (around 25nm).

Neurofilament proteins are considered to be major constitutional proteins of the neuronal cytoskeleton. They are localized in large myelinated axons and large neurons (Fride and Samorajski, 1970). Their role is considered to be supportive and structural – providing the axons with cytoskeletal support, diameter length, and possibly communication with other neural structures such as microtubules.

The exact role of neurofilaments is yet to be discovered, but for now, we know they are important in both normal and pathological conditions.

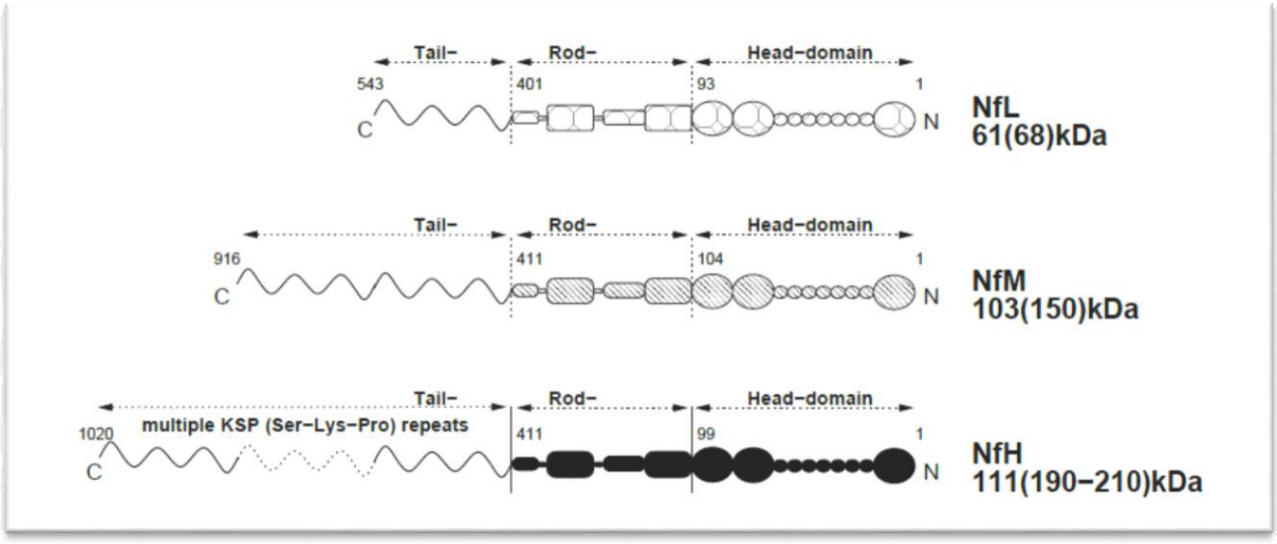
Neurofilament proteins consist of a total of 6 subunits, 3 of which are polypeptides: neurofilament light chain (NfL) with a diameter of 68kDa, neurofilament medium chain (NfM) with a diameter of 160 kDa and neurofilament heavy chain (NfH) with a diameter of 111 kDa. It is believed that each of the presented subunits has a different functional role, especially in pathological brain conditions.

Structure of neurofilaments

The structure of a neurofilament is a tripartite construction. It is constituted of an alpha-helical rod domain which is important for the formation of oligomers, the core of each Nf subunit is bound by the head called the N-terminus and the tail called the C-terminus. The head and the tail domains are responsible for the functional properties of these proteins such as interactions with other proteins and organelles (Petzold, 2004).

The function of the C-terminal tail domain lies in differentiating between neurofilament subunits, as the length of the tail domain determines the molecular mass and further specifies the three subunits. Therefore, the molecular mass of the neurofilament light chain (NfL) is 68kDa, the molecular mass of the neurofilament medium chain is around 160kDa and the heaviest among them is the neurofilament heavy chain (NfH) which has a molecular mass of 111kDa (Fuchs and Cleveland, 1998).

Neurofilament light proteins are often times called the "backbone" among all other neurofilament proteins. The reason behind this nickname lies in outnumbering other subunits, but also due to its smaller size.



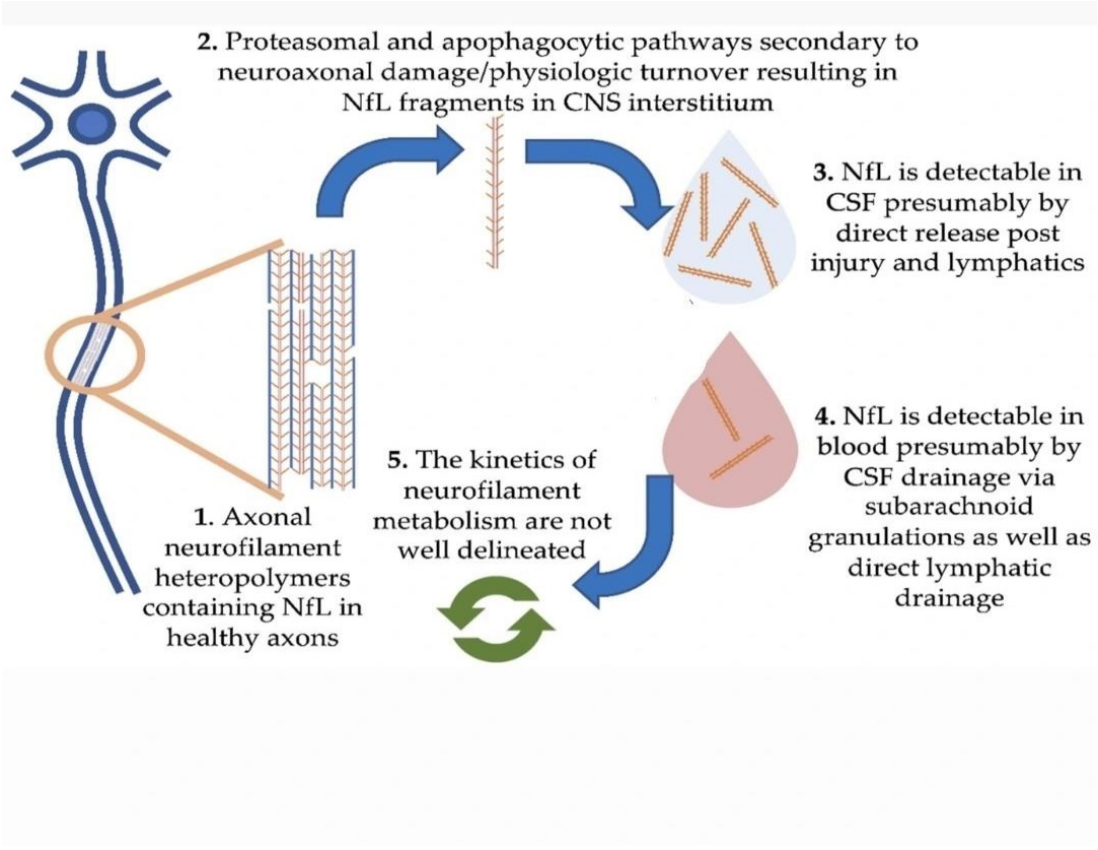
Neurofilament proteins – light (61kDa), medium 103 (kDa) and heavy (111 kDa)

Degradation of neurofilaments

The breakdown of neurofilaments is caused by protease digestion. Based on the work done by Lee and Cleveland, the lifetime of neurofilaments is believed to be around 1-2 years. After this period passes, it is believed that neurofilaments arrive at the axon terminus where they are further broken up (Lee and Cleveland, 1996).

However, in an abnormal brain environment that can be caused by oxidative stress, inflammation, or neurodegeneration, it is believed that neurofilaments are disturbed and their process of breakage is disrupted. As a consequence of normal neurofilament breakage, they will start to accumulate and form a pathogenic assembly which will then cause an axonal pathology.

As mentioned in the works done by Petzold, not all neurofilament subunits are suspectable to protease digestion. It is believed that neurofilament light chain protein has a potential to polymerise on its own, which might be the reason of the NfL accumulation in certain diseases (Petzold, 2004)



Pathophysiology of neurofilament light chain in blood and cerebrospinal fluid (CSF)

Adapted from Thebault, 2020

Measurement of NfLs

There exist a number of different valuable methods for quantification of neurofilament proteins. Here are presented the most used methods in the last twenty years.
In order to correctly determinate the concentration of neurofilament light chain in serum is to use immunoassays for quantitifcation.

In summary, three generations of NFL testing were proposed and all three are still used in both laboratory and clinical practice.
First generation was based on immunoblots, but significantly lacked sensitivity. Second generation solved the sensitivity problem by using a novel method called electrochemiluminescence assay technology (ECL). Third generation is by far the most widely used technique and it is a single – molecular assay technology which has the most sensitive and reliable measurement for NFL (Khalil, 2018).

Enzyme-linked immunosorbent assay (ELISA)

The first ever authors to use ELISA method in order to quantify the concentrations of neurofilament light chain proteins in CSD were Karlsson, Rosengren and colleagues. Their work was published in 1989 and they were able to detect the increase of this biomarker by immunoblot methodology (Karlsson, Rosengreen, 1989).
The Lars Rosengren research group was among the first to investigate the increase of NfLs in the human body using an ELISA methodology. This immunoassay methodology was used in order to compare normal levels of neurofilament light chain with patients suffering from neurodegenerative diseases (such as amyotrophic lateral sclerosis ALS and Alzheimer's disease AD).
They were among the first who discovered a multilevel higher increase of NfLs in cerebral white matter in comparison with grey matter, as they researched patients with ALS (Rosengren, 1996).
In 2018, Gaetani and colleagues have published a study focusing on novel ELISA methodology

for measuring the levels of NfLs in CSF. Authors of this study proposed and confirmed two new antibodies – NfL21 and NfL23 that are specific for neurofilament light chain proteins and applied this novel immunoassay in order to measure the presence of NfLs in the CSF of patients with different neuropathological backgrounds (Gaetani, 2018).
It is important to note that sandwich method ELISA provides good specificity of biomarkers in CSF, but the lacks sufficient sensitivity. This is the reason why researchers opt to use other methods of quantification other than ELISA.

Electrochemiluminescence assay technology (ECL)

ECL technology is considered to be a more sensitive technique of measuring the levels of NfLs in CSF than ELISA. This technique is not that often used as it doesn't have a great discriminatory ability for detecting the lowest concentrations of NfLs in blood.

Single molecule array technology (SiMoA)

SiMoA was developed as a way of measuring the levels of neurofilament light chain proteins in the blood. Studies have confirmed that levels of NfLs in blood correlate with levels of NfLs in the CSF. This technique provides a faster and less invasive quantification as it doesn't require lumbar puncting for the sampling.
The SiMoA technique is an ultasensitive technique that can detect even lowest concetrations of NfLs in the blood and is often times used in longitudinal research.
A study done in 2016 by Kuhle and colleagues compared the three above-mentioned immunoassay techniques used for measurement of NfLs in the system and discovered that SiMoA assay technology is by far the most sensitive assay among the three. The results in their studies confirmed that levels of NfLs in blood reflect the ones in the CSF as well and they highlight the importance of using SiMoA method in future study as it is coast effective, less invasive, more sensitive and faster method of obtaining such quantification (Kuhle, 2016)

CSF analysis

Extraction of CSF was the first method ever to be done in order to measure the levels of neurofilament light chain, but also of other structural proteins as well (such as tau, beta – amyloid, etc).

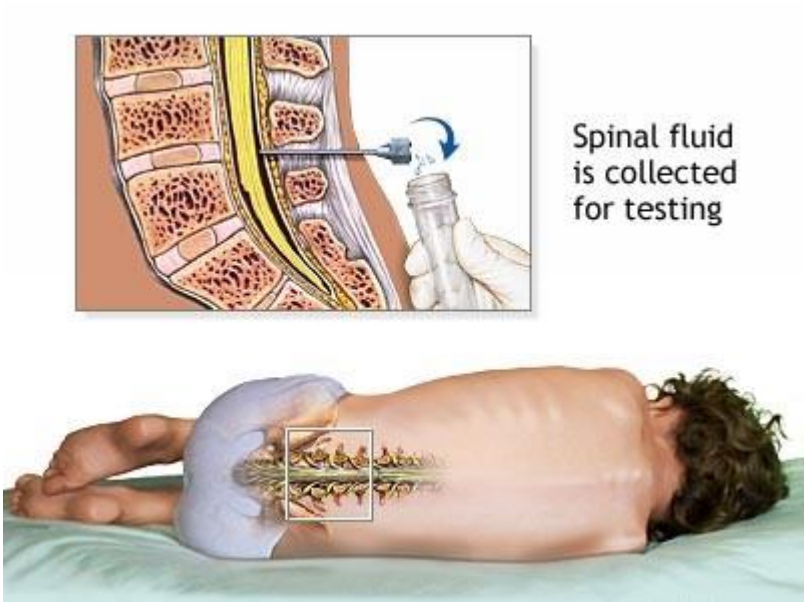
Cerebrospinal fluid (CSF) is considered of highest value for quantification of biomarkers in neurological diseases since it is located so close to the brain and therefore is the best in reflecting pathological processes.

The method relies on extracting the CSF via lumbar puncture at the L3/L4 or L4/L5 interspace. The collected sample was then mixed and left in storage for further biochemical analyses. Numerous studies have confirmed that CSF best reflects the increase of NfLs in the system, yet this procedure is considered invasive, difficult to perform and somewhat expensive.

This is the reason why researching of concentrations of NfLs have lead us to use blood samples instead of CSF samples. Even though the concentration of neurofilament light chain protein in blood is not the same as in CSF, many researchers have confirmed the levels of serum and levels of blood correlate and could therefore help us still in revealing if the increase is present in the system.

Another reason why researchers today opt to use blood samples of NfLs over CSF sample is the option of retesting over the period of time. Longitudinal measures of NfLs are what is missing in current scope of research and what can provide us with more answers about the timeline of structural and chemical changes in the brain.

In order to successfully perform longitudinal studies, researchers must be able to extract a sample of NfLs over some time, without causing further harm or discomfort to the patient.



CSF extraction from lumbar puncture

Blood analysis

When the idea of using blood for measurement of NFL was first proposed, it was quickly hinder by the lack of a blocker for heterophilic antibodies. Moreover, detection levels for this biomarker were not sensitive enough to be measured in blood.

Thanks to the Blennow and Zetterberg who were the inventors of SiMoA technology, a neuroscientific community reached a breakthrough. By using single - molecule array technology higher sensitivity of measurement for NFL levels in the blood was reached.

The SiMoA technology is nowadays mostly used as the safest, quickest and most reliable method of measuring NfL in the blood of both patients, but also in healthy controls.

Following the damage to axons and neuronal degeneration, a significant amount of neurofilament proteins are being released from the axonal membrane into the interstitial fluid. From the interstitial fluid compartment of NF proteins will reach the cerebrospinal fluid (CSF) and later blood.
Even though the presence of neurofilament subunits is better reflected in the CSF, Gaiottino showed in their study how blood samples of patients suffering from neurodegenerative disorders can also positively reflect the increases of neurofilament proteins.

As Gaiottino noted in their study, obtaining blood samples is much easier as the procedure of collecting is simplified and less invasive for the patient. Gaiottino is not the only author to measure NFLs by blood samples, several others have also choosen the route for using peripheral blood levels as quantifying measures of neurofilament proteins (Gaiottino, 2013; Lewis, 2008, Boysan 2013).

There exist a number of both physiological and pathological factors that influence the increase of NFL in the blood.
Increasing the NFL levels in blood could be attributed to neurodegenerative diseases (such as AD, PD), but it could also be due to the head impact during sports. Other factors such as age or cardiovascular problems have been noted to contribute to the neuronal damage. Neurofilament light chain can also be increased due to infarctions in the peripheral nervous system.

One of the reasons why NFL tends to increase with age might be due to the disruption of blood brain barrier. As the nervous system ages, so does the BBB change its permability, thus allowing for more permeable transport.

Barro and colleagues investigated the correlation between NFL levels in CSF and blood, their finding suggested that blood NFL accurately reflects the levels of NFL in CSF as well. However, this correlation is somewhat weakened when taken into the account other factors such as BMI that decrease the levels of NFL in blood.
One study found that an increase of 10% in CSF NFL for MS patients was almost half the measurement for the same group of patients that used blood samples. Alongside of this finding, the prediction and detection of any consecutive changes was better in samples that used CSF NFL.
Conditions that usually show lower levels of NFLs like Parkinson's disease will have a weaker correlation between the CSF and the blood measurement (Barro, 2021)

Neurofilaments as biomarkers

Biomarkers are biologically objective indicators of medical signs that point out the health status or illness of a patient.
A definition proposed by WHO states that biomarkers are "any substance, structure or process that can be measured in the body or its products and influence or predict the incidence of

outcome or disease" (WHO International Program on Chemical Safety, 2001).

Today this definition is widely used both in theory and clinical practice, as it allows us to manage the outcomes of diseases, effects of treatments, and interventions.

Biological markers could be anything ranging from blood pressure or pulse through basic chemical tests of other bodily tissues. Defining, quantifying and/or treating an illness or a disease utilizing laboratory-based biomarkers is considered a somewhat young procedure, as the best approaches are still being developed into practice.
The most important feature of using biomarkers in laboratory and clinical settings is determining the relationship between specific biomarkers and any relevant clinical endpoints. Today, numerous body of evidence exists that proves how levels of NfLs in both CSF and blood may reflect neuronal degeneration in various neurological states.
Blennow (Blennow, 1995) has described how pathological brain states come to be – when the pathological state starts affecting the structure of axons and neurons, it will produce a leakage of different kinds of cellular constituents, one of them being structural proteins. This leakage enters the extracellular fluid of the brain which is connected to the cerebrospinal fluid (CSF) and thus leads to consecutive change in other brain regions. As the CSF circles around the brain and its cavities, rich in these structural proteins, the extraction of CSF would reveal how high is the concentrations of these chemical changes that might reflect structural changes in the brain at the cellular level.
Blennow believed that neurofilaments had a double role – both in pathogenesis of neuronal damage, but also serving as potential markers for disease progression (Blennow, 1995).

Structural changes reflect increase in NFL

One study done in 2001 by Sjogren and colleagues, found that an increase in levels of neurofilament light chain correlated with the presence of white matter changes and this was especially prominent in patients with dementia. The results of their research showed a positive correlation between CSF NfL concentrations and the degree of white matter changes, leading us to conclude that neurofilaments might reflect pathophysiological changes in the brain.

As we know from the previous studies – neurofilament light chain proteins are most abundant

in large myelinated axons (Friede and Samorajski, 1970) and as the disruption of these axons progresses so it becomes evident in concentrations of NfLs in CSF. The authors suspected the process is somehow associated with demyelination which is supported by other studies done on patients with multiple sclerosis (MS) in active phase of the disease when their levels of NfLs were most prominent and highly visible of brain MRI (Lycke et al.,1998).
A possibility of underlying mechanisms responsible for both brain pathology and neurofilament increases has often times been mentioned in scientific circles. Unfortunately, the pathological processes behind this mechanism is still widely unknown and left to be answered in future studies.

Interesting point to make is that NFL levels also tend to rise in patients that survived a stroke. Studies have confirmed in the first few day post-stroke, patients will have a subsequent increase of NFL in blood and serum which might reflect the neuronal disruption happening as one of the consequence of a stroke. Yet, in these patients it was noted that NFL start to come back to normal, baseline levels they had pre-stroke after the few month post incident.

It seems like certain neuronal repair mechanisms are taking place in these specific conditions, taking care of the acute damage following the stroke and thus providing the stopping of the leakage. Interestingly enough, this phenomenon could not be seen in patients with neurodegenerative disease. It seems like as the brain undergoes the pathological states of neuronal degeneration (that's happening in PD), it also disrupts the repair processes that could help the brain patch itself post infraction.

NFL as a diagnostic procedure

Numerous authors agree that the best way of utilizing the knowledge we have on neurofilaments should be without categorizing it in specific diseases, but rather focusing on the symptoms that are present and associated with those neurological diseases (Barro, 2021).

Neurofilaments have proven their worth in a spectrum of neurologic injuries and diseases. For example, we have found out an association between NFLs in inflammatory diseases such as

multiple sclerosis (MS), traumatic brain injuries (TBI), but also in patients who survived strokes. An interesting point to make here is that in patients who had acute neuronal injuries, the increases of NFL were almost immediate, following the occurrence of said injury. Researcher believe the increases that we see might be because of subacute phases of the event.

In one study, patients who had intensive epileptic seizures or survived an event of a stroke, showed significant levels of NFLs over the course of next few days followed by a mild cognitive decline. The cognitive decline, as confirmed by the increases of neurofilaments, was present for some weeks and less often, months after the event (Barro, 2021).

In contrast, chronic neurological condition such as neurodegenerative diseases like Alzheimer's disease or Parkinson's diseasae would also show us increases of NFLs. The main difference lies in the factor of time – while acute injuries would cause an immediate neuronal damage, chronic conditions present slight increases over a period of few years. Explanation of this notable differences lies in the ongoing neuronal pathological changes that influence the micro and macrological structures of the brain. Tau accumulation, alpha synuclein plaque formation all require time to develop. Unfortunately, by the time these pathological changes show a significant increase of NFL levels, symptoms are usually already present and the neurodegenerative disease has entered its active phase.

Until recently the main diagnostic procedure were focused on clinical testing, screening the patients and using the neuroimaging techniques. Yet, the possibility of a misdiagnosis for certain area of neurodegenerative disease were still high. The need for reliable testing that would point to a neurodegenerative disease that is causing the pathophysiological changes in the brain, have long been pointed out.

In recent years, the scope of the research has focused on finding a reliable biological marker for various neuropathological states.
Numerous studies have pointed out the importance of tissue fluid biomarkers in neurodegenerative disorders that can shine the light on microscopic changes happening in axons. Focus is currently held on neurofilament light chain protein and its sample that could be derived either from blood of a patient or from CSF (Pafiti, 2023).

Gaetanni was among the first authors who pointed out two primary reasons for neurofilament light chain protein to serve as a potential biomarker. As he staded in his study done in 2019,

first reason focuses on the clinical implication of these biomarkers; meaning we can use them in a clinical setting for improving the rates of differential diagnoses making, making a good prediction based on the levels of these biomarkers or simply using it for therapeutical purposes. Yet, another point has been brought to light by Gaetanni – their possibility of uncovering the true nature of ongoing neurodegenerative diseases (Gaetanni, 2019).

NFL as a prognostic measure

Neurofilaments are neuronal proteins that have more than one purpose in clinical practice. Apart from serving as a valuable diagnostic tool, neurofilaments can also be used as part of a prognostic procedure.

Measuring the levels of neuorfilament light protein at disease onset can serve as a baseline for future trajectory purposes, allowing us to monitor possible changes of this biomarker over time.

Many studies have also used NFL as part of their longitudinal repetitive measure as they observed their elevation in certain neurodegenerative diseases.

In terms of Parkinson's disease, NFL can serve as a good prognostic indicator in terms of cognitive decline and the occurrence of dementia. As it is widely known, certain group of PD patients will have a more aggressive forms of disease trajectory, showing symptoms of

cognitive difficulties. Gaetanni proposed that based on their NFL levels, a group of PD patients will turn into PDD patients presenting with symptoms of cognitive decline and dementia over the course of 5 to 9 years (Gaetanni, 2018).

Another study reported significant correlations between NFL levels at baseline and cognitive and motor worsenings over time.

In summary, NFL can serve as a good prognostic biomarker in Parkinson's disease and atypical parkinsonian disorders, but it is important to note that this biomarker has the best prognostic capabilities when accompanied by other protein biomarkers such as phosphorylated tau.

Parkinson's disease

Parkinson's disease is one of the leading neurodegenerative diseases commonly described by motor and non-motor symptoms. PD is considered to be the most common form of parkinsonism, which is defined as a group of neurological disorders with movement control problems that resemble Parkinson's disease. Other forms of parkinsonism are drug-induced parkinsonism, vascular parkinsonism, and other neurodegenerative diseases such as progressive supranuclear palsy, multiple system atrophy and corticobasal syndrome (Jankovic, 2008).

In order to make a diagnosis of PD, a patient needs to be assessed based on his history and examination of presented motor symptoms. The presence of motor symptoms that define the diagnosis of PD are bradykinesia, tremor, rigidity, and postural instability. Besides the mentioned motor problems, patients may also experience other accompanying motor difficulties such as freezing of gate, camtocormia which is a state of severe forward flexion of the trunk, Pisa syndrome, scoliosis, and antecollis (Armstrong, 2020).

Furthermore, patients will experience a spectrum of non-motor problems, most notable ones being autonomic dysfunctions, sleep disorders, anosmia, cognitive difficulties, and psychiatric problems.

Parkinson's disease affects around 3% of the population by the age of 65 and up to 5% of the global population over the age of 85, thus making around 6 million individuals suffering from this neurodegenerative disorder worldwide (Armstrong, 2020).

Pathogenesis of Parkinson's disease

The main cause of Parkison's disease is death of dopaminergic neurons located in the substantia nigra. Formation of α – synuclein protein aggregations called Lewy bodies is considered to be the main pathological hallmark of Parkison's disease.

Braak was the first one to introduce the model of neuropathological progression of Parkison's disease. This model suggests that Parkinson's disease development and progression can be divided into six main stages, each stage classified with specialized neuropathological locations and accompanying symptoms (Braak, 2007).

At the start of the development of this neurodegenerative disorder (stages I and II) inclusions of Lewy bodies can be located in the medulla and the olfactory bulb. This specific site of insult is associated with showing of first prodromal symptoms such as rapid eye movement sleep behavior disorder and decreased sense of smell. At this stage, the diagnosis of Parkinson's disease has still not been made, as the main motor symptoms develop in the later stages.
Disease progression follows in stages III and IV when pathology spreads to other brain areas such as substantia nigra pars compacta, midbrain structures, and basal forebrain structures.
At this stage, the first motor symptoms emerge and usually, the diagnosis of Parkinson's disease is made.
In the final stages of PD (V and VI), the pathology has spread to the cerebral cortices and the main symptoms such as cognitive impairment, cognitive decline, dementia, and hallucination emerge (Braak, 2007).

Trajectory of the disease

Parkinson's disease is considered to be a progressive neurodegenerative disease, with various motor and non-motor symptoms emerging over the course of time.

Distinguishing different stages of PD is based on its gradual development of neuropathology in the brain and autonomic, motor, and non-motor problems patients report having. Making a categorization of a course of disease is important due to developing certain disease-modifying treatments and research focus.

The prodromal stage is the first stage of development of Parkinson's disease when most of the non-motor difficulties are present. The presence of non–motor symptoms in the prodromal stages of PD can be explained by the growing accumulation of Lewy bodies in the midbrain, specifically in the nuclei of the glossopharyngeal, vagal, and olfactory nerves. It is believed that symptoms showcased in this stage can last up to 20 years before the occurrence of motor symptoms.

The non–motor symptoms that are often described in the prodromal stages of this neurodegenerative disorder are loss of sense of smell (hyposmia), REM sleep disorder, depression, anxiety, and autonomic dysfunction (constipation, urinary retention). Patients are usually the first to notice these changes, yet they fail to mention them unless specifically queried. Failure to report the aforementioned problems is often due to embarrassment or patient unawareness these problems could be related to a wider spectrum of a disorder.

Clinical features of Parkinson's disease

The four cardinal features of Parkinsonism are bradykinesia, rigidity, tremor, and postural instability. Together they form an acronym TRAP: Tremor at rest, Rigidity, Akinesia (or bradykinesia) and Postural instability.

Bradykinesia is one of the cardinal motor symptoms of Parkinson's disease. It is described as slowness of movement and may have various features such as decreased dexterity, decay with repetitive movements, imprecision, and lack of synchrony with coordinated movements. Besides the slowness of the movement, bradykinesia also accompanies lower amplitude in movements which is called *hypokinesia*. It is not yet clearly defined when one can expect bradykinesia to occur, as previous research has reported certain difficulties in establishing early

symptoms of bradykinesia in the prodromal stages of PD. The cause of bradykinesia occurs due to dopaminergic denervation of the basal ganglia and the dysfunction of the striatal–cortical circuits (LeWitt, 2020).

Tremor is seen as one part of the triad of parkinsonian symptoms. Tremors tend to be unilateral and more prominent in the distal part of an extremity. There has been a growing interest in researching this symptom in patients and results have led to the discovery of different types of tremors that might be present in PD patients. One of the most prominent is classic tremor, also known as tremor-at-rest. It can involve muscles of the chin, jaw, lips, and legs, but rarely occurs in the neck. Hand tremor, also called „pill-rolling“ can spread from one hand to the other. Another type of tremor is the re-emergent tremor which can be seen when a patient is in action. Besides these subtypes, there are also essential tremors, dystonic tremors, and an exaggerated physiological tremor. Several types of tremors are believed to be due to the disruption of different neural networks (Chen, 2022).

Rigidity presents as increased resistance and stiffness that may occur in proximal limbs (neck, shoulders, hips) and distal limbs (wrists, ankles). Axial rigidity of the neck and trunk may lead to postural deformities such as anterocollis and scoliosis (Jankovic, 2007). Patients who experience rigidity often complain of feelings of pain, particularly located in the shoulders.

Gait disturbances stand out as one of the primary indications of Parkinson’s disease and they can usually be seen in later stages of the disease. As the disease progresses, the neuropathology of the brain also undergoes further changes, thus affecting extrapyramidal pathways in the basal ganglia. The patient starts walking slower and the amplitude of his arm swings and axial movements reduces.
The patient’s posture is also due to change with a prominent flexion of the elbow, trunk, and knees. Patients may experience locomotion disturbances in the form of hesitation when starting movements, freezing on turns, and festinating gait.

Cognitive status in Parkinson’s disease

As previously mentioned, one of the most deterimental non - motor symptoms of Parkinson's disease are cognitive impairment and dementias.

Cognitive difficulties in Parkinson's disease range from mild cognitive impairment to disabling cognitive problems that eventually turn into dementia. Mild cognitive impairment in Parkinson's disease is clinically presented as manifestation of executive dysfunctions, memory deficits and problems with visuospatial abilities (Pagonbarraga, 2022). Impaired cognition is usually in areas of working memory, attention and information processing speed (Watson, 2010).

Attention and frontal executive function

Certain authors believe that PD patients with MCI have difficulties in complex attention tasks. By definition, complex attention tasks assess divided attention and therefore the best cognitive test for assessing is trail-making test, part B (Willson, 2010).

Other than attention, PD patients might show poorer performance on tests assessing planning, response inhibition and mental flexibility. Poor performance in mentioned skills represents a challenge for PD patients as it signals a possible decline in their strategic everyday skills and adaptive behavior.

Memory

Interestingly, memory problems haven't been associated with patients suffering with Parkinson's disease as much as it has with Alzheimer's disease populations. Nevertheless, evidence suggest that mild cognitive impariment in PD patients shows signs of memory decline as well.
Willson has shown impairments in immediate and delayed story recall in patients with PD. This evaluation is focused on problems with explicit and implict memory in PD patients. Implicit memory consists of procedural memory, language learning, motor memory, priming and mirror learning (Willson, 2010). On the other hand, explicit memory includes episodic memory and semantic memory.

Visuospatial skills

Visuospatial skills are compromise of a number of cognitive abilities connected to the processing of visual information. As such it includes facial recogntion, constructional abilities and spatial analysis.

The brain area responsible for visuospatila abilities is believed to be posterior cortical areas such as occipital, parietal and temporal lobes.
Some studies done on neuroimaging have found evidence of posterior cortical dysfunction in PD patients with MCI.

Scientific findings thus far suggest of a complex and variable cognitive profile of impairment in patients with Parkinson's disease. While some patients might show isolated impariments in memory, others will present with a widespread frontal - executive dysfunction.

Neuropathological studies have focused on finding Lewy body related pathology in certain brain areas which might lead to a definitive cognitive profile of PD patients, yet a consensus has not been reached (Willson, 2010).

As it is still unknown what pathological processes are underlying for cognitive impairment in PD patients, the possibility of uncovering this lies in using blood-based biomarkers such as NFL.
This could possibly provide us with a better understanding and subsequently better characterization of cognitive impairment in PD, with the ultimate goal of treating this pathological processes before they reach the dementia stages.

The pathophysiology of cognitive impairment in PD

Disease progression will start widespread through other brain areas, thus affecting fronto-striatal and posterior-cortical networks (Pagonbarraga, 2012) . Micro- and macrodegeneration of mentioned networks would lead to presented clinical features and manifestations (Hwang, 2013). For example, disruption of frontal and striatal brain areas often causes executive dysfunction syndrome, while posterior and cortical deteoriration leads to visuospatial and

language disabilities (Williams, 2019).

White matter lesions

Hansson and colleagues have discovered associations between white matter lesions (WML) and levels of neurofilament light protein. In their research, the group found positive correlation between Fazekas score and blood levels of NfLs only in patients with Parkinson's disease. The same finding was not present in healthy control nor in atypical parkinsonian patients. This finding suggest that greater white matter lesions might be showed not only by MRI scans, but also by increased levels of neurofilaments in the blood (Hansson, 2017).

Brain atrophy

Another study conducted a research where they looked at brain atrophy and neurofilaments. The results showed that levels of CSF NfLs had a negative correlation with baseline hippocampal volume. On the controrary, a positive correlation was noted as the levels of neurofilaments started increasing and more hippocampal atrophy was noted. In conclusion, the levels of NfLs in CSF served as a good predictor of hippocampal atrophy (Hall, 2012).

Neurofilaments and cognitive decline in Parkinson's disease

In clinical practice, a regular examination of cognitive status in PD patients is based on cognitive assessments and testing, and in some cases neuroimaging techniques.
Still, a rate of misdiagnosis of mild cognitive impairments in PD is still high compared with some other neurodegenerative disorders.

Therefore, the need for a reliable blood-based biomarker that will further confirm the diagnosis of MCI is urgently needed.

In order to use neurofilament light chain as a biomarker in clinical practice for patients with Parkinson's disease, certain requirements should be first met (Buhman, 2023):

Firstly, a blood-based biomarker such as NFL needs to reflect the changes in the central nervous system. Following this, a second demand for a blood based biomarker is the accurate quantification of any neuronal damages undergoing in the brain. Third and final remark for a biomarker to be used in a PD diagnosis, is to show an association between clinical features and other available diagnostic methods.
A numerous body of research has confirmed neurofilaments status as a blood based biomarker fulfilling all of the above requested requirements (Hansson et al. 2017; Bacioglu et al. 2016, Dickamper et al. 2021).

Utilizing neurofilaments as a blood-based biomarker in patients with Parkinson's disease has been the center of recent studies of biological indicators and neurodegenerative diseases. While some research has found a positive association between blood NFLs levels in Parkinson's disease (Chen, 2020; Hansson, 2017), unfortunately others have not (Lin, 2018).

Another study has found positive indications between NFL levels in blood and disease duration and PD stages in patients with advanced Parkinson's disease (Lin, 2019, Niemann 2021). Yet, some other researchers have showed no significant correlations between disease stage and blood levels of NfLs (Wang, 2019).

Unfortunately, mentioned research are not singular cases of opposing results in the same area of research interest. In the past decades, the status of neurofilaments has been long questioned and studied and it seems like no significant and universal results have been found.

The Amsterdam cohort study was conducted in 2020 and revealed that serum NFL levels were associated with cognitive functioning. In contrast to this study (Oostervald, 2020) another research conducted in Taiwan in year prior has found no significant correlations (Lin, 2019). Similarly, researchers from USA have found no correlations between either plasma or serum NFLs levels and cognitive impairments in PD patients (Aamodt, 2021).
By contrast, a group of researchers from Germany has found significant correlations between MOCA scores and blood levels of NfLs (Niemann, 2021).

Based on the presented studies and their results, we can conclude that a relationship between

cognitive status (cognitive impairment) in patients with Parkinson's disease and levels for neurofilaments does exist - yet it is weak and possibly dependent on other factors.
Important point to make here is the difference of methodologies used in all above mentioned studies. The Amsterdam group used Mini Mental State Examination (MMSE) scales, while the US group used Dementia Rating Scale (DRS-2). Additionally, the study cohort in Germany used Montreal Cognitve Assessment scales (MOCA).
Despite the fact that all of the mentioned cognitive assessment tests are widely used and standardized, it is important to make a distinction of the methodology used in these studies as they might contribute to the confounding factors.

Pagonbarraga conducted a study on blood-based NFLs and cognitively impaired patients with Parkinson's disease with the aim to differentiate the PD patients from healthy controls. In his study, his primary focus was blood-based NfL as a biomarker, yet the group used p-tau 181 as a mean of double confirmation.

The results obtained showed it was possible to make a distinction only based on the presented biomarkers, between the healthy control group and the PD patients with mild cognitive impairment. In addition, the group has found a significant prognostic value of neurofilament light proteins as good predictor of MCI progressing to dementia in 4 year follow up (Pagonbarraga, 2022).

Neurofilaments as differential diagnosis biomarker

Parkinson’s disease is characterized by proteinaceous aggregates and as such it requires a protein biomarker that could be used in various forms of diagnosis-making, prognostic assessment or treatment response monitoring. Many proteins such as alpha-synuclein, tau, and neurofilaments have been proposed as potential biomarkers and in recent years the scope of research seems to be focused on neurofilament protein as a possible biomarker with the highest sensitivity and selectivity. Still, the work of making the neurofilament protein as a potential biomarker for Parkinsonian disorder is in the research stadium.

Atypical parkinsonian disorders is a group of heterogeneous neurodegenerative disorders that are considered to be different than PD but still share some of the central characteristics. In the clinical picture of atypical parkinsonian disorder the presence of certain features that are

different from PD can be noted - early autonomic failures, cerebellar and pyramidal signs (present in multiple system atrophy), supranuclear gaze palsies and executive syndrome (present in progressive supranuclear palsy), myoclonus, cortical sensory loss (present in corticobasal degeneration) and overall a more rapid deterioration.

Diagnosis lies upon noting the clinical features, using neuroimaging techniques and other neurocognitive assessments. Unfortunately, this is not enough as misdiagnosis is still quite present, especially in the early stages when the clinical symptoms often overlap.
The need for a reliable biological tissue marker is urgently expressed in the area of distinguishing between typical PD profile and atypical parkinsonian disorders. The role of neurofilament has been introduce as one of the possible biomarkers that could help in differential diagnosis.

Hall and colleagues have found that higher levels of NfLs will correlate more with a more severe disease stage in patients with PD, PSP and AD. This might reflect an increase in neuroaxonal pathology over time. In this sense, neurofilament light chain protein levels can be indicative as a biomarker of disease severity rather than diagnostic abilities.

Currently, there are no established diagnostic methods that can help us distinguish among Parkinoson's disease and atypical parkisonian disorders.

Hansson and his research group has long been focused on resolving this issue by using NfLs as biological indicators and distinguisher among the two similar pathological states.
His most recent study showed that higher levels of blood NfL were associated with lower levels of CSF alpha – beta 42 and higher levels of CSF tau.
Alongisde of this findings, Hansson and colleagues found an association between clinical symptoms of Parkinson's disease and atypical parkisonian disorders. Blood levels of NfLs positively correlated with disease duration, Hoehn and Yahr stage (H&Y), Unified Parkinson's Disease Rating Scale (UPDRS). Among all of them, there was a positive correlation between blood levels of NfLs and more aggravated motor and cognitive symptoms in Parkinson's disease patients (Hansson, 2017).
On the other side, results obtained from patients with atypical parkinsonian disoders showed a different picture – blood levels of NfLs correlated only with Hoehn and Yahr stage (H&Y) and UPDRS-III motor score. This group didn't reach significant correlations between other clinical

assessments, disease duration and the blood levels of NfLs (Hansson, 2017).

One research group has found a positive association between NFL levels and disease severity in patients with atypical parkinsonian symptoms. The research was conducted on patients with PSP and CBD and the results showed a significant and progressive increase of NFL levels just in the course of one year. It could be that in case of atypical parkinsonian features, neuronal degeneration is more widespread and has a rapid incline, which causes a significant rise in NFL levels (Magdalinou, 2015).

A study assessing 5 CSF biomarkers done in 2012 has found significant results regarding the differential diagnosis of parkinsonism and atypical parkinsonian syndromes. The Hall research group found out that NfL levels in CSF were substantially increased in patient groups suffering from atypical parkinsonian syndromes such as CBD, PSP, and MSA (Hall, 2012).

This neurofilament light protein biomarker alone could differentiate PD from atypical Parkinsonian disorders, with an area under the curve of 0.93 (Hall, 2012).

Conclusion

In summary, Parkinson's disease is a heterogenic neurodegenerative disease with an area of variable symptoms that are presented throughout the whole disease trajectory. The existence of mild cognitive impairment that has a possibility of developing into dementia in Parkinson's disease is yet another factor that challenges clinical practitioners, movement disorder experts, and scientists.

Even though we as a scientific community have come thus far with clinical and scientific findings about neurodegenerative disease, it seems like reliable and universally accepted definitions are still lacking.

Cognitive decline in Parkinson's disease is a quantifiable measurement of brain degeneration with an area of indicators in almost all cognitive subsets. Oftentimes we will have PD patients and their caregivers who complain of memory problems, attentional dysfunctions, language problems and visuospatial disabilities.

Nevertheless, a clear, universally accepted and scientifically proven profile of the impairment is missing.

The reason behind this might be many. It could be a problem of neuropsychological tests used to measure the impairment, or due to the unknown pattern of brain pathophysiology causing the cognitive problems. It could also be a timing problem – as we know thus far, Parkinson's disease has a slow progression. The existence of atypical parkinsonian disease is yet another confounding factor that delays the definitive diagnoses of MCI and therefore, delays the applicable treatments and therapies as well.

For this reason, the application of biological biomarkers such as neurofilament light chain is considered a burning question in neuroscience today.
Neurofilaments have proven their utility in diagnosis making, prognosis monitoring and treatment response modifying in several neuroimmunological conditions (multiple sclerosis), neurodegenerative (Alzheimer's disease, multiple system atrophy, Parkinson's disease) and neurological injuries (TBI, epileptic seizures).
As best indicators of neuroaxonal injuries, neurofilaments can help us reveal the neurological pathophysiology happening in Parkinson's disease.
Moreover, by having a reliable and quantifiable measure of micrological and macrological structural changes in the brain following the neurodegeneration, a possibility of slowing down or completely halting the disease progression exists. Furthermore, by identifying the factors influencing the neurodegeneration, lies a possibility of reversing the disease trajectory by inducing certain treatment and therapeutical actions.

The starting steps of implementing neurofilaments as valuable biomarkers in Parkinson's disease lies in reaching the consensus of its utility and practice in scientific community. We are in need of baseline quantification of NfLs not only in clinical populations facing the neurodegeneration, but also in healthy populations as means of comparable measurements.

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