

# The role of neurofilament light chain in COVID-19: A potential prognostic biomarker

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Coronavirus disease 2019 (COVID-19) is an unprecedented global health concern that was declared a pandemic in March 2020. Although primarily recognized by respiratory symptoms, growing evidence suggested the causal relationship between the infection with the disease agent, namely severe acute respiratory coronavirus 2 (SARS-CoV-2), and neurological manifestations. Given that the virus-induced neurological involvement is associated with a poorer prognosis, persistent neurological sequelae, and a more severe form of the disease, efforts have been made to introduce a biomarker to recognize neurological abnormalities early in the course of the disease. Studies indicate a significantly higher concentration of neurofilament light chain (NFL) in cerebrospinal fluid or blood of COVID-19 patients *versus* adjusted controls. It has also been reported that COVID-19 patients suffering from the severe form of the disease had higher NFL levels than patients with mild to moderate forms. Moreover, elevated NFL levels at hospital admission in patients who did not present primarily with neurological expressions could predict the emergence of neurological symptoms during the hospital stay. The early recognition of neurological abnormalities using the NFL biomarker could lead to escalated medical care limiting the progression of SARS-CoV-2-induced central nervous system pathogenesis, resulting in a significant amelioration in disease outcome. Nevertheless, NFL assessment integrated with the evaluation of other neurodegenerative biomarkers and factors indicating disease prognosis could provide a more comprehensive estimate of disease prognosis and the extent of neurological involvement.

**Key words:** COVID-19, SARS-CoV-2, neurological involvement, neurofilament light chain, neurological manifestations

## INTRODUCTION

Severe acute respiratory coronavirus 2 (SARS-CoV-2), the infecting agent of coronavirus disease 2019 (COVID-19), was first detected in December 2019 in the city of Wuhan in China (Zhu et al., 2020). Within a short period after its emergence, the virus spread dramatically to almost all countries, areas, and territories until the World Health Organization (WHO) declared its pandemic on 11 March 2020. As of WHO reports of 3 June 2022, this exponentially growing virus infected more than 528 million individuals and took at least 6 million lives

worldwide, making it an unprecedented global health concern.

The most observed symptoms of COVID-19 are reported as fever, cough, fatigue, dyspnea, and myalgia (Alimohamadi et al., 2020). Although the disease primarily involves the respiratory system, patients could also present multiple neurological manifestations, increasing the chance of their mortality or long-term neurological sequelae (Bhola et al., 2022). In this regard, clinical features of the central nervous system (CNS) and peripheral nervous system (PNS) involvement have been respectively evident in 36.4% and 8.9% of patients with COVID-19 in a study (Mao et al., 2020). CNS-relat-

ed manifestations mainly include headaches, dizziness, anosmia, hypogeusia, encephalitis, encephalopathy, cerebrovascular events, and seizures (Roy et al., 2021). Additionally, PNS abnormalities may manifest with myalgia, myopathy, or Guillain-Barré syndrome (GBS) (Roy et al., 2021, Bhola et al., 2022). Moreover, psychological manifestations, including anxiety and fatigue, are increasingly reported in the acute and post-infectious phases of the disease (Ayaz et al., 2020; Carfi et al., 2020; Edén et al., 2021b; Cénat et al., 2021). Currently, the precise mechanisms causing the neurological involvement during acute or post-infectious phases of COVID-19 are not sufficiently elucidated. However, the CNS-affecting consequences of the body's anti-virus inflammatory responses, such as cytokine release syndrome (CRS), as well as the direct interaction of the virus with neurons or vascular cells residing in the CNS, and the toxic and metabolic effects of the virus on the respiratory system resulting in hypoxic brain injuries are the leading candidates (Roy et al., 2021).

Neurofilaments are a class of intermediate filaments constituting the major cytoskeletal backbone of neurons in both CNS and PNS (Hsieh et al., 1994; Gafson et al., 2020). They are primarily located in axons, with a low concentration in cell bodies and dendrites. They are heteropolymers consisting of four subunits with various molecular weights, including light, medium, and heavy chains, along with an  $\alpha$ -internexin (INA) subunit in the CNS or peripherin subunit in the PNS (Verde et al., 2021). Neurofilaments leak into the cerebrospinal fluid (CSF), followed by the blood following CNS degeneration or physiological turnover of neurons (Verde et al., 2021, Thebault et al., 2020). The neurofilament light chain (NFL) is the most abundant subunit released following neuronal and axonal damages (Varhaug et al., 2019). In this light, the increased CSF or blood levels of NFL can be translated into the existence of a disorder, exerting neurovirulence effects. For instance, neurodegenerative diseases like amyotrophic lateral sclerosis (ALS) have been identified as a cause of high CSF or blood NFL levels (Forgrave et al., 2019). Nevertheless, any disorder with the potential to damage the neurological integrity can cause NFL leakage from neurons, followed by NFL-level rise. For instance, several viral diseases like varicella-zoster virus (VZV) infection (Tyrberg et al., 2020) or autoimmune diseases like systemic lupus erythematosus (Tjensvoll et al., 2021) could reportedly lead to neurological sequelae, resulting in increased peripheral NFL concentrations.

COVID-19, a viral infection with the potential to involve the nervous system, could also lead to neuronal degeneration followed by the release of neurodegenerative biomarkers like NFL. Although COVID-19 has

newly emerged, attempts to find a potential biomarker indicating neurological involvement in COVID-19 have led to a significant body of literature. Accordingly, research quantifying the peripheral levels of NFL biomarker as a clue for neuronal damage in COVID-19 patients is rapidly evolving. In this regard, this study aimed to evaluate the potential utility of the NFL as a biomarker indicating neurological damage and disease prognosis and severity in patients with COVID-19.

### Neurological involvement in patients with COVID-19

Although the CNS is a highly protected body system with various layers of defensive barriers, several viruses, such as the SARS-CoV-2, have shown their ability to penetrate the defensive layers of this system and exert their neurovirulence effects (Desforges et al., 2014; DosSantos et al., 2020). In this regard, Moriguchi et al. (2020) were the first, who reported the presence of the ribonucleic acid (RNA) of SARS-CoV-2 in a CSF of a patient with COVID-19 and encephalopathy, which is then followed by the recognition of several symptoms and complications related to nervous system involvement caused by the SARS-CoV-2 infection (Harapan and Yoo, 2021).

SARS-CoV-2 can access the CNS using either hematological/lymphatic or neurological transmission routes (Harapan and Yoo, 2021). After entering the body by infecting the air tracts, SARS-CoV-2 can join the bloodstream by passing through the epithelial barrier of the alveoli. Through the bloodstream, the virus reaches the blood-brain barrier (BBB) and may subsequently invade endothelial cells of the BBB in the choroid plexus, facilitating its abluminal recruitment into the parenchyma or interstitial fluid of the CNS (Desforges et al., 2019a). Moreover, SARS-CoV-2 can enter the CNS by infecting the peripheral white blood cells (WBCs) and exploiting these cells as its transporters to the CNS. This strategy is known as the Trojan horse mechanism (Desforges et al., 2019b). On the other hand, the neuronal access routes used by the virus are classified into two main categories, namely the transcribrial route and axonal or trans-synaptic transmission (Saper et al., 1987). Angiotensin-converting enzyme 2 (ACE2) and the transmembrane protease serine 2 (TMPRSS2) receptors are essential receptors interacting with the spike protein of SARS-CoV-2, which are expressed on a wide variety of cells, including neural and epithelial cells (Nejadghaderi et al., 2020; Sarker et al., 2021). As the olfactory bulb is adjacent to the olfactory epithelium, which is a convenient target site to be attacked by SARS-CoV-2 because of its abundance in ACE2 and

TMPRSS2 receptors, the olfactory nerve is considered a significant peripheral nerve candidate for transmitting the virus to the CNS (Reza-Zaldívar et al., 2021). Following infecting the olfactory nerve, the virus transmits retrogradely through the cribriform plate of the ethmoidal bone to the subarachnoid space and contacts second-order neurons, namely spherical glomeruli (Reza-Zaldívar et al., 2021). This pathway from the olfactory nerve through the cribriform plate to the CNS is known as the transcribrial route, allowing the virus to spread to different brain zones (Chen et al., 2021) (Fig. 1).

Similarly, the axonal and trans-synaptic transmission refers to the viral transportation to the CNS by infecting different peripheral nerve terminals, including the vagus and the trigeminal nerves located in the gastrointestinal or respiratory tracts (Chen et al., 2021). These peripheral nerve involvements could be followed by the transneuronal transmission of the virus to different brain zones using anatomical interconnections between neurons (Saper et al., 1987) (Fig. 1).

Regardless of the type of access route the SARS-CoV-2 uses to reach the brain, in the CNS, the spike protein of the virus interacts with the host cellular ACE2 receptors, expressed on neurons and glial cells of various brain regions, including the cerebral cortex, hypothalamus, the striatum, brain stem, and substantia nigra (Saper et al., 1987; Benedetti et al., 2021). This attachment causes the processing and priming of the S protein by the TMPRSS2 host cell receptors, leading to the fusion of the viral and host cellular membranes (Hoffmann et al., 2020). The fusion facilitates the virus's entry to the target neurons, thereby inducing the viral neurotoxic effects. Hence, the presence of double-positive ACE2<sup>+</sup> and TMPRSS2<sup>+</sup> neural cells predisposes the CNS to be invaded by SARS-CoV-2 (Hoffmann et al., 2020). Moreover, neuropilin-1 (NRP1) receptors expressed on neuronal cells play supporting roles in viral entry to these cells (Mayi et al., 2021; Zalpoot et al., 2022).

Evidently, extraction of the SARS-CoV-2 from the CSF (Moriguchi et al., 2020), co-localization of the virus in the olfactory system (Reza-Zaldívar et al., 2021), evidence of viral budding in the neurons of the frontal lobe (Baig, 2022), and different neurological symptoms and complications of the disease (Harapan and Yoo, 2021) are substantial pieces of evidence to prove, at least in part, the viral infection of some brain regions during the course of COVID-19.

On the other hand, the virus can also cause neurological consequences without having to physically achieving the CNS. Several factors are associated with the indirect effects of COVID-19 on the CNS, resulting in neurotoxicity. These factors include immune-mediated

pathogenesis resulting in the systemic inflammatory response to the virus (Moradian et al., 2020; Kunal et al., 2022), disorganization in lung-brain dialogue leading to hypoxic-ischemic encephalopathy, coagulation dysfunctions manifested with high D-dimer, extended prothrombin time, and low platelet levels predisposing the patient to the cerebrovascular accident (CVA) (Henry et al., 2020; Asakura and Ogawa, 2021), impaired gut-brain-axis resulted from malfunctioned gut microbiome due to COVID-19-induced gastrointestinal infection resulting in viral transmission through the vagus nerve (Wong et al., 2020), modified metabolism of glucose and lipids during the course of the disease (Byambasuren et al., 2020; Hussain et al., 2020), and the presence of cardiovascular comorbidities like hypertension (Li et al., 2020a).

As a prominent example, a systemic inflammatory reaction of the body to SARS-CoV-2 could be manifested as CRS (Basiri et al., 2021), having the ability to increase the permeability of the BBB, thereby inducing a state of neuroinflammation within the CNS (Moradian et al., 2020). This increased permeability facilitates the viral entry to the CNS on the one hand and increases the recruitment of the inflammatory cells to the brain on the other hand. This neuroinflammation can consequently result in neurodegeneration. Therefore, CRS could play an essential role in exerting the harmful effects of the SARS-CoV-2 on the nervous system (Moradian et al., 2020; Li et al., 2020b).

Collectively, it seems that both the mentioned viral indirect mechanisms for affecting the neurological system and the direct viral invasion of the CNS play their part in explaining the neurological manifestations of COVID-19.

Evidently, these neurological manifestations were observed in 42% of the patients as early presentations of the disease, in 63% during the hospital stay, and in 82% at any time throughout the disease course in several hospital studies in Chicago (Liotta et al., 2020). Similarly, 36% of hospitalized COVID-19 patients in China and 60% of COVID-19 patients in Europe showed neurological manifestations of the disease (Harapan and Yoo, 2021). Gustatory and olfactory dysfunctions are leading neurological symptoms of the disease, with 38.5% and 35.8% pooled prevalence rates in a systematic meta-analysis study (Favas et al., 2020). Other frequent neurological symptoms of COVID-19 are myalgia, headache, impaired cognition, dizziness, nausea, vomiting, neuralgia, ataxia, myoclonus, and diplopia (Harapan and Yoo, 2021). Among the neurological complications of the disease, CVA is estimated to occur in COVID-19 patients with a pooled prevalence rate of 2.0%, according to a systematic review and meta-analysis (Misra et al., 2021).

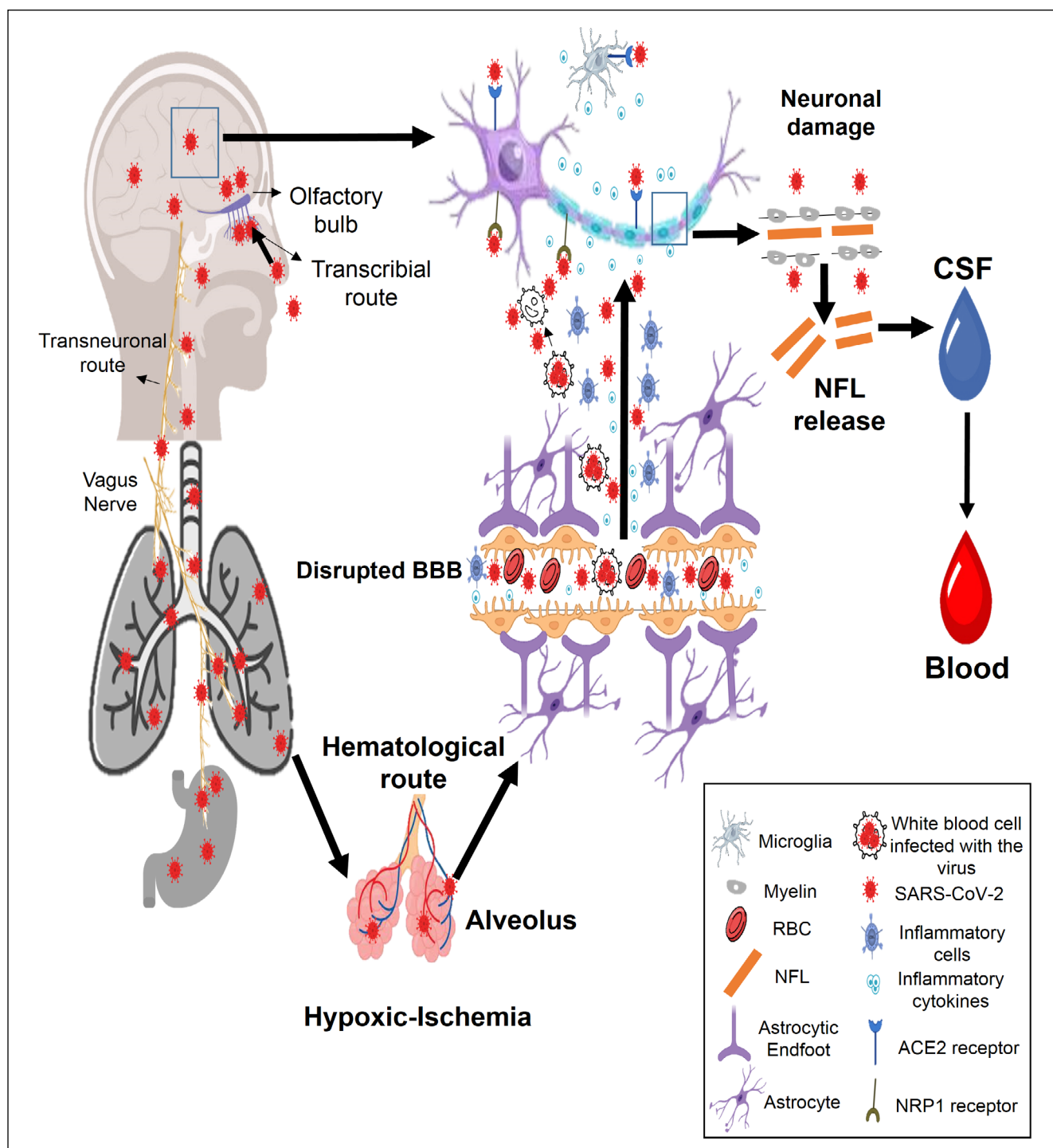


Fig. 1. SARS-CoV-2 uses either neurological or hematological routes to gain access to the CNS. Neurological access routes classify into transcribrial and trans-synaptic pathways. By transcribrial route, the virus achieves the olfactory bulb by infecting the olfactory epithelium through coupling its spike protein with host ACE2 receptors. After crossing the cribriform plate, the virus could spread to different brain regions. Trans-synaptic pathway refers to the retrograde transmission of the virus from peripheral nerves in gastrointestinal or respiratory tracts like the vagus nerve to the CNS. On the other side, the virus can gain access to the bloodstream by infecting the alveolus. Afterward, SARS-CoV-2 reaches the BBB and can join the CNS through damaging endothelial cells of BBB or using WBC as its carrier. In the CNS, the virus degenerates neurons by attaching to their ACE2 or NRP1 receptors, which is followed by NFL release into the interstitium, CSF, and then the blood. Moreover, cytokine storm syndrome induced by the immune system's response to the virus can also disrupt the BBB and has degenerative effects on the neurons. Whether the direct or indirect effects of the virus on the CNS play more critical roles in neurodegeneration remains to be elucidated in future research. ACE2: angiotensin-converting enzyme 2, BBB: blood-brain barrier, RBC: red blood cell, NFL: neurofilament light chain, SARS-CoV-2: severe acute respiratory coronavirus 2, NRP1: neuropilin 1.

Other neurological complications include epilepsy and seizure, cerebral venous thrombosis, meningitis, encephalitis, GBS, miller fisher syndrome (MFS), encephalopathy, CNS vasculitis, and movement disorders (Harapan and Yoo, 2021). Despite significant explorations in the field, more studies are still needed to determine which of these symptoms or complications are the results of the direct invasion of the virus to the CNS and which of them are due to the body's inflammatory response, to shed light on the precise pathogenesis of neurological consequences, resulting in designing novel target-specific therapeutic approaches for SARS-CoV-2.

### NFL as a useful biomarker for neurological involvement

Neurofilament proteins (NFPs) are cylindrical structures exclusively found in neurons' cytoplasm, responsible for providing structural stability, radial growth, and effective message conduction of neurons (Disanto et al., 2017; Zanardini et al., 2022). NFPs are predominantly present in axons with much lower concentrations in neuronal soma and dendrites (Gaetani et al., 2019). Since the diameter of neurofilaments (~10 nm) is between actin filaments (microfilaments) (~7 nm) and microtubules (~25 nm), they are classified as intermediate filaments (IF) (Gaetani et al., 2019). It should be noted that the width of IFs is usually below myosin filaments, which are formed by conventional myosin isoforms, although the myosin filaments are very diverse in their width dependent on various factors, including the type of myosin isoforms in their structure and their ionic conditions. NFPs are hetero-polymers consisting of up to five different subunits, including NFL (68 kDa), neurofilament medium chain (NFM) (150 kDa), neurofilament heavy chain (NFH) (200 kDa),  $\alpha$ -internexin (INA) (68 kDa), and peripherin (57 kDa), each of which is expressed by a different gene (Yuan et al., 2012, Lobsiger and Cleveland, 2009). All NFPs' subunits found in CNS, namely, NFH, NFL, NFM, and INA, pose a conserved domain of  $\alpha$ -helical rod and variable amino-terminal and carboxy-terminal zones, resulting in their varied molecular structures and weights (Gaetani et al., 2019). Under physiological conditions, there is a constant release of low amounts of NFPs from neurons into the interstitial fluid, which is freely in contact with the CSF and blood (Gaetani et al., 2019). The quantity of NFPs released into biological fluids has been reported to correlate positively with aging (Gaetani et al., 2019). Following axonal damages arising from inflammatory, neurodegenerative, vascular, traumatic, or infection insults,

NFPs are dramatically released into the CSF, followed by blood.

Neurofilaments are categorized as class-IV IF specific to neurons. Like other IF proteins, neurofilaments have a tripartite structure, consisting of a conserved  $\alpha$ -helical rod at the center which is surrounded by two variable domains at its C- and N-terminal regions, forming the head and tail sections (Herrmann and Aebi, 2016). In this regard,  $\alpha$ -helix serves as the filament backbone, and C- and globular N-terminal domains play their roles in regulating polymerization and interactions (Yuan and Nixon, 2021). The construction of IFs within nerve cells highly relies on the cell type and the developmental stage. For instance, at the earliest stages of infancy, nestin, a type-VI IF, along with vimentin, a type-III IF, constitute the major IF proportion in the precursor nervous cells. During neuronal development, vimentin is gradually dominated by peripherin, NFM, NFL, and IA, and a substantial increase in neuronal NFH expression occurs postnatally, making neurofilaments the prominent IF in the mature neurons (Yuan and Nixon, 2021). Neurofilament monomers interact by tight hydrophobic reactions through their rod domains to form a dimer. The antiparallel aggregation of dimers leads subsequently to the formation of tetramers, which are believed to be the primary subunit of neurofilaments consisting usually of NFL along with other NFPs (Herrmann et al., 2007; Qin et al., 2009; Yuan and Nixon, 2021). Afterward, eight tetramers contribute to building a unit-length filament (ULF), which encounters radial compaction resulting in the formation of a mature hetero-polymer assembly with a 10-nm diameter (Herrmann et al., 2007; Qin et al., 2009; Yuan and Nixon, 2021) (Fig. 2). It has been shown that in the CNS, the ratio of NFL : INA : NFM : NFH in an assembled neurofilament is 4 : 2 : 2 : 1, respectively, whereas in the PNS, the ratio of NFL : NFM : peripherin : NFH is 4 : 2 : 1 : 1, respectively. NFPs are synthesized and assembled in the neuronal cell bodies and then transferred in the forms of hetero-oligomers and short filaments to the axons and dendrites. NFPs found within neurons are mostly in the form of stable hetero-polymers rather than soluble NFPs (Yuan and Nixon, 2021).

NFPs are mainly responsible for regulating the neuronal cellular shape and neurite outgrowth, determining the caliber of axons, and maintaining signal transmission (Liem and Messing, 2009). NFPs contribute to forming the neuronal cytoskeleton in conjunction with microtubules (~25nm) and microfilaments (~7nm) (Yuan and Nixon, 2021). Several neuronal functions of neurofilaments are conducted by their specific subunits. For instance, NFL has significant roles in the transport of synaptic vesicles by the interaction

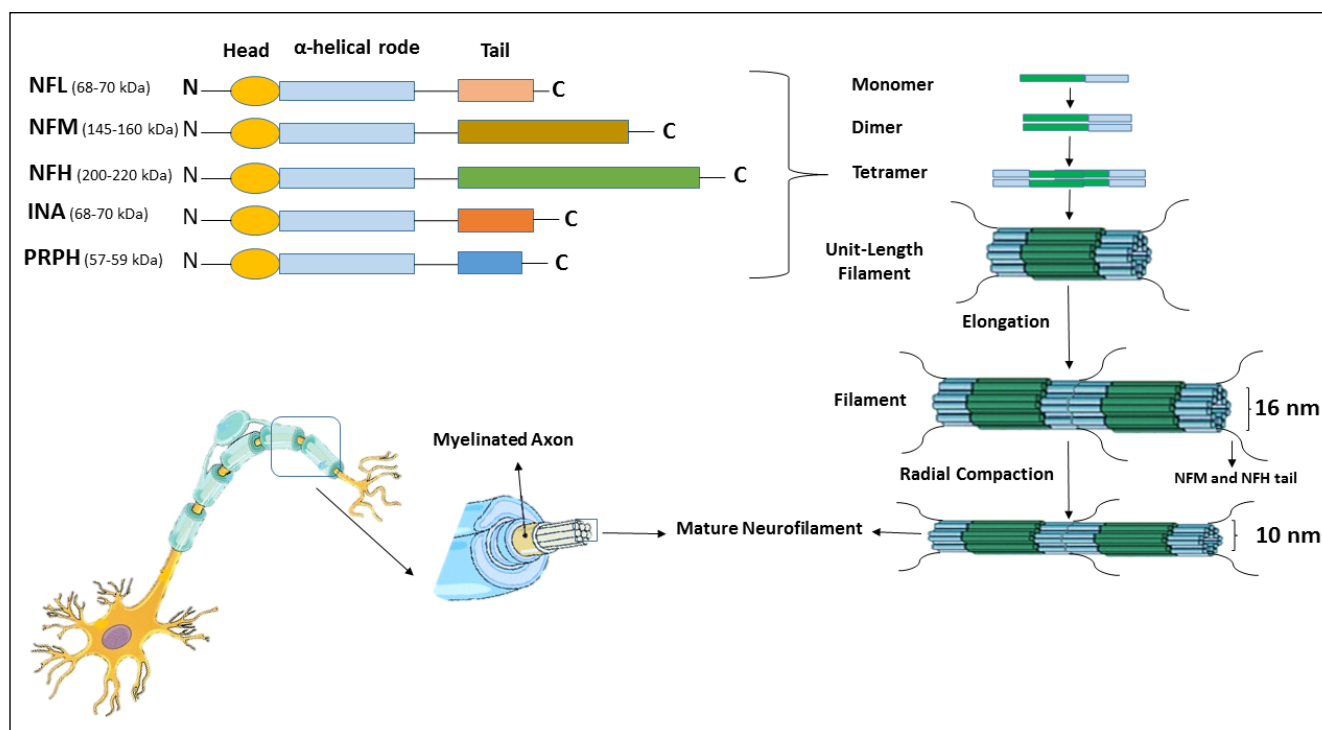


Fig. 2. Different subunits of neurofilaments and the specific arrangement of neurofilaments within neurons. Neurofilaments are composed of various subunits with different molecular masses, including NFL, NFM, NFH, INA, and peripherin. Each subunit is composed of a constant  $\alpha$ -helical rod surrounded by variable C- and globular N-terminal zones. Neurofilament monomers interact by tight hydrophobic reactions through their rod domains to form a dimer. The antiparallel aggregation of dimers leads to the formation of tetramers. Subsequently, eight tetramers contribute to building a ULF, experiencing radial compaction resulting in the formation of a mature hetero-polymer assembly with a 10-nm diameter. NFL: neurofilament light chain, NFM: neurofilament medium chain, NFH: neurofilament heavy chain, PRPH: peripherin, ULF: unit-length filament.

with myosin Va (Rao et al., 2011), and NFM can modify the surface expression of  $D_1$  dopamine receptors by binding to them (Kim et al., 2002). NFH can regulate microtubule polymerization by attaching to the tubulin's C-terminal domains (Miyasaka et al., 1993). Moreover, soluble short oligomers of neurofilaments can bind to the N-methyl-D-aspartate and other surface neuronal receptors to adjust synaptic functions (Yuan and Nixon, 2016). Clinical studies demonstrated that the presence of sufficient amounts of NFPs, as well as their proper structure and assembling play significant roles in human health. In this regard, impaired NFP accumulation, distribution or integrity contributes to underlying pathological processes of many nervous disorders (Hamberger et al., 2003). For example, reduced expression of NFLs resulting from NFL gene loss of function mutations in neuropathies can impair axonal calibers and lead to sensorimotor and cognitive dysfunctions (Sainio et al., 2018; Yuan and Nixon, 2021). Moreover, NFPs constitute the inner component of neurofibrillary tangles in Alzheimer's disease, which is believed to have primary roles in the disease pathogenesis (Rudrabhatla et al., 2010).

Abnormal NFPs aggregation has also been related to ALS pathogenesis (Cleveland and Rothstein, 2001). In this regard, the lower molecular mass of NFL (68 kDa), compared to NFM (150 kDa) and NFH (200 kDa), could facilitate the easier leakage of this subunit to body fluids (Yuan and Nixon, 2021).

Therefore, the CSF or blood concentrations of NFL could serve as a potential biomarker with the ability to give clues on the type of disorder and estimate the prognosis and severity of the neuro-axonal injury (Saak et al., 2021).

The NFL protein is considered the most abundant and soluble neurofilaments' subunit, making it the most reliable and easy-to-quantify subunit to be measured in biological fluids (Saak et al., 2021). NFL levels are normally measured in the CSF, since the NFL concentrations of CSF are about 40 times higher than its concentrations in the blood (Disanto et al., 2017). Nevertheless, the recent successes in developing a novel ultrasensitive method have enabled the blood quantification of NFL, serving as a convenient tool for estimating neuro-axonal damages without having to conduct a lumbar puncture (Tyrberg et al., 2020).

Apart from neurological disorders with neurodegenerative effects, in which the NFL concentrations typically rise, infections with neurotropic agents could also lead to CNS damage followed by NFL levels elevation. For example, the CSF level of NFL in patients with meningitis resulting from an infection with community-acquired bacterial agents like *Streptococcus pneumoniae*, *Neisseria meningitidis*, and *Listeria monocytogenes* correlates significantly with their adverse outcomes and the presence of focal cerebral deficits leading to their poor prognosis (Chekrouni et al., 2022). Moreover, the NFL has been verified to be a sensitive biomarker, which can be independently used to estimate neuronal injury in various stages of the human immunodeficiency virus (HIV) infection (Yilmaz et al., 2017). Besides, in another study on HIV patients, the CSF level of NFL witnessed a significant reduction following a highly active antiretroviral treatment, proposing the potential implementation of this biomarker in evaluating the efficacy of the antiretroviral therapy in HIV patients (Mellgren et al., 2007).

Moreover, a study on patients who had CNS infection with VZV demonstrated a strong correlation between CSF and serum levels of NFL (Tyrberg et al., 2020), which is in line with previous observations in patients with CNS injury by HIV (Gisslén et al., 2016), multiple sclerosis (Disanto et al., 2017), and traumatic brain injury (Shahim et al., 2016). Additionally, patients with VZV-induced encephalitis reportedly had higher NFL concentration in CSF and serum compared to patients with VZV-induced meningitis or Ramsay Hunt syndrome. This observation is justifiable as, in encephalitis, a higher number of neurons are affected than in meningitis. However, patients with VZV infection without CNS manifestations also had elevated NFL serum levels, which might be due to virus-induced PNS damage complicating the interpretation of NFL serum levels in these patients (Tyrberg et al., 2020).

### The utility of potential biomarkers in COVID-19-induced neurological abnormalities

As many cases of COVID-19 are neurologically affected, which can exacerbate the disease prognosis, scientists attempted to introduce useful neurological biomarkers for early detection of neurodegenerative changes in COVID-19 to treat the disease more efficiently and to prevent its long-term sequelae. In this regard, biomarkers that were extensively validated for cell death and CNS injury in well-known neurodegenerative diseases were proposed as potential biomarkers for evidencing neurological abnormality in COVID-19.

Although various mechanisms contribute to neuronal involvement during the COVID-19 infection, evidence indicates that the body's immune reaction against the virus is of primary importance. In this regard, biomarker studies in COVID-19 patients with neurological symptoms and also studies on autopsies reported the signs of immune activation, like the elevation of inflammatory cytokines' levels in the CSF and blood (Edén et al., 2021b). The increase in CSF inflammatory cytokines like IL-6, IL-8, TNF- $\alpha$ , and IL-18 could serve as potential biomarkers demonstrating the disease activity in the brain, predisposing patients to neuronal injury (Edén et al., 2021b; Guasp et al., 2022). In this context, the pro-inflammatory cytokine profile of CSF in COVID-19 patients could give clues on the likelihood of virus-induced neuronal injury. However, a study has reported that this index cannot predict the long-term outcome of COVID-19 patients (Guasp et al., 2022). It seems that these biomarkers do not have the required specificity for labeling the patients with neuro-axonal injury; however, their presence in CSF can make the patients vulnerable to developing neurological involvement.

On the other hand, the activation of glial cells like astrocytes and microglia in response to the invasion of the virus or the state of inflammation within the CNS leads to the release of glial and cellular activation biomarkers (Heidari et al., 2021), including glial fibrillary acidic protein (GFAP), neopterin, and soluble triggering receptor expressed on myeloid cells 2 (sTREM2), which were reported to be elevated in COVID-19 patients with CNS involvement (Edén et al., 2021a, Pilotto et al., 2021). S100 calcium-binding protein B (S100B), an astrocyte-derived biomarker, showed also to increase and correlate with IL-6 levels in intensive care unit (ICU)-admitted COVID-19 patients, who had no other evidence of brain injuries. Moreover, a case-control study on serum GFAP levels in COVID-19 patients has shown that this biomarker's level correlates with the disease severity but not the neurological symptoms (Sahin et al., 2022). As the protoplasmic astrocytes are the most abundant cells within the brain, astrocyte-derived biomarkers could be potentially more sensitive biomarkers for demonstrating CNS injury in different neuronal diseases compared to neuron-specific biomarkers (Garden and Campbell, 2016). The usefulness of these biomarkers for clueing on the neurological involvement was subject to some inconsistencies in various studies and needs to be more precisely evaluated in future research. However, these biomarkers could have the potential to be used along with conventional biomarkers as predictive factors of the disease prognosis.

Neurodegenerative biomarkers other than NFL, including total tau, phosphorylated tau-181, ubiqui-

tin carboxy-terminal hydrolase L1, amyloid beta 40 (A $\beta$ -40), and A $\beta$ -42 have also the potential to be applied along with NFL and conventional biomarkers as factors estimating neurological involvement and prognosis. Evidently, total tau, phosphorylated tau, and GFAP were found to be significantly higher at hospital admission in COVID-19 patients who had encephalopathy or died in the hospital than in other COVID-19 hospitalized patients (Frontera et al., 2022). Accordingly, total tau, GFAP, and NFL were found to be significantly lower in patients who were discharged home (Frontera et al., 2022). However, the precise efficacy of broadly using neurodegenerative biomarkers in monitoring the neurological abnormalities of COVID-19 patients remains to be elucidated in future studies. These biomarkers' utility in predicting the disease severity and neurological involvement is improved when they are interpreted in the context of other neurological and conventional biomarkers and also the patient's clinical evaluations.

### Evidence of NFL levels' alterations in COVID-19

As NFL is one of the most studied neurodegenerative biomarkers in COVID-19 patients and it has an acceptable sensitivity and specificity for neurodegenerative changes, we aimed to review studies focusing on NFL levels alterations during this disease in the following paragraphs.

### Blood NFL levels in COVID-19 patients compared to controls

Sutter et al. (2021) compared the serum NFL (sNFL) concentrations in 259 healthy controls, 29 critically ill patients with COVID-19, and 10 critically ill patients without COVID-19 within 48 hours following admittance to the ICU. After adjustment for age and pre-existing comorbidities, they found that ICU-admitted patients with COVID-19 had significantly higher levels of sNFL vs. the equivalently-affected ICU patients without COVID-19 (2.6 times higher levels of sNFL,  $P=0.010$ ) and healthy controls (5.7 times higher levels of NFL,  $P<0.001$ ). The higher NFL values in ICU-admitted COVID-19 patients vs. other critically ill patients in this study suggest that COVID-19 infection is an additional factor leading to neuronal injury in critically ill patients. Moreover, this study showed that sNFL levels were significantly correlated with poorer outcomes and more prolonged ICU stay in all critically ill patients. Chung et al. (2021) compared plasma NFL (pNFL) levels between patients suffering from

COVID-19-induced pneumonia and bacterial pneumonia who had the same disease severity. In contrast to Sutter's findings, the authors reported a significantly lower pNFL level in patients with COVID-19-induced pneumonia than those with bacterial pneumonia. It should be noted that NFL levels were quantified nearly three days after the diagnosis of sepsis in participating patients in this study. These inconsistent results might have arisen because the clinical severity of COVID-19 patients in the mentioned two studies was not equal; in other words, the Sutter's studied COVID-19 patients were in more critical phases than the patients from Chung's study (Leppert et al., 2021). Other possible reasons for this discrepancy might be different time points for quantifying NFL levels during the disease course in the two studies (Leppert et al., 2021) and different study cohort compositions, such as the presence of delirium and acute kidney injury in COVID-19 patients studied in Sutter's study, which might have contributed to a more dramatic NFL rise in Sutter's COVID-19 samples (Chung et al., 2021). These studies did not compare the course of the disease between male and female participants.

A case-control study comparing sNFL levels of 142 hospitalized COVID-19 patients and 55 controls demonstrated also a significantly higher sNFL levels in COVID-19 patients than controls (29.4 pg/ml vs. 10.9 pg/ml). This significant difference was repeated after adjusting for sex and age. Moreover, the prevalence of COVID-19 patients with elevated sNFL levels, based on the cut-off point of three standard deviations higher than the mean sNFL levels in controls, was reached about 34% in this study (Prudencio et al., 2021). Additionally, the findings indicated an association between elevated sNFL concentrations and worse clinical outcomes in this study (Prudencio et al., 2021).

Another case-control study compared pNFL levels of 47 COVID-19 patients with severe, moderate, and mild forms of the disease and 33 age-matched controls. The results illustrated significantly higher values of pNFL in patients with severe COVID-19 than controls (32.7 pg/ml vs. 13.1 pg/ml). Moreover, despite the constant plasma concentration of NFL in moderate and mild patients during the course of the disease, patients with severe COVID-19 witnessed a significant rise in pNFL levels in the follow-up measurement (from 20 pg/ml to 32 pg/ml). The authors also found a strong correlation between the age and pNFL levels in both COVID-19 patients and controls ( $r=0.62$ ,  $P<0.001$ ), but there was no significant correlation between pNFL and C-reactive protein (CRP) levels in this study (Kanberg et al., 2020).

A prospective study investigated 100 healthcare workers in Germany, with 28 individuals having positive reverse transcription polymerase chain reaction

test for SARS-CoV-2 and 72 negative-tested individuals serving as controls. All COVID-19 patients in this study had mild to moderate forms of the disease, of whom 21 patients also manifested mild neurological symptoms. The 100 participants were divided into six groups based on their age and COVID-19 status, and the median values of sNFL levels were measured for each group. In COVID-19 patients, the median sNFL values were 4.5 pg/ml for the age group of 18 to 35 years, 9.6 pg/ml for the age group of 36 to 50 years, and 11.6 pg/ml for the age group of 51 to 65 years, which was significantly higher than controls with the same age group except for the age group of 18 to 35 years (Ameres et al., 2020). In this regard, multivariable linear regression showed that both factors of age and COVID-19 status were significantly correlated with the sNFL levels in this study, which was in accordance with previous observations (Ameres et al., 2020). Moreover, sNFL levels of COVID-19 patients correlated significantly with the disease status after controlling for sex and age. Another case-control study comparing nine hospitalized patients with COVID-19-induced encephalopathy, nine post-hospitalized COVID-19 patients with post-acute neurological sequelae, 38 non-hospitalized patients with COVID-19 and neurological symptoms, and eight healthy controls reported that hospitalized patients with COVID-19 and encephalopathy were older and had higher pNFL levels than the other groups (Hanson et al., 2022). Although the extrapolation of the results should be with caution because of the small sample size, this study also provided other clues to the association between NFL levels and higher ages or acute neurological symptoms in COVID-19. Another study investigating 27 COVID-19 patients admitted to ICU and 19 ICU controls did not show a significant correlation between pNFL levels and the presence of delirium and the score of intensive care delirium screening checklist in patients with COVID-19. These findings persisted after adjusting for sex and age. Although there was no significant difference between NFL levels in COVID-19 patients and controls acutely, pNFL levels tended to increase in patients with COVID-19 between days one and seven in this study (Cooper et al., 2020).

Another case-control study assessed 251 hospitalized COVID-19 patients without a history of dementia and 161 controls who were either cognitively normal or suffered from mild cognitive impairment or Alzheimer's disease. The findings indicated that sNFL levels were significantly higher in COVID-19 patients than pNFL levels in controls. Moreover, sNFL levels at admission were significantly higher in COVID-19 patients with encephalopathy and in COVID-19 patients

who died in hospital vs. in COVID-19 patients without encephalopathy and COVID-19 survivors, respectively (Frontera et al., 2022). Furthermore, COVID-19 patients who developed new neurological symptoms during hospitalization had higher sNFL levels than COVID-19 patients without in-hospital developed neurological manifestations. What is more, sNFL levels were correlated with disease severity in this study. These data confirmed previous research on the association between sNFL concentrations and poorer outcomes in hospitalized COVID-19 patients (Frontera et al., 2022).

Collectively, the majority of mentioned studies indicated significantly higher blood levels of NFL in patients with COVID-19 than controls, suggesting neurodegenerative impacts of SARS-CoV-2 (Table 1).

### NFL as a predicting factor of mortality and severe illness in COVID-19

The pNFL concentrations at hospital admission in COVID-19 patients demanding hospital stay could reportedly serve as a helpful prognostic biomarker. Apart from the aforementioned case-control studies showing the association between high NFL levels and worse clinical outcomes, some other studies were conducted to assess this association (Sutter et al., 2021; Prudencio et al., 2021; Frontera et al., 2022). In this regard, De Lorenzo et al. (2021) measured the pNFL levels in 104 hospitalized patients with COVID-19 in Italy. They demonstrated that patients with fatal outcomes had significantly higher pNFL values at the beginning of hospitalization than those who survived (36.1 pg/ml vs. 17.1 pg/ml). Moreover, patients requiring ICU care during their hospital stay had significantly higher pNFL levels at admission than those without a need for ICU stay (26.3 pg/ml vs. 13.9 pg/ml). Additionally, pNFL levels were strongly correlated with the factors conventionally used for evaluating COVID-19 prognosis, such as the pressure of arterial oxygen to fractional inspired oxygen concentration ( $PaO_2/FiO_2$ ), lymphocyte count, CRP, lactate dehydrogenase (LDH), and creatinine, suggesting that neuronal injury might occur secondary to damages to other vital organs (De Lorenzo et al., 2021). There was no comparison in NFL values based on sex in this study.

Another study investigated the prognostic value of serum or plasma NFL levels in predicting mortality in 338 COVID-19 patients categorized into three cohorts in Italy. In cross-sectional samples of cohort one, the authors reported significantly higher NFL levels in critically-ill COVID-19 patients vs. controls. Longitudinal assessment of NFL in cohort two showed that NFL

Table 1. Differences in blood NFL levels between patients with COVID-19 and controls.

Reference	Sample	Number of cases	Number of controls	NFL level in cases (pg/ml)	NFL level in controls (pg/ml)	P value	Most Notable findings
(Sutter et al., 2021)	Serum	29	259 (healthy controls) 10 (critically ill controls)	36.1 pg/ml	6.3	P<0.001	Increased sNFL was associated with unfavorable outcomes, sNFL levels in COVID-19 patients were higher than critically-ill controls after adjusting for pre-existing comorbidities and age (no sex segregation)
(Prudencio et al., 2021)	Serum	142	55	29.4	10.9	P<0.001	Higher sNFL levels were associated with worse clinical outcomes, Remdesivir treatment in 100 hospitalized COVID-19 patients led to sNFL levels reduction
(Kanberg et al., 2020)	Plasma	47	33	Severe: 32.7 Moderate: 19.3 Mild: 9.5	13.1	Severe COVID-19 cases vs. controls: P<0.001	Age correlated positively with pNFL levels (r: 0.62, P<0.001), Severe cases of COVID-19 had a significantly higher pNFL levels than controls and moderate and mild COVID-19 cases, Severe cases showed a significant rise in pNFL during the disease course (no sex segregation)
(Ameres et al., 2020)	Serum	28	72	Age (18-35): 4.5 Age (36-50): 9.6 Age (51-65): 11.6	Age (18-35): 4.4 Age (36-50): 6.8 Age (51-65): 9.6	For age groups 36-50 and 51-65: P<0.001	Age and COVID-19 status correlated positively with sNFL levels (P=0.001 and P=0.005), COVID-19 cases had a significantly higher sNFL levels than controls in the age groups (36-50) and (51-65), but not in the age group (18-35) (no sex segregation)
(Hanson et al., 2022)	Plasma	56: 8 CE* 9 PNP 38 NNP	8	CE (age>50): 75.6, CE (age<50): 186.3, PNP (age>50): 14, PNP (age<50): 5.1, NNP (age>50): 8.11, NNP (age<50): 5.4	HC (age>50): 9.25, HC (age<50): 5.4	NA	CE patients older than 50 had the highest pNFL levels, age was correlated with pNFL levels (no sex segregation)
(Cooper et al., 2020)	Plasma	27	19	36.7	32.9	P=0.19	sNFL levels had no significant association with delirium and ICDSC score in patients with COVID-19, There was not any significant difference between sNFL levels of cases and controls, elevated NFL levels were unrelated to respiratory function and peripheral cytokines, pNFL levels witnessed an increase in COVID-19 patients between days one and seven (no sex segregation)

NFL: neurofilament light chain, sNFL: serum NFL, pNFL: plasma NFL, CE: COVID-19 encephalopathy, HC: healthy control, PNP: post-hospitalization neuro-PASC (PNP), NNP: non-hospitalized neuro-PASC, PASC: post-acute sequelae of severe acute respiratory coronavirus type 2.

risers in late stages during the disease course following a reduction in absolute lymphocyte count (ALC) and an elevation in LDH (De Lorenzo et al., 2021). Cross-sectional samples of cohort three was used to validate the observations in cohort one and two. This study showed a significant correlation between ALC, LDH, and NFL abnormalities in later phases of the disease, suggesting neuro-axonal damage as a probable result of multi-organ failure in COVID-19 later stages. The authors concluded that blood NFL, LDH, and ALC abnormalities increase with COVID-19 severity and correlate with mortality. This study also indicated that female participants were more likely to survive COVID-19 in cohorts one and two. The combined assessment of these biomarkers might have the potential to differentiate COVID-19 from other acute respiratory diseases causing ICU admission. Although in this study, NFL showed clinical prognostic value only close to death, which is very late to alter medical management, simultaneous assessment of NFL, LDH, and ALC might still identify patients at high risk of COVID-19 mortality at a time that there is still hope of the effectiveness of escalated medical care (Masvekar et al., 2022).

Another study investigating 111 COVID-19 patients with severe illness demanding ICU care demonstrated that 11 patients who developed critical illness neuropathy (CIN) or critical illness myopathy (CIM) had higher pNFL levels, more severe illness, more prolonged ICU stay, and a higher likelihood of thromboembolic events compared with those who did not develop CIN/CIM (Frithiof et al., 2021). Moreover, higher pNFL concentrations were also correlated with more prolonged ICU stay in this study, which was in line with previous observations (Frithiof et al., 2021). Additionally, male patients with COVID-19 had a significantly higher risk for developing CIN or CIM in this study (100% of patients with CIM and CIN were male). In summary, most of the present research indicated that higher NFL levels in COVID-19 patients correlated with more intense severity of the disease and poorer clinical outcomes, including higher mortality rate, more extended ICU stay, and a higher likelihood of adverse effects.

### CSF NFL levels in COVID-19

Given the recent advancements in technology allowing the blood measurement of NFL, most studies on COVID-19 patients assessed the blood levels of this biomarker, as blood sampling is a more convenient and tolerable procedure for patients. However, depending on patients' status, some studies have quan-

tified this biomarker in CSF to provide more sensitive evidence of neurological consequences of COVID-19, as NFL presents at higher concentrations in CSF than in blood. For instance, in a study of 19 hospitalized COVID-19 patients with neurological symptoms, 12 patients (63%) were found to have elevated levels of NFL in CSF, which was more pronounced in those with central neurological symptoms (Virhammar et al., 2021). This study showed that CSF NFL levels correlated significantly with higher disease severity, duration of ICU stay, and impaired consciousness status (Virhammar et al., 2021). A case-control study investigating CSF levels of NFL in 18 COVID-19 patients with neurological complications and 82 controls demonstrated a higher CSF NFL levels in COVID-19 patients with stroke or with a critical form of the disease compared to controls and patients in other categories of disease severity (Garcia et al., 2021). These findings indicate more pronounced neuro-axonal injury in the mentioned groups of COVID-19 patients (Garcia et al., 2021). Nevertheless, low detected amounts of pro-inflammatory cytokines and absence of SARS-CoV-2 RNA in CSF of COVID-19 subjects in this study were in contrast to the hypothesis of neuroinflammation and direct neurovirulence of the virus as causative factors of neuro-axonal injury (Garcia et al., 2021).

Another study comparing CSF NFL levels between COVID-19 patients with encephalitis, non-COVID-19 encephalitis patients, and healthy controls illustrated that patients with COVID-19-related encephalitis had significantly higher NFL values in CSF than the healthy controls, but not than non-COVID-19 encephalitis patients (Pilotto et al., 2021). This study showed CSF NFL rise only in severe cases of COVID-19, although the concentrations of pro-inflammatory cytokines and markers indicating damage to astrocytes and microglia like GFAP were increased in all but one COVID-19 subjects (Pilotto et al., 2021). The observed alterations in neuroinflammatory markers in this study were highly suggestive of CRS as responsible for COVID-19-related neuro-virulence (Pilotto et al., 2021).

On the other side, a case report study of a female with COVID-19 and acute necrotizing encephalopathy demonstrated extreme elevation of NFL levels in CSF as well as detection of SARS-CoV-2 RNA on day 19 after the symptoms' onset following two times of negative tests, emphasizing the neurotropic features of the virus in causing neurodegeneration (Virhammar et al., 2020). A case series on six patients with moderate to severe COVID-19 and neurological manifestations including suspected encephalopathy, suspected meningitis, and dysgeusia also showed raised NFL levels in CSF in two of the mentioned subjects (33%).

Although soluble markers of neuroinflammation were increased in this study, SARS-CoV-2 could not be detected and there were no elevations in WBC count and other immunological responses as opposed to typical viral CNS infections of CSF, underlying distinguishing features of SARS-CoV-2-induced neurological involvement (Edén et al., 2021a). Besides, a well-conducted systematic review of the studies investigating CSF biomarkers of COVID-19 patients with neurological manifestations including seizure, stroke, encephalitis, encephalopathy, inflammatory syndrome, headache, and meningitis illustrated that 71% of these patients had elevated CSF levels of NFL biomarker, pointing out to COVID-19-related neurodegeneration (Domingues et al., 2022). The mentioned studies on CSF did not investigate whether the course of the disease varies in male and female participants.

Whilst, the extrapolation of the results should be cautiously due to the low number of studies on CSF NFL levels in COVID-19 patients and small sample sizes, the majority of mentioned research indicated increased levels of CSF NFL, which were in line with the observations in blood NFL levels, translating into neuro-axonal damage in COVID-19.

### NFL levels in children with COVID-19

A study in Germany investigated 2652 children, including 147 asymptomatic to mild COVID-19 cases diagnosed with positive anti-SARS-CoV-2 antibody, of whom 47 subjects (31.8%) had neurological symptoms. This study did not show any significant difference in sNFL levels between children with positive SARS-CoV-2 antibodies and those without (Geis et al., 2021). Besides, multivariate regression analysis indicated age as an independent predicting factor for sNFL level. In contrast, anti-SARS-CoV-2 antibody level, qualitative antibody status, and clinical severity of the disease did not correlate with sNFL values in children. This study also did not show any significant differences between the gender of children and their COVID-19 antibody status. These findings might be interpreted as that children are less susceptible to neuro-axonal injury following COVID-19 than adults (Geis et al., 2021), as the children population of this study showed no signs of neuro-damage. However, one should consider that all children participating in this study had asymptomatic or mild forms of the disease, which can limit the results' generalization to all COVID-19 children. In this regard, there is a substantial need for more studies on children with various severities of COVID-19 to more accurately elaborate on the status of neurological involvements and the utility

of neurodegenerative biomarkers like NFL as a prognostic factor.

### NFL levels after recovery from COVID-19

The neurological symptoms of COVID-19 might still be persistent in patients after full recovery from the acute phase of the disease. In this regard, several studies quantified the NFL levels after disease recovery to determine whether active neuro-axonal damage is responsible for manifesting such neurological symptoms chronically. For instance, a follow-up study in Italy demonstrated that COVID-19-related neurological symptoms were present in 49 out of 107 COVID-19 patients after recovery. According to this study, the most common persistent neurological symptoms were hyposmia, fatigue, impaired memory, and myalgia. However, the follow-up sNFL levels were within normal ranges in all except for five patients in this study. Moreover, sNFL values could not differentiate patients who have neurological manifestations from those who have not. Furthermore, in 29 patients with available sNFL levels at disease onset, sNFL values witnessed a significant reduction in the follow-up measurement (Bozzetti et al., 2021). This study did not indicate a difference in the pattern of NFL reduction in follow-up evaluations between males and females. This data might suggest that despite the persistence of neurological symptoms in patients who recovered from COVID-19, there is no ongoing neuro-axonal damage in this phase of the disease (Bozzetti et al., 2021).

In line with that, another follow-up study on 24 mild, 28 moderate, and 48 severe COVID-19 patients illustrated that patients with severe COVID-19 had significantly higher pNFL values than patients with mild or moderate forms of the disease in acute phases (Kanberg et al., 2021). Although, after six months from the disease onset, the pNFL concentrations were normalized in the mentioned COVID-19 patients regardless of their disease severity, 50 patients (50%) still complained of persistent neurological symptoms like fatigue, cognitive changes, and brain fogs (Kanberg et al., 2021). These persistent neurological symptoms were not correlated with the level of pNFL during the acute phase of the disease. These findings similarly indicate that post-COVID-19 neurological symptoms might not be associated with active CNS injury (Kanberg et al., 2021). This study also revealed that the male sex could be a risk factor for developing the moderate or severe forms of the disease, as the majority of patients classified in these groups were male.

A follow-up multicentric study investigated 152 individuals surviving the severe form of COVID-19,

enrolled between 90 and 120 days after hospital discharge. The findings revealed an inverse relationship between pNFL levels and episodic memory, global cognition, and executive functions (Serrano-Castro et al., 2022). It should be noted that most of the COVID-19 subjects enrolled in this study had chronic neurological abnormalities, which could have considerably affected the findings (Serrano-Castro et al., 2022). Another follow-up case-control study compared sNFL levels between 45 post-COVID-19 individuals with neuropathic pain and 45 healthcare workers who recovered from COVID-19 without neuropathic pain. The results indicated that individuals with post-COVID-19 pain had significantly higher sNFL levels than controls (11.34 pg/ml vs. 7.64 pg/ml,  $P=0.029$ ), suggesting sNFL as a potential biomarker for predicting neuropathic pain after recovery from COVID-19 (Magdy et al., 2022). The result also indicated that the gender has no effect on developing neuropathic pain in this study. On the other hand, a study comparing sNFL levels of patients who recovered from COVID-19 with symptoms of myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) and those without ME/CFS did not demonstrate any significant difference in sNFL levels, indicating that neuro-axonal injury might not play an influential role in inducing ME/CFS-like symptoms in COVID-19-recovered patients. Gender has no significant influence on developing ME/CFS in this study. However, the findings are recommended to be confirmed by studies with larger sample sizes (Mantovani et al., 2021).

In a prospective cohort study, sNFL levels of 39 patients with mild to moderate COVID-19 and 14 patients with severe COVID-19 were evaluated at enrolment and  $28 \pm 7$  days later. The results showed that sNFL levels were significantly increased in the follow-up measurement in individuals with severe COVID-19. At the same time, patients with mild to moderate COVID-19 did not show sNFL elevation during their follow-up evaluation. Moreover, elevated sNFL levels in individuals with severe COVID-19 were correlated with anti-spike IgG and neutralizing antibody levels in this study (Hirzel et al., 2022). This study did not indicate a significant deviation in COVID-19 severity between male and female participants. A case-report study of a female with COVID-19 and MFS demonstrated a high blood NFL level which did not normalize in follow-up measurements 7 and 23 days later, indicating MFS contributing roles in neuro-axonal damage after COVID-19 recovery (Senel et al., 2020). To summarize, most of the research body indicated that despite the persistence of neurological manifestations after recovery from the disease in many subjects with COVID-19, there is no ongoing neuro-axonal damage

in this phase of the disease. In other words, NFL levels tend to normalize after recovery from COVID-19 in a high proportion of patients.

## CONCLUSION

SARS-CoV-2 has shown its potential to exert detrimental effects on the nervous system. The virus can affect the neurological system by directly entering the CNS through the blood-brain barrier. On the other hand, the virus can trigger the body's inflammatory response, such as cytokine release syndrome, allowing immune cells and pro-inflammatory cytokines to recruit to the CNS and degrade neurons. NFL is an intermediate filament mostly found in myelinated axons. As NFL releases into the CSF and the blood following any degenerative insults to neurons, the CSF or blood concentrations of this neurofilament are conventionally used as a sensitive and specific biomarker to give clues on the degree of neurodegeneration and the disease severity.

Given that the nervous system involvement caused by SARS-CoV-2 could significantly weaken the disease prognosis and might also result in persistent neurological sequelae, scientists proposed measuring NFL concentrations in different biological fluids of patients with COVID-19 to early indicate neurological involvement in these patients to prevent the progression of neurodegeneration and improve the prognosis. In this regard, the majority of case-control studies indicated significantly higher blood or CSF levels of NFL in COVID-19 patients compared with controls. Studies have also shown that NFL level is significantly correlated with disease severity indicators like prolonged hospital or ICU stay, high likelihood of adverse effects, and mortality rate. Moreover, high NFL levels at hospital admission in patients without early-onset neurological symptoms could predict the development of neurological manifestations during hospitalization. Despite the persistent neurological symptoms in some cases of COVID-19 after recovery, NFL levels return reportedly to the normal range in this population, suggesting that there is no ongoing neuro-axonal damage in this phase of the disease.

All in all, it seems that NFL could serve as a useful biomarker with acceptable sensitivity to be widely used in COVID-19 patients, especially those who suffer from the severe form of the illness. However, one should consider that the longitudinal assessment of NFL biomarker integrated with other existing neurodegenerative biomarkers and conventional factors showing neurological consequences and disease severity like GFAP, CRP and lymphocyte count could pro-

vide a more accurate estimate of the degree of neurodegeneration and the prognosis in COVID-19 patients. In conclusion, more longitudinal studies with large sample sizes on various groups of COVID-19 patients are needed to provide more validated information on the cost-effectiveness and utility of NFL assessment as a single neurodegenerative biomarker in predicting the disease prognosis and severity.

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