REVIEW

Review of Neurofilaments as Biomarkers in Sepsis-Associated Encephalopathy

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Abstract: Sepsis is a common and fatal disease, especially in critically ill patients. Sepsis-associated encephalopathy (SAE) is a diffuse brain dysfunction with acute altered consciousness, permanent cognitive impairment, and even coma, accompanied by sepsis, without direct central nervous system infection. When managing SAE, early identification and quantification of axonal damage facilitate faster and more accurate diagnosis and prognosis. Although no specific markers for SAE have been identified, several biomarkers have been proposed. Neurofilament light chain (NFL) is a highly expressed cytoskeletal component of neurofilament (NF) proteins that can be found in blood and cerebrospinal fluid (CSF) after exposure to axonal injury. NFs can be used as diagnostic and prognostic biomarkers for sepsis-related brain injury. Phosphorylation of NFs contributes to the maturation and stabilization of cytoskeletal structures, especially axons, and facilitates axonal transport, including mitochondrial transport and energy transport. The stability of NF proteins can be assessed by monitoring the expression of NF genes. Furthermore, phosphorylation levels of NFs can be monitored to determine mitochondrial axonal transport associated with cellular energy metabolism at distal axons to assess progression during SAE treatment. This paper provides new insights into the biological characteristics, detection techniques, and scientific achievements of NFs, and discusses the underlying mechanisms and future research directions of NFs in SAE.

Keywords: neurofilament light chain, phosphorylated neurofilaments, cognitive dysfunction, mitochondria, axonal transport

Introduction

Sepsis is life-threatening organ dysfunction caused by dysregulated host response to infection.[1] It is also the main cause of morbidity and mortality in critically ill patients.[2] The central nervous system (CNS) is one of the most vulnerable organs to systemic lesions caused by sepsis. Sepsis-associated encephalopathy (SAE) is one of the most serious clinical manifestations seen in patients with sepsis.[3] SAE is often accompanied by delirium and various degrees of cognitive dysfunction, including chronic neurological dysfunction.[4] Its surveillance, screening, and early diagnosis are crucial for improving prognosis and treatment management. However, despite monitoring methods and comprehensive treatment strategies that have been summarized after years of research on sepsis, sensitive and specific biomarkers for detection of SAE are lacking.[5]

Neurofilament light chain (NFL) has been identified as a prognostic and diagnostic biomarker for neurological disorders, including multiple sclerosis, Alzheimer's disease, postoperative delirium, and traumatic brain injury.[6–9] Research confirmed that NFL in CSF and blood has similar pathophysiological processes and identical prognostic effects on cognitive function and neurodegeneration. Progression of NFL levels can predict conversion from the pre-symptomatic to symptomatic stage and time of survival.[10,11] In patients with SAE, elevated NFL levels indicate adverse neurological outcomes such as cognitive impairment. The close link between NFL, reduced brain volume, and neuronal damage also supports the feasibility of monitoring NFL levels to predict neurological damage in SAE patients.[12,13] Moreover, elevated plasma NFL values were significantly correlated with SAE severity and poorer neurocognitive outcomes. However, plasma NFL is consistent with CSF-NFL; elevated levels in both plasma and CSF represent poorer clinical outcomes.[14]

Graphical Abstract
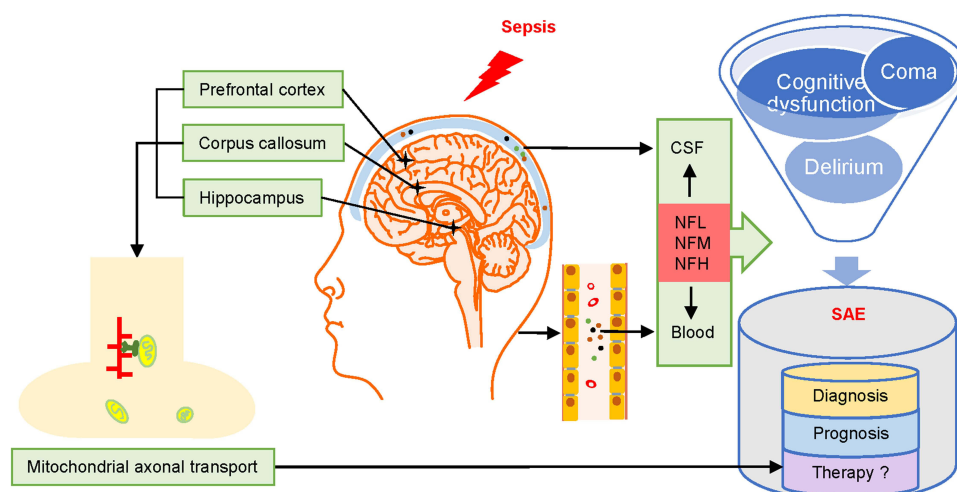
In this paper, we reviewed the biological structure, type of measurements, and research background of neurofilaments (NFs). We also discussed the mechanisms of NFs, phosphorylation changes, and their association with mitochondria during SAE. In addition, the potential implications of NFs in the surveillance of SAE are discussed.

Biological Structure and Measurements of NFs

NFs, a basic component of the cytoskeleton of neurons, provide the structural basis for axons and regulate axonal diameter to affect neuronal conduction velocity and the transportation of essential organelles.[15–17] NFs belong to the intermediate filaments (IFs) family, with diameters ranging between actin (6 nm) and myosin (15 nm).[18] They are major neuronal supporting proteins comprising five subunits (neurofilament triplet proteins): neurofilament light chain (NFL, 68 kDa), neurofilament medium chain (NFM, 160 kDa), neurofilament heavy chain (NFH, 200 kDa), α-internexin (66 kDa), and peripherin (57 kDa).[19] The NF protein is rigorously assembled from dimer to tetramer, then an octamer, forming a mature long NF polymer of 10 nm diameter (Figure 1).[20] All NF subunits contain a conserved A-helical rod domain connected by several helical coils, a variable amino-terminal head region, and a carboxy-terminal tail region. Different types of NFs have different number of repeats containing Lysr-Ser-Pro sequences at the carboxy terminal. Epigenetic modifications of NF genes include methylation, acetylation, and phosphorylation.[21–23] Phosphorylation of NF subunits is the most important factor in stabilizing the axonal cytoskeleton.

NFL, as the most abundant quantifiable subunit in NFs, can be measured in blood and cerebrospinal fluid (CSF).[24] Kuhle et al suggested using blood NFL levels for real-time monitoring of disease activity and response to drugs.[25] Previous studies have analyzed three approaches to reliably measure NFL levels in blood samples. The fourth-generation single-molecule array (Simoa) assay for NFL was found to be 126-fold more sensitive than the conventional ELISA and 25 times more sensitive than electrochemiluminescence (ECL).[26] Continuous measurement of blood NFL is clinically feasible, with minimal collateral damage to the patient. It also provides more possibilities for the early diagnosis and prognostic analysis of neurological diseases. However, Shahim et al observed the dynamic release of NFs in serum and CSF, which increased over the first 12 days post-injury.[27] The mean NFs in patients with SAE showed a time-dependent increase from day 1 to day 7.[14] Therefore, these studies suggested that the measurement of NFs should be initiated from the first day after injury and may be influenced by time. Timely repeat measurements are of great significance for assessing NF levels.
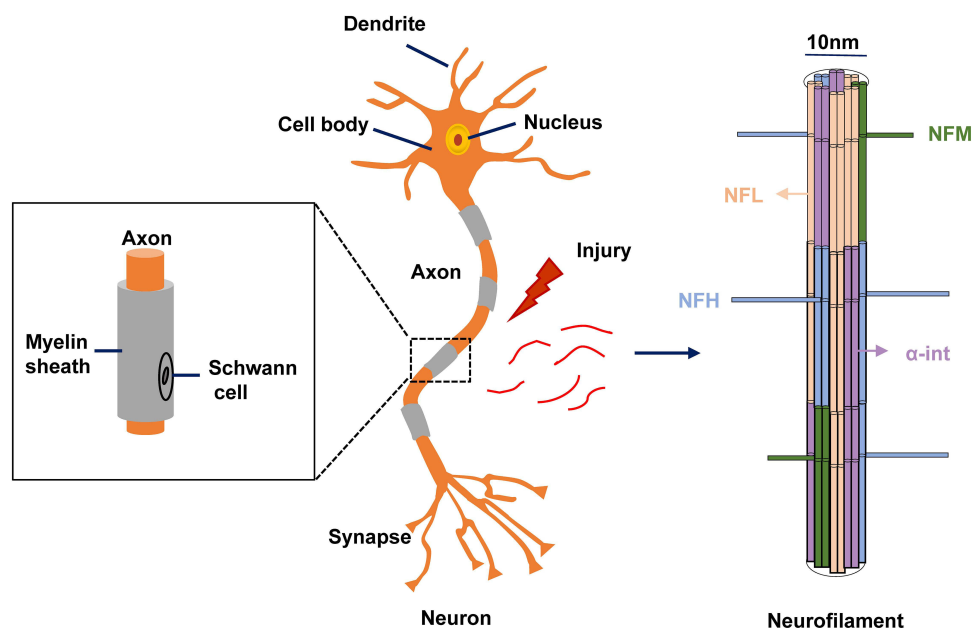
Figure 1 Neurofilament assembly in CNS. The mature long NF polymer has a diameter of 10 nm. NFL (yellow) is the most abundant quantifiable subunit. Tail domains of NFM (green) and NFH (blue) protrude outward from the neurofilament core for phosphorylation of the carboxy-terminal tail region. In addition to the neurofilament triplet proteins (NFL, NFM, and NFH), α-internexin (purple) is also present in the CNS. A magnified image of myelinated axon is shown on the left.

NF Response to Nerve System Injury
Clinical Research

Several studies provided evidence that disorganization of the neuronal IF network may be directly involved in neuronal dysfunction. Depending on the extent of neuronal dysfunction, NFs can easily diffuse into the extracellular fluid and subsequently into CSF and peripheral blood via the blood–brain barrier (BBB).[25]

Recently, the application of NFs in SAE has attracted extensive interest. The elevated NFL value and soluble triggering receptor expressed on myeloid cells 2 (sTREM2) in CSF predicted early brain damage caused by sepsis and neuroinflammation before the onset of SAE symptoms. Moreover, a positive correlation was found between CSF NFL and sTREM2 levels ($p = 0.005$, $R = 0.706$).[28] Thus, NFL and sTREM2 levels have been suggested as diagnostic biomarkers for early brain damage and prognostic biomarkers for long-term cognitive dysfunction in SAE patients. Besides NFL, human septic brains postmortem demonstrated that the deterioration of brain homeostasis could contribute to diffuse and/or ischemic axonal damage during SAE. The immunohistochemical staining for non-phosphorylated NFH SMI32 revealed numerous axonal endbulbs accompanied by bead-like swelling, indicating axonal degeneration.[29] Moreover, Ehler et al found elevated levels of NFL and NFH in plasma and CSF of SAE patients. The plasma NFL values were significantly related to the severity of SAE ($R = 0.534$, $p = 0.022$) and a poorer functional outcome after 100 days ($R = -0.535$, $p = 0.0003$). Also, CSF NFL levels were significantly correlated with the last lifetime ($R = -0.932$, $p < 0.0001$) and functional outcome after 100 days ($R = -0.749$, $p < 0.0001$) in contrast to CSF NFH levels (Table 1).[14] This prospective, longitudinal study suggested for the first time that NF levels in plasma and CSF provide prognostic information on the severity of SAE and long-term cognitive outcome in survivors.

NFL is a novel and less invasive biomarker for clinical assessment of patients with neurodegenerative diseases.[30,31] However, NFL levels are affected by factors such as age, renal function, surgery, and anesthesia.[32–36] In addition, recent evidence reported a correlation between NFL and SARS-COV-2 infection. However, whether NFL can be used as a definitive biomarker for COVID-19 patient needs to be further explored.[37] Therefore, severe sepsis patients with the above-mentioned high-risk factors may be more likely to develop delirium and SAE.

NF is a neuronal cytoskeletal protein that increases in certain PNS lesions, such as amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD), Charcot–Marie–Tooth (CMT) disease, giant axonal neuropathy, and COVID-related

Table 1 Research of NF Response to SAE in Clinical and Preclinical Findings

Reference	Species	Sample Source	Detection Methods	Conclusion
[14]	Human	CSF, Plasma	ELISA	Increased levels of NFL and NFH in CSF and plasma provide prognostic information for the severity of SAE and long-term cognitive outcome in survivors
[28]	Human	CSF	ELISA	The elevated NFL value and sTREM2 can predict cognitive dysfunction and support the pathological process of microglial activation and neuroaxonal degeneration in SAE
[29]	Human	Postmortem brain tissue	Immunohistochemistry	The staining for non-phosphorylated NFH^{SMI32} indicates the presence of axonal endbulbs due to axonal degeneration
[29]	Animal, rat	Brain tissue	ELISA	The increased NFH^{SMI35} level predicts the time from sepsis onset
[44]	Animal, rat	Brain tissue	Western blot	Decreased expression of NFL, NFM, and NFH proteins is associated with axonal injury
[44]	Animal, rat	Primary cultured cortical neurons	Western blot, Immuno-cytochemical staining	IL-1 β activates the phosphorylation of the P38-MAPK signaling pathway to inhibit the protein expression of NFL, NFM, NFH, and synaptophysin
[45]	Animal, rat	Brain tissue	Western blot	Decreased expression levels of NFL, NFM, and NFH result in axonal impairment and neurological deterioration
[46]	Animal, rat	Brain tissue	Immuno-fluorescence, Western blot	Downregulated of NFL, NFM, and NFH expressions indicate that the axon development is disrupted after LPS injection

Abbreviations: ELISA, enzyme-linked immunosorbent assay; sTREM2, soluble triggering receptor expressed on myeloid cells 2; P38-MAPK, P38 mitogen-activated protein kinase.

critical neuropathy/myopathy.[20,38] However, NFL can reflect the intensity of neurodegeneration in PNS and neurodegeneration-related cognitive decline in CNS.[39] The abnormal accumulation of NFs is consistent with indicators such as GFAP and Tau proteins.[40,41] Thus, we concluded that the measurement of NFs in CNS and PNS diseases is equally reliable. Therefore, monitoring NFs to reflect neuronal damage may be useful for individual clinical treatment decisions and prognostic assessment.

Preclinical Research

The use of lipopolysaccharide (LPS) and cecal ligation and puncture (CLP) are the two most common methods used to simulate sepsis models. CLP-induced SAE model is characterized by septic brain injury with cognitive impairment.[42] However, no studies have investigated the role of NFs in the CLP-induced SAE model.

Due to the altered regulation of NFL gene expression caused by experimental LPS administration, resulting in structural abnormalities in neurons and synapses, the application of LPS in models involving the study of NFs may have certain advantages.[43] In the LPS-induced sepsis model, the expression of NFL, NFM, and NFH proteins was significantly decreased in the corpus callosum, while the axonal injury involved decreased mean axonal diameter, decreased axonal myelination levels, and even no myelin sheaths. Additionally, in primary cultured cortical neuron models with IL-1β treatment, IL-1β activates the phosphorylation of the P38-MAPK signaling pathway to inhibit the protein expressions of NFL, NFM, NFH, and synaptophysin.[44] In vivo, IL-1β produced by activated microglia enters the brain through BBB and induces the decrease of NF expression and synaptic damage through the inflammatory pathway. Similarly, the significantly reduced expressions of NFL, NFM, and NFH in neonatal rats induced by LPS injection resulted in reduced number of myelinated axons and impaired nodes of Ranvier, which were associated with axonal development disorders manifested as movement disorders in later life.[45] Furthermore, in an LPS-induced neonatal rat model of sepsis, significant down-regulation of NFL, NFM, and NFH expressions in the prefrontal cortex and hippocampus was associated with axonal dysfunction, which may be related to motor dysfunction and cognitive dysfunction.[46] In addition to the LPS modeling, there are two types of ischemic and diffuse axonal damage in the septic rat model induced by intraperitoneal injection of fecal slurry. The mild correlation between NFH^{SMI35} levels and the time to sepsis induction accompanied by abnormal staining of β-amyloid precursor protein (βAPP) and severe disruption of the β-tubulin network

was reported (Table 1).[29] The above preclinical studies provided evidence at the protein level for NFs as a non-specific biomarker for supplementary monitoring to predict the occurrence of axonal damage and related neurocognitive dysfunction during sepsis. However, the gene transcription or mRNA stability is responsible for normal levels of NF proteins.[47–49] Current samples are insufficient to provide direct evidence of reduced NF gene transcription or mRNA stability in SAE patients. Therefore, more information is needed from subsequent experiments in different sepsis models in order to translate discoveries into human setting.

Pathological Mechanisms Associated with NFs and SAE

Current hypotheses suggest that neuroinflammation, activation of microglia cells, destruction of BBB, mitochondrial dysfunction, dysregulation of oxygen and energy metabolism, and neuroaxonal damage can lead to cerebral dysfunction in sepsis.[29,50–55] Besides the pro-inflammatory cytokines that are produced during sepsis can inhibit the expression of NF subunits while penetrating the BBB and activating microglia.[44,56] NF-related axonal transport mechanism contributes to mitochondrial translocation and energy transport that are worth exploring in SAE.

The oxidative stress in sepsis affects the intra-mitochondrial biogenesis, which finally causes reduced energy production.[54] Since synapses consume most of the energy in the brain and synaptic energy is mainly provided by mitochondria,[57] transporting mitochondria to distal neuronal axons may be an important measure to improve the synaptic energy supply and even reverse the SAE.

The transport of mitochondria in CNS involves neuronal cytoskeletal components such as microtubules, actin and NFs, the driving force system, and the mitochondrial membrane potential. Here, we focused on how NF injury affects mitochondrial axonal transport in SAE. First, the driving force for NFs to participate in axonal transport comes from the combination of dynein/dynactin, kinesin, myosin and NFs, based on the microtubules.[58–61] In addition, mitochondrial association with IFs alters mitochondrial membrane potential, energy production and transport.[62] The phosphorylated NFLs are more likely to bind stationary mitochondria with higher membrane potentials.[63] Furthermore, the phosphorylation of NFH and NFM protrude their side arms from the trunk to increase the negative charge and then form the regular cross-bridge between mitochondria and NFs, promoting the translocation of mitochondria to distal axons and facilitating the acquisition of energy in neurons, which is required for maintaining neuronal resting potential, synaptic transmission and ATP for the action potential[63,64] (Figure 2). Therefore, efficient binding of NFs to mitochondria mediated by phosphorylated NFs is essential for normal mitochondrial translocation in axons. In sepsis, the disruption of NFs leads to reduced axonal diameter, atrophy of NFs and decreased conduction velocity, which contributes to axonal transport disorder. Therefore, monitoring NFs, even the phosphorylation status, may effectively assess the treatment of SAE, which is helpful for recovery.

NF is a biomarker that contributes to the diagnosis of SAE and the prediction of disease severity. Maintaining mRNA stability by targeting miRNA is conducive to normal protein expression and reduced abnormal aggregation of NFs.[65] Meanwhile, reversal of translational dysregulation of NF mRNAs in the ALS/frontotemporal dementia (FTD) mouse model contributes to improving cognitive impairment associated with restored axonal integrity and synaptic function.[66] Moreover, assessment of mitochondrial function related to NFs may be useful for assessing brain energy metabolism.[67] These studies support the importance of repair of neuroaxonal function associated with mitochondrial transport mediated by NFs and provide a new idea for NFs as potential therapeutic targets for SAE.

Conclusions

NFs can be used as diagnostic and prognostic biomarkers for SAE to assess cognitive function. Monitoring mRNA expression levels of NFs is useful for assessing neuronal cytoskeleton stability. Monitoring NF phosphorylation levels may be used to assess mitochondrial axonal transport, which has important implications for cellular energy metabolism at distal axons. The next step is to use NFs as biomarkers in practical studies and combine with pathophysiological changes under different etiologies, to explore whether NF monitoring can play a more active role in the clinical management of SAE.
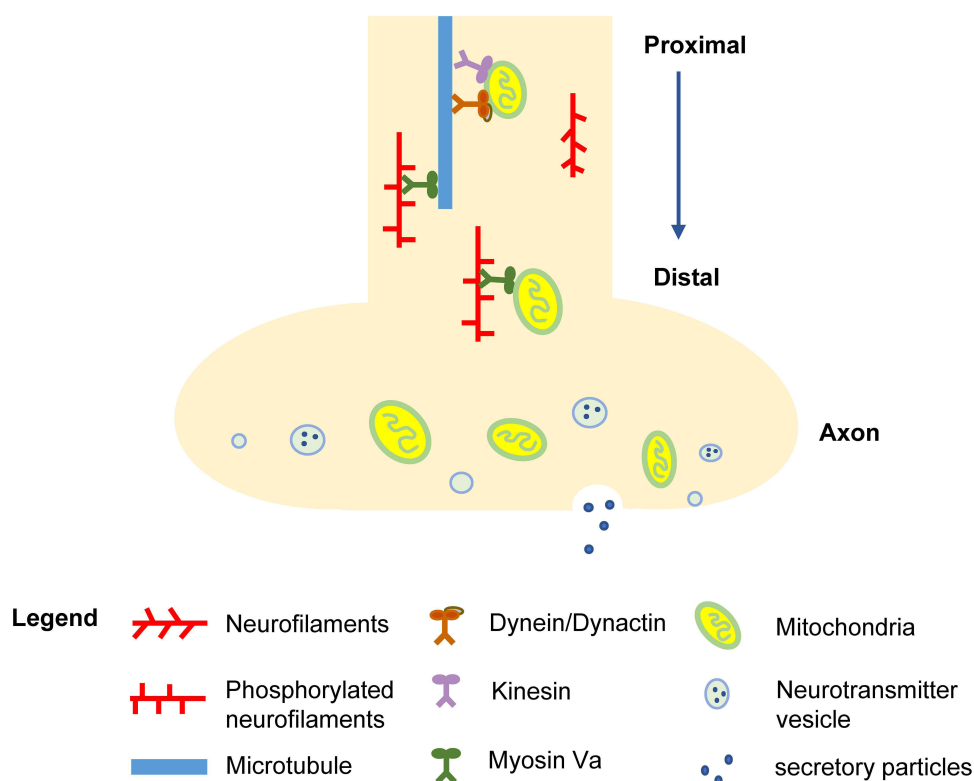
Figure 2 Mitochondrial axonal transport associated with NFs. The blue arrow indicates the anterograde axonal transport of mitochondria towards the distal axon. The driving force comes from the combination of dynein/dynactin, kinesin, myosin and phosphorylated NFs based on the microtubules. The elements that participate in the rapid axonal transport include mitochondria, neurotransmitter vesicles, and secretory particles. In addition, the direct combination of NFL and myosin Va plays a critical role in slow axonal transport for local mitochondrial movement.

Disclosure

The authors report no conflicts of interest in this work.

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