REVIEW ARTICLE

The multifaceted role of neurofilament light chain protein in non-primary neurological diseases

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Abbreviations: AUC = area under the curve; BMI = body mass index; bvFTD = behavioral variant of frontotemporal dementia; COVID-19 = coronavirus disease 19; ELISA = Enzyme-Linked Immunosorbent Assay; GFAP = glial fibrillary acidic protein; HbA1c = glycosylated hemoglobin; ICU = intensive care unit; MS = multiple sclerosis; NfL = neurofilament light chain protein; NfH = neurofilament heavy chain protein; NfM = neurofilament medium chain; Nfs = neurofilaments; NSE = neuron-specific enolase; OHCA = out-of-hospital cardiac arrest; S100B = S100 calcium-binding protein B; SARS-CoV-2 = severe acute respiratory syndrome coronavirus type 2; SBP = systolic blood pressure; Simoa = single molecule array; SSEP = somatosensory-evoked potentials; t-tau = total-tau; WMH = white matter hyperintensities

Abstract

The advancing validation and exploitation of cerebrospinal fluid and blood neurofilament light chain protein as a biomarker of neuroaxonal damage has deeply changed the current diagnostic and prognostic approach to neurological diseases. Further, recent studies have provided evidence of potential new applications of this biomarker also in non-primary neurological diseases. In the present review we summarise the current evidence, future perspectives, but also limitations, of neurofilament light chain protein as a cerebrospinal fluid and blood biomarker in several medical fields, including intensive care, surgery, internal medicine and psychiatry. In particular, neurofilament light chain protein is associated with the degree of neurologic impairment and outcome in patients admitted to intensive care units or in the perioperative phase and it seems to be highly interconnected with cardiovascular risk factors. Beyond that, interesting diagnostic and prognostic insights have been provided by the investigation of neurofilament light chain protein in psychiatric disorders as well as in the current coronavirus disease 19 (COVID-19) pandemic and in normal aging. Altogether, current data outline a multifaceted applicability of cerebrospinal fluid and blood neurofilament light chain protein ranging from the critical clinical setting to the development of precision medicine models suggesting a strict interplay between the nervous system pathophysiology and the health-illness continuum.

Introduction

The European Medical Agency and the Food and Drug Administration define a biomarker as a biological molecule accessible in body fluids or tissues that can be exploited to follow physiological, disease and pharmacological processes in humans and animals.[1–3] Accordingly, a biomarker could fall into several, not mutually exclusive, categories depending on its diagnostic, prognostic, predictive or pharmacodynamic role.[1–3] Therefore, advances in biomarker research have historically been critical to progress in a broad spectrum of clinical conditions. The past decade has witnessed an explosion of interest in neurofilament light chain protein (NfL) as a biomarker reflecting neuroaxonal damage in neurological disorders.[4,5] Notably, the introduction of ultra-sensitive immune assays, having a severalfold higher analytical sensitivity than for examples standard enzyme-linked immunosorbent assay (ELISA) methods,[6–9] has represented a substantial advancement into the biomarker field, especially in the assessment of NfL concentration, given the possibility to obtain rapid and robust quantification in blood samples.[10] So far, results from hundreds of studies have supported the multifaceted role of CSF and blood NfL, showing diagnostic, prognostic and even pharmacodynamic utility in most neuroinflammatory, neurodegenerative, cerebrovascular and traumatic disorders.[4,5,11–22]

Nevertheless, there is also growing evidence that NfL may be able to track a subclinical neuronal damage even in non-primary neurological diseases, which is likely associated with the development of short- or long-term neurological sequelae. In this regard, the association between NfL and the degree of neurologic impairment and outcome in patients admitted to intensive care units (ICU) or in the perioperative phase along with the complex interplay existing between cardiovascular risk factors and NfL levels are informative. Moreover, the raising concern from worldwide healthcare systems for psychiatric disorders as well as the current coronavirus disease 19 (COVID-19) pandemic provided two emerging settings in which NfL has been investigated. Furthermore, some very recent pieces of evidence opened a new perspective in terms of the possible prognostic implication of NfL in healthy elderly individuals. Finally, few reports assessed NfL as a biomarker for neuronal injury in disorders due to physical agents (i.e. decompression sickness, etc.) as well as in gynecological and dermatological conditions.

In the present review we provided an up-to-date and comprehensive overview of the current evidence, future perspectives, and limitations of NfL as a CSF and blood biomarker in non-

primary neurological disorders and in normal ageing, taking into account the lessons obtained from this biomarker application in the field of neurological diseases.

Biological and analytical aspects of neurofilament light chain protein

Neurofilaments (Nfs) are exclusively expressed in mature neurons where they form fibrillary networks representing the major cytoskeletal component, thus providing structural stability and resistance against mechanical stress.[23,24] Nfs are particularly abundant in the large-myelinated axons, such as those of motor neurons, where they are involved in the radial growth of axons and dendritic branching, in axonal transport, and they act as scaffold for microtubules to regulate positioning of cellular organelles.[23,24] NfL, a class IV intermediate filament protein of 68 kDa encoded by the *NEFL* gene located on chromosome 8 (8p21.2) represents the most abundant intermediate filament protein in neurons.[22,23,25,26] To build up Nfs, NfL protein assembles into heteropolymers together with neurofilament medium chain (NfM) and neurofilament heavy chain (NfH) proteins.[23,25–27] The physiological turnover of Nfs is very slow[28] and might be regulated by phosphorylation of NfM and especially NfH subunits.[29,30] The proteasome system is considered to be involved in the degradation of Nfs, although little is known about Nf proteolysis under disease conditions.[24]

While NfL is secreted to, and readily measurable in CSF of healthy young people[31] and children [32], after processes that cause neuroaxonal damage, increased amounts of NfL are released to the CSF and then are drained into the blood.[4,5] NfL release might occur as consequence of membrane disintegration as well as through regulated secretion of exosomes.[33] Nevertheless, data concerning the influence of blood-brain barrier permeability on blood NfL concentrationsare still to be fully elucidated.[34,35] NfL levels can reliably be quantified in the CSF using commercially available sandwich ELISAs. The CSF NfL mean concentration in individuals without neurodegenerative diseases is about a few hundred pg/ml up to 1 ng/ml with the levels increasing with age (refer chapters below).[16,36] Correspondingly, under non-pathological conditions, NfL blood concentration is approximately 15-60 fold lower than in CSF and below the detection limit of classical sandwich ELISAs. [10,37] However, this issue has been recently overcome with the introduction of new analytical techniques. A first effort in this direction was based on an electrochemiluminescence assay (Meso Scale Discovery platform).[38] Some years later, sensitivity was further improved by the implementation of digital ELISA technology by means of the single molecule array (simoa,

Quanterix),[10] which enabled a reliable measurement of both serum and plasma NfL. Notably, a comparison of NfL concentrations determined by these two different approaches is not possible and even measurements obtained with simoa can vary according to different assays. For example, the current version of the commercial simoa assay (NF-light Advantage kit, Quanterix, US) with ready-to-use calibrators yields twice as high CSF NfL concentrations compared to the previous version that contains a lyophilized calibrator stock. Furthermore, home-brew versions generally obtain higher NfL concentrations compared to commercial assays due to different blocking buffers or other technical modifications. Finally, a technology dependent bias might be considered in the analytical process.[10,35] Other recently introduced NfL ultrasensitive assays are based on microfluidic ELISA systems (ELLA, Proteinsimple)[8,39] and advanced acridiniumester-(AE-) technology (ADVIA Centaur CP System, Siemens Healthineers).[9,40] Here, a strong correlation between the simoa and the ELLA platforms has been reported.[8] Taking into account all these issues, and for the successful implementation of NfL as biomarker for clinical routine applications, a first step is international multicenter round robin studies, with the aim to compare assay performance and correlations across assays. Here, a first such study has recently been published.[41] In the second phase, the development of certified reference materials is needed, meaning large pools of plasma/serum that have been aliquoted into high numbers of samples with specified NfL levels, optimally three different with low/medium and high levels, that can be distributed to kit vendors for quality assurance and harmonization of levels across assays and platforms.

Confounding factors influencing neurofilament light chain protein levels

Beside neuroaxonal damage, several physiological and pre-analytical factors might also influence NfL levels, especially in blood. Although many mechanisms are yet not fully understood, recent pieces of evidence highlighted the significant confounding role of these variables, which should be carefully considered when measuring NfL in both healthy and primary non-neurological conditions (**Fig. 1**). First, a significant inter-individual variability has been reported in normal individuals, especially in the healthy elderly.[42,43] Biological fluctuations may explain variations between consecutive blood NfL measurements in both healthy controls and patients.[44] However, blood NfL seems to be not subject to diurnal changes, as differentially reported for other biomarkers.[42,45] Furthermore, the biological matrix and the type of tube can affect blood NfL measurements with slightly higher values reported in serum compared to plasma samples[46] and with concentrations in citrate plasma >

20% lower than in EDTA plasma.[47] Concerning demographical variables, it is well-known that NfL levels increase with age in both patients and healthy controls, whereas sex does not impact NfL levels in the latter.[43] Both ageing and age-related comorbidities might be responsible of this phenomenon (refer to chapter below).[43] Accordingly, there have been recent efforts to establish age-related reference values for blood NfL.[48–50] Other important counfonding factors are blood volume and body mass index (BMI), which are inversely associated with blood NfL, probably due to a dilution effect driven by blood volume increase.[51] Conversely to blood NfL levels, CSF concentrations were not influenced by blood volume.[51] Taking into account the strong effect of these confounders, recent papers highlighted the importance of age and/or BMI-adjustment of blood NfL values, to improve the biomarker use at an individual level, especially in diseases like multiple sclerosis (MS) with more subtle increases of NfL levels.[50,52] Here, the usage of percentiles (or Z scores which are interchangeable) based on large, international healthy control cohorts allows the quantitative assessment of deviation from normal NfL concentrations.[50] In pregnancy, despite the expanded blood volume, a progressive rise in blood NfL levels until delivery has been reported.[53,54] It remains still unclear whether this increase is driven by structural brain changes (e.g. peri-partum transient grey matter atrophy)[55] or by NfL release from other tissues during pregnancy (e.g. highly innervated uterine muscle).[56] Glycosylated hemoglobin (HbA1c) has been also shown to significantly explain the variability on blood NfL levels.[57] Furthermore, renal function influences blood NfL, with a respective increase in creatinine values (or decrease in glomerular filtration rate) associated with higher biomarker levels.[46,57–59] However, the mechanisms underlying this association are still to be fully elucidated.[58] On another issue, an increase in blood NfL levels has been described in sports associated with even different degrees of traumatic brain injuries, such as football, hockey and boxing.[60–62]

Methods

The methods of this review followed Preferred Reporting Items for Systematic Reviews and meta-analysis (PRISMA) guidelines for scoping reviews. Five reviewers (SAR, AA, MF, LDA, MR) systematically searched PubMed for studies reporting on NfL and non-primary neurological conditions from last database opening on 28th February 2022. The search strategy was performed using specific search terms (Supplementary Methods). Titles and abstracts were screened to select articles relevant to the purpose of the review. Their

reference lists were also hand-searched to increase the identification of useful data. We selected studies assessing the diagnostic and/or prognostic value and/or dynamic of NfL (in CSF and/or blood) in primary non-neurological conditions, including intensive and perioperative care, atrial fibrillation and cardiovascular risk factors, COVID-19, HIV, psychiatric disorders, normal aging and other minor non-primary neurological conditions. The selection was shared among all co-authors. Only studies on humans and articles in English language were included (Supplementary Figure 1).

Neurofilament light chain protein as a biomarker in adult and pediatric intensive care units

Post-anoxic encephalopathy after resuscitated out-of-hospital cardiac arrest (OHCA) is one of the leading causes of long-term neurological disability and mortality in patients admitted to ICUs.[63] To date the prognostic evaluation of neurologic outcome after OHCA is still complex and have poor accuracy, and relies typically on a multimodal approach, including clinical examination, neuroradiological investigations, EEG, somatosensory-evoked potentials (SSEP), and biofluid markers.[64,65] In this regard, neuron-specific enolase (NSE) is currently the only blood biomarker recommended by European and American guidelines for post-cardiac arrest care.[64–68] However, false positives are common as a consequence of haemolysis and only samples taken 3 days after the event may show a prognostic value.[69,70] In the very last years, NfL emerged as a feasible and accurate marker for prediction of survival and neurological outcome after cardiac arrest in both adult and pediatric patients. In this regard, small cohort studies provided already in the early 2010s preliminary data of interest by analysing neurofilament proteins in CSF or blood using standard immunoassays[71–73] (**Table 1 and Supplementary Table 1**). In a huge cohort study based on the Target Temperature Management After Cardiac Arrest trial NfL levels were measured at 24, 48 and 72 hours after OHCA in 717 patients, of whom 360 (50%) showed poor neurological outcome 6 months later. Blood NfL concentrations increased in the first-time interval but remained stable at 72 hours and were significantly higher at all time points in patients with poor compared to those with good clinical outcome, reaching overall an optimal accuracy [area under the curve (AUC) 0.94] in the prediction of poor neurologic outcome at 6 months.[64] Several other studies[65,68,74,75] confirmed the same findings and provided new insights, such as steadily high NfL concentrations even at late time points suggesting the potential use of NfL as both early

and late prognostic marker.[65,76] Overall NfL showed a clearly higher prognostic value than head CT, SSEP, EEG, bedside clinical tests[64] or other serum biomarkers (tau protein, NSE and S100 calcium-binding protein B - S100B),[64,65,68] implicating that the current recommendations in the prognostic evaluation after OHCA could be improved by upgrading with blood NfL.[65,68,74] In this regard, NfL might be a more suitable predictive marker compared to NSE in the early course of the disease.[74,75] Indeed, a multivariable model integrating clinical variables, NSE after 72 hours and NFL on any of the three first days showed a very promising prognostic performance with low risk of false-positive predictions although retaining a low number of false-negative predictions.[75] On another hand, given the high prevalence of electrographic status epilepticus after cardiac arrest and the current debate about its pathogenic role,[77] the finding of higher serum NfL in subjects with electrographic status epilepticus compared to those without the syndrom and similar initial brain injury might suggest an additional secondary brain structural injury due to status epilepticus itself.[77]

Sepsis-associated encephalopathy represents another important contributor of mortality and long-term morbidity in the ICU setting[78,79] Sepsis-associated encephalopathy is defined as a diffuse brain dysfunction related to sepsis without evidence of primary CNS infection.[80] Differently from classic biomarkers, such as S100B protein, and NSE, which generated contradictory findings,[81,82] preliminary data concerning CSF and serum NfL in sepsis-associated encephalopathy were promising. In this regard, NfL seemed to correlate with disease severity, poorer functional outcome after 100 days and also with the extent of MRI brain damage (white matter hyperintensities – WMH).[83]

Among other common complications in the ICU setting, the ICU–acquired weakness shows an incidence of 40% and includes several entities such as critical illness myopathy, critical illness polyneuropathy or critical illness neuromyopathy.[84] Nevertheless, while Fisse *et al*. did not find significant differences in blood NfL levels between patients with and without critical illness polyneuropathy/neuromyopathy at any timepoint,[76] a recent paper disclosed higher blood NfL values in COVID-19 patients with critical illness polyneuropathy/neuromyopathy.[85] Most interestingly, in a cohort of patients admitted to ICU with a broad spectrum of different neurological and non-neurological diseases, NfL maintained the association with outcome irrespective of the underlying disease.[76] Considering the high specificity of NfL for neuroaxonal damage and the difficult assessment of neurological involvement in analgosedated patients, NfL might be thus adopted as a surrogate marker of CNS involvement in all patients admitted to ICU.[76]

In the field of pediatric intensive care, likewise in adults, blood NfL seems to increase more in nonsurvivors compared to survivors after cardiac arrest.[86] Moreover, few pilot studies found elevated blood NfL concentrations in newborns with hypoxic-ischemic encephalopathy to be associated with insult severity evaluated through brain imaging[87–90] and with poor outcomes in those subjects, who undergone mild therapeutical hypothermia, currently a recognised standard care of hypoxic-ischemic encephalopathy.[89,90] Similarly, in preterm infants with neonatal brain injury serum NfL dynamics appeared predictive of motor outcome.[91]

The potential role of neurofilament light chain protein in the perioperative care

The perioperative period can be associated with a wide range of neurological short and long-term sequelae.[92] Clinical and radiological investigations could help to assess major postoperative complications (i.e. stroke), while other subtle manifestations such as post-operative cognitive decline are difficult to diagnose and currently underrecognized.[93] A sensitive, easily accessible and longitudinally measurable biofluid marker for neurological damage might represent a valuable addition to the current diagnostic tools, given that the use of CT and MRI might be limited by logistics, limited sensitivity and spatial resolution.

In this regard, several studies revealed no significant changes in CSF NfL concentrations up to 48 hours after surgery in the absence of neurological complications and regardless of type of surgical intervention.[94–98] Contrary, blood NfL levels seem to show a more dynamic profile. Indeed, a 67% elevation of blood NfL has been reported postoperatively at 48 hours in the study by Evered *et al*.[99] Of note, in around 77% of the patients included in this study major lower limb surgery with general anesthesia and spinal and/or perineural blockade were performed, which might be associated with incidental subclinical nerve fiber injury. Other studies examining other surgery branches, revealed conflicting results. From one side a marked increase in blood NfL has been documented during the procedure and up to seven days postoperative in patients who underwent cardiac surgery, specifically in the group having extracorporeal circulation[100] as well as in cases with perioperative myocardial injury.[101] Furthermore, although a mild increase in NfL 3-5 days post-surgery, no association has been reported between blood NfL and cerebral oxygenation saturation[102] or mean arterial pressure values[101,103] in different types of surgery. On the other side, one study[104] reported an increase of blood tau but not of blood NfL over a follow-up period up to 3 months after major cardiac surgeries. Nevertheless, none of the above-mentioned studies could conclude if the

elevation of NfL (if present) was solely attributed to the surgical intervention and not to the general anesthesia. However, in the study by Deiner *et al*. no elevation of blood NfL has been detected up to 5 hours after inhalation anesthesia without a following surgery, rendering a structural neurotoxic effect, as assessed by NfL, of general inhalation anesthesia to be neglectable.[105]

Among postoperative sequelae ischemic complications represent a significant portion. Here, an early (till post-operative day 3) increase in CSF NfL has been documented in patients with postoperative symptomatic spinal cord ischemic lesions compared to controls in the studies from Winnerkvist *et al*.[106] and Merisson *et al*.[96] Beyond that, post-operative neurocognitive disorders, and among them delirium, are currently underdiagnosed, undertreated, only partially preventable complications, which are associated with long-term morbidity and mortality and a significant financial consequence of up to $17 275 in payments in the one year after the index surgical procedure.[107,108] Bearing in mind that delirium is a manifestation of a putative neurological demise, several studies reported a more accentuated blood or CSF NfL increase in delirium patients compared to those who did not develop delirium,[109–111] peaking at day two and month one[112] and correlating with the degree of MRI white matter injury[109] (**Table 2**). Of interest, cases with higher NfL levels during the preoperative assessment (fourth quartile) had almost 4 times (3.71 OR; 95% CI: 1.09–12.58) higher risk to develop postoperative delirium compared to patients with lower NfL levels (first quartile).[112] Therefore, two main underlying pathophysiological mechanisms might be responsible for postoperative delirium: 1) structural alterations associated with neuroaxonal loss 2) concomitant neurodegeneration (reflected by higher NfL levels) predisposing or concurring to the development of delirium. On another issue, NfL was also explored in post-operative cognitive decline, which can be usually diagnosed between 30 days and 12 months after surgery.[99] Here a couple of studies reported a post-operative increase in serum NfL without any significant correlation with cognitive performance after 3 months.[98,102] Likewise, CSF NfL measurement collected during spinal anesthesia failed to predict delayed post-operative neurocognitive disorders.[113]

Associations between atrial fibrillation, other cardiovascular risk factors and neurofilament light chain protein

Patients with atrial fibrillation show a higher incidence of both ischemic stroke and cognitive impairment compared to the non-atrial fibrillation population.[114–117] In this regard, several possible pathogenetic mechanisms might be responsible, ranging from subclinical embolic ischemic infarcts, or chronic cerebral hypoperfusion to shared cardiovascular risk factors.[118] Given its sensitivity for neuroaxonal damage, blood NfL has been recently evaluated in patients with atrial fibrillation, with the aim to identify possible interconnections between atrial fibrillation, neuronal damage and dementia.[119] Hence, Polymeris *et al.*[119] found an independent positive association between CHA2DS2-VASc score[114] and serum NfL in atrial fibrillation patients without recent stroke or transient ischemic attack. Higher CHA2DS2-VASc score was in turn related with an increased risk of dementia in these groups,[120] with higher NfL levels associating with a worse cognitive performance.[119] The authors suggested that a complex relationship between cerebral ischemia and hypoperfusion (as suggested by the associations between NfL, white matter lesion volume at MRI, heart failure and lower mean arterial pressure) and neuronal damage (caused by ageing and brain atrophy) may be involved in the pathogenesis of cognitive impairment in patients with atrial fibrillation.[119,121]

As concern cardiovascular risk factors, serum NfL levels have been investigated in patients with diabetes mellitus, hypertension and habitual cigarette smoking.[35] Diabetes mellitus has been found to independently associate with higher serum NfL levels in atrial fibrillation patients, with increased levels of HbA1c positively correlating with serum NfL concentration.[119,122] Furthermore, a cross-sectional study showed increased blood NfL levels in patients with type 1 diabetes mellitus and impaired awareness of hypoglycemia,[123] and an association between high marker levels, reduced cerebral gray matter volume and higher degree of hypoglycemic severity unawareness. Indeed, small vessel disease and non-ischemic glycemic dysregulation-induced neuroaxonal central nervous system injury may be responsible for the increase in serum NfL levels observed in diabetes mellitus patients.[119,124,125]

Data concerning blood NfL levels in patients with arterial hypertension appear discordant. Few studies recently reported higher blood NfL levels in hypertensive than normotensive patients[122,126] while CSF NfL levels were similar between these groups.[126] On the other side, lower mean arterial pressure resulted to be inversely related with blood NfL concentration in atrial fibrillation patients, perhaps as a consequence of cerebral hypoperfusion.[119] These

findings may be explained by the neuropathological evidence of a higher rate of brain infarcts in subjects showing either late-life higher average systolic blood pressure (SBP) or faster decline in SBP.[127] Given that elevated SBP has been linked to the pathogenesis of atherosclerosis and arteriolosclerosis,[127] serum NfL levels were found to be increased in patients with MRI signs of small vessels disease[128] or with recent small subcortical infarcts.[129] On the counterpart, faster decline in SBP may cause cerebral hypoperfusion, which is a well-known risk factor for accelerated cognitive decline, potentially increasing serum NfL levels.[127,130]

Finally, the relationship between cigarette smoking habit and NfL needs to be further investigated. Indeed, a recent longitudinal study found 20% higher serum NfL levels in smoker patients with multiple sclerosis included in the BENEFIT trial at long-term assessment (11 year) when compared to nonsmokers.[131] On another hand, in patients with atrial fibrillation, past smoking status was negatively associated with serum NfL levels.[119]

Neurofilament light chain protein in major human infectious diseases

COVID-19

Since severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) pandemic outbreak in January 2020 a plethora of neurologic syndromes have been described in association with coronavirus diseases 2019 (COVID-19).[132–137] Furthermore, general neurologic symptoms (e.g. delirium, headache, dizziness, anosmia) are reported in 30% of all COVID-19 cases,[134,138] although subtle CNS involvement might be also more frequent and still underrecognized.[139] Central and peripheral nervous system damage in COVID-19 may be driven by multiple mechanisms encompassing direct viral invasion, cytokines storm, para- or post-infectious autoimmunity and secondary effects of severe systemic disorder.[140,141] Taking in to account these findings, NfL might help to clarify the pathophysiological mechanisms underlying neurological complications of COVID-19, as well as the presence of subtle neuro-axonal injury. In this regard, the marker levels have been assessed in COVID-19 patients with encephalopathy,[142–145] stroke,[144] Guillain Barré-syndrom spectrum,[145,146], headache[145], critical illness polyneuropathy/neuromyopathy[85] and multisystem inflammatory syndroms[147,148] (**Supplementary Table 2**). In some reports, patients with COVID-19 encephalopathy showed a CSF profile more consistent with a neuroinflammatory process rather than with a neuro-axonal demise (e.g. normal NfL levels, but increased concentrations of neuroinflammatory

biomarkers).[142,143] Similarly, prominent neuroinflammatory changes, along with increased biomarkers of glial activation, were disclosed in 13 cases with COVID-19 related encephalitis, while only patients with severe neurological symptoms exhibited very high CSF levels of NfL and total tau (t-tau).[149] Conversely, other studies[144,145] found in the same diagnostic groups increased CSF NfL and t-tau levels as neurochemical evidence of widespread damage following systemic concurrent processes, such as hypoxia, sepsis and hypercoagulability. Here, neuroinflammatory changes were observed only in COVID-19 stroke patients.[144] Furthermore, when compared to COVID-19-related encephalopathy or headache, COVID-19-associated inflammatory neurological conditions (e.g. meningitis, encephalitis, myelitis, acute disseminated encephalomyelitis) showed higher median CSF NfL concentrations, maybe due to a more severe axonal injury in the presence of an exacerbated inflammatory process.[145,150]

By stratifying patients according to disease course, mild-to-moderate COVID-19 cases with only minor (e.g. anosmia, headache) or without neurological symptoms showed higher blood NfL levels compared to age-matched control subjects, suggesting the presence of a subtle neuro-axonal damage in the former group.[151,152] Interestingly children with asymptomatic to moderate COVID-19 showed no increase in blood NfL values, in line with the higher rate of neurologic complications in older COVID-19 patients.[147] Otherwise, patients with severe COVID-19 seem to display early peak in blood glial fibrillary acidic protein (GFAP) levels and sustained sNfL increase during disease course, reflecting an early astrocytic response followed by a lagged neuro-axonal damage.[154–156] However, apart from one study[156] the persistence of neurological symptoms or chronic fatigue after recovery from SARS-CoV-2 infection seems not to be associated with a significant neuroaxonal damage, as suggested by several studies.[155,157,158] In particular, blood NfL normalized at 6 months of follow-up with no association between the initial increase in the acute stage and the presence of long-term symptoms.[155]

From the prognostic point of view, NfL might play a predictive role for COVID-19 severity and outcome, as suggested by either cross-sectional or prospective studies.[152,154,155,159–161] Specifically, elevated CSF or blood NfL levels seem to be associated with a higher rate of ICU admission and mechanical ventilation,[152,159,162] prolonged ICU stay,[159] as well as severe disease course and unfavorable clinical outcome.[160–163] Moreover, NfL concentrations in non-survivors seems to increase over repeated measurements, whereas levels in survivors might be stable over time.[163] Of note, a single study showed a trend toward lower serum NfL levels

in hospitalized COVID-19 patients treated with remdesivir, potentially opening future perspectives in the monitoring of treatment response.[164]

HIV

NfL may represent a valuable tool to evaluate ongoing CNS injury during HIV infection with potential applications either in clinical and research settings.[6,165] Indeed, increased levels of CSF NfL have been widely reported in HIV-patients, not only in those with HIV-associated dementia (where the marker reached the highest levels) but also in a proportion (about 30%) of untreated "neuro-asymptomatic" patients and in those with reduced (<250 μ/L) blood CD4+ cell count.[6,165] Furthermore, antiretroviral therapy seems to potentially reduce CSF NfL values,[6,165] although treated and virologically suppressed patients may exhibit persistently increased CSF NfL levels, suggesting a subtle but significant HIV-related neuronal pathogenicity.[165] Finally, preliminary data concerning the role of plasma NfL as a pharmacodynamic marker for antiretroviral drugs showed no biomarker evidence of neuro-axonal injury when switching tenofovir to a different formulation with potentially increased CNS exposure.[166]

The role of neurofilament light chain protein in primary psychiatric disorders

One of the reasons influencing the current underestimation of the true global burden due to mental illness disease might rely on the great overlap between psychiatric and neurological disorders.[167] In this regard, several studies investigated the potential value of NfL, on either CSF or blood, across the heterogeneous spectrum of primary psychiatric disorders and in the differential diagnosis with primary neurologic diseases with conflicting results (**Supplementary Table 3**). Firstly, few studies[168–171] demonstrated higher CSF NfL concentrations in patients with bipolar disorder, schizophrenia and depression compared with control groups. Interestingly the association between the marker and decreased cognitive performance in patients with bipolar disorder[169] seems to be in accordance with structural imaging studies,[172,173] which showed an association between cognitive decline and deep white matter hyperintensities. Similarly, the increase of CSF NfL in patients with schizophrenia and depression might support the notion that both diseases may incorporate neurodegenerative aspects.[174] Very recently, NfL has been found to be elevated also in blood samples of patients

with anorexia nervosa[175–177] with a negative correlation with BMI[176] and a tendency to decreased levels after weight restoration.[175,177] These results may support the thesis of ongoing mild neuronal injury during the underweight phase of anorexia nervosa, a phenomenon that is also highlighted by the evidence of MRI structural grey matter or white matter changes, with many of these findings appearing to reserve upon recovery.[178] Of note, in these studies the degree of NfL increase was relatively minor in all psychiatric disorders compared to other diseases.

On another issue, there is accumulating evidence that both CSF and blood NfL might be a potential discriminative biomarker between primary psychiatric disorders and neurodegenerative disorders[179,180], such as frontotemporal dementia,[181,182] which may share a significant clinical overlap.[183] Few preliminary studies[180,184] documented significantly lower CSF NfL levels in patients with several types of primary psychiatric disorders compared to patients with neurodegenerative [e.g. behavioral variant of frontotemporal dementia (bvFTD)] or neurological disorders and a high diagnostic accuracy for the marker in the discrimination between the two groups.[180] Similarly, Al Shweiki *et al*. was the first to report elevated plasma NfL in bvFTD compared to schizophrenia, depression, and bipolar disorder and being discriminative from these disorders with AUCs ranging between 0.89 and 0.94.[185] A similar discriminative value (AUC = 0.83) was also documented by Katisko *et al.*[186]

Neurofilament light chain protein in other non-primary neurological conditions

NfL levels have been sparsely investigated in other non-primary neurological conditions, including gynecological and dermatological disorders, as well as in diseases related to physical agents. Preliminary findings from available studies are reported in Supplementary Text.

The interplay between normal ageing and neurofilament light chain protein

Normal aging is associated with gradual atrophy and microstructure alterations of grey and white matters.[43,187] Thus, several morphologic and biochemical instruments have been investigated with the attempt to accurately estimate these processes.[43,187] In this regard, several cross-sectional, longitudinal and population-based studies revealed a marked progressive increase of CSF and blood NfL with ageing and a significantly higher variability of the biomarker levels in older subjects, suggesting from one side the accelerated neuronal

injury at older age, and from the other, the possible contribution of subclinical brain tissue damage driven by ageing-related comorbidities.[18,43,65,188–192] Nevertheless, besides neuronal damage, a reduced CSF turnover may contribute to NfL rise with ageing.[43] Moreover, the marker has been variably associated with ageing-related brain white matter alterations, brain volume changes and neuropsychological performances among different studies,[43,190,191] confirming similar results in CSF[193] and supporting the existence of a continuum between physiological aging and incipient, subclinical pathology, and manifest disease.[191] In line with this theory, in cognitively unimpaired subjects elevated CSF NfL levels have been strongly associated with risk of mild cognitive impairment and hippocampal atrophy independently from Alzheimer's disease pathology.[194,195] However, a recent wide cohort study found no association between plasma NfL and cognitive decline or incipient Alzheimer's disease,[190] not excluding, however, a possible contribution of NfL in predicting cognitive impairment other than Alzheimer's disease.

So far, two recent population-based studies scrutinized the significance of NfL for the prediction of survival in elderly healthy individuals.[191,192] In the first, lower serum NfL levels were associated with longer survival in nonagenarians and centenarians and predicted mortality with a better performance compared to previously described multi-item scales of cognitive or physical functioning.[192] In the same paper, mice undergoing a low-calorie diet, a regimen that has been associated with extended survival showed attenuated age-related increase in plasma NfL and lived longer than mice in a control group.[192] The second work focused on elderly individuals without pre-defined neurological conditions, and revealed that serum NfL, but not tau, was a significant prognostic marker for all-cause mortality if no detailed clinical history or MRI data was available.[191]

Discussion

Close may be the time where biofluid biomarkers and, especially NfL, can represent an easily accessible, cheap and reproducible tool to investigate the neurological involvement in human subjects in different clinical fields. Here, we provided an extensive overview of currently available data on CSF and blood NfL in non-primary neurological conditions, including diseases mostly belonging to the intensive care, surgery, cardiology, psychiatry and infectious disease panoramas together with physiological states such as normal ageing (**Fig. 2**, **Fig. 3**).

Firstly, evidence suggests the potential value of blood NfL as a useful test in the prognostic evaluation in hypoxic-ischemic encephalopathy mostly after OHCA with relevant clinical implications. Indeed, the marker might help clinicians to stratify subjects with the aim to take rapid, safe and ethical decisions and carry on early therapeutic interventions in patients with good prognosis and to avoid prolonged and futile treatment in those without chance of adequate survival.[70,76,196] Indeed, more than 80% of patients admitted to ICU after resuscitation in case of OHCA develop hypoxic-ischemic injury and 2/3 of them die after withdrawal of life-sustaining treatment due to a predicted poor neurological outcome.[63] In this regard, NfL assessment might improve the prognostic accuracy of current clinical scores and multivariable algorithms and, interestingly, seem to outperform not only the performance of classic fluid markers (e.g. NSE) but also that of neurophysiological and neuroimaging investigations. In the era of COVID-19 pandemic this issue could be also relevant with the view to optimize the high occupation rate of ICUs.

Strictly connected to the above-mentioned topic is the investigation of the perioperative dynamic of NfL, which might provide valuable clues on understanding the effect of surgery, surgical procedures, such as extracorporeal circulation during cardiac surgery, and anaesthetic drugs and anesthesic regimens (such as blood-pressure control) on neuronal health. Given the lack of an elevation in CSF NfL (at least over the short period of follow-up time) both after surgery and inhalation anesthesia, an early relevant CNS structural damage associated with uncomplicated non-neurological surgeries appears to be less likely. Hence, the reported high blood NfL levels after major cardiac and orthopaedic surgeries might be potentially explained by the damage of peripheral nerves during surgical manipulations, or by the post-surgical temporary distribution of the renal functions. Indeed, an acute kidney injury typically occurs within the first few days in up to 30% of patients undergoing cardiac surgery,[197] which partially coincides with the reported post-operative dynamics of NfL in the studies mentioned above. One hint supporting this notion is also the association between NfL levels and blood loss volume during surgery.[101,109] Moreover, the finding of elevated blood NfL concentrations in postoperative delirium has some relevant implications. From one side it supports the notion that postsurgical delirium is accompanied by a structural neuronal damage. On the other side, since preoperative blood NfL values seem to be predictive for postoperative delirium, adding NfL measurements to the standard presurgical evaluation might help the clinicians to identify subjects who are more vulnerable to this complication with the aim to early initiate appropriate preventive strategies and to better select patients in clinical trials. In contrast to these promising potentials, NfL seems to not predict long-term

postoperative cognitive impairment, suggesting that subtle central nervous system injury should take place over the prolonged postoperative period.

In the cardiological field the evidence of associations between NfL and most cardiovascular risk factors is growing and support the well-known notion of a strict interplay between heart, vascular and brain diseases. In this regard, the potential application of NfL rely on its usefulness to detect subclinical neuronal damage in patients with cardiovascular risk factors and to stratify patients with atrial fibrillation at risk of cognitive impairment, allowing the initiation of intense preventive and follow-up measures.[121]

On another issue, the current experience of COVID-19 pandemic has prompted the scientists to reconsider the management of infectious diseases as a priority in worldwide healthcare systems. The data collected in one year of pandemic together with those in recent years on other endemic diseases, such as HIV, suggested that NfL measurement in biofluids might be useful to track pathophysiological changes in nervous system (e.g. infection-associated neuroaxonal damage concomitant or after neuroinflammation, underlying mechanisms of long-COVID syndrome) and to monitor disease trajectory and long-term complications even in subjects without early and full-blown neurological manifestations. In this regard, higher levels of CSF and/or blood protein might predict a higher grade of disease severity and/or unfavorable outcome. Notably, due to the limited accessibility for current neurophysiological and neuroimaging investigations in the context of COVID-19 pandemic-imposed restrictions, easily measurable blood markers can also be advantageous by providing useful diagnostic and prognostic information by overcoming the limit of medical isolation procedures.

Concerning the potential role of NfL in psychiatric diseases current data are discordant. From one side some pieces of evidence revealed higher concentrations of the marker in those conditions, such as schizophrenia, bipolar disorder, depression and anorexia nervosa, where a neurodegenerative process, even subtle, could not be excluded. From the other side, many studies supported the role of NfL in the biochemical discrimination between primary psychiatric disorders and neurodegenerative disorders, such as bvFTD. Nevertheless, the pathophysiological processes underlying NfL raise in patients with primary psychiatric comorbidities is still partially unclear and should be further addressed in future studies.

In other conditions belonging to the fields of gynecology, dermatology and disorders due to physical agents, evidence on NfL is still limited and should be addressed in further studies. Nevertheless, promising findings concern the potential role of NfL as an accurate predictor of preeclampsia development.

In the present review we decided to include a short focus on normal ageing, given its relevance as a unique physiological entity in the continuum between healthiness and neurodegeneration. In this regard, recent studies showed a significant role for blood NfL in the prediction not only of future cognitive decline but also of mortality in the elderly. Here, given that MRI and neuropsychological evaluations are often not available in the routine due to costs and time-consume, blood NfL might be a feasible tool measured in the next future within a prognostic check-up in elderly individuals.

Although the very promising results, the application of NfL in the routine setting of all above-mentioned fields is currently hampered by some unresolved issues and limitations which deserve further analyses. First, in the studies concerning OHCA, surgery and COVID-19, many patients could have suffered from age-related neurological comorbidities such as Alzheimer's disease, and cerebrovascular disease, which are notoriously associated with increased NfL levels, therefore influencing the final diagnostic and prognostic values. However, given the relatively low blood marker concentrations in these diseases the effect on prognostication is, from our point of view, quite unlikely.[64] Similarly, renal excretion dynamics and medications used in ICU and anesthesia might also influence NfL levels. Nevertheless, the decrease in NfL concentrations after 5 h from general anesthesia inhalation compared to baseline may suggest that NfL levels in ICU patients might be even larger in magnitude but partially masked by anesthesia-induced decrease.[105,163]

Second, the marker cut-offs maximizing the diagnostic and prognostic accuracies, even when analysed at the same time-points, are heterogenous across studies. Possible contributors in these terms might also be the relatively lack of longitudinal evaluations compared to cross-sectional analyses, different cohort selection criteria and assay types. Here, large studies in control populations have recently been published or are under way to develop age and BMI-adjusted reference ranges and to allow the evaluation of NfL values independently of these confounding factors. The aim is to establish reference values to understand which levels are in the normal range and how strongly individual values may deviate from the physiological state to better estimate which NfL changes might be considered clinically relevant. Nevertheless, the definition of an optimized clinically useful cut-off value or meaningful change in NfL levels that would be applied to more than one condition remains to date challenging. For example, in the Target Temperature Management After Cardiac Arrest trial, the difference in biomarker medians between subjects with poor and good outcome was 3290 pg/ml (3344 vs 54 pg/ml) at 72 h after OHCA.[64] Conversely, in MS, a change of 5 pg/ml (from 10 pg to 15 pg/ml) in a 40-year-old patient leads to a change in age- and BMI-adjusted

Z score from 1.3 (90.3 percentile) to 2.1 (98.2 percentile), which is a level that is associated with a 3.15-fold increase in risk for developing disease activity.[50] Thus, in subjects after OHCA, an increase of just 5 pg/ml is not expected to have relevant clinical meaningfulness. Moreover, it is still a matter of debate whether single or repeated measurements with trends over time might further improve the sensitivity of this biomarker to predict neurological outcome in non-primary neurological diseases. Hence, further studies are needed to evaluate the inclusion of NfL in validated predictive multi-modal models, which include clinical scores, electrophysiological and neuroimaging investigations. In addition, the NfL increase in chronic conditions (i.e., subclinical neurodegeneration, minor cerebrovascular damage, etc.) could make the interpretation of levels following acute injuries misleading. Moreover, although the availability of many commercial kits, NfL measurements are not yet standardized and worldwide accessible given the significantly high costs.

Taking into account all these issues, it is likely that an estimation of how strongly individual NfL measurements deviate from normal levels based on large and adequately adjusted (e.g. age, BMI, renal function, etc.) reference datasets reflecting the general population, might be an important advancement compared to the mere analysis of raw and unadjusted levels.[48,50,57] In this regard, NfL levels might be a general marker of CNS health and thus, may play a potential role in complex frailty indices for geriatric patients that suffer on comorbidities, which affect NfL levels (i.e., neurodegeneration, renal and cardiovascular diseases, diabetes, etc.).

In conclusion, available data outline a multifaceted applicability of CSF and blood NfL measurement, first as an independent marker of neuroaxonal damage and, more generally, of nervous system involvement in several primary non-neurological diseases. The great diagnostic and prognostic performances of NfL might be soon exploited to accelerate the diagnostic ascertainment in critical clinical settings, optimise a precision medicine approach, simplify patient recruitment in clinical trials and test the potential benefit of new therapeutical agents.

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Supplementary material

Supplementary material is available at *Brain* online.

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Figure legends

Figure 1 Confounding variables affecting blood NfL levels in healthy subjects and patients. Blood NfL may be affected by several physiological and pre-analytical confounding factors which can increase (red area: aging, pregnancy, sports injury, increased glycated hemoglobin – HbA1c) or decrease (blue area: increased body mass index and blood volume) its levels. Other variables, including renal function, biological matrix of the sample, biological fluctuations and inter-individual variability, can influence NfL blood levels bidirectionally (yellow area). Created with BioRender.com

Figure 2 Most promising applications of CSF and blood NfL in non-primary neurological diseases and current limitations. The figure shows the most promising applications of CSF and blood NfL in the medical fields of intensive care, surgery, internal medicine and psychiatry. Furthermore, major limitations of the marker hampering its clinical utilization on a wide scale are displayed. Created with BioRender.com

Figure 3 Distribution of blood NfL levels in most important non-primary neurological diseases. The figure shows blood NfL elevation in several non-primary neurological diseases which have been examined in the present review. Narrows represent approximate mean fold increases of blood NfL in each condition compared to controls. We obtained the approximate mean fold increase values based on available blood NfL values reported in papers included in the review.[64,65,68,74,100,154,161,164,176–178,186,187] Created with BioRender.com

Table 1 Main studies exploring NfL performance in the adult and pediatric ICU setting and mostly in patients after cardiac arrest

Paper	Country of studied population	Examined biofluid	Assay	*n*	Primary outcome and timing	Good/poor Outcome, *n*	Sample timing (after CA)	Best AUC (sample timing)	Sens (%)	Spec (%)	Cut-off
Anderss on *et al.*[75]	Multicentric: Europe and Australia	Serum	Simoa	939 adults with OHCA	CPC at 6 months	440/492	24, 48, 72 h	0.92 when NfL analyzed alone.	-	-	-
Disanto *et al.*[65]	Switzerland	Serum	Simoa	14 adults with OHCA	Death at 1 month	7/7	Median 2 (1–3) days, range (0–17 days)	0.98	83	100	3437 pg/ml
Fisse *et al.*[76]	Germany	Serum	Simoa	35 adults admitted to ICU	mRS at end of follow-up (median follow-up time 26 days)	-	Day 1 and every 7 days during ICU treatment	-	-	-	-
Goeral *et al.*[91]	Austria	Serum	Simoa	48 preterm infants with PIVH	Neurodevelopment (motor and cognitive skills) or death at 2 years	22/24	From 3 to 7 time points (not specified time)	0.71	-	-	-
Hunzike r *et al.*[74]	Switzerland	Serum	Simoa	164 adults with OHCA	CPC at hospital discharge	66/98	Day 1	0.77	72	82	50 pg/ml
Kirsche n *et al.*[86]	USA	Serum	Simoa	32 children with ARDS and OHCA; 18 healthy controls	Survival	13/19	Within 24 h (from ARDS onset)	0.78	-	-	-
Moseby-Knappe *et al.*[64]	Multicentric: Europe and Australia	Serum	Simoa	717 adults with OHCA	CPC at 6 months	357/360	24, 48, 72 h	0.94 (Day 3)	70	99	1122 pg/ml
Rana *et al.*[73]	Germany	Serum	ELISA Thermo-Fisher	85 adults with OHCA	mGOS at 6 months	43/18	Days 1 (within 2 h), 2, 3, 5, 7	0.99 (Day 7)	94	100	252 pg/ml
Rosén *et al.*[71]	Sweden	CSF	ELISA	22 adults with OHCA	GOS at best-recorded result at any of the time points (12 days -1 year)	10/12	Mean 17.5, range 12–30 days	-	92	90	9622 µg/l
Rosén *et al.*[72]	Sweden	CSF	ELISA Uman Diagnostic	21 adults with OHCA	GOS at best-recorded result at any of the time points (12 days -1 year)	10/11	12–14 days	-	-	-	-
Shah *et al.*[89]	UK	Plasma	ELISA IBL	26 newborns with hypoxic-ischemic encephalopathy treated with therapeutic hypothermia for 72 h	MRI pattern predictive of outcome	13/13	At target temperature, prior to rewarming, and after rewarming	0.97 (after rewarming)	92	92	417 pg/ml

Wilshesari *et al.*[68]	Finland and Denmark	Plasma	Simoa	112 adults with OHCA	CPC at 6 months	73/39	Admission, 24, 48, 72 h	0.98 (Day 3)	85	99	344 pg/ml

See also Supplementary Table 1. ARDS = Acute respiratory distress syndrome; AUC = area under the curve; CA = cardiac arrest; CPC = cerebral performance categories scale; CSF = cerebrospinal fluid; ELISA = Enzyme-Linked Immunosorbent Assay; GOS = Glasgow Outcome scale; ICU = intensive care unit; mGOS = modified Glasgow Outcome Score; mRS = modified Rankin Scale; NfL = neurofilament light chain protein; OHCA = out of hospital cardiac arrest; PIVH = peri/intraventricular hemorrhage; Sens = sensitivity; Spec = specificity; - = not reported.

Table 2 Blood NfL in post-operative delirium

Study	Examined biofluid	Assay	n	Design	Incidence of delirium in the study	Serum NfL levels in both groups (Delirium versus no delirium, respectively) (postoperative day, total follow-up days)	Postoperative NfL dynamics in blood	Correlation between NfL and Delirium scores	Factors affecting NfL concentration
Casey *et al.*[109]	Plasma	Simoa	102 patients[b]	prospective observational study	36.1%	Higher levels in delirium patients compared to no-delirium group $P < 0.001$	Mean difference pre-post operative 0.240log10(pg/ml) (POD1) with more pronounced increase in the delirium group	Improvement of the overall model fitness by the addition of NfL ΔR2 = 0.199	IL-8, IL-10 and IL-1B concentrations; duration of operation and volume of blood loss
Fong *et al.*[112]	Plasma	Simoa	108 patients[a]	Nested matched case-control as a part of a prospective observational study	50%	Median paired difference 16.2 pg/ml and 13.6 pg/ml (POD2, 30, respectively)	Remained elevated at Day30	Change in NfL level from preoperative to POD2 correlated with delirium severity (rank correlation coefficient= 0.31)	-
Halaas *et al.*[110]	Serum and CSF	ELISA	314 patients with hip fracture and 135 healthy controls[b]	Prospective observational study	51.6%	94 versus 54 pg/ml 135 versus 92 pg/ml (preoperative, POD5)	-	-	-
Saller *et al.*[111]	Serum	Simoa	9 patients	Case series	33.3%	No significant difference between both groups (n = 6 versus n = 3)	In the group developing delirium, up to a seven-fold increase in NfL at the time of discharge (POD4-30)	-	-

CSF = cerebrospinal fluid; ELISA = Enzyme-Linked Immunosorbent Assay; IL = interleukin; POD = postoperative day; - = not reported

[a]Age-matched control group.

[b]Age-adjusted analysis, or age in control group did not differ statistically from patient group.

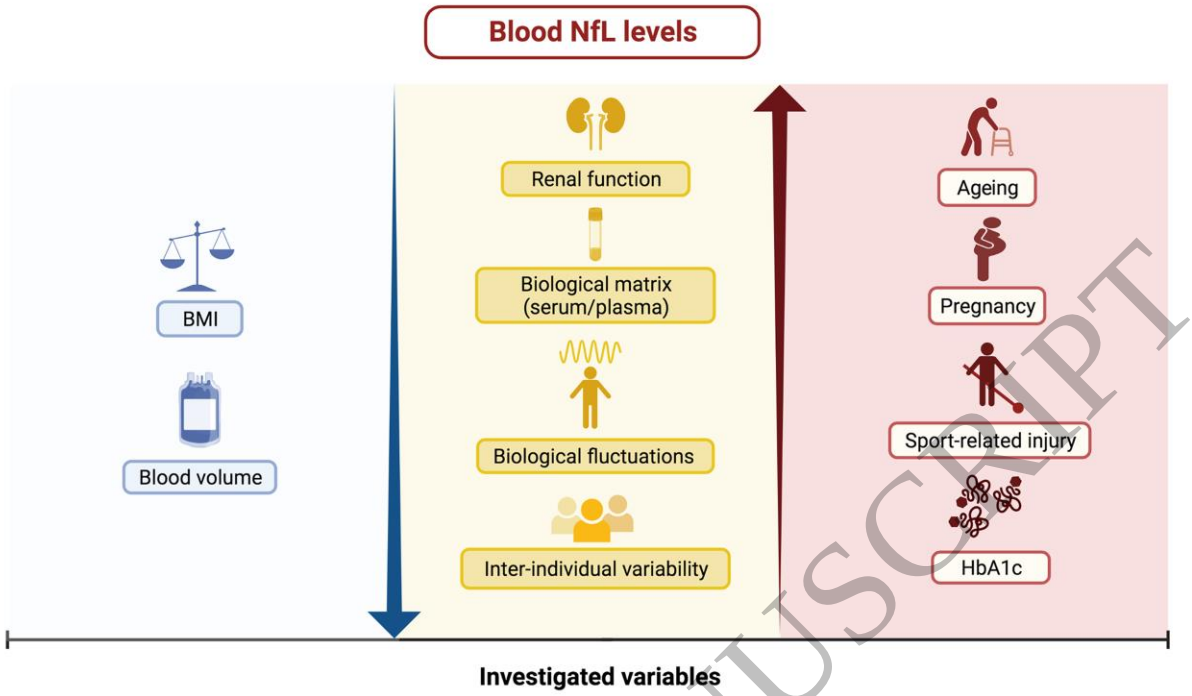

Figure 1
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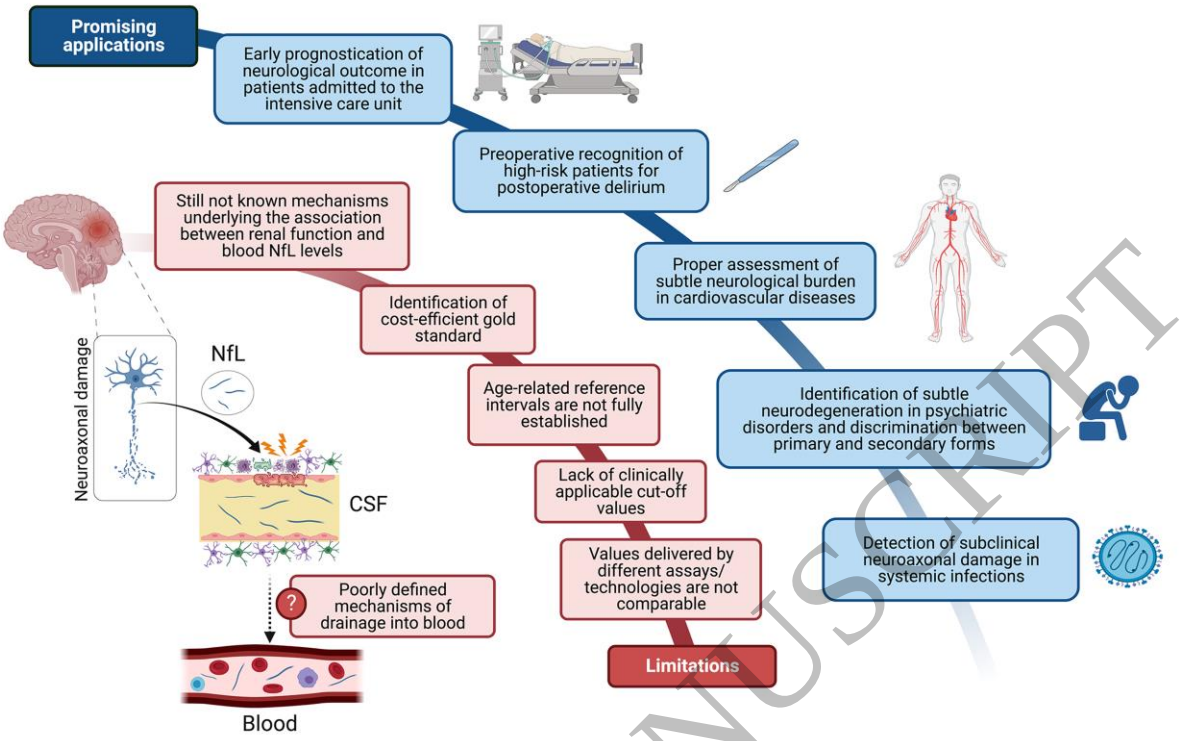

Figure 2
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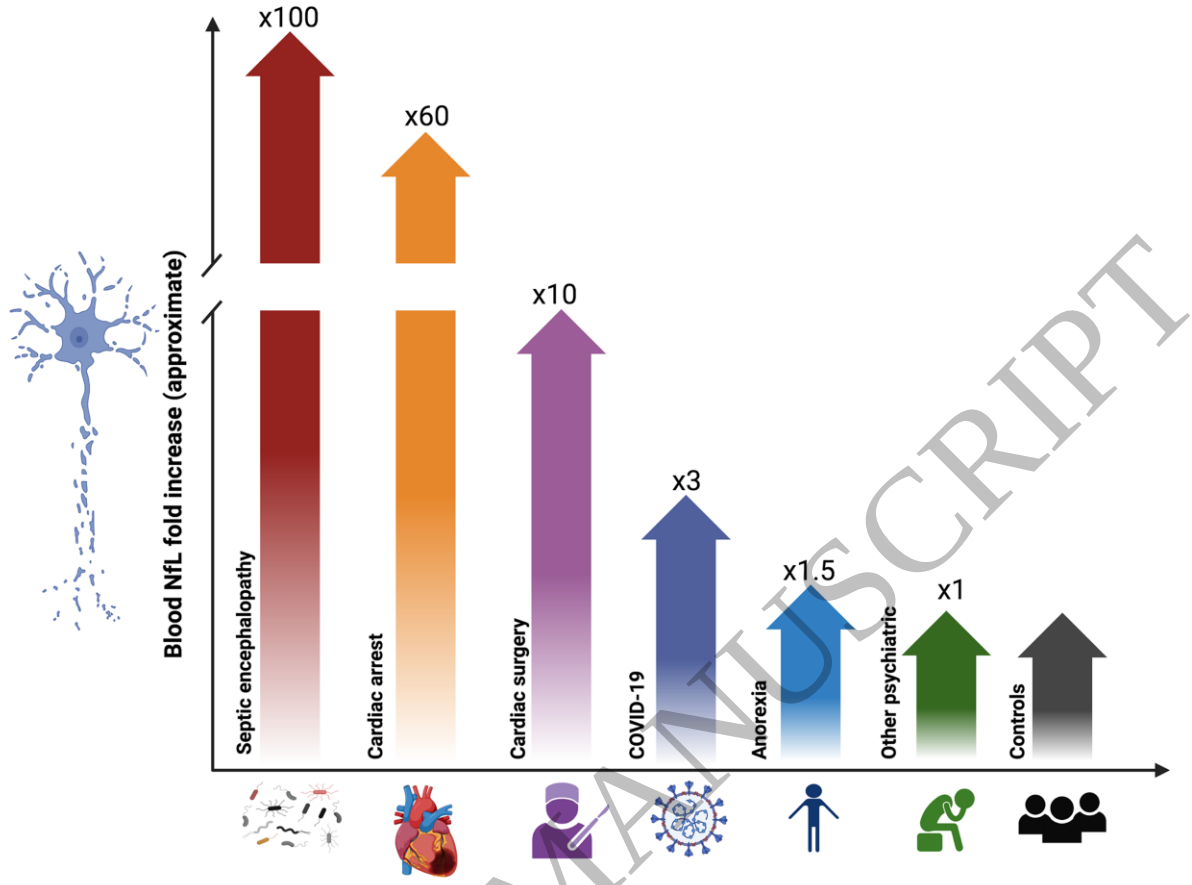

Figure 3
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