

Title: Blood Neurofilament Light Chain Measurement toward Clinical Application: A Review

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Abstract

Biomarkers are necessary for the evaluation of disease activity and treatment effects in neurological diseases. However, current tests such as cerebrospinal fluid analysis or imaging cannot be used widely because of several limitations, such as high invasiveness, high costs, and a limited number of tests per day. The blood neurofilament light chain (NFL) assay is a promising test for neuronal injury with low invasiveness and high scalability and has attracted a great deal of attention. It became more precise and accurate because of the newly developed ultrasensitive immunoassay called the Simoa assay. Blood NFL assay could reportedly track disease activity, monitor treatment response, and predict clinical outcomes in various neurological diseases such as multiple sclerosis, amyotrophic lateral sclerosis, dementia, stroke, and traumatic brain injury. In this review, we describe the scope and limitations of blood NFL assay in neurological diseases and future research directions needed for clinical application.

Keywords: Neurofilament light chain; Single-molecule array assay; Blood assay; Neuronal injury; Renal function

Introduction

It is essential to evaluate disease activity to determine the effect of treatment and predict prognosis in managing neurological diseases. To date, neurologists have evaluated the extent of neuronal injury by cerebrospinal fluid (CSF) analysis or imaging methods, such as computed tomography, magnetic resonance imaging (MRI), 18F-fluorodeoxyglucose positron emission tomography, and N-isopropyl-4-[123I] iodoamphetamine single-photon emission computed tomography. These methods provide valuable information but require large and expensive instruments and have low feasibility for the number of tests performed per day. Blood tests have advantages in diagnostic methods requiring repeated measurements because of their low invasiveness and high scalability. In recent years, blood tests for nerve damage assessment have been gaining attention, with blood neurofilament light chain (NFL) currently being the most reliable biomarker.

NFL in the blood

NFL is a neuronal cytoskeletal protein that is primarily expressed in large-caliber myelinated axons. [1,2] When a neuroaxonal injury occurs, NFL proteins are released into the interstitial space through the disrupted cell membrane and diffuse into the CSF and blood; hence, its increased concentration is detected in these fluids. Conventional enzyme-linked immunosorbent assay (ELISA) and electrochemiluminescence methods can quantify NFL levels in the CSF but not in blood, because the amount of NFL in blood is only 1/500 of that in the CSF. Ultrasensitive immunodetection methods enabled reliable quantification of NFL in blood. The most rigorous and reliable method is the single-molecule array (Simoa) assay, also called digital ELISA. This method is based on microbeads (2.7µm in diameter) and microwells (4.25µm in diameter), which are far smaller than those used in conventional ELISA methods (~7 mm in diameter), achieving a very low background level. Furthermore, it detects a signal from only one antibody-antigen complex, thus requiring a much smaller number of NFL molecules and having high analytical sensitivity to the femtomol/L range. The NFL assay on the Simoa platform showed a strong correlation between NFL levels in the CSF and serum or plasma, which

paved the way for assessing neuroaxonal injury using blood tests [3-5].

Blood NFL levels in neurological diseases

According to previous reports, blood NFL levels are elevated in various neurologic conditions, including multiple sclerosis (MS), stroke, amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), and traumatic brain injury (TBI). Blood NFL levels can also be used to monitor disease severity and predict long-term outcomes. In specific situations, it may help support a differential diagnosis. The most studied neurological disease with blood NFL analysis is MS. The current role of biomarkers to monitor neuronal injury is limited. Usually, it is evaluated by MRI, but its high cost and long scan time limit its utility. Blood NFL level elevation is reported to be correlated with relapse of symptoms, as well as new lesions on MRI scans, in both progressive and relapsing MS in many studies [3, 5-10]. The decrease in blood NFL levels was observed after treatment, indicating its potential for monitoring treatment effect. [8,11,12] Blood NFL level responds to anti-inflammatory therapies 3 to 6 months after their initiation, with its degree of difference reflecting treatment efficacy. [7] Blood NFL level is also elevated after stroke. It takes several days to rise, is correlated with symptom severity and lesion size, and can predict functional outcomes and the onset of postinfarct depression. The elevation can take several hours to more than a week, and it remains elevated for several months. Patients with ALS have higher blood NFL levels 1 year before symptom onset, thus helping in predicting incidence. Blood NFL levels can differentiate ALS from its mimics, and it can be an independent predictor of clinical prognosis. In FTD, a higher blood NFL level is

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Received: 03-Jan-2022, Manuscript No. jcen-21-25152; **Editor assigned:** 05-Jan-2022, PreQC No. jcen-21-25152(PQ); **Reviewed:** 19-Jan-2022, QC No. jcen-21-25152; **Revised:** 24-Jan-2022, Manuscript No. jcen-21-25152(R); **Published:** 31-Jan-2022, DOI: 10.4172/jcen.1000139

Citation: Akamine S, Kudo T (2022) Title: Blood Neurofilament Light Chain Measurement toward Clinical Application: A Review. J Clin Exp Neuroimmunol, 7: 139.

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observed at symptom onset when compared to that in healthy controls. It can be used as a predictor of prognosis and differentiate FTD with behavioral symptoms from primary psychiatric disorders. TBI is also reported to be related to elevated blood NFL levels. In this case, blood NFL correlates with the number of head impacts and is associated with the outcome after a year. An increase in blood NFL is also observed in patients with Parkinson's disease (PD) and Alzheimer's disease (AD); however, the overlap of the levels with healthy controls is relatively broad. Atypical parkinsonian syndromes, such as progressive supranuclear palsy and multiple system atrophy, can reportedly be differentiated from PD using blood NFL. In both sporadic and familial AD, higher blood NFL levels are observed compared to those in healthy controls, and they correlate with loss of cortical thickness and cognitive function. In large-scale studies of familial AD, asymptomatic carriers were found to have lower blood NFL levels than symptomatic carriers; however, this abnormal increase starts about 22 years before onset. Moreover, blood NFL elevation is also observed in peripheral neuropathies, such as Charcot-Marie-Tooth disease and Guillain-Barré syndrome. As described above, blood NFL reflects neuroaxonal injury; thus, it is not a disease-specific biomarker. It can be used as a general indicator for neurodegeneration, as with C-reactive protein for inflammation.

Factors influencing blood NFL level other than neurodegeneration

Currently, numerous reports show the promising potential of blood NFL by case-control comparison. However, due to the relatively wide overlap with healthy controls, the efficacy of personalized evaluation of blood NFL is limited. This may be due to the wide range of inter-individual variability, which is influenced by many factors such as age, body mass, and renal function. For clinical use, a more in-depth understanding of NFL dynamics in the blood is needed. It is widely accepted that blood and CSF NFL levels increase with age, at an average of 2-3 % per year [5]. Elevated blood NFL levels become more evident at an age over 60 years. The inter-individual variability also increases at this point, which may be due to increased comorbidities associated with aging. There is no significant difference in blood NFL levels between sexes [5]. In a report assessing the blood from patients with MS, body mass index (BMI) and blood volume, both calculated from height and weight, had weak negative correlations with plasma NFL level. In addition, renal function is a potential influencing factor. Two reports about the relationship between renal function and blood NFL are available; however, these results are conflicting. Recently, we investigated the association between renal function and blood NFL levels in two different cohorts. We found a significant negative correlation between serum creatinine levels (or estimated glomerular filtration rate) and blood NFL levels in healthy controls (Pearson's $\rho=0.50$, $p=0.0007$) and patients with type 2 diabetes (Pearson's $\rho=0.56$, $p<0.0001$). Even after adjusting for age, sex, and BMI, the association remained statistically significant, indicating that this association is independent of known physiological conditions ($p=0.0001$ and <0.0001 , respectively). To standardize the measurement value of blood NFL for personalized medicine, age, BMI, and renal function should be considered. Some researchers have pointed out the influence of blood-brain barrier (BBB) permeability on the level of NFL in blood because most of the NFL in blood comes from the central nervous system [8]. There are several reports assessing the relationship between BBB permeability and its correlation with blood and CSF NFL; however, the results are inconsistent. Pre analytical conditions also influence the measured value of the NFL level. NFL levels in serum may be measured slightly higher than in the plasma. Freeze-thaw and storage at 4°C or room temperature for one week resulted in a 7-8% decrease.

Clinical application of Blood NFL

Although blood NFL has shown its potential in various neurological diseases, there are still difficulties in interpreting blood NFL levels in individual patients because of the large inter-individual variability. When a patient exhibits high blood NFL levels at the first visit, it is difficult to determine whether the level is physiological or pathological. Very little knowledge about the dynamics of blood NFL is available. Factors affecting blood NFL levels such as age, BMI, and renal function, as well as their effect size, should be determined. It is also necessary to establish standard operation procedures, specify reference materials, and determine inter-facility variability. There are other blood NFL assays using the Simoa platform beside the commercially available kit from Quanterix [5]. They are not directly comparable because the calculated concentrations are different by approximately 2-fold due to differences in assay reagents (e.g., blocking agents). Efforts are underway to integrate and standardize blood NFL assays across multiple sites.

Conclusion

Blood NFL assay is a promising test for detecting neuronal injury with low invasiveness and high scalability and has attracted a great deal of attention. It has the potential to be particularly useful in clinical practice; however, some factors influencing blood NFL levels other than neuronal injury must be considered as well. It is necessary to elucidate the pathophysiological dynamics further to better interpret blood NFL measurements.

Competing interests

The authors declare no competing interest.

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