Neurofilament light chain (NfL)

There are numerous and varied pathologies that can cause axonal damage. Having a simple, affordable biomarker to quickly and accurately assess neuronal injury and neurodegeneration has been long sought to expedite time to diagnosis, monitor disease progression, and evaluate drug treatment efficacy. For the past few years, neurofilament light chain (NfL) has become the subject of investigation as the primary biomarker candidate for this purpose.

What Are Neurofilaments?

Neurofilaments are cell-specific structural proteins found in abundance in the axonal interior of healthy, myelinated neurons[1] (Figure 1). They are comprised of heavy, medium, and light chain components, that form heteropolymers to facilitate axonal diameter determination and facilitate attachment by organelles.[2] Neurofilaments are constantly released from neurons into the extracellular space and make their way into the cerebral spinal fluid and peripheral blood, providing for a basal level of detectable neurofilament light chain proteins in both cerebral spinal fluid (CSF) and blood. Serum NfL (sNfL) levels in healthy individuals are lower than those in CSF but correlate (reviewed in 3). New technologies have enabled NfL to be reliably measured in plasma and serum.[4] Levels of sNfL rise above normal in response to neuronal injury and neurodegeneration independent of cause, making its potential utility as a biomarker both obvious but also inherently context dependent on clinical assessment.

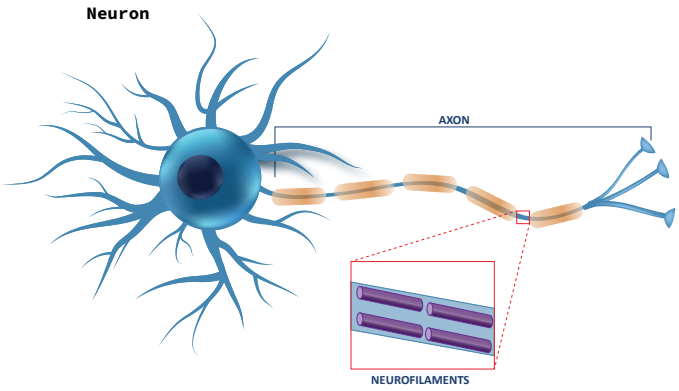

Figure 1: Neurofilment light chain is a neuron specific protein that lines the axons of healthy neurons.

Areas of Clinical Study for NfL

NNfL has been studied as a neurodegenerative or neuronal injury biomarker in a variety of diseases and conditions,[3] including:

- Multiple Sclerosis(MS): sNfL has been widely studied as a marker of disease progression, treatment efficacy, and clinical outcomes for MS.[3] sNfL levels are increased in early relapsing MS and have been shown to correlate with markers of disease severity.[5] Treatment with disease modifying therapy has been reported to be associated with lower sNfL levels compared to untreated individuals.[6] A key challenge with having more definitive clinical guidance for sNfL in MS patients had been the lack of wide availability of an sNfL assay.
- Alzheimer's Disease(AD): The application of sNfL as a potential biomarker for AD has been extensively investigated.[3,7-10] Elevated NfL levels have been associated with the presence of beta-amyloid plaques in pre-symptomatic individuals and with the level of tau in symptomatic patients.[9] Additionally, elevated sNfL levels can be a predictor of disease progression in symptomatic patients with subjective cognitive decline.[10]
- Huntington's Disease(HD): sNfL levels were significantly higher in patients with HD than in healthy controls,[11] and increased sNfL levels were found in young adult carriers of HD gene mutation years before the clinical onset of symptoms.[12]
- Parkinson's Disease: sNfL levels have been shown to correlate with disease severity and motor and cognitive decline.[13,14]
- Amyotrophic Lateral Sclerosis (ALS): Serum NfL levels can have diagnostic and prognostic value for symptomatic patients[15] and distinguish early onset ALS patients from those with other neurologic diseases.[16]
- Spinocerebellar Ataxias: NfL can help stratify pre-ataxic individuals with regard to onset and facilitate early detection of neurodegeneration.[17]
- Concussion recovery: NfL can be used in conjunction with clinical observation, as a primary biomarker to assess "return to play" in athletes with sports-related concussion.[18-20]
- Oncology: Use of NfL testing prior to immunotherapy infusions may permit for early identification of patients at risk for immune effector cell-associated neurotoxicity syndrome.[26]

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Reference Intervals

NfL levels in healthy patients are known to generally increase with age throughout adulthood (21,22). Younger children have higher NfL levels than older children reaching a nadir between the ages of 10 to 15 years, then increasing in a linear fashion until the age of 60 years and accelerating non-linearly thereafter (22-24). Also, this is a new assay that does not have traceability to a WHO or other appropriate standard. For these reasons, Labcorp generated de novo a new reference interval for the primary specimen type (red tiger-top serum). Discard specimens of the primary specimen type (gel-separated serum from an SST tube) were stored in a deep frozen environment (< -70°C) and used to establish analyte reference intervals. At least 120 samples were obtained for each age decade between 20 and 79 years old (i.e. 20–29, 30–39, 40–49, 50–59, 60–69, and 70–79). At least 120 samples were measured for the following will be age ranges: 0–4, 5–9, 10–14, and 15–19. In addition, at least 40 samples were measured for individuals 80 years or older.

Reference intervals were generated by measuring a total of 1,634 gel-separated serum samples from individuals representing the range of ages mentioned above. Chauvenet's criterion were utilized to remove 109 results that were determined to be outliers for each of the age ranges listed. A Box-Cox transformation was then applied to transform the data for each age range into a Gaussian model and allow the determination of the upper end of the reference interval. As the disease state indication is increased levels of NFL, only an upper reference interval value is employed (Table 1).

Table 1

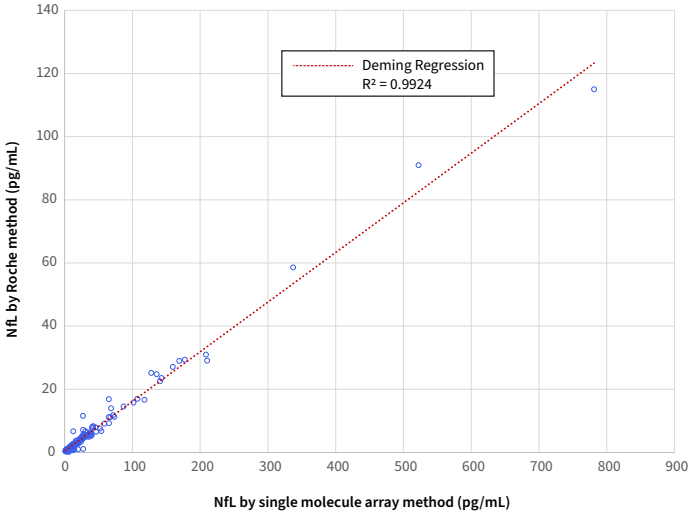
Age Range	Reference Interval (pg/mL)
4 years or younger	≤ 1.973
5 to 9 years	≤ 1.639
10 to 14 years	≤ 1.434
15 to 19 years	≤ 1.603
20 to 29 years	≤ 1.648
30 to 39 years	≤ 1.878
40 to 49 years	≤ 2.139
50 to 59 years	≤ 3.794
60 to 69 years	≤ 4.620
70 to 79 years	≤ 7.653
> 79 years	≤ 11.56

Limitations

NfL levels may vary between different labs depending on which methodologies are employed and which platforms are used to perform the assay. Care must be taken when interpreting results obtained from different studies.

Labcorp performed studies comparing our methodology to the single molecule array technology used frequently in literature studies. Initial method comparison results using native serum samples demonstrated a correlation of 0.9924 (Figure 2).

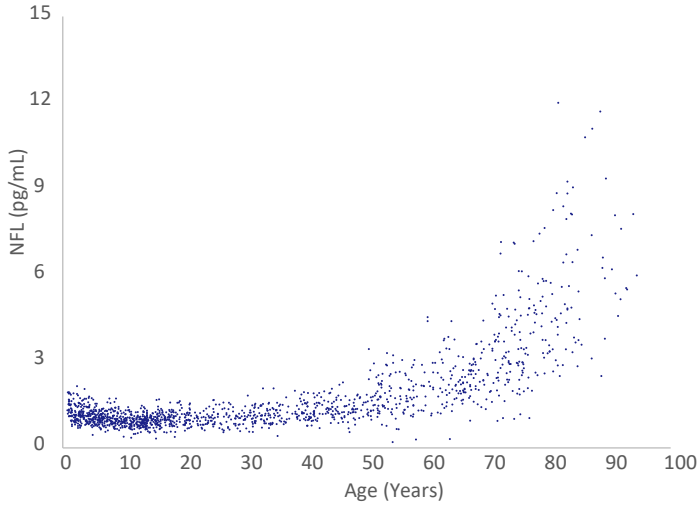
Figure 2



Precision

Imprecision of the NFL assay was investigated by measuring serum samples and QC materials with a range of NFL concentrations over a period of several days (≥ 5 days) with several replicates (total n ≥ 25 for each sample and material). Results indicated CVs less than 8.0% for NFL concentrations within reference intervals (i.e. < 11.56 pg/mL) while elevated NFL levels had CVs ≤ 2.0% (Figure 3).

Figure 3



Lower Limit of Quantification

The lower limit of quantitation (LLOQ), or the lowest analyte concentration that can be reproducibly measured with an intermediate concentration ≤ 25.0%, was determined to be 0.227 pg/mL. Linearity was from below the LLOQ to greater than 1,000 pg/mL with biases ≤ 4.4%. The analytical measurement range of this assay is 0.227 to 1,000 pg/mL.

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