Blood Neurofilament light chain (NfL) analysis.

BACKGROUND
Neurofilament light chain (NfL) is a major component of the axonal cytoskeleton and has been identified as a sensitive biomarker of neuronal damage. Recent evidence has shown utility in various conditions including multiple sclerosis (MS) [1-5], Alzheimer’s disease[6], Parkinson disease[7], and amyotrophic lateral sclerosis (ALS) [8,9]. The largest body of evidence has been generated in MS patients with its main utility in prognosis and follow-up, but of increasing importance in monitoring response to therapy.

REGULATORY
EORLA holds a clinical diagnostic licence to provide neurofilament light chain testing from the Ontario Ministry of Health and Long-term Care.

SPECIMEN REQUIREMENTS
Specimen: Serum (red top or SST) acceptable. Plasma not acceptable. Shipping and storage: Store and send frozen. Turn-around-time: Maximum 30 days (Analytical runs are done 1/month)

For questions, please contact:
Dr. Ronald A. Booth, FCACB, FACB
Clinical Biochemist | Div. of Biochemistry | EORLA & The Ottawa Hospital
Associate Professor | Dept. of Pathology & Lab. Medicine | University of Ottawa
Tel: 613-737-8899 Ex. 79095 | rbooth@eorla.ca

NfL UTILITY IN MS
Multiple sclerosis is a chronic disease of the central nervous system characterized by loss of motor and sensory function resulting from immune-mediated inflammation, demyelination and subsequent axonal damage. This disease-related axonal damage releases NfL into the CSF and blood proportional to the amount of axonal damage.

Blood NfL concentration:
- Can identify acute and chronic neuronal damage in early MS
- Increasing NfL can predict disease worsening and brain & spinal cord atrophy in MS
- Correlates with baseline load of T2-weighted MRI lesions
- Correlates with disability as assessed by EDSS score
- Higher baseline values are predictive of worse disease
- Decrease in NfL correlates with positive response to treatment

- It can be used to monitor patients, reducing the need for yearly MRI in stable patients and importantly select those in need of a more urgent MRI.
- It may also have the potential to identify very early MS patients at risk of imminent progression and allow for earlier treatment, as well as identifying poor prognostic patients earlier, affording them the opportunity for more aggressive therapy, when it is most likely to have the greatest impact.
LABORATORY MEASUREMENT of NfL using SIMOA TECHNOLOGY

- SIMOA is a high sensitivity digital ELISA, allowing measurement of protein biomarkers in the fg/mL range with precision acceptable for clinical use.

![Diagram showing comparison between Traditional (Analog) and Simoa (Digital) methods.]

- Reaction volume = 100 \times 10^{-6} \text{ L}
- Diffusion = dilution = low sensitivity
- Millions of molecules needed to reach detection limit

- Reaction volume = 50 \times 10^{-15} \text{ L (2 billion times smaller)}
- Diffusion defeated = single molecule resolution = ultimate sensitivity
- One molecule needed to reach detection limit
SELECTED MS PUBLICATIONS

High serum neurofilament light chain normalizes after hematopoietic stem cell transplantation for MS. Thebault, et al. [16]. In-house study comparing CSF and serum NfL in patients that underwent bone marrow transplant (BMT) for treatment of MS. Following BMT, high pretreatment NfL levels significantly reduced in serum and CSF. Baseline NfL levels were associated with worse pretreatment disease measures, relapses, MRI lesions. Elevated baseline NfL levels were associated with persistently worse indices of disease burden post-BMT.

**Figure 1** Serum and CSF neurofilament levels following Immunoablative Autologous Haematopoietic Stem Cell Transplant

Baseline NfL levels (n = 23) were significantly elevated relative to controls (n=33) in (A), serum (p = 0.0001, 95% CI 13.33-32.38) and (B), CSF (p = 0.0001, 95% CI 1122-2638). Following IAHSCT, levels significantly reduced in both compartments (serum p=0.0023, 95% CI 6.39-26.94; CSF p = 0.0068, 95% CI 52.0-2141). Post-ASCT NfL levels (12 and 36m) in both serum and CSF were not significantly different from controls (p ≥ 0.05). Serum and CSF NfL levels were correlated (p = 0.0001, Spearman r = 0.76). 12m: 12 months post IAHSCT. 36m: 36 months post IAHSCT. Error bars represent the standard error of the mean. CI = confidence interval; IAHSCT = immunoablation followed by autologous hematopoietic stem cell transplantation; NfL = neurofilament light chain.
Identification of acute and chronic neuronal damage in early MS – Siller et al. [5] using the Simoa assay showed that baseline serum NfL correlated with MRI T2 lesion volume, and higher serum NfL levels positively correlated with patients that progressed. Disease-modifying treatment decreased serum NfL levels.

**Figure 4.** Effect of immunomodulatory therapy on sNfL levels. (a) 14 therapy-naive patients who started an immunomodulatory therapy in comparison to 7 naive patients staying without therapy, each with a consecutive serum sample (both groups median 13 months after baseline; t0 = baseline, t1 = follow-up). Immunomodulatory therapy was started median 12 months before second serum sample. (b) sNfL decreases significantly after initiation of any immunomodulatory therapy (**p = 0.0074, obtained by Mann–Whitney U test).
Prediction of disease worsening and brain & spinal cord atrophy in MS – Barro et al. [4] using the Simoa NfL assay using 259 patients showed that serum NfL was higher in patients with CIS or relapsing remitting multiple sclerosis as well as in patients with secondary or primary progressive multiple sclerosis than in healthy controls. They also showed that patients above the 90th percentile of healthy controls was an independent predictor of disease worsening in the subsequent year. Finally, they showed that blood NfL correlated with concurrent and future clinical and MRI measures of disease activity and severity with high serum NfL levels associated with both brain and spinal cord volume loss.

Figure 3 Estimated mean change in brain and spinal cord volume over 2 and 5 years against sNfL dichotomized based on age-corrected percentile curves from healthy controls. The estimated mean percentage of brain volume change in patients with sNfL above the respective age corrected percentiles gradually increased with increasing sNfL percentile category over 2 (A) and 5 years (B) of observation time. The mean reduction in spinal cord volume over 2 (C) and 5 years (D) gradually increased with increasing sNfL percentile category. For example, patients with sNfL above the 97.5th percentile had on average a 1.7% and 2.5% lower spinal cord volume at 2 (C) and 5 years (D) of follow-up as compared to those below the same percentile, respectively. Numbers in the figure denote the number of samples above or below the respective percentiles of healthy controls.
Monitoring MS disease activity with NfL – Novakova et al. [11] measured paired serum and CSF samples from 286 MS patients, 45 other neurological conditions and 42 healthy controls. Serum NfL was measured using the Simoa assay. In MS patients, the correlation between serum and CSF NfL was $r = 0.62$ ($p<0.001$). Serum concentrations were significantly higher in patients with relapsing-remitting MS and in patients with progressive MS than in healthy controls. Treatment significantly reduced median serum NfL levels ($p<0.001$). Patients with relapse or with radiologic activity had significantly higher serum NfL levels than those in remission ($p<0.001$) or those without new lesions on MRI ($p<0.001$).

Correlation of NfL with brain atrophy and disability in MS – Kühle et al. [2] compared patients enrolled in a clinical treatment trial and showed changes in NfL were correlated with clinical change ($p=0.009$) and neuropsychological outcomes. Brain volume decreased more rapidly in patients with high baseline NfL ($p=0.05$ at 12 months and $p=0.008$ at 24 months) and this relationship became stronger at 24 months ($p=0.024$ for interaction). Higher and increasing NfL predicted higher number of gadolinium-enhancing MRI lesions ($p<0.001$ for both).
Monitoring of therapy using NfL in MS patients - Piehl et al. [12] using the Simoa assay showed plasma NfL was highly correlated to CSF NfL. Both CSF and plasma NfL decreased after switching to highly effective MS therapy. They suggest blood NfL measurement can be considered as a biomarker for MS therapy response.

**Figure 3.** Serum neurofilament light chain (NFL) levels in a cohort of patients (n = 243) switching from injectable therapies to fingolimod, with sampling at baseline and at 12 and 24 months after start of therapy with fingolimod. At the group level, mean plasma NFL demonstrated a significant 34% reduction at 12 months compared to baseline, where levels remained similar at 24 months compared to 12 months.
**Blood neurofilament light chain as a biomarker of MS disease activity and treatment response** – Kuhle et al [17] measured NFL in blood samples from 589 patients with relapsing-remitting multiple sclerosis (from phase 3 studies of fingolimod vs placebo, FREEDOMS and interferon [IFN]-β-1a, TRANSFORMS) and 35 healthy controls and compared NFL levels with clinical and MRI-related outcomes. High vs low baseline NFL levels were associated (estimate [95% confidence interval]) with an increased number of new or enlarging T2 lesions (ratio of mean: 2.64 [1.51–4.60]; p = 0.0006), relapses (rate ratio: 2.53 [1.67–3.83]; p < 0.0001), brain volume loss (difference in means: −0.78% [−1.02 to −0.54]; p < 0.0001), and risk of confirmed disability worsening (hazard ratio: 1.94 [0.97–3.87]; p = 0.0605). Fingolimod significantly reduced NFL levels already at 6 months (vs placebo 0.73 [0.656–0.813] and IFN 0.789 [0.704–0.884]), which was sustained until the end of the studies.

**Figure 4** Effect of fingolimod on NFL levels in blood, (A) compared with placebo, FREEDOMS study; (B) compared with interferon-β-1a, TRANSFORMS study.

![Figure 4](image)

The figure shows geometric means of NFL with 95% confidence intervals and statistical tests from mixed models for repeated measurements of post-baseline NFL. Dotted line represents plasma NFL (pg/mL, geometric mean) concentrations in healthy controls. **p < 0.0001, n = number of patients with evaluable data. NFL = neurofilament light chain.
OTHER NEUROLOGICAL CONDITIONS

Neurofilaments in early symptom onset amyotrophic lateral sclerosis – Feneberg et al. [13] measured serum NFL in early ALS and showed it was able to discriminate early ALS patients with early symptom onset from those with other neurologic diseases and motor neuron disease mimics with high sensitivity (88% to 100%) and specificity (90% to 92%). It did not vary between clinical diagnostic categories of ALS in the early symptomatic phase group.

Blood NFL in diagnosis of parkinsonian disorder – Hansson et al. [7], using >300 patients from 3 different cohorts showed that blood NFL could accurately distinguish Parkinson disease from atypical parkinsonian disorders (area under the curve [AUC] 0.91) with similar results seen in both the London cohort (AUC 0.85) and the early disease cohort (AUC 0.81).

Blood NFL as a potential biomarker in Alzheimer’s disease - NFL concentrations were measured in 99 subjects with Alzheimer’s disease at the stage of mild cognitive impairment (MCI-AD; n = 25) or at the stage of early dementia (ADD; n = 33), and in nondemented controls (n = 41) by Lewczuk et al. [6]. NFL was significantly higher in the MCI-AD group (38.1 ± 15.9 pg/mL, p < 0.005) and even further elevated in the ADD group (49.1 ± 28.4 pg/mL; p < 0.001) when compared to controls (22.0 ± 12.4 pg/mL). Using a cut-point of 25.7 pg/L, unconditional sensitivity, specificity, and accuracy for detection of Alzheimer’s disease were 0.84, 0.78, and 0.82, respectively.

REFERENCES


