658.05

Monitoring Axonal Damage in Neurological Disorders: Detection and Quantification of Neurofilaments Light and Heavy Chain (NF-L/ pNF-H) as Potential Biomarkers of Neurodegeneration

Y Noam[1], TA Libermann[2], ST Dillon[2], V Sheen[2], SA Karumanchi[3], E Schoell[1], D Perregaux[1], N Steere[4], T Munn[4], P Younge[4], I O'Brien[4], M Anderson[4]

[1]ProteinSimple, Wallingford, CT [2]Beth Israel Deaconess Med. Center, Harvard Medical School, Boston, Massachusetts [3]Cedars-Sinai Med. Center, Los Angeles, CA [4]R&D Systems, Minneapolis, MN

Abstract

Axonal damage is a hallmark of neurodegenerative disorders. In various neuropathologies, injured or degenerated axons release their intracellular components into the extracellular space, resulting in circulation of these molecules in the cerebrospinal fluid (CSF) and peripheral blood. Consequently, monitoring the levels of these molecules in biological fluids can serve as a potent biomarker for the diagnosis, prognosis, and evaluation of treatment in neurodegenerative diseases.

Of the various axonal molecules, neurofilaments are major structural components of the cytoskeleton. There are three distinct axonal neurofilament subunits: light (NF-L, ~68 kDa), medium (NF-M, ~145 kDa), and heavy/phosphorylated (pNF-H, ~200 kDa). In recent years, neurofilaments (and in particular NF-L and NF-H) have emerged as promising biomarkers across a wide range of neurological disorders, including neurodegenerative dementia, multiple sclerosis, Parkinson's disease, traumatic brain injury, stroke, and amyotrophic lateral sclerosis (ALS). The ability to detect neurofilaments not only in CSF but also in blood is important in order to allow minimally-invasive diagnosis and monitoring of disease progression. However, whereas neurofilaments are readily detected in the CSF, their substantially lower levels in blood pose a challenge for standard immunoassay-based methods.

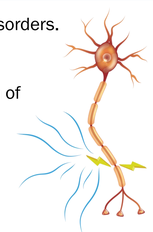
Here, we employed the Ella™ platform to establish a novel, microfluidics-based assay for detecting and quantifying NF-L and pNF-H in CSF and blood samples. The automated nature of the assay facilitated a high degree of precision and reproducibility across trials, while requiring ≤ 25 ul of sample per measurement. Applying our assay to a physiologically-relevant context, we first studied the role of neurofilaments in aging by assessing NF-L and pNF-H in blood and CSF samples of healthy controls across ages. We next applied our assay to evaluate the potential of NF-L/ pNF-H as non-invasive bio-markers by measuring their levels in blood samples of patients diagnosed with neurodegenerative diseases. Taken together, our results support a role for neurofilaments as valuable markers of neurodegeneration and provide a novel and efficient platform for their detection in various biological samples.

Rationale & Objective

Axonal damage is a common feature across many neurological disorders.

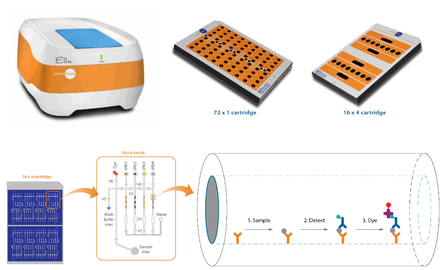
Minimally-invasive assessment of axonal damage can be highly beneficial for the diagnosis, monitoring, and treatment evaluation of neurological disease.

Here, we establish novel, microfluidic assays for the sensitive and accurate detection of the axonal molecules Neurofilament Light (NF-L) and Heavy (pNF-H) in blood and CSF.

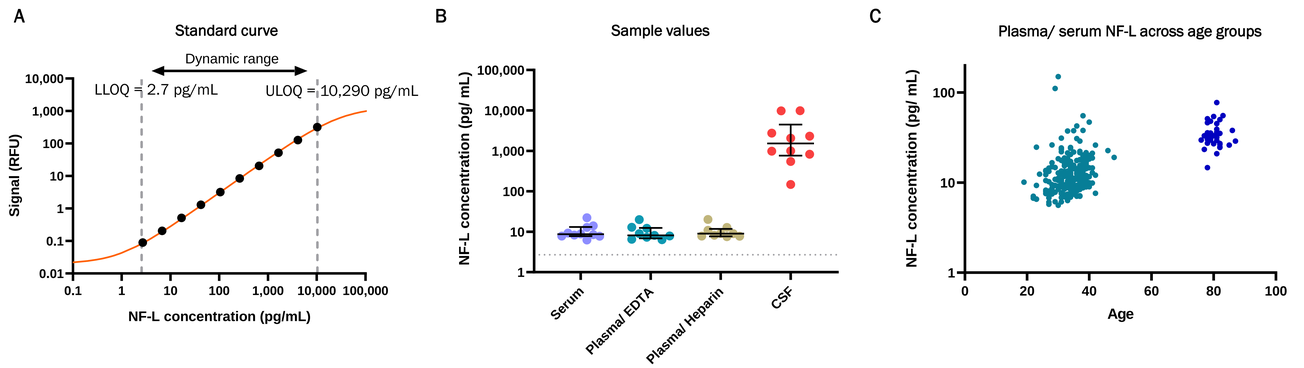


We apply NF-L and pNF-H assays to explore their potential as bio-markers in mild cognitive impairment (MCI) and preeclampsia.

The Ella™ workflow



A Novel Microfluidic Assay for NF-L Detection



D

NF-L Precision

Parameter	Low QC	High QC
Intra-mean (pg/ mL)	76.6	3309
Intra-assay SD	3.64	154
Intra-assay CV (%)	4.8	4.7
Inter-Mean (pg/ mL)	80.5	3476
Inter-assay SD	8.35	267
Inter-assay CV (%)	10.4	7.7

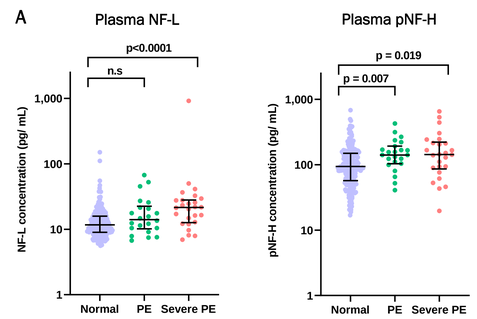
NF-L Recovery

Sample type	AVG %	Range %
Serum (n=4)	108	95-115
EDTA plasma (n=4)	93	86-99
Heparin Plasma (n=4)	101	93-106

(A) Curve, limits of quantitation, and dynamic range of the NF-L assay. **(B)** Control samples of plasma/serum and CSF sample types were readily detectable (Serum and CSF 100% detectable, Plasma samples 90% detection). **(C)** The graph depicts NF-L values of two cohorts: preeclampsia controls (turquoise, N=224) and asymptomatic, older controls from the NCRAD repository (dark blue, N=40). In accord with published literature, NF-L plasma/serum levels positively correlated with age. Sample detection rate was 95%. **(D)** NF-L assay precision and recovery properties. Recovery was measured at three different spiked concentrations within the assay range.

Neurofilaments as potential biomarkers of preeclampsia

In a prospective cohort of >250 pregnant women, NF-L/ pNF-H levels were measured at delivery and compared between normal pregnancy and preeclampsia samples

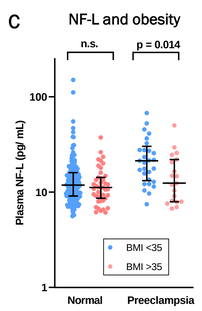


B

Group	N	Median NF-L (pg/ mL)	Interquartile range (Q3-1)	P-value (vs control)
Normal pregnancy	224	11.7	9.0 – 15.9	-
Preeclampsia	22	14.1	10.1 – 22.5	0.169
Severe Preeclampsia	26	21.5	12.7 – 28.1	<0.0001*

Group	N	Median pNF-H (pg/ mL)	Interquartile range (Q3-1)	P-value (vs control)
Normal pregnancy	230	93.6	57.1 - 149	-
Preeclampsia	23	141	104 - 192	0.007*
Severe Preeclampsia	26	143	86.4 - 222	0.019*

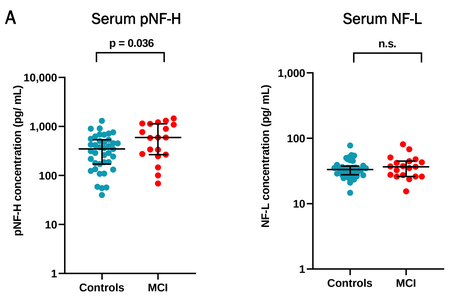
* Statistical significance was assessed using Kruskal-Wallis non-parametric test, followed by Dunn's post-hoc test



Neurological complications in sub-populations of preeclampsia can lead to seizures, coma, and death. Therefore, identifying patients at high-risk for developing cerebral complications is of high importance.

(A) Neurofilament plasma levels were elevated in women with preeclampsia; whereas pNF-H levels were elevated in both mild and severe preeclampsia, NF-L levels were specifically augmented in severe preeclampsia. (B) Summary of the results. (C) Unexpectedly, high BMI was found to be negatively correlated with NF-L levels, suggesting complex relationship between obesity and neurological vulnerability in preeclampsia.

Neurofilaments in Mild Cognitive Impairment



B

Group	Median pNF-H (pg/ mL)	Interquartile range (Q3-1)
Controls	347	171 - 537
MCI	593*	266 - 1133

*= P<0.05, Mann-Whitney test.

Group	Median NF-L (pg/ mL)	Interquartile range (Q3-1)
Controls	33.1	27.8 – 37.5
MCI	36.6	26.1 - 44.6

Assessment of Serum pNF-H and NF-L levels in older individuals with mild cognitive impairment (MCI). (A, B) pNF-H and NF-L serum levels were measured in samples from asymptomatic controls (n=40, Mean age 80.1) and individuals with MCI (n=20, Mean age 79.2). An increase in pNF-H levels was observed in the MCI group. Analyte detection rate in all experimental groups was >=95%.

Summary

- The Ella™ platform provides a novel assay for neurofilaments detection with high sensitivity and precision.
- NF-L and pNF-H are readily detectable in blood samples, rendering them attractive bio-markers for axonal injury using minimally-invasive procedures.
- Both plasma NF-L and pNF-H levels were elevated in preeclampsia, suggesting their potential as biomarkers for early detection of neurological complications in preeclampsia.
- Elevated pNF-H levels were found in a pilot cohort of 20 patients with mild cognitive impairment.
- Further studies may shed light on implications for other neurodegenerative conditions, including multiple sclerosis, ALS, Alzheimer's disease, and other dementias.