Neurofilament Light: a Dynamic Cross-Disease Fluid Biomarker for Neurodegeneration

Henrik Zetterberg[1,2,3,*]

[1]*Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, the Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden*
[2]*Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden*
[3]*Department of Molecular Neuroscience, UCL Institute of Neurology, Queen Square, London, UK*

*Correspondence: henrik.zetterberg@clinchem.gu.se; h.zetterberg@ucl.ac.uk

In the current issue of *Neuron*, Bacioglu, Maia and colleagues show that neurofilament light concentrations in body fluids reflect pathology and symptoms in mouse models of cerebral proteopathies and that these findings translate to human disease (Bacioglu et al., 2016).

There are two major types of intermediate filaments in the nervous system: neurofilaments (NFs) and glial filaments. NFs exist as 10-nanometer filaments in the axoplasm of neurons, where they give tensile strength to dendrites and axons. NFs share several features with other intermediate filaments, such as being resistant to extraction at physiological pH and having a high degree of helicity. They are composed of three major polypeptides with molecular masses of 200, 150 and 68 kilodaltons (kD), respectively. As the name implies, neurofilament light (NfL) is the lightest of the three components.

In 1987, Swedish researchers in the team of legendary Kenneth G. Haglid managed to obtain pure fractions of the different NFs from bovine brain (Karlsson et al., 1987), which was the basis for the generation of polyclonal rabbit antisera specific against the individual NF polypeptides (Karlsson et al., 1989). The most promising combination of these was developed into the first enzyme-linked immunosorbent assay (ELISA) for NfL (Rosengren et al., 1996). Rosengren and colleagues showed that CSF NfL concentration was increased in amyotrophic lateral sclerosis (ALS), particularly so in patients with pyramidal tract involvement, and that increased concentrations also characterized Alzheimer's disease (AD), vascular dementia and normal pressure hydrocephalus, but with lower magnitude of the rise compared with that seen

in ALS (Rosengren et al., 1996). The authors concluded that CSF NfL was a promising biomarker for neurodegeneration in general; a conclusion that has later been confirmed, *e.g.*, in studies examining atypical parkinsonian disorders (Hall et al., 2012; Magdalinou et al., 2015) and frontotemporal dementias (Scherling et al., 2014).

Monoclonal antibodies against NfL were developed (Norgren et al., 2002), and a new NfL ELISA that did not depend on exhaustible antisera was established (Norgren et al., 2003). Given the high expression of NfL in large caliber myelinated axons, studies on multiple sclerosis (MS) soon followed. Researchers found that CSF NfL is increased in both relapsing-remitting and primary progressive MS, that CSF NfL concentration indicates ongoing axonal injury and reflects the intensity of the process, that CSF NfL concentration normalizes within 6-12 months in MS patients following initiation of clinically effective treatment and that CSF NfL thus is a promising biomarker for disease intensity and progression, as well as for treatment response (Teunissen and Khalil, 2012). Similar results on CSF NfL dynamics have been obtained in stroke, TBI, HIV-associated dementia and a broad range of other neuroinfectious conditions.

None of the clinical studies discussed above, however, have addressed the relationship between CSF NfL and neuropathology. To that end, joint first authors Bacioglu and Maia with colleagues performed a remarkable set of experiments in which they collected CSF (not the regular lumbar puncture and not the volumes clinical neurochemists are used to!) and blood over time in three transgenic mouse models of neurodegenerative proteopathies (P301S-tau mice as a model for tau pathology, APPPS1 mice as a model for amyloid β [Aβ] pathology, and A53T-α-synuclein mice as a model for α-synuclein pathology). Using the same reagents that constitute the basis for the standard NfL ELISA discussed above but transferred onto a platform with electrochemiluminescent detection that yields a 5-fold increase in analytical sensitivity compared with the ELISA, they measured CSF and serum/plasma NfL (Bacioglu et al., 2016) (Figure 1).

In the mouse models, CSF NfL increased in parallel or slightly before protein deposition. Intriguingly, in all three models, CSF NfL increased months before the first symptoms appeared, suggesting it might serve as a preclinical marker. By the time symptoms developed, CSF NfL concentrations in APPPS1 and P301S-tau mice were 10-20 times those in wild-type animals, whilst CSF NfL concentration in A53T-αS mice was a 1000-fold higher. Plasma NfL

followed the same pattern; the correlation coefficients with CSF NfL were as high as 0.86-0.94 in the transgenic animals. However, in wild-type mice the correlation coefficient was lower, 0.47, and the increase in plasma NfL before symptom onset was less clear compared with CSF NfL. The two latter results may be explained by overall lower concentrations of NfL in wild-type mice and in the early phase of protein deposition in the transgenic models; these concentrations may simply be closer to the limit of quantification of the assay and thus more variable, suggesting that even more sensitive tests would be of value.

Next, Bacioglu, Maia and colleagues performed a series of experiments in which the pathology load in the models was modified and the effects of these modifications on NfL concentrations were examined. Using a β-secretase inhibitor (one of the enzymes essential for Aβ production from amyloid precursor protein, APP), they lowered Aβ production in APPPS1 mice over six months. Both CSF and plasma NfL concentrations decreased in parallel with Aβ load. Thus, the neuronal reaction to the treatment-induced reduction of Aβ pathology could be monitored using CSF and plasma NfL. In a differently designed experiment, the authors worsened the α-synuclein pathology of A30P-αS mice by seeding the pathology in young animals with brain extract from aged A30P-αS animals. Lesions and symptoms developed more rapidly in seeded mice and CSF and plasma levels of NfL increased more rapidly, in parallel with the number and size of the α-synuclein inclusions, corroborating the direct relationship between protein deposition and fluid NfL concentration.

Finally, the researchers measured CSF and serum NfL concentrations in neurodegenerative diseases, resembling the three mouse models they examined: Parkinson's disease (PD), dementia with Lewy bodies (DLB) and multiple system atrophy (MSA) that are synucleinopathies; progressive supranuclear palsy (PSP) and corticobasal syndrome (CBS) that are tauopathies (although the latter diagnosis is very hard to make *in vivo*); and AD that is the classical cerebral β-amyloidosis. The authors found CSF NfL concentrations to be increased over controls in all disease groups except PD. Overall, the NfL increase in the patients was smaller than in the mouse models, from 1.5- to 5.5-fold over healthy controls in both CSF and serum, but they were still clearly discernible and in agreement with previous studies (Hall et al., 2012; Magdalinou et al., 2015).

Altogether, the paper by Bacioglu, Maia and colleagues clarifies several outstanding issues in regards to the potential use and interpretation of CSF and plasma/serum NfL as a biomarker for neurodegeneration. The clinical data support CSF NfL as a general marker of neurodegeneration and the plasma/serum results suggest that similar information can be gained through a simple blood test (a major step forward). In the clinical work-up of suspected neurodegenerative disease, NfL will most likely be of limited value from a differential diagnostic perspective (with the differentiation of typical idiopathic PD from atypical parkinsonian disorders being one potential exception) but could instead be used to determine disease intensity and predict progression, and also identify disease onset in autosomal dominant forms of neurodegenerative disease. Uniquely, in the mouse models, the data show that CSF and plasma NfL concentrations predict onset of neuronal dysfunction in response to pathology and reflect disease modification when pathology load is altered by treatment. Taken together, the data speak for a potential clinical scenario in which CSF and/or plasma/serum NfL may bridge preclinical research and be used in trials to detect treatment effects of novel disease-modifying drug candidates in patients with increased NfL concentrations at baseline, and in the clinic to facilitate treatment selection and optimize dose-finding faster than what would be possible using clinical assessment and/or neuroimaging.

In the field of neurodegeneration, any research team who could report treatment-induced changes in NfL, similar to what has been reported after initiation of successful treatment against MS (Gunnarsson et al., 2011), would be saluted. The encouraging results by Bacioglu, Maia and colleagues suggest that disease modification in neurodegeneration is a reachable goal and that biomarkers like NfL may be an additional tool to help us achieving it.

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Figure legend

Fig. 1: Schematic drawing of a neuron with the pathologies Bacioglu, Maia and colleagues examined in relation to cerebrospinal fluid (CSF) and plasma/serum concentrations of neurofilament light (NfL) in animal models of and patients with proteopathic neurodegenerative diseases (Bacioglu et al., 2016). Intraneuronal inclusions of tau and α-synuclein (α-syn, Lewy bodies) are depicted along with extracellular aggregates of amyloid β (Aβ). NfL is a protein highly expressed in large caliber myelinated axons. Upon axonal injury, irrespective of cause, the protein leaks out into the brain interstitial fluid that communicates freely with the CSF and eventually ends up in the blood from which it is cleared by unknown mechanisms.