

Comment on Gunnarson *et al.* “Axonal Damage in Relapsing Multiple Sclerosis is Markedly Reduced by Natalizumab” Ann Neurol 2011;69:83-89

For the past decade both biomarker research and treatment strategies in multiple sclerosis were focused on neurodegeneration, the main cause for irreversible disability in patients.

We would therefore like to congratulate Gunnarson *et al.* on their pioneering work establishing the neurofilament light chain (NfL) as a biomarker for monitoring axonal damage in multiple sclerosis: “In this context, analysis of NfL levels in the CSF have the potential to provide vital information regarding treatment efficacy in the short-term perspective and to facilitate treatment decisions”¹.

A requirement for any biomarker to be provide reliable data is stability^{2,3}. If a biomarker is not stable and rigorous sample collection and handling cannot be guaranteed, then there is a risk to obtain unreliable data²⁻⁴. We found stability to be a particular problem for NfL.

Figure 1 illustrates the problem. Linear regression analysis showed a significant decrease of NfL levels at 4°C (p<0.01) and room temperature (RT) (p<0.01) from a baseline concentration of 1.4 ng/mL in native CSF (ELISA¹). NfL became essentially non-detectable within 4 days. Consistent with the ELISA data the immunoblot analysis showed that NfL could only be detected in spiked buffer solution if analysed immediately, but not from native or spiked CSF (Figure 1, inset) and was lost at RT after one overnight incubation. Analysing the same samples, there was no significant decrease for NfH^{SMI35} at 4°C over 7 days (ELISA⁵) from the baseline concentration (0.48 ng/mL). At RT there was about a 5% decrease (p<0.05). Long term storage (6.5 years) of native CSF at -80°C did not lead to a significant decrease of NfH from a baseline concentration of 8 ng/mL (Figure 1, right part). In line with the ELISA data the NfH^{SMI35} band remained stable in the

immunoblot.

Taken together the quantitative and qualitative data shows that NfL is not stable in native CSF. Because quantification of NfH from the same samples did not show a significant decrease this problem is specific to NfL, possibly related to protease activity (reviewed in reference ²).

In order to recommend CSF NfL as a biomarker to be used in clinical trials and for treatment decisions we would like to ask the authors to share their own experience on the stability of NfL. If our concern were to be correct, what are the author's recommendations to avoid pre-analytical problems?

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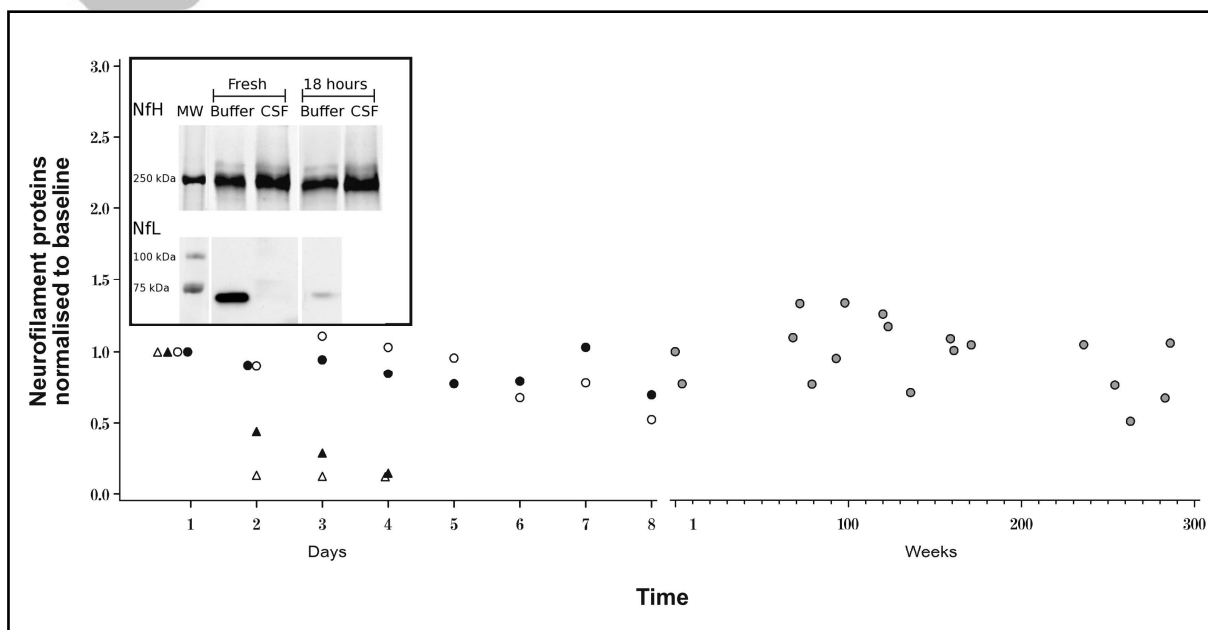


Figure 1. The neurofilament light chain (NfL, triangles) is not stable. At room temperature (RT), the concentration of NfL (open triangles) almost disappears within a day if stored either at RT (open triangles) and decreases by about 50% if stored at 4°C (closed triangles). The poor stability is a specific problem with the NfL protein and not due to poor sample processing as shown by the concentration of NfH measured from the same samples. At 4°C, NfH was stable for at least 7 days (closed circles). At RT there was a small, but significant decrease of NfH after a 5 day incubation period. At -80°C, NfH (grey shaded circles) remains stable for at least 6.5 years. For ease of comparison the Figure shows all values normalised to baseline (the absolute values are given in the text). The inlay shows the immunoblot for NfL and NfH spiked into buffer or CSF if measured immediately (fresh) and after an 18 hour incubation. MW = molecular weight marker.

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