Neurofilaments (Nfs) are type IV intermediate filaments and a main component of the axonal cytoskeleton. They are released into the cerebrospinal fluid (CSF) when axonal damage occurs. In multiple sclerosis (MS), several studies have demonstrated higher levels of both light (NFL) and heavy (NfH) sub-units in the CSF of patients with higher clinical or radiological activity, even during early phases of the disease, whereas others have shown associations with disability accumulation or changes in brain volume, particularly in progressive forms of MS. More specifically, NFL appears to be more associated with acute axonal damage, whereas NfH has stronger associations with disability accumulation. Therefore, their levels are thought to reflect the degree of axonal damage occurring in the central nervous system (CNS), and these findings have led to the consideration of Nf levels in CSF as potential biomarkers of treatment response, both to monitor axonal damage and to investigate possible neuroprotective effects of different MS treatments. NFL and NfH levels in the CSF have been measured in patients treated with natalizumab, prior to and up to 12 months after treatment initiation. NfH and NFL levels decreased by 73.3% and 90.0%, respectively, following natalizumab treatment. Using NFL levels, similar results were found in patients treated with mitoxantrone or rituximab and, more recently, with fingolimod.

While these studies may support the role of Nfs as biomarkers for treatment response in MS, there are still a few technical hurdles and issues regarding their use as a treatment efficacy outcome. With reference to technical difficulties, an important concern is the reproducibility of Nf quantification due to variations in assay characteristics and protein stability. For instance, in a validation study of the NFL enzyme-linked immunosorbent assay (ELISA) carried out by Petzold and colleagues, a high inter-laboratory coefficient of variation was found, mainly secondary to a lack of preparation of accurate and consistent protein standards. This issue should be solved before Nfs are to be extensively used as biomarkers of treatment response in MS. An additional issue is the need to perform at least two lumbar punctures to determine treatment-related changes in Nf levels. Although CSF is in close proximity to the sites of disease pathology in MS making it ideal for monitoring disease activity, obtaining samples through a lumbar puncture is a relatively invasive procedure that is carried out in some centers for diagnostic purposes only. For this reason, some attempts have been made to determine Nf levels in serum; in a phase II lamotrigine trial in secondary progressive MS, no difference in reduction of NFL levels was observed between lamotrigine and placebo arms, although there was a significant reduction in patients with adherence to lamotrigine, by serum levels, compared to non-compliant patients. These results underscore the current lack of sensitivity of Nf serum assays, particularly when performing longitudinal assessments.

In the matter of Nf levels as a treatment efficacy outcome, there are a number of considerations to take into account. Firstly, although NfH might be associated with chronic axonal damage and disability accumulation, there is, to date, more evidence regarding NFL as a biomarker of treatment response in both relapsing–remitting MS (RRMS) and progressive forms. Secondly, the predominant underlying pathogenic mechanisms during clinically isolated syndromes or RRMS and progressive forms differ, and therefore measures of treatment efficacy would be expected to change accordingly. In inflammatory phases there is, to date, a much larger evidence base supporting the role of magnetic resonance imaging (MRI) as a robust, noninvasive procedure to measure treatment response. Clinical trials have demonstrated its usefulness as an outcome measure in terms of number of gadolinium-enhancing lesions or of new lesions in T2-weighted images as well as changes in...
lesion volume, all of which are deemed good markers of inflammation. In clinical practice, the number of new T2 or of gadolinium-enhancing lesions can be used in combination with clinical parameters like number of relapses and disability accumulation to assess treatment response. In this scenario, the added value of Nfs has not been demonstrated to be superior to the above-mentioned parameters. However, in MS phenotypes with predominant neurodegeneration, diffuse parenchymal inflammation predominates, which cannot be assessed with conventional MRI and outcomes such as changes in Expanded Disability Status Scale (EDSS) scores and MRI atrophy measures have yielded heterogeneous results. In this setting, Nf levels may have a more relevant role as biomarkers of treatment response. Nevertheless, the use of less-invasive T2-weighted fluid-attenuated inversion recovery MRI sequences, performed after gadolinium injection, has recently shown promising results in detecting inflammation in the leptomeningeal compartment, which is more commonly affected in progressive MS forms.

Therefore, in order to consider Nfs as useful measures of treatment response in MS, rather than assessing correlations, their performance should be directly compared to the best existing clinical and MRI outcomes according to the MS phenotype. Only if they prove to be superior would the invasiveness of a lumbar puncture be justified. Additionally, the consistency of treatment effects on Nf levels should be reproduced in further studies. Finally, their widespread use in clinical trials or in daily clinical practice is limited by the lack of assay standardization and the need to perform repeated lumbar punctures. In conclusion, rather than being the only certain measure of response to treatment, Nf levels, here and now, appear to be a complementary marker to MRI and clinical parameters that, in the future, may prove to be more relevant as an outcome measure of neuroprotection.

Conflicts of interest
G Arrambide has received compensation for consulting services from Biogen-Idec.
C Espejo has nothing to declare.
M Tintore has received compensation for consulting services and speaking honoraria from Bayer-Schering, Merck-Serono, Biogen-Idec, Teva, Sanofi-Aventis, Roche, Genzyme, and Novartis.

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