Clinical Commentary

Neurofilament light and heavy subunits compared as therapeutic biomarkers in multiple sclerosis


Background – Neurofilaments are promising biomarkers in multiple sclerosis (MS) and increased levels in cerebrospinal fluid (CSF) indicate axonal damage or degeneration. In a previous study, neurofilament light chain (NfL) levels in CSF of relapsing remitting (RR) patients with MS were normalized by natalizumab treatment.

Aims of the study – We compared the coherence between NfL and neurofilament heavy chain (NfH \textsuperscript{SMI35}) levels in longitudinal CSF samples in a subset of these patients.

Methods – In 30 patients with RRMS, CSF was obtained prior to and following 12 months of natalizumab treatment. NfH \textsuperscript{SMI35} was measured by an electrochemiluminescence-based immunoassay. NfL levels were determined previously by the UmanDiagnostics NF-light\textregistered assay.

Results – NfH \textsuperscript{SMI35} decreased in 73.3% and NfL in 90% of the patients following natalizumab treatment (32.4 vs 27.4 pg/ml, \(P = 0.002\) and 820 vs 375 pg/ml, \(P < 0.0001\)). Patients experiencing a relapse showed higher NfH \textsuperscript{SMI35} levels compared with patients in remission (47.7 vs 27.6 pg/ml, \(n = 8, P = 0.001\)). This difference was less obvious for NfL (1055 vs 725 pg/ml, \(P = 0.256\)). In patients in remission, NfL levels were lower following natalizumab treatment (830 vs 365 pg/ml, \(n = 20, P = 0.0002\)), whereas the same comparison failed significance for NfH \textsuperscript{SMI35} (28.3 vs 26.9 pg/ml, \(P = 0.086\)).

Conclusions – We confirm previous findings, indicating reduced axonal damage under natalizumab treatment by measuring NfH \textsuperscript{SMI35}, using an assay with independent methodology. In comparison with NfH \textsuperscript{SMI35}, NfL changes were more pronounced and the treatment effect also included patients in remission. Our results suggest that NfL is superior over NfH \textsuperscript{SMI35} as therapeutic biomarker and is a promising candidate to measure neuroaxonal damage in MS treatment trials.

Key words: cerebrospinal fluid; multiple sclerosis; natalizumab; neurofilament heavy chain; neurofilament light chain

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Introduction

Neurofilaments (Nf) are specifically expressed in axons and dendrites and are composed of four subunits: the triplet of the Nf-light (NfL), Nf medium (NfM) and heavy (NfH) chains and \(\alpha\)-internexin in the central, or peripherin in the peripheral nervous system (1). They have emerged as promising biomarkers for neurodegeneration experimentally and clinically in a range of neurological disorders. Reliable body fluid biomarkers are needed to quantify disease activity and progression, and thus to adopt them as surrogate endpoints in trials on potentially disease modifying agents. Increased NfL and NfH levels have been found in all stages of multiple sclerosis (MS) with
the highest levels during relapses and in relation to contrast enhancing lesions on MRI (2–5). Natalizumab binds to α4β1-integrin, preventing lymphocyte transmigration into the CNS and is approved as monotherapy for relapsing remitting multiple sclerosis (RRMS) (6).

The UmanDiagnostics NF-light® (Umeå, Sweden) assay uses two highly specific non-competing monoclonal antibodies to quantitate NfL in human body fluids (7). Employing this assay in patients with RRMS showed that natalizumab treatment normalized cerebrospinal fluid (CSF) NfL levels (8). We have recently developed a sensitive electrochemiluminescence (ECL)-based solid-phase sandwich immunoassay for NfH (9).

In this study, we wanted to confirm and compare the results obtained with the NfL assay by determining CSF NfH levels in a subset of the patients who had previously shown significantly reduced NfL levels after natalizumab treatment (8).

Patients and methods

Patients, CSF samples and Nf assays

Cerebrospinal fluid was consecutively collected from 30 patients by lumbar spinal taps before (pre-Nat) and after (post-Nat) 12 months of natalizumab treatment [all samples from the Sahlgrenska Academy, University of Gothenburg that were included in (8)]. All patients [median age 35.0 (IQR 28.8–40.5) years; disease duration 6.5 (4.0–9.3) years, Expanded Disability Status Scale (EDSS) 3.5 (2.0–6.0)] were in the RRMS stage of the disease. CSF NfH levels were determined with an ECL-based solid-phase sandwich immunoassay, which has been described in detail elsewhere (9). CSF NfL levels were measured with the UmanDiagnostics NF-light® ELISA as described in (8). The investigators who conducted the NfH measurements had no access to the clinical data.

Standard protocol approvals, registrations and patient consents

Samples were collected after written patient informed consent, and the study was approved by the regional ethical board of Uppsala University, Sweden.

Statistical evaluation

Neurofilament levels were compared by Wilcoxon matched pairs test or Mann–Whitney U-test. Correlation analysis was carried out by Spearman rank correlation coefficient (r). Partial correlations adjusted for age were computed by first regressing the two variables on age and then determining the Spearman rank correlation coefficient (r) of the respective residuals. A two-sided P-value < 0.05 was considered as significant. All statistical analyses and graphs were prepared using SPSS (Version 15.0; SPSS, Chicago, IL, USA) and Graph Pad Prism 5.02 for Windows (GraphPad Software, San Diego, CA, USA).

Results

Effect of natalizumab treatment

22/30 patients (73.3%) had lower NfH levels in post-Nat compared with pre-Nat [median 820 (405–1300) pg/ml vs 27.4 (21.0–34.7) pg/ml, P = 0.002, Fig. 1]. In comparison, 27/30 (90%) patients had lower NfL levels in post-Nat compared with pre-Nat [820 (405–2130) pg/ml vs 375 (293–575) pg/ml, P < 0.0001, Fig. 1].

Age association

NfH showed a moderate correlation with age at both time points (pre-Nat: r = 0.38, P = 0.038; post-Nat: r = 0.399, P = 0.029), conversely this was not seen for NfL (pre-Nat: r = −0.07, P = 0.73; post-Nat: r = 0.20, P = 0.299).

Disability and disease activity

NfH and NfL did not correlate with EDSS score after age correction at either of the two time points (NfH: pre-Nat: r = 0.29, P = 0.123; post-Nat: r = 0.05, P = 0.794; NfL: pre-Nat: r = 0.001, P = 0.997; post-Nat: r = 0.058, P = 0.761).

Eight of the 30 patients experienced a relapse within 3 months prior to pre-Nat and three patients had a relapse whilst receiving natalizumab prior to sampling (post-Nat) (one patient experienced a relapse prior to both samplings). NfH levels were significantly higher in pre-Nat patients experiencing a relapse (47.7 pg/ml, n = 8) vs those in remission (27.6 pg/ml, n = 22, P = 0.001), whereas this difference was not significant for NfL (relapse: 1055 pg/ml, remission: 725 pg/ml, P = 0.256). The duration since onset of relapse and NfH correlated (R = 0.73, P = 0.04); this was less clear for NfL (R = 0.64, P = 0.091).

After exclusion of the 10 patients experiencing a relapse in the 3 months prior to or after starting natalizumab, median NfL levels dropped from 830 to 365 pg/ml (P = 0.0002) after natalizumab
treatment. This was less clear for NfH$_{SMI35}$ levels (pre-Nat: 28.3 pg/ml; post-Nat: 26.9 pg/ml, $P = 0.086$), that is 90% of the patients experienced a reduction of NfL, whereas this was only seen in 65% of the patients for NfH$_{SMI35}$. NfL/NfH$_{SMI35}$ levels in the 10 patients experiencing a relapse were clearly lower in post-Nat compared with pre-Nat (775 pg/ml vs 550 pg/ml, $P = 0.013$ and 45.6 pg/ml vs 32.2 pg/ml, $P = 0.013$).

**Discussion**

Neurofilament heavy chain is the most extensively phosphorylated protein of the human brain with regulatory influences on cell structure homeostasis and axonal transport (10), whilst NfL is the most abundant and essential component of the Nf core, acting as the backbone to which NfM and NfH copolymerize (11). Persisting neurological deficits in MS likely emerge as a consequence of accumulating axonal injury starting in the very early phase of the disease. Due to the lack of reliable, quantitative biomarkers, the effect of immunomodulatory treatments on neuroaxonal damage and degeneration has been difficult to assess. Increased CSF levels of Nf reflect ongoing axonal deterioration, the culprit of disability development in MS. Several other body fluid biomarkers mirror different parts of the actual immune activity in MS. In comparison with these inflammatory biomarkers, the therapeutic impact from immunomodulatory drugs on Nf levels add essential information about the effects on neuroaxonal damage.

A recent study in 92 patients with RRMS showed that natalizumab treatment for 6–12 months reduced NfL levels from a mean of 1300–400 pg/ml ($P < 0.0001$). Post-natalizumab treatment values were similar to levels from healthy subjects (350 pg/ml) (8). In this study, the mean NfL concentration in patients with a recent relapse was 2300 pg/ml ($n = 30$), as compared with 860 pg/ml in patients in remission ($n = 62$, $P < 0.038$). Importantly, when analysing exclusively the patients in remission, NfL levels were still significantly reduced following natalizumab treatment ($P < 0.001$). We confirm these findings by independent measurements of NfH$_{SMI35}$ in a subset of 30 patients from this cohort ($P = 0.002$). Despite losing significance for the reduction of NfH$_{SMI35}$ after excluding 10 of the 30 patients that experienced a relapse within 3 months of sampling, these results support and confirm mitigation of neuroaxonal damage or degeneration by natalizumab treatment.

Of note, the NfL measurements seemed to be more treatment responsive than NfH$_{SMI35}$: the mean post-treatment reduction of NfL was 45% as opposed to 11% for NfH$_{SMI35}$. This suggests that measurements of NfL could be superior over NfH$_{SMI35}$ to detect treatment effects in the CSF of patients with MS.

Previous studies have reported relatively weak correlations of NfL with the EDSS (12) and with age in controls (13). Similar findings have been reported for the NfH protein in CSF of patients with MS (5). Sample access in our study was limited to 30 CSF pairs and the follow-up was limited to 12 months. Besides the fact that measured Nf values likely reflect the rate of actual ongoing axonal destruction, we feel this to be a possible explanation for the lack of correlation of the investigated Nf proteins and the EDSS scores.

Taken together, we confirm CSF Nf as promising candidates to measure neuroaxonal damage or degeneration in MS treatment trials. In comparison with the ECL-NfH$_{SMI35}$ assay, the responsiveness of the NF-light ELISA to natalizumab treatment was more pronounced, proposing the UmanDiagnostics NF-light$^{TM}$ assay as the preferred assay for future investigations.
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Conflict of interest

J. Kuhle receives research support from Novartis Pharma, Protagen AG, Roche and served in scientific advisory boards for Genzyme/Sanoﬁ-Aventis, Merck Serono and Novartis Pharma used exclusively for research. His work is supported by an ECTRIMS Research Fellowship Programme and by the ‘Forschungsfonds’ of the University of Basel, Switzerland. C. Malmeström receives non-conditional research grants from Biogen Idec, lecture honoraria and travel grants from Biogen Idec and Merck Serono and serves in scientific advisory boards for Novartis Pharma. M. Axelsson reports no disclosures. K. Plattner reports no disclosures. O. Yaldızlı has received advisory, consulting and lecture fees, travel expenses for attending meetings and financial support for research from Bayer Schering, Biogen Idec, Merck Serono, Novartis Pharma and Teva Neurosciences. Advisory, consulting and lecture fees were exclusively used for education and research at the neurological department (University of Basel). T. Derfuss serves on scientific advisory boards for Novartis Pharma, Merck Serono, Biogen Idec, Genzyme, Mitsubishi Pharma and Bayer Schering Pharma; has received funding for travel and/or speaker honoraria from Biogen Idec, Novartis Pharma, Merck Serono and Bayer Schering Pharma; and receives research support from Novartis Pharma, Merck Serono, the German Research Foundation, the European Union and the Swiss MS Society. G. Giovannoni serves on scientific advisory boards for Merck Serono and Biogen Idec and Vertex Pharmaceuticals; served on the editorial board of Multiple Sclerosis; has received speaker honoraria from Bayer Schering Pharma, Merck Serono, Biogen Idec, Pfizer Inc; Teva Pharmaceutical Industries Ltd.–Sanoﬁ-aventis, Vertex Pharmaceuticals, Genzyme Corporation, Ironwood and Novartis Pharma; has served as a consultant for Bayer Schering Pharma, Biogen Idec, GlaxoSmithKline, Merck Serono, Protein Discovery Laboratories, Teva Pharmaceutical Industries Ltd.–Sanoﬁ-aventis, Vertex Pharmaceuticals, Genzyme Corporation, Ironwood and Novartis Pharma; has served as a consultant for Bayer Schering Pharma, Merck Idec, GlaxoSmithKline, Merck Serono, Protein Discovery Laboratories, Teva Pharmaceutical Industries Ltd., Sanoﬁ-Aventis, UCB, Vertex Pharmaceuticals, GW Pharma, Novartis Pharma and FivePrime; serves on the speakers bureau for Merck Serono; and has received research support from Bayer Schering Pharma, Biogen Idec, Merck Serono, Novartis Pharma, UCB, Merz Pharmaceuticals, LLC, Teva Pharmaceutical Industries Ltd, Sanoﬁ-Aventis, GW Pharma and Ironwood. L. Kappos has participated in the last 24 months as principal investigator, member or chair of planning and steering committees or advisory boards in corporate-sponsored clinical trials in multiple sclerosis and other neurological diseases. The sponsoring pharmaceutical companies for these trials include Abbott, Actelion, Advancell, Allozyme, BaroFold, Bayer Health Care Pharmaceuticals, Bayer Schering Pharma, Bayhill, Biogen Idec, BioMarin, CSL Behring, Elan, Genmab, GeNeuro SA, Genmark, GlaxoS aith Kline, Lilly, Merck Serono, Novartis Pharma, Novonordisk, Peptimmune, Sanoﬁ-Aventis, Santher-a, Roche, Teva, UCB and Wyeth. He has also lectured at medical conferences or in public on various aspects of the diagnosis and management of MS. In many cases, these talks have been sponsored by non-restricted educational grants to his institution from one or another of the above-listed companies. Honoraria and other payments for all these activities have been exclusively used for funding of research of his department. Research and the clinical operations (nursing and patient care services) of the MS Centre in Basel have been supported by non-restricted grants from one or more of these companies. J. Lycke serves on scientific advisory boards for Teva, Biogen Idec and Genzyme/ Sanofi-Aventis; has received speaker honoraria and travel grants from Bayer Schering Pharma, Biogen Idec, Novartis Pharma and Sanofi-Aventis; serves on the editorial board of the Acta Neurologica Scandinavica and receives non-conditional research grants from Biogen Idec and Novartis Pharma.

References