

Short Report

Serum Neurofilament Light Chain in NMOSD and Related Disorders: Comparison According to Aquaporin-4 and Myelin Oligodendrocyte Glycoprotein Antibodies Status

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Abstract

Background: Neurofilament light chain (NF-L) levels reflect axonal damage in different conditions, including demyelinating disorders.

Objectives: We aimed to compare serum NF-L levels in patients with aquaporin-4 antibodies (AQP4-Ab), myelin oligodendrocyte antibodies (MOG-Ab) and seronegative cases with neuromyelitis optica spectrum disorders and related disorders.

Methods: We analysed AQP4-Ab and MOG-Ab with cell-based assay and NF-L with ultrasensitive electrochemiluminescence immunoassay.

Results: Median NF-L levels were increased in 25 AQP4-Ab-positive patients (59 pg/ml) as compared with 22 MOG-Ab-positive cases (25 pg/ml), 52 seronegative patients (18 pg/ml), 25 multiple sclerosis patients (12 pg/ml) and 14 healthy controls (12 pg/ml).

Conclusions: Increased serum levels of NF-L in patients with AQP4-Ab or MOG-Ab might reflect an ongoing axonal damage and a more malignant disease course.

Keywords: Neurofilament light chain, optic neuritis, myelitis, neuromyelitis optica (NMOSD), aquaporin-4, myelin oligodendrocyte glycoprotein

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Introduction

Cerebrospinal fluid (CSF) neurofilament light chain (NF-L) has been proposed as potential biomarker of disease activity in different conditions, including multiple sclerosis (MS) and clinically isolated syndrome (CIS).¹ The strong correlation between CSF and serum NF-L, detected with ultrasensitive single-molecule array, supports their value in monitoring tissue damage and treatment response.² Although considered a useful biomarker in demyelinating disorders, serum NF-L concentration has never been investigated in neuromyelitis optica spectrum disorders (NMOSD) according to antibody status. Our objective was to analyse serum NF-L in patients with NMOSD and related disorders and to compare its level in cases with autoantibodies to aquaporin-4

(AQP4-Ab), myelin oligodendrocyte antibodies (MOG-Ab) and seronegative patients.

Materials and methods

We identified patients referred for serum AQP4/MOG-Ab analysis to the Laboratory of Neuropathology, University of Verona, between May 2014 and May 2017. Of the 454 consecutive serum samples that were analysed, nine were found to be AQP4-Ab positive and MOG-Ab negative, and 22 MOG-Ab positive and AQP4-Ab negative. To extend the analysis, we retrospectively identified 16 AQP4-Ab positive cases among patients referred for AQP4-Ab analysis between April 2012 and April 2014. Among seronegative cases, we excluded those with a final diagnosis of non-inflammatory neurological disorders, other defined inflammatory

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disorders, CIS or MS. All samples obtained from AQP4-Ab-positive and seronegative patients were collected at disease onset, in the absence of any disease-modifying treatment. Among MOG-Ab positive cases, 12 samples were obtained at onset, seven during relapses, one in the course of progression and two in the chronic stage (median time from disease onset 0 months, range 0–264 months). Only one MOG-Ab positive patient was under disease-modifying treatment (mycophenolate mofetil) at the time of sample collection. As controls we included 25 MS patients and 14 healthy subjects. Five cases with MS were under relapse at the time of NF-L analysis, and 14 patients, all with a relapsing course, had been undertaking treatment for at least 6 months. Median time from disease onset was 193 months (range 1–445) in patients with relapsing–remitting MS (RRMS), and 219 months (range 75–274) in those with progressive MS.

A commercial cell-based assay (Euroimmun, Lübeck-Germany) was used for the detection of AQP4-Ab. Seronegative cases with a history suggestive for NMOSD were tested for AQP4-Ab using a live cell-based assay at the Neurological Research Laboratory of Innsbruck. The presence of MOG-Ab was analysed by three independent investigators (SF, SM, AF) at the Verona Neuropathology Laboratory using recombinant live cell-based immunofluorescence assay with HEK293A cells transfected with full-length MOG (human MOG alpha-1 EGFP fusion protein) and incubated with CyTm 3-conjugated goat anti-human IgG antibody (H+L, Jackson ImmunoResearch Laboratory, West Grove, PA, USA; diluted 1:200 in PBS/10%FCS), as previously described.^{3,4} Data were collected as part of standard clinical practice, and patients consented to diagnostic procedures and sample storage at Verona Neuropathology Laboratory. According to available medical records, analysed patients were classified into five diagnostic categories: NMOSD;⁵ idiopathic optic neuritis (ON); idiopathic acute myelitis (AM); ON and AM; other demyelinating disorders. Idiopathic ON and/or AM were defined as acute/subacute optic neuropathy and/or myelopathy of inflammatory origin not fulfilling other established diagnostic criteria. Other demyelinating disorders were characterized by inflammatory conditions with clinical, CSF and radiological evidence, not included in the disorders previously mentioned.

Serum NF-L levels were quantified by an investigator blinded to patient data (AF), using a highly sensitive electrochemiluminescence based

immunoassay as previously reported.⁶ Briefly, diluted sera and standards were incubated in duplicate in Meso Scale Discovery (MSD) plates previously coated (mAB 47:3, UmanDiagnostics). After blocking and washing, biotinylated secondary antibody (mAB 2:1, UmanDiagnostics) was added. SULFO-TAG_{TM}-labelled streptavidin and ECL read buffer (MSD) were added and signal was measured with QuickPlex SQ120. Data were analysed by Discovery Workbench 4.0 software, MSD, using a four-parameter weighted logistic curve. The lower detectable value was 12 pg/ml, according to the standard curve.

Statistical analysis was performed using IBM SPSS, release V.24.0. We compared clinical, demographic and serological data using the Kruskal–Wallis test with Dunn’s multiple comparison test, Chi-square test and binary logistic regression analysis (enter model). Statistical significance was defined as a two-sided *p*-value of <0.05 and *p*-values were corrected for multiple comparison using Bonferroni’s correction if applicable. Sex and age were used as covariates, in accordance to previous reports.²

Results

All patients with AQP4-Ab had a final diagnosis of NMOSD. The most frequently observed final diagnosis among MOG-Ab-positive patients was ON (10), followed by other demyelinating disorders (6), ON+AM (3), AM (2) and MS (1). A monophasic course was observed in 45.5% of patients, a relapsing one in 50% and a gradually evolving course in 4.5%. Seronegative cases had a final diagnosis of ON (22), AM (23) and NMOSD (7). Among patients with MS the course was relapsing–remitting in 18, primary progressive in three (PPMS), and secondary progressive in four (SPMS) cases. The median Expanded Disability Status Scale score at the time of sampling was 2.75 (range 1–6) in patients with RRMS and 6.5 (range 6–8.5) in cases with progressive MS.

Median serum NF-L concentration was significantly higher in patients with AQP4-Ab (59 pg/ml) compared with healthy controls (12 pg/ml), and was also increased in MOG-Ab positive (25 pg/ml) and seronegative patients (18 pg/ml), although the latter did not reach significant difference (Table 1). Detectable levels of NF-L (≥ 15 pg/ml, 12 pg/ml + 20% to correct for measurement error) were more frequently observed in patients with AQP4-Ab (68%) and MOG-Ab (70%) as compared with seronegative cases (57%), patients with MS (44%) or healthy

Table 1. Serum neurofilament light chain levels in patients with NMOSD and related disorders, MS and healthy controls according to antibody reactivity to AQP4 and MOG.

	NMOSD and related disorders			Controls		<i>p</i> -value
	AQP4-Ab positive	MOG-Ab positive	Seronegative	MS	HC	
Number	25	22	52	25	14	
Females	23 (92%)	12 (54%)	34 (65%)	16 (64%)	9 (64%)	0.064 ^a
Age	51 (31–79)	40 (15–64)	44 (21–69)	44 (28–67)	37 (26–62)	0.095 ^b
Serum NF-L (pg/ml)	59 (12–994) [*]	25 (12–140)	18 (12–540)	12 (12–1464)	12 (12–788)	0.021 ^b
Serum NF-L ≥15 pg/ml (detectable levels)	17 (68%) <i>p</i> = 0.034 ^c	14 (70%) <i>p</i> = 0.019 ^c	31 (57%) <i>p</i> = 0.077 ^c	11 (44%) <i>p</i> = 0.417 ^c	4 (29%) reference	0.018 ^c

Groups were statistically compared with ^aChi-square test, ^bKruskal–Wallis test (data shown as median with 9th–95th percentile range) and ^cbinary logistic regression analysis (enter model) with age and sex as covariates. ^{*} Statistically significant difference to HC (corrected *p*-value <0.05).
 Ab: antibody; AQP4: Aquaporin-4; HC: healthy controls; MOG: myelin oligodendrocyte glycoprotein; MS: multiple sclerosis; NF-L: neurofilament light chain; NMOSD: Neuromyelitis optica spectrum disorders.
 AQP4-ab positive: NMOSD (25); MOG-Ab positive: ON (10), AM (2), ON+AM (3), other demyelinating disorders (6), MS (1); seronegative: ON (22), AM (23), NMOSD (7); MS: RRMS (18), SPMS (4), PPMS (3).

controls (29%). Overall, NF-L levels were significantly increased in AQP4-Ab and MOG-Ab positive patients as compared with healthy controls (Table 1).

Discussion

In patients with demyelinating disorders, increasing attention has recently been paid to biomarkers which reflect neural tissue damage and predict long-term disability. In particular, glial fibrillary acid protein (GFAP) had been indicated as a biomarker of astrocytic damage and clinical severity in patients with NMOSD.⁷ Moreover, it was shown that myelin basic protein is elevated in the CSF of both AQP4-Ab and MOG-Ab-positive patients as compared with MS patients, while GFAP is higher only in AQP4-Ab-positive ones, reflecting either myelin injury or astrocyte damage, respectively.⁸ In this scenario, the role of axonal damage reflected by elevation of neuron-specific biomarkers located in axons is unclear. An increase of NF-heavy and light chains has been demonstrated in CSF of patients with NMOSD compared with MS cases, suggesting the implication of axonal injury.⁹ However, previous reports were mainly focused on CSF analysis, and did not account for the presence of specific antibodies which might induce prominent damage at their target site. We here report the first observation of increased serum NF-L levels in patients with AQP4-Ab and MOG-Ab, compared with seronegative ones. Our study is limited by the relatively small number

of patients and by the partial availability of clinical data that did not allow an extensive comparison of clinical, radiological and laboratory findings in the whole cohort. Compared with previous studies, we observed lower NF-L levels in patients with MS, probably related to the absence of recent relapses in most of them, and to the fact that most of these patients were under treatment.² In contrast, NF-L levels observed in healthy controls were comparable.^{2,6} The high NF-L levels associated with AQP4-Ab might in part explain the more severe clinical phenotype observed in these patients, reflecting the axonal injury induced by astrocytic and complement-mediated cellular damage. On the other hand, in MOG-Ab positive patients, the relatively increased levels of the axonal biomarker NF-L could reflect the long-term disability described in many MOG-Ab-positive cases, suggesting that myelin and neuronal damage may coexist in this condition.

Conclusion

Our data demonstrate that serum NF-L concentration is elevated in patients with MOG-Ab and, prominently, with AQP4-Ab-associated NMOSD and related disorders, possibly reflecting axonal injury. Future studies correlating NF-L levels, disease course and therapeutic effectiveness are required in order to establish the role of NF-L as a biomarker of disease activity in these conditions.

Conflicts of interest

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: Alberto Gajofatto received speaking honoraria and travel support to attend scientific meeting by Merck and Novartis. Ruggero Capra received lecture fees from Novartis, Biogen, Teva, Genzyme and Sanofi-Aventis. The University Hospital, and Medical University of Innsbruck (Austria, Markus Reindl) receives payments for antibody assays (NMDAR, AQP4, and other autoantibodies) and for MOG and AQP4 antibody validation experiments organized by Euroimmun (Luebeck, Germany). Markus Reindl is an academic editor for *PLoS One*. The other authors declare that there is no conflict of interests.

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References

1. Håkansson I, Tisell A, Cassel P, et al. Neurofilament light chain in cerebrospinal fluid and prediction of disease activity in clinically isolated syndrome and relapsing-remitting multiple sclerosis. *Eur J Neurol* 2017; 24: 703–712.
2. Disanto G, Barro C, Benkert P, et al. Swiss Multiple Sclerosis Cohort Study Group. Serum Neurofilament light: A biomarker of neuronal damage in multiple sclerosis. *Ann Neurol* 2017; 81: 857–870.
3. Mader S, Gredler V, Schanda K, et al. Complement activating antibodies to myelin oligodendrocyte glycoprotein in neuromyelitis optica and related disorders. *J Neuroinflammation* 2011; 8: 184.
4. Di Pauli F, Mader S, Rostasy K, et al. Temporal dynamics of anti-MOG antibodies in CNS demyelinating diseases. *Clin Immunol* 2011; 138: 247–254.
5. Wingerchuk DM, Banwell B, Bennett JL, et al. International Panel for NMO Diagnosis. International consensus diagnostic criteria for neuromyelitis optica spectrum disorders. *Neurology* 2015; 85: 177–189.
6. Gaiottino J, Norgren N, Dobson R, et al. Increased neurofilament light chain blood levels in neurodegenerative neurological diseases. *PLoS One* 2013; 8: e75091.
7. Takano R, Misu T, Takahashi T, Sato S, Fujihara K and Itoyama Y. Astrocytic damage is far more severe than demyelination in NMO: A clinical CSF biomarker study. *Neurology* 2010; 75: 208–216.
8. Kaneko K, Sato DK, Nakashima I, et al. Myelin injury without astrocytopathy in neuroinflammatory disorders with MOG antibodies. *J Neurol Neurosurg Psychiatry* 2016; 87: 1257–1259.
9. Wang H, Wang C, Qiu W, Lu Z, Hu X and Wang K. Cerebrospinal fluid light and heavy neurofilaments in neuromyelitis optica. *Neurochem Int* 2013; 63: 805–808.
1. Håkansson I, Tisell A, Cassel P, et al. Neurofilament light chain in cerebrospinal fluid and prediction of