Neuroinflammation and neuroaxonal damage in multiple sclerosis: a cross-sectional cerebrospinal fluid-based proteomic study

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INTRODUCTION AND AIM

Neuroaxonal damage is strongly related to disease progression in multiple sclerosis (MS) [1]. In MS, axonal loss is considered the detrimental consequence of central nervous system (CNS) inflammation [2]. While several treatments are effective in reducing the inflammatory activity of the disease, no therapy is available to directly counteract axonal damage [3]. The study of cerebrospinal fluid (CSF) inflammatory markers closely related to axonal damage can help to identify novel immunological pathways responsible for a more severe neuronal injury.

The aim of this study was to explore the correlations between a panel of CSF inflammation-related proteins (IRPs) and a well-established marker of neuro-axonal damage, namely CSF neurofilament light (NfL).

PATIENTS AND METHODS

The levels of NfL and of 92 IRPs were determined in the CSF of patients with radiologically isolated syndrome **(RIS, n=6),** clinically isolated syndrome (**CIS, n=32**), relapsing remitting MS (**RRMS, n=51**), progressive MS (**PMS, n=8**) and in the CSF of patients with other neurological diseases as control group (**OND, n=36**). NfL was assessed through a newly developed in-house ELISA while the 92 IRPs were determined with a proximity extension assay (PEA) using the Proseek Multiplex Inflammation I kit (Olink Bioscience, Uppsala, Sweden) **(Figure 1) (Figure 2).**

FIGURE 1. Study design. Collection of CSF samples and subsequent analyses.

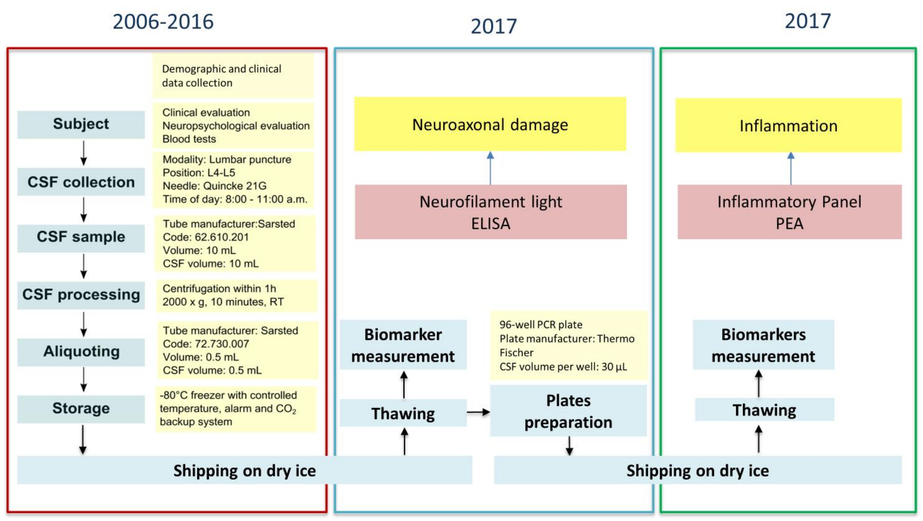
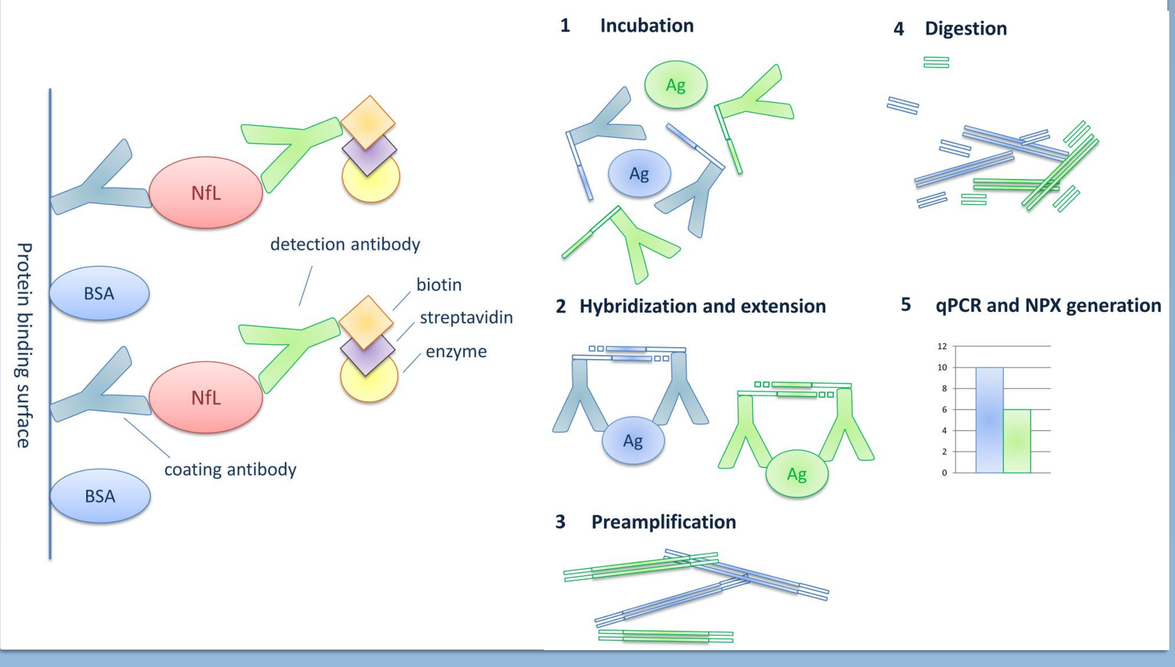


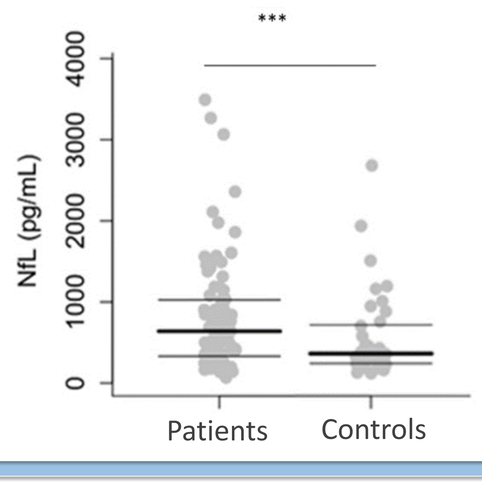
FIGURE 2. ELISA for NfL (left) and proximity extension assay analysis (PEA) (right).



RESULTS - NfL

- **CSF NfL levels were significantly higher in RIS, CIS, RRMS and PMS patients as compared to controls (p<0.001) (Figure 3).**
- No significant differences in CSF NfL values were found between RIS, CIS, RRMS and PMS patients.

FIGURE 3. CSF NfL values in patients vs ONDs.



RESULTS - IRPs

- Out of the 92 IRPs, 41 were excluded from the analysis because of a call rate < 75% (>75% of all the patients had values below the lower limit of detection).
- 44 IRPs were not significantly different between patients and controls.
- 8 proteins (listed according to p-values from p<0.0001 to p=0.049: CD5, IL12B, TNFβ, MIP1α, TNFSF14, TNFRSF9, CXCL11) were significantly increased in the CSF of MS patients **(Figure 4) (Figure 5).**

FIGURE 4. IRPs fold **change** in MS patients as compared to OND controls. The arrows indicate the significant differences (Adjusted ANOVA + Tukey contrasts)

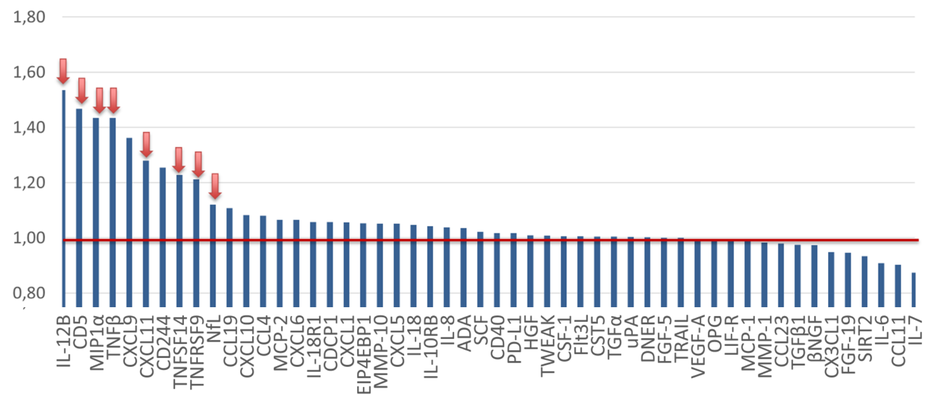
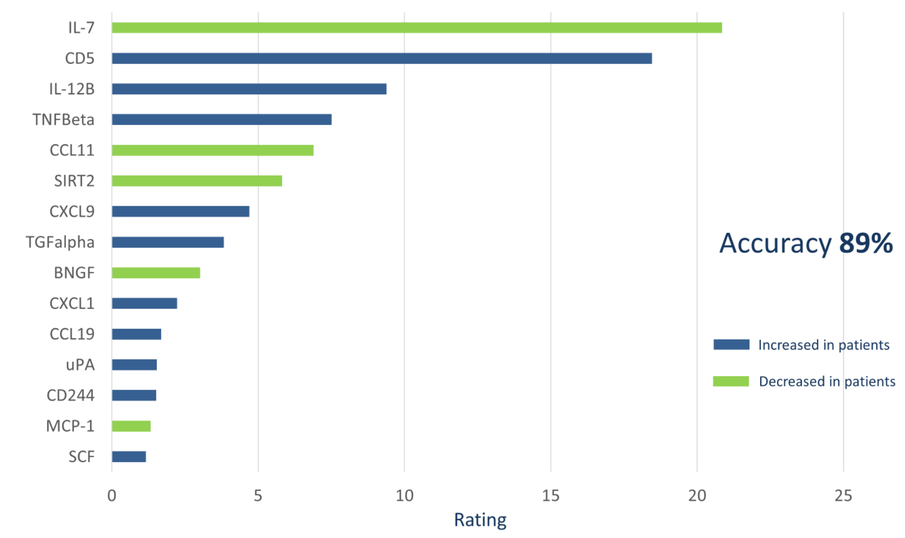


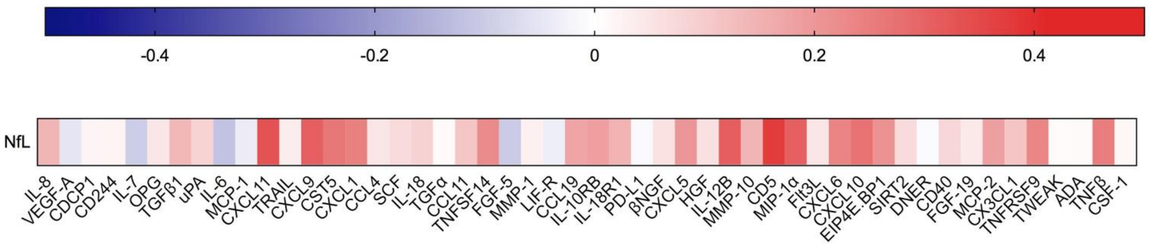
FIGURE 5. Stochastic gradient boosting rating values of each of the IRPs in the discrimination between MS patients and OND controls. The model reaches a high diagnostic accuracy (89%).



RESULTS – IRPs and NfL

- 15 proteins (including the 7 proteins with higher concentrations in the CSF of MS patients plus CXCL1, CXCL6, CXCL9, CXCL10, CCL23, CCL28, CST5, EIF4EBP1) positively correlated with the levels of CSF NfL in MS patients **(Figure 6).**

FIGURE 6. Spearman's rank correlation coefficents for the correlation between IRPs and NfL.



CONCLUSIONS

- In MS patients **several IRPs are increased** as compared to controls.
- Several IRPs **positively correlate with the degree of neuroaxonal damage**.
- The IRPs we have found to be increased in MS and to correlate with neuroaxonal damage reflect different immunological pathways including **B cell activity and lymphoid neogenesis.**

References.
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2. Dendrou CA, Fugger L, Friese MA (2015) Immunopathology of multiple sclerosis. Nat Rev Immunol 15: 545-558.
3. Thompson AJ (2017) Challenge of progressive multiple sclerosis therapy. Curr Opin Neurol 30: 237-240.