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Cerebrospinal fluid biomarkers as a measure of disease activity and treatment efficacy in relapsing-remitting multiple sclerosis

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Abbreviations used:

AD Alzheimers disease

CCL2 C-C motif chemokine ligand 2

CIS clinically isolated syndrome

CHIT1 chitotriosidase

CHI3L1 chitinase-3-like protein 1

CSF Cerebrospinal fluid

CXCL13 C-X-C motif chemokine 13

DMT disease modifying therapy

EDSS Expanded Disability Status Scale

ELISA enzyme-linked immunosorbent assay

Gd gadolinium

GFAP glial fibrillary acidic protein

JC John Cunningham

LLoQ lower limit of quantification

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MCI mild cognitive impairment

MRI magnetic resonance imaging

MS Multiple Sclerosis

MSSS Multiple Sclerosis Severity Score

NA not applicable

NFL neurofilament light protein

NGRN neurogranin

PML progressive multifocal leukoencephalopathy

RRMS Relapsing-remitting Multiple Sclerosis

Abstract

Cerebrospinal fluid (CSF) biomarkers can reflect different aspects of the pathophysiology of relapsing-remitting multiple sclerosis (RRMS). Understanding the impact of different disease modifying therapies on the CSF biomarker profile may increase their implementation in clinical practice and their appropriateness for monitoring treatment efficacy. The present study investigated the influence of first-line (interferon beta) and second-line (natalizumab)

therapies on seven CSF biomarkers in RRMS and their correlation with clinical and radiological outcomes.

We included 59 RRMS patients and 39 healthy controls. The concentrations of C-X-C motif chemokine 13 (CXCL13), C-C motif chemokine ligand 2 (CCL2), chitinase-3-like protein 1 (CHI3L1), glial fibrillary acidic protein (GFAP), neurofilament light protein (NFL), and neurogranin (NGRN) were determined by ELISA, and chitotriosidase (CHIT1) was analyzed by spectrofluorometry.

RRMS patients had higher levels of NFL, CXCL13, CHI3L1, and CHIT1 than controls ($p<0.001$). Subgroup analysis revealed higher NFL, CXCL13 and CHIT1 levels in patients treated with first-line therapy compared to second-line therapy ($p=0.008$, $p=0.001$ and $p=0.026$, respectively). NFL and CHIT1 levels correlated with relapse status, and NFL and CXCL13 levels correlated with the formation of new MRI lesions. Furthermore, we found an association between inflammatory and degenerative biomarkers.

The results indicate that CSF levels of NFL, CXCL13, CHI3L1, and CHIT1 correlate with the clinical and/or radiological disease activity, providing additional dimensions in the assessment of treatment efficacy.

Introduction

Accumulating evidence suggests that, in relapsing-remitting multiple sclerosis (RRMS), early signs of CNS degeneration are dependent on inflammatory activity (Malmestrom et al., 2003, Lycke et al., 1998, Gunnarsson et al., 2011). The levels of several cerebrospinal fluid (CSF) biomarkers seem to reflect disease activity, and CSF levels decrease towards normality during intervention with disease modifying therapies (DMTs) (Gunnarsson et al., 2011, Kuhle et al., 2015a, Khademi et al., 2011, Conductier et al., 2010, Comabella et al., 2010, Malmestrom et al., 2014, Verbeek et al., 2010, Lycke et al., 1998, Axelsson et al., 2014, Franciotta et al., 2001, Axelsson et al., 2011, Sellebjerg et al., 2009, Olsson et al., 2012). Previous investigations of CSF biomarkers revealed altered immune cell (Sospedra and Martin, 2005)

and protein profiles, reflecting different aspects of the immunopathogenesis of MS (Axelsson et al., 2011, Malmestrom et al., 2003, Sellebjerg et al., 2009).

Neurofilament light protein (NFL) is a major component of the cytoskeleton of the axon, and elevated levels of NFL in the CSF of MS patients indicate ongoing neuro-axonal degeneration (Malmestrom et al., 2003, Lycke et al., 1998). NFL levels are increased in the CSF during all stages of MS, with the highest levels following acute relapses (Malmestrom et al., 2003) or during contrast enhancement of lesions on MRI (Axelsson et al., 2014). DMTs reduce the CSF levels of NFL (Gunnarsson et al., 2011, Kuhle et al., 2015a, Axelsson et al., 2014).

Chitinase-3-like protein 1 (CHI3L1, also known as YKL-40) is expressed by microglia, macrophages, epithelial cells, and astrocytes and is involved in the pathogenesis of chronic autoimmune disorders (Bonneh-Barkay et al., 2012). High CSF levels of CHI3L1 have been associated with disability progression and, together with NFL, are higher in patients with shorter time to conversion to MS from a clinically isolated syndrome (CIS) (Comabella et al., 2010, Martinez et al., 2015). CSF CHI3L1 levels are reduced by DMTs (Malmestrom et al., 2014). Glial fibrillary acidic protein (GFAP) is also expressed by astrocytes. The concentration of GFAP in the CSF is thought to reflect astrogliosis, and increased concentrations of GFAP and CHI3L1 have been associated with the progression of disability in MS (Axelsson et al., 2011, Malmestrom et al., 2003) (Martinez et al., 2015).

Chitotriosidase (also known as chitinase 1, CHIT1) is a marker of microglial activation (Olsson et al., 2012, Verbeek et al., 2010). Elevated CHIT1 levels are associated with conversion to MS in patients with CIS (Mollgaard et al., 2016). Treatment with natalizumab reduces CHIT1 levels (Olsson et al., 2012).

C-X-C motif chemokine 13 (CXCL13) is a B-cell chemokine. The concentration of CXCL13 in CSF is increased in patients with active MS and in patients converting from CIS to MS (Khademi et al., 2011). CXCL13 levels are reduced by natalizumab treatment (Sellebjerg et al., 2009).

C-C motif chemokine ligand 2 (CCL2), also known as monocyte chemoattractant protein-1 (MCP-1), is an important chemokine for the recruitment of monocytes and macrophages to the CNS. The CNS levels of CCL2 are reduced during increased disease activity in MS (Franciotta et al., 2001, Mahad and Ransohoff, 2003).

Neurogranin (NGRN) is a postsynaptic protein enriched in dendritic spines. Elevated levels of NRGN have been found in subjects with mild cognitive impairment (MCI) and Alzheimer's disease (AD) (Thorsell et al., 2010, Kester et al., 2015). In CSF, NGRN is a marker of synaptic integrity and its levels are influenced by neurodegeneration (Portelius et al., 2015).

In the present study, we explored the possible relationships between these CSF biomarkers of inflammation and degeneration in RRMS compared to healthy controls, as well as the influence of different DMTs on biomarker levels. A better understanding of the relationships between these biomarkers could lead to their implementation in clinical practice and treatment monitoring.

Material and methods

Patients and healthy controls

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Patients with RRMS fulfilling the revised McDonald criteria (Polman et al., 2011) who had an indication to switch therapies because of breakthrough disease activity (n=40), tolerability/adverse events (n=2), or risk of progressive multifocal leukoencephalopathy (PML) due to JC virus antibody positivity during natalizumab therapy (n=17) were consecutively enrolled in the study at the MS centers of Sahlgrenska University Hospital, Gothenburg (n=17), and Karolinska University Hospital, Stockholm, Sweden (n=42). A total of 59 patients were enrolled, 7 were treatment naïve (n=4) or without DMT for one year or more, 33 were on first-line (interferon beta) and 19 were on second-line treatment (natalizumab) for at least 1 year before the study.

Thirty-nine blood donors and university students served as healthy controls. None of the controls had any neurological signs or a history of neurological disease. The demographic and clinical characteristics of the patients and controls are presented in Table 1.

Clinical assessments and MRI

Patients underwent a clinical neurological examination, MRI, and lumbar puncture at one time point (Table 1). A relapse was defined as an episode of neurological disturbance lasting for at least 24 h (McDonald et al., 2001). The neurological examinations were performed by trained neurologists, disability measured using the Expanded Disability Status Scale (EDSS) (Kurtzke, 1983), and disease severity with the Multiple Sclerosis Severity Score (MSSS) (Roxburgh et al., 2005). The MRI scans of the brain and cervical column were performed with 1.5 or 3.0 Tesla machines using a 3 mm slice thickness. A standard protocol for MS was applied with T1- and T2-weighted sequences, FLAIR, and T1 following a standard dose of intravenous gadolinium (Gd) contrast. Because Gd-enhancement on MRI appears in the majority of cases during a period of 6 weeks, mean 3.07 weeks (Cotton et al., 2003) we choose to include only MRI performed 6 weeks before or after lumbar puncture in order to evaluate correlation between MRI measures and CSF biomarker concentrations. New disease activity on MRI compared to a previous MRI was defined as a new Gd-enhanced lesion or new or enlarged T2 lesion.

Blood tests and CSF sampling

Peripheral blood and CSF were sampled at the clinical assessment. The CSF samples were handled according to the consensus protocol of the BioMS-EU network for CSF biomarker research in MS (Teunissen et al., 2009). Increased concentrations of NFL in CSF were expected from relapse onset and approximately 100 days thereafter (Lycke et al., 1998). In line with this observation we considered CSF to be obtained during relapse if lumbar puncture was performed within this period of time.

Biomarker analysis

All analyses were performed by board-certified laboratory technicians at the Clinical Neurochemistry Laboratory of Sahlgrenska University Hospital according to protocols approved by the Swedish Board for Accreditation and Conformity Assessment. CSF NFL levels were measured using a sensitive sandwich ELISA method (NF-light® ELISA kit, UmanDiagnostics AB, Umeå, Sweden). The intra- and inter-assay coefficients of variation were < 10%. The lower limit of quantification (LLoQ) of the assay was 31 pg/mL. GFAP was

measured in the CSF by ELISA as previously described in detail (Rosengren et al., 1994). The LLoQ of the GFAP assay was 16 pg/mL. CSF CXCL13 levels were measured by ELISA (Human CXCL13/BLC/BCA-1 Immunoassay, R&D Systems Inc., Abingdon, United Kingdom) according to the manufacturer's instructions. The average intra- and inter-assay coefficients of variation were ≤ 10% and the LLoQ was 7.8 pg/mL. CHI3L1 in the CSF was analyzed with solid phase sandwich ELISA (Human Chitinase 3-like 1 Quantikine ELISA Kit, R&D Systems Inc., Minneapolis, MN). The intra-assay coefficient of variation was < 7% and the LLoQ was 8.15 pg/mL. CSF CCL2 levels were also analyzed by solid phase sandwich ELISA (Human CCL2/MCP-1 Quantikine ELISA Kit, R&D Systems Inc., Minneapolis, MN). The intra- and inter-assay coefficients of variation were ≤ 8% and the LLoQ was 10 pg/mL. CSF levels of NGRN were measured using an in-house ELISA with the monoclonal antibody Ng7 (BioLegend, CA, USA) as the capture antibody and the rabbit anti-NGRN antibody (Upstate, MA, USA) as the detecting antibody as described previously (Kvartsberg et al., 2015). The LLoQ was 125 pg/mL and the mean intra-assay coefficient of variation 17%. Chitotriosidase activities in the CSF were measured by an in-house method as described previously (Novakova et al., 2016). The LLoQ was 0.2 nkat/L and intra- and inter-assay coefficients of variation <10%. CSF samples below the LLoQ were designated as having the value of the LLoQ.

Statistical analysis

Visual inspection of the data and Shapiro-Wilk test of normality did not show a normal distribution of the biomarkers; therefore, non-parametric tests were used in this study. The Mann-Whitney U test was used for unpaired data and Kruskal-Wallis H test was used to analyze several independent samples. Correlations between biomarkers were analyzed by the Spearman's rank correlation test. Multiple regression analysis was performed to test the influence of gender, age, EDSS, and MSSS on the biomarkers. Statistical calculations were performed in SPSS Statistics 22 software.

Ethics

All patients and controls voluntarily participated in the study and informed consent was obtained from all subjects after oral and written information was provided. The regional ethical review boards in Uppsala (Dnr 2005:253) and Stockholm (Dnr 2009:2107) approved the study.

Results

The influence of DMTs on biomarker concentrations

RRMS patients had significantly higher mean CSF levels of NFL, CXCL13, CHI3L1, and CHIT1 than controls ($p<0.001$), but the mean CSF levels of GFAP, CCL2, and NRGN did not significantly differ between patients and controls (Table 2). Excluding patients with relapse (n=15), the mean CSF levels of NFL, CXCL13, CHI3L1, and CHIT1 were still higher than in controls ($p=0.002$, $p<0.001$, $p=0.005$, and $p<0.001$, respectively). The CSF levels of NFL, CXCL13, CHI3L1, and CHIT1 were also significantly higher in untreated patients and in patients treated with interferon beta compared to controls ($p<0.001$). In patients treated with natalizumab, only CHI3L1 and CHIT1 were significantly elevated in the CSF compared to

controls (p=0.001 and p=0.009, respectively). Patients treated with interferon beta had significantly higher levels of NFL (p=0.008), CXCL13 (p=0.001), and CHIT1 (p=0.026) than those treated with natalizumab. We found no significant difference in the levels of GFAP, CHI3L1, NGRN, or CCL2 between patients treated with interferon beta and natalizumab (Table 2).

Clinical and radiological characteristics of patients

Patients without treatment or on first-line treatment had more disease activity than those on second-line treatment based on both clinical and radiological measures (Table 3). Within 100 days (mean 51, range 4-98 days) prior to lumbar puncture, 15 patients without treatment or on interferon beta treatment had relapse, and 17 patients had ≥1 Gd-enhanced lesion, whereas no patient on natalizumab treatment had relapse and only one of them had one Gd-enhanced lesion. The median EDSS and MSSS in the group without treatment, on interferon beta and on natalizumab was 1.5 (range 0-5.0) and 2.44 (0.67-9.18), 2.0 (range 0-6.5) and 3.05 (range 0.17-9.09), and 3.5 (range 0-7.5) and 3.46 (range 0.10-9.13), respectively. We found no significant group difference regarding EDSS and MSSS. The mean age was lower in patients on interferon beta (36 years, range 19-55 years) compared to patients on natalizumab (42 years, range 23-59 years, p=0.017), and the disease duration was shorter in patients on interferon beta (6 years, range 1-15 years) compared to patients on natalizumab (13 years, range 4-23 years, p<0.001).

Relationships between biomarker concentrations and clinical and radiological disease activity

The CSF levels of NFL, CHI3L1, and CHIT1 were higher in patients with a clinical relapse (n=15) than patients in clinical remission (p=0.002, p=0.006, and p=0.006, respectively). MRI was performed within an average of 23 days (range 1-42 days) before or after lumbar puncture (n=25) and compared to a previous MRI performed on average 578 days (range 81-1294 days) ago. The CSF levels of both NFL and CXCL13 were higher in patients with new T2 lesions (n=16) than patients without new T2 lesions (n=9, p=0.007 and p=0.014, respectively). When only selecting patients with ≥1 Gd-enhanced lesion (n=7), the levels of CXCL13 and NGRN were higher and the levels of CCL2 were lower than in patients without Gd-enhanced lesions (n=18, p=0.003, p=0.049 and p=0.029, respectively). Note that no differences were observed in NGRN concentrations between patients and controls or between first- and second line treatments.

NFL, CHI3L1, and CHIT1 levels correlated with the clinical relapse rate (r=0.414, p=0.001; r=0.358, p=0.0015; and r=0.359, p=0.005, respectively). The level of CHI3L1 was related to disability and disease severity based on the EDSS and MSSS, respectively (r=0.274, p=0.036 and r=0.297, p=0.022). CXCL13 and CCL2 levels correlated with the presence of Gd-enhanced lesions (r=0.6114, p=0.0001 and r=-0.445, p=0.026, respectively) and with the number of Gd-enhanced lesions (r=0.622, p=0.001 and r=-0.505, p=0.010, respectively). CXCL13 and NFL were associated with new or enlarged T2 lesions (r=0.502, p=0.011 and r=0.555, p=0.004, respectively). No other significant correlations were found between biomarker concentrations and clinical or MRI measures.

Biomarker concentrations in patients and healthy controls

The levels of NFL, GFAP, CHI3L1, and CCL2 were detectable in all patients and controls. The levels of CXCL13 were below the LLoQ in 39 controls and 38 patients, the levels of CHIT1 were below the LLoQ in 13 controls and 5 patients, and the levels of NGRN were below the LLoQ in 14 controls and 21 patients. The mean concentrations of CSF biomarkers in healthy controls are presented in Table 2.

The NFL, GFAP, CHI3L1, and CCL2 levels were age-dependent; therefore, all of the correlation analyses were adjusted for age. We found no influence of gender on the analyzed biomarkers. Except for a significant relationship between GFAP and CHI3L1 ($r=0.552$, $p<0.001$), no correlations were found between the analyzed biomarkers in healthy controls. We found correlations between biomarkers of inflammation and degeneration in patients (Table 4); GFAP correlated with CHI3L1 ($r=0.352$, $p=0.007$), NFL correlated with CHI3L1 ($r=0.330$, $p=0.011$), and NFL correlated CHIT1 ($r=0.298$, $p=0.023$). CHIT1 correlated with two other inflammatory biomarkers, CHI3L1 and CXCL13 ($r=0.499$, $p<0.001$ and $r=0.298$, $p=0.023$, respectively).

Discussion

The influence of DMTs on the concentrations of seven CSF biomarkers that reflect different aspects of the immunopathogenesis of RRMS was explored in this study. We show that B-cell regulation (CXCL13), microglial activation (CHIT1, CHI3L1), and axonal damage (NFL) are all associated with disease activity in RRMS. Higher levels of these CSF biomarkers were observed in patients treated with interferon beta compared to those treated with natalizumab, suggesting that the efficacy of therapeutic intervention can be monitored by these biomarkers.

In previous studies, CSF levels of NFL and CXCL13 were shown to be associated with disease activity (Malmestrom et al., 2003, Khademi et al., 2011). Thus, NFL and CXCL13 were increased during relapse (Khademi et al., 2011, Malmestrom et al., 2003) and in the presence of contrast enhancing lesions on MRI (Axelsson et al., 2014). In the present study, we confirmed the association between NFL and CXCL13 and ongoing clinical and MRI activity, but we also showed that these biomarkers correlate with new or enlarged T2 lesions in clinically stable patients. Thus, even new or enlarging lesions that do not disrupt the blood-brain barrier influence the rate of axonal damage. We did not have access to spinal cord MRI scans and cannot exclude the possibility that occasional patients could have had enhancing cord lesions. However, clinically silent enhancing cord lesions are rare and likely do not account for this finding at an aggregate level.

Glial activation occurs during inflammation or damage of the CNS (Bonneh-Barkay et al., 2012, Eng and Ghirnikar, 1994). In previous studies, increased levels of CHI3L1, CHIT1, and GFAP were associated with disease activity (Malmestrom et al., 2014, Olsson et al., 2012) or disease progression (Modvig et al., 2013, Martinez et al., 2015, Axelsson et al., 2011, Burman et al., 2016, Comabella et al., 2010). Although CHI3L1 correlated with GFAP, only CHI3L1 correlated with clinical measures of progression (i.e., EDSS) and disease severity (i.e., MSSS). However, in previous studies, the correlation between GFAP and EDSS or MSSS (Malmestrom et al., 2003, Axelsson et al., 2011) was limited to patients with progressive MS (Axelsson et al., 2011) or associated with earlier neurological deterioration and EDSS progression (Martinez et al., 2015). Thus, the selection of RRMS patients and the cross-sectional approach in the present study probably influenced the results.

In contrast to previous studies on MCI and AD (Thorsell et al., 2010, Kester et al., 2015, Portelius et al., 2015), NGRN levels were normal in our patients with RRMS. The NGRN level increases as the dendritic spines degenerate and is associated with cognitive decline and the intensity of synaptic and neuronal degeneration in MCI and AD (Portelius et al., 2015). Although axonal damage may become extensive in RRMS, we did not find signs of dendritic spine involvement in our patients.

In the present study, we confirmed that natalizumab-treated patients have CSF NFL and CXCL13 levels similar to those of healthy controls as previously reported [2, 18], whereas the CSF concentration of CHI3L1 (Malmestrom et al., 2014) and CHIT1 (Olsson et al., 2012) do not reach normal levels. Thus, a low grade microglial activation seemed to be present even in natalizumab-treated RRMS patients who were clinically and radiologically stable. In RRMS patients, DMTs decreased CSF concentrations of NFL, CXCL13, CHIT1, and CHI3L1, whereas GFAP, NGRN, and CCL2 were unaffected. However, because the CSF concentrations of CXCL13 and CHIT1 were frequently below the detection levels of the immunoassay, their usefulness for monitoring therapeutic efficacy seemed limited.

In order to correctly interpret CSF biomarker data in relation to disease activity or treatment efficacy, certain aspects must be considered. Firstly, the treatment duration may influence biomarker levels. Both first- and second-line MS therapies usually achieve an effect on MRI and clinical measures after several months of treatment. However, none of our patients were on DMTs for less than 1 year. Secondly, some of the biomarkers are age-dependent e.g. NFL (Malmestrom et al., 2003, Lycke et al., 1998, Modvig et al., 2013), GFAP (Malmestrom et al., 2003, Rosengren et al., 1994), CHI3L1 (Modvig et al., 2013), and CCL-2 (Conductier et al., 2010)). In the present study all correlations were adjusted for age. Moreover, in the subgroup analysis based on the treatment, the group treated with natalizumab was older. However, the expected influence of age seemed limited, as significantly lower biomarker levels were found in this sub-group. Thirdly, repeated lumbar puncture may not be feasible in some patients due to side effects or a fear of the sampling procedure. Therefore, the possibility of clinically monitoring therapeutic interventions using CSF biomarkers is limited. Results from analyses of CSF biomarkers may essentially contribute to the evaluation of new DMTs in phase II trials or in difficult therapeutic decisions for selected patients. However, NFL and CXCL13 have been reported to be measures of the disease activity of MS in serum (Festa et al., 2009, Kuhle et al., 2015b). Thus, the evolution of extremely sensitive assays may make it possible to replace CSF with serum or plasma, even for CNS-specific antigens or inflammatory disorders of the CNS. Further studies of biomarkers obtained from peripheral blood are highly warranted in MS to establish them as biomarkers of disease progression, disease activity, and treatment efficacy.

In conclusion, this study showed that CSF biomarkers related to different pathological processes involved in MS reflect both disease activity and DMT efficacy. The association between neurodegenerative and inflammatory biomarkers confirms the hypothesis regarding inflammatory-induced degeneration, at least in the relapsing-remitting phase of the disease (Owens, 2003, Stadelmann et al., 2011). The determination of CSF NFL and CHI3L1 levels may be useful for measuring treatment efficacy in clinical trials and can also be used to monitor DMTs in clinical practice to provide an additional dimension in the assessment of treatment efficacy.

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Tables

Table 1. Baseline demographic and clinical characteristics of patients with relapsing-remitting multiple sclerosis and healthy controls.

	Patients (n=59)	Healthy controls (n=39)
Gender, Men/Women, no[a]	23/36	25/14
Mean age, years (range)[b]	37 (17-59)	34 (21-56)
Median/Mean EDSS (range)	2.5/2.48 (0-7.5)	NA
Median/Mean MSSS (range)	3.10/3.68 (0.10-9.18)	NA
No treatment	7	NA
First-line treatment = interferon beta	33	NA
Second-line treatment = natalizumab	19	NA

DMT=disease modifying therapy; EDSS=Expanded Disability Status Scale; MSSS=Multiple Sclerosis Severity Score; NA not applicable; [a] p=0.015 patients vs. healthy controls; [b] p=0.075 patients vs. healthy controls

Table 2. Mean concentrations of CSF biomarkers

	No treatment (n=7)	First-line treatment (n=33)	Second-line treatment (n=19)	Healthy controls (n=39)
NFL (pg/mL)	1900 (297-7185)[a]	1319 (165-13237)[a,b]	707 (161-5852)	364 (78-1287)
CXCL13 (pg/mL)	30.9 (7.8-160)[a]	29 (7.8-226)[a,b]	8.2 (7.8-16)	7.8 (7.8)
CHI3L1(ng/mL)	173(78-261)[a]	132 (50-260)[a]	120 (44-207)[a]	85 (24-159)
GFAP (pg/mL)	561 (344-1414)	519 (169-867)	603 (199-1556)	465 (55-922)
CCL2 (pg/mL)	410 (242-662)	386 (204-1057)	424(250-639)	373 (250-756)
NGRN (pg/mL)	206 (125-531)	189 (125-534)	219 (125-414)	241 (125-937)
CHIT1 (nkat/L)	4.8 (0.2-13.7)[a]	1.8 (0.2-6.3)[a,b]	1.2 (0.2-6.2)[a]	0.4 (0.2-1.6)

NFL=neurofilament light; CXCL13=C-X-C motif ligand 13; CHI3L1=chitinase-3-like protein 1; GFAP=glial fibrillary acidic protein; MCP-1=monocyte chemoattractant protein-1; NGRN=neurogranin; CHIT1=chitotriosidase; [a] $p<0.05$ patients vs. healthy controls; [b] $p<0.05$ first-line vs second-line treatment; () range

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Table 3. Influence from treatment on clinical and MRI outcome in relapsing-remitting multiple sclerosis.

	No treatment	First-line treatment	Second-line treatment
Number of Patients	7	33	19
Gender F/M	5//2	19//14	12//7
Age, mean (range)	37 (17-49)	35 (19-55)	42 (23-59)
Disease duration, mean (range)	6.6 (0-18)	6.4 (1-15)	12.7 (4-23)
EDSS mean/median (range)	2.0/1.5 (0-5.0)	2.17/2.0 (0-6.5)	3.21/3.5 (0-7.5)
MSSS mean/median (range)	3.08/2.44 (0.67-9-18)	3.83/3.05 (0.17-9.09)	3.65/3.46 (0.10-9.13)
Relapse, n	3	12	0
Valid MRI scan, n	7	32	19
Patients with Gd+ lesions, n (%)	2 (29)	15 (46)	1 (5)
Patients with new or enlarging T2 lesions, n (%)	5 (71)	29 (88)	10 (53)

F=female; M=male; EDSS=Expanded Disability Status Scale; MSSS=Multiple Sclerosis Severity Score; MRI=magnetic resonance imaging; Gd+=gadolinium enhancing

Table 4. Relationships between biomarkers of inflammation and degeneration

	NFL	GFAP	CXCL13	CHI3L1	NGRN	CHIT1	CCL2
NFL							
GFAP	r=0.109 (p=0.417)						
CXCL13	r=0.01 (p=0.939)	r=-0.007 (p=0.96)					
CHI3L1	**r=0.33 (p=0.011)**	**r=0.352 (p=0.007)**	r=0.242 (p=0.067)				
NGRN	r=0.12 (p=0.369)	r=0.015 (p=0.91)	r=-0.016 (p=0.903)	r=-0.084 (p=0.529)			
CHIT1	**r=0.298 (p=0.023)**	r=0.161 (p=0.227)	**r=0.298 (p=0.023)**	**r=0.499 (p<0.001)**	r=-0.001 (p=0.995)		
CCL2	r=-0.016 (p=0.905)	r=0.221 (p=0.096)	r=-0.13 (p=0.331)	r=0.205 (p=0.122)	r=-0.146 (p=0.274)	r=0.152 (p=0.254)	

The correlations between CSF biomarker levels are shown. The correlations in bold letters were significant ($p<0.05$). Statistical testing was done by Spearman's rank correlation analysis. All listed correlations and p-values have been corrected for age. r=Spearman's rho.

Figure legend

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Figure 1. Levels of NFL in cerebrospinal fluid in MS patients compared with levels obtained in healthy controls. The box indicates the interquartile range (IQR), bar indicates the median, and whiskers indicate the 95% CI. Extreme values are marked with open dots (±1.5 x IQR) or with asterisks (±3 x IQR).

Figure 2. Levels of CXCL13 in cerebrospinal fluid in MS patients compared with levels obtained in healthy controls. The box indicates the interquartile range (IQR), bar indicates the median, and whiskers indicate the 95% CI. Extreme values are marked with open dots (±1.5 x IQR) or with asterisks (±3 x IQR).

Figure 3. Levels of CHI3L1 in cerebrospinal fluid in MS patients compared with levels obtained in healthy controls. The box indicates the interquartile range (IQR), bar indicates the median, and whiskers indicate the 95% CI. Extreme values are marked with open dots (±1.5 x IQR) or with asterisks (±3 x IQR).

Figure 4. Levels of CHIT1 in cerebrospinal fluid in MS patients compared with levels obtained in healthy controls. The box indicates the interquartile range (IQR), bar indicates the median, and whiskers indicate the 95% CI. Extreme values are marked with open dots (±1.5 x IQR) or with asterisks (±3 x IQR).