

Biomarkers as measures of treatment efficacy

Cerebrospinal fluid biomarkers of inflammation and degeneration as measures of fingolimod efficacy in multiple sclerosis

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Abstract

BACKGROUND:

The disease-modifying therapies (DMTs) in relapsing-remitting multiple sclerosis (RRMS) vary in their mode of action and when therapies are changed, the consequences on inflammatory and degenerative processes are largely unknown.

OBJECTIVE:

We investigated the effect of switching from other DMTs to fingolimod on cerebrospinal fluid (CSF) biomarkers.

METHODS:

43 RRMS patients were followed up after 4-12 months of fingolimod treatment. Concentrations of C-X-C motif chemokine 13 (CXCL13), chemokine (C-C motif) ligand 2 (CCL2), chitinase-3-like protein 1 (CHI3L1), glial fibrillary acidic protein (GFAP), neurofilament light protein (NFL), and neurogranin (NGRN) were analyzed by enzyme-linked immunosorbent assay (ELISA), while chitotriosidase (CHIT1) was analyzed by spectrofluorometry.

RESULTS:

The levels of NFL, CXCL13, and CHI3L1 decreased ($p < 0.05$) after fingolimod treatment. Subgroup analysis revealed a reduction in NFL ($p < 0.001$), CXCL13 ($p = 0.001$), CHI3L1 ($p < 0.001$), and CHIT1 ($p = 0.002$) in patients previously treated with first-line therapies. In contrast, the levels of all analyzed biomarkers were essentially unchanged in patients switching from natalizumab.

CONCLUSION:

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We found reduced inflammatory activity (CXCL13, CHI3L1, and CHIT1) and reduced axonal damage (NFL) in patients switching from first-line DMTs to fingolimod. Biomarker levels in patients switching from natalizumab indicate similar effects on inflammatory and degenerative processes. The CSF biomarkers provide an additional measure of treatment efficacy.

KEYWORDS: Multiple sclerosis; biomarkers; cerebrospinal fluid; fingolimod; treatment response

Introduction

Fingolimod is an inverse agonist for sphingosine-1-phosphate receptors, which reduce the number of circulating memory T cells and their infiltration of the central nervous system (CNS),¹ and it may also influence regeneration of damaged CNS parenchyma.² Fingolimod is mostly used as a second line disease-modifying therapy (DMT) in patients with relapsing-remitting multiple sclerosis (RRMS). In two randomized clinical phase III trials (RCT), fingolimod reduced the relapse rate and lesion formation compared with placebo (FREEDOMS³ and FREEDOMS II⁴), and based on these outcomes, it was superior to interferon beta 1a (TRANSFORMS⁵).

Previous investigations of cerebrospinal fluid (CSF) biomarkers revealed altered profiles of immune cells and the proteins that reflect different aspects of the immunopathogenesis of multiple sclerosis (MS). We have demonstrated that natalizumab treatment of RRMS reduced the CSF level of the neurofilament light protein (NFL) to levels found in healthy controls (HC).⁶ Recently, fingolimod showed similar effects on axonal damage in a subgroup of patients participating in one of the pivotal RCTs for this treatment.^{3,7} In these patients, the reduction of the CSF NFL level correlated with improvements in relapse and MRI findings.⁷

In the present study, we investigated the concentration of biomarkers in the CSF from RRMS patients who switched to fingolimod from first line therapies (interferon beta, glatiramer acetate, and teriflunomide) because of breakthrough disease activity or second line therapy (natalizumab) to reduce the risk of progressive multifocal leukoencephalopathy (PML) in patients positive for the JC virus. We used biomarkers that reflect leukocyte trafficking: C-X-C motif chemokine 13 (CXCL13), chemokine (C-C motif) ligand 2 (CCL2), also known as

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Monocyte Chemoattractant Protein-1, MCP-1); microglial activation: chitinase-3-like protein 1 (CHI3L1, also known as YKL-40) and chitotriosidase (also known as chitinase 1, CHIT1); axonal degeneration: neurofilament light protein (NFL); synaptic degeneration: neurogranin (NGRN); and astrocytic reaction: glial fibrillary acidic protein (GFAP).

Except for NGRN, all of these biomarkers have previously been associated with disease activity⁸⁻¹⁷ or progression^{11, 12, 18} in MS, and DMTs have reduced the CSF levels of NFL,^{6, 15} CHI3L1,¹⁹ CHIT1,²⁰ and CXCL13.²¹ The major component of the cytoskeleton of the axon, NFL, is released into the CSF with neuroaxonal degeneration^{12, 14} and is increased during all stages of MS, with the highest levels following acute relapses¹² or contrast enhancing lesions.¹⁵ The biomarker CHI3L1 is expressed on macrophages, epithelial cells, and astrocytes, and is involved in the pathogenesis of chronic autoimmune disorders.²² High CSF levels of CHI3L1 were associated with disability progression and shorter time to conversion from clinically isolated syndrome to MS.¹¹ The biomarker NGRN is a postsynaptic protein that is enriched in dendritic spines and elevated levels were found in subjects with mild cognitive impairment (MCI) and Alzheimer disease (AD).^{23, 24} In CSF, NGRN is a marker of synaptic integrity and its levels are influenced by neurodegeneration.²⁵ A better understanding of these biomarkers and their response to DMTs could lead to their implementation in clinical practice and treatment monitoring.

Material and methods

Patients and healthy control subjects

Patients with RRMS fulfilling the revised McDonald criteria²⁶ were consecutively enrolled in the study at the MS centers of Sahlgrenska University Hospital, Gothenburg (n=17) and

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Karolinska University Hospital, Stockholm, Sweden (n=16). In total, 43 patients were included, 3 treatment naïve and 40 patients switching from another DMT. Treatment with oral fingolimod, 0.5 mg once daily, started at baseline. Patients were prospectively followed and had a second assessment, including lumbar puncture after 4 (n=4), 6 (n=18), 9 (n=3), or 12 (n=18) months. According to their previous treatment, these patients were divided into first-line (n=26, interferon beta, glatiramer acetate, and teriflunomide) and second-line treatment groups (n=14, natalizumab). The reasons for switching to fingolimod from first-line treatments were evidence of breakthrough disease (n=23), insufficient compliance (n=1), and undocumented (n=2). All of the second-line treated patients were serum positive for the JC virus antibody (JCV+) and had been on natalizumab treatment for 25 months or more and switched because of the increased risk for PML. The median wash-out period before starting fingolimod treatment was 22 days (range 0-1107; four patients had a treatment interruption for >1 year) for the first-line treated patients, and 81 days, (range 42-134) for second-line treated patients. Thirty-nine blood donors and university students served as age-matched HC. None of them had any neurological signs or a history of neurological disease. Demographic and clinical characteristics of the cohorts of patients and HC are presented in Table 1.

Clinical assessments and MRI

Patients underwent a clinical neurological examination, MRI scanning, and lumbar puncture at baseline and after 4-12 months of fingolimod treatment. A relapse was defined according to McDonald criteria as an episode of neurological disturbance lasting for at least 24 h.²⁷ The neurological examinations were performed by trained neurologists and disability was measured using the Expanded Disability Status Scale (EDSS)²⁸ and disease severity with the

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Multiple Sclerosis Severity Score (MSSS).²⁹ 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, and in close association with the clinical neurological examinations and lumbar puncture (median 44 days, range 0-379). 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. New disease activity on MRI was defined as a new Gd-enhanced lesion or a 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.³⁰ Disease activity (relapse, contrast enhancing T1, and new or enlarged T2) was recorded at baseline and at follow-up (Table 2). At follow-up, none of patients had relapse prior to lumbar puncture.

Biomarker analysis

All analyses were performed by board-certified laboratory technicians at the Clinical Neurochemistry Laboratory, at the Sahlgrenska University Hospital, according to protocols approved by the Swedish Board for Accreditation and Conformity Assessment. The NFL protein was measured with a sensitive sandwich ELISA method (NF-light® ELISA kit, UmanDiagnostics AB, Umeå, Sweden). Intra- and inter-assay coefficients of variation were below 10%. The lower limit of quantification (LLoQ) of the assay was 31 pg/mL. The GFAP was measured by ELISA, as previously described.³¹ Briefly, ELISA plates were coated with hen anti-

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GFAP IgG followed by incubation with CSF samples for 2 h, then rabbit anti-GFAP IgG was added as a secondary antibody and incubated for 1 h, both at room temperature. Detection was performed with peroxidase-conjugated donkey anti-rabbit IgG and 3,3',5,5'-tetramethylbenzidine substrate. The absorbance was read at λ 490 nm, and the sensitivity of the GFAP assay was 16 pg/mL. The CXCL13 was 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 \leq 10%. The LLoQ was 7.8 pg/mL and CSF samples below that level were designated the value 7.8 pg/mL. The CHI3L1 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 below 7% and the LLoQ was 8.15 pg/mL. The CCL2 was analyzed by solid phase sandwich ELISA (Human CCL2/MCP-1 Quantikine ELISA Kit, R&D Systems Inc., Minneapolis, MN). Intra- and inter-assay coefficients of variation were \leq 8% and the LLoQ of the assay was 10 pg/mL. The CSF NGRN was measured using an in-house ELISA with the monoclonal antibody Ng7 (BioLegend) as the capture antibody and the rabbit anti-NGRN antibody (Upstate) as the detecting antibody. The LLoQ was 125 pg/mL and the mean intra-assay coefficient of variation was 17%. The CSF chitotriosidase activities were measured with an in-house method in which 100 μ L of a fluorescence-linked substrate, 4-MU- β -D-N,N''-triacetylchitotrioside (Sigma M5639) was added to 100 μ L CSF and incubated for 15 min at 37°C. In the presence of chitotriosidase, the β -chain cleavage leads to release of the fluorescence element into the samples, which is quantified by spectrofluorometry (Jasco, Cremella, Italy). A serial dilution of pooled reference plasma was used as a standard from which activities were calculated. The LLoQ was 0.2 nkat/L and intra- and inter-assay coefficients of variations were $<$ 10%.

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Statistical analysis

The Shapiro-Wilk test of normality did not show normal distribution of our data; therefore, non-parametric tests were used in this study. Wilcoxon signed ranks test were used for paired data and the Mann-Whitney U test for unpaired data. Correlations between biomarkers were analyzed with Spearman's rank correlation test. Multiple regression analysis was performed to test the influence of gender, age, disease duration, EDSS, and MSSS on the biomarkers. Statistical calculations were performed in SPSS Statistics 21 software.

Ethics

All patients and HC 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, Sweden (Dnr 2005:253) and Stockholm (Dnr 2009:2107) approved the study.

Results

Biomarker concentrations after fingolimod treatment

The patients who were followed-up after 4-12 months of fingolimod treatment (n=43) had significantly reduced CSF levels of NFL, CXCL13, and CHI3L1 compared with their baseline. The mean pre-treatment level of NFL, 1 183 pg/mL (SD 2 135), decreased to 654 pg/mL (SD 448; $p=0.036$) after treatment, the CXCL13 level decreased from 19.0 pg/mL (SD 25.9) to 8.1 pg/mL (SD 1.2; $p=0.001$), and the CHI3L1 level decreased from 144 ng/mL (SD 67) to 119 ng/mL (SD 40; $p<0.001$, Fig 1-3). The mean post-treatment levels of NFL and CHI3L1 were significantly higher than in HC ($p<0.001$ for both); whereas, the mean post-treatment level of CXCL13 was

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similar to that of HC (Figures 1-3). The mean levels of CCL2 and GFAP did not significantly change prior to and after the treatment with fingolimod; in fact, both baseline and follow-up concentrations were similar to those of HC. Although patients had lower pre- and post-fingolimod treatment levels of NGRN ($p=0.059$ and $p=0.020$, respectively) and higher levels of CHIT1 ($p<0.001$ and $p<0.001$, respectively) compared with HC, fingolimod did not influence their mean levels. The levels of NFL, GFAP, and CCL2 were detectable in all patients and HC. The levels of CXCL13 were below the LLoQ in 39 HC and 27 patients, the levels of CHIT1 were below the LLoQ in 12 HC and 6 patients, and the levels of NGRN were below the LLoQ in 14 HC and 20 patients.

The influence of previous DMT on biomarker concentrations

At baseline, patients previously treated with first line therapies had significantly higher levels of NFL ($p=0.006$), CXCL13 ($p=0.005$), CHI3L1 ($p=0.025$), and CHIT1 ($p=0.005$) compared with those previously treated with natalizumab, a second line therapy; whereas, their NGRN levels were lower ($p=0.067$). There was no difference in the pre-fingolimod treatment levels of GFAP and CCL2 between first and second line treatment groups. Fingolimod treatment decreased the levels of NFL, CXCL13, CHI3L1, and CHIT1 ($p<0.001$, $p=0.001$, $p<0.001$, and $p=0.002$, respectively) in patients who switched from first line therapies. However, the levels of these biomarkers were still significantly higher after fingolimod treatment compared with controls ($p<0.001$, $p=0.031$, $p<0.001$, and $p<0.001$, respectively). In contrast, the levels of all analyzed biomarkers were essentially unchanged by fingolimod treatment in patients who switched from natalizumab treatment (Table 2 and Fig 1-3). Only CHIT1 was significantly increased in patients treated with natalizumab compared with HC ($p=0.032$).

The influence of fingolimod treatment duration on biomarker concentrations

Patients were dichotomized into those who had fingolimod treatment for less or more than 6 months. Subgroup analysis revealed that fingolimod treatment for 6 months or less, reduced CSF levels of NFL ($p=0.002$), CXCL13 ($p=0.012$), and CHI3L1 ($p=0.001$). There were no significant differences between biomarker levels from patients who were followed for up to 6 months and those who were followed for more than 6 months (Table 3).

Clinical and radiological effects of fingolimod treatment

Prior to fingolimod treatment, patients on first line treatments had more disease activity as determined by both clinical and radiological measures, than those on second line treatment (Table 4). Within 3 months prior to switching to fingolimod, 5 patients on first line treatment had a relapse and 10 patients displayed ≥ 1 Gd-enhanced lesion; while, only one patient on second line treatment had ≥ 1 Gd-enhanced lesion with no relapse. At follow-up and while treated with fingolimod, no patient experienced relapses in either group and one patient in each group displayed ≥ 1 Gd-enhanced lesion. The number of new T2 and Gd-enhancing lesions were decreased significantly in patients previously on first line treatments ($p=0.007$ and $p=0.001$, respectively). In contrast, fingolimod had no significant effect on disability or disease severity during follow-up. The median pre-treatment EDSS was 2.5 (0-6.5) in the group switching from first line treatments and 3.25 (0-6.5) in the group on second line treatment. The median post-treatment EDSS values were 2.5 (0-6.5) and 3.0 (0-6.5), respectively. The median pre-treatment MSSS was 3.58 (0.17-8.15) in the group on first line treatment and 3.38 (1.04-7.41) in the group on second line treatment and the median post-treatment MSSS values

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were 3.00 (0.16-8.14) and 3.34 (1.04-7.14), respectively. There were no significant group difference regarding EDSS and MSSS at baseline and the multiple regression analysis excluded the influence of gender on the biomarker levels of MS patients.

Correlations between clinical and radiological outcomes and biomarkers

The decrease of the relapse rate correlated with the decrease of the CHIT3L1 level ($r=0.491$, $p=0.001$), decrease of Gd-enhanced and T2 lesions ($r=0.401$, $p=0.010$ and $r=0.329$, $p=0.038$, respectively). There was a lack of correlation between the biomarker levels and EDSS and MSSS. The decrease of Gd-enhanced lesions correlated with the CHIT3L1, NFL, and CXCL13 levels ($r=0.409$, $p=0.009$; $r=0.396$, $p=0.011$; and $r=0.345$, $p=0.029$; respectively) and the decrease of T2 lesions correlated with the CHIT3L1, NFL, and NGRN levels ($r=0.383$, $p=0.015$; $r=0.424$, $p=0.008$; and $r=-0.341$, $p=0.031$; respectively).

Discussion

In the present study, we demonstrated that fingolimod treatment reduced biomarker concentrations in CSF, reflecting reduced microglial activation (CHI3L1, CHIT1), B-cell activity (CXCL13), and axonal damage (NFL) in patients with RRMS. This effect was observed in patients previously treated with first line therapies, and the majority of these patients switched to fingolimod because of breakthrough disease activity. In contrast, the biomarker levels were essentially stable in JCV+ patients previously treated with natalizumab. The switch to fingolimod in these patients was motivated entirely by being JCV+ and was a measure to reduce the risk of PML. In contrast to the former group, these patients were all clinically and

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radiologically stable before the switch. Thus, based on CSF biomarker concentrations, the influence of fingolimod on pathological processes of RRMS seemed to be superior to first line therapies and equal to natalizumab.

However, even on fingolimod treatment, CHI3L1, CHIT1, CXCL13, and NFL concentrations did not reach the levels observed in age-matched HC. This is in contrast to previous reports on natalizumab treatment of RRMS patients that showed decreased NFL and CXCL13 levels compared with those observed in HC.^{6, 21} In the present study, we confirmed these biomarker levels in natalizumab treated patients. Of the analyzed biomarkers, only CHIT1 was increased compared with HC in natalizumab treated patients prior to switching to fingolimod. Thus, although the biomarker levels of patients previously treated with natalizumab did not increase significantly compared with baseline levels and generally remained at low levels after fingolimod treatment, the CHI3L1, CHIT1, and NFL levels were still significantly higher than those of HC. Fingolimod treatment reduced the relapse rate and formation of new lesions on MRI. Again, this effect was entirely attributed to the group of patients previously on first-line drugs. The measures of decreased disease activity correlated with reduction of these biomarkers.

The marker GFAP indicates MS progression, correlates with EDSS and MSSS,^{12, 18} and is believed to reflect astrogliosis.¹⁸ Our result is in line with this assumption since no clinical neurological deterioration was observed and the GFAP level remained essentially unchanged. However, in previous studies, the highest correlation was confined to patients with progressive MS who had a mean follow-up of 9 years.¹⁸ Thus, the selection of patients and the limited time of follow-up in the present study might also have influenced the results.

Astrocytes are the major CNS source of CCL2 in both experimental autoimmune encephalomyelitis and MS,^{10, 17} but it is also produced by endothelial cells.³² We could not show that fingolimod influenced the CCL2 level in CSF. In previous studies, CSF levels of CCL2 were lower in active MS, with a median EDSS of 5.0,^{9, 16} but did not differ significantly in stable MS with a low EDSS (1.5-3.0) compared with controls.¹⁶ The temporal decrease of CCL2 is related to clinical relapse onset and is increased thereafter (analyzed after 5 and 15 weeks).³³ Thus, the less disabled patients and CSF sampling essentially during clinically stable periods probably explain why CCL2 levels were not significantly different compared with HC.

In contrast to previous studies on MCI and AD,²³⁻²⁵ NGRN levels were higher in HC than in RRMS patients and were not influenced by the fingolimod treatment. In MCI and AD, the dendritic spines are degenerated and the NGRN level increases and is correlated with cognitive decline.²⁵ We show for the first time that the NGRN concentration is decreased in RRMS; although, the reason for this reduction is unknown. However, the mean CSF NGRN level was not significantly different between RRMS patients previously treated with first-line compared with second line treatments, and fingolimod did not influence the level. Thus, the rate of neuroinflammation did not seem to involve degeneration of dendritic spines in RRMS.

The treatment duration and length of wash-out periods may influence biomarker levels at baseline. However, the mean wash-out period was longer in the group on second line treatment so it cannot explain the higher levels of biomarkers found in patients on first line treatment at baseline. There were four patients who switched to fingolimod from first line treatment with treatment interruptions of more than one year. However, excluding these patients from the analysis did not significantly change the results.

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Some of the biomarkers (NFL, GFAP, CHI3L1, and CCL-2) are age-dependent. In the subgroup analysis based on the previous treatment, the group previously treated with second line treatments was older than the group previously treated with first line treatments. If there was a positive correlation between those biomarkers and age, there would be increased levels in the second line treatment (natalizumab) group. However, we did not find any significant increase in these biomarkers. The biomarkers were not gender dependent and there was no relationship between the biomarker levels and EDSS or MSSS.

In conclusion, our study confirms that CSF biomarkers reflect both disease activity and DMT efficacy. These findings add to those obtained from clinical and MRI measures, since the influence of DMT on CSF biomarkers is selective, i.e. different parts of the immunopathogenesis of MS are involved. Fingolimod reduced B-cell activity (CXCL13), markers of glial activation (CHI3L1, CHIT1), and axonal degeneration (NFL) in RRMS patients with breakthrough disease while on first-line DMTs; while, the levels of the analyzed biomarkers were essentially unchanged in patients who switched from natalizumab. The determination of these biomarkers in CSF may be a measure of treatment efficacy in clinical trials and can also be used for monitoring DMTs in clinical practice.

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Author Contributions

LN, MA, MK, HZ, KB, CM, FP, TO, and JL participated in the primary data acquisition. LN, MA, CM, FP, TO, and JL participated in the study concept and design. TO and JL supervised the study. LN did the statistical analysis. LN and JL drafted the report and all authors participated in the critical revision of the final version.

Conflicts of interest

LN, MK, HZ, KB declare no competing interests and no disclosures.

MA has received compensation for lectures and/or advisory boards from Biogen, Genzyme, and Novartis.

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Biomarkers as measures of treatment efficacy

Figure 1.

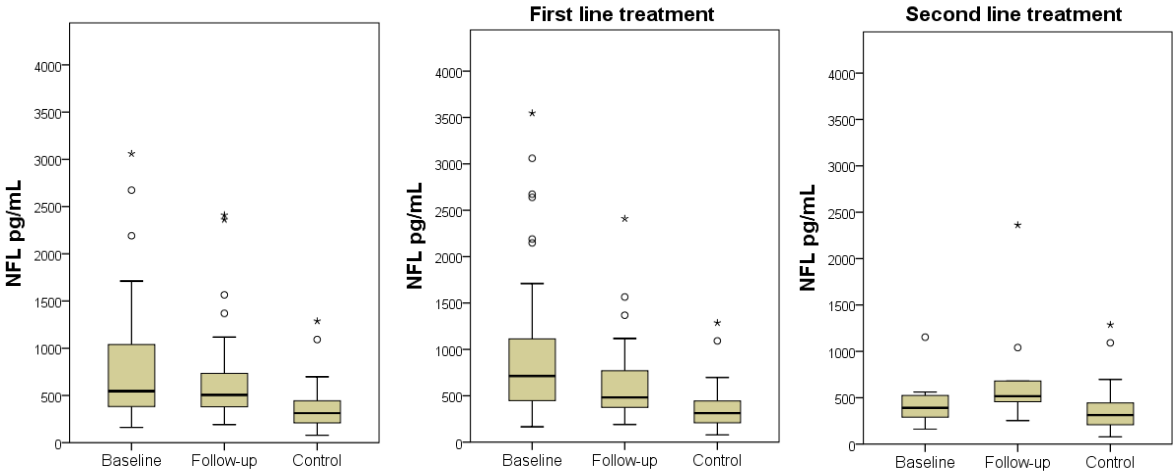


Figure 2.

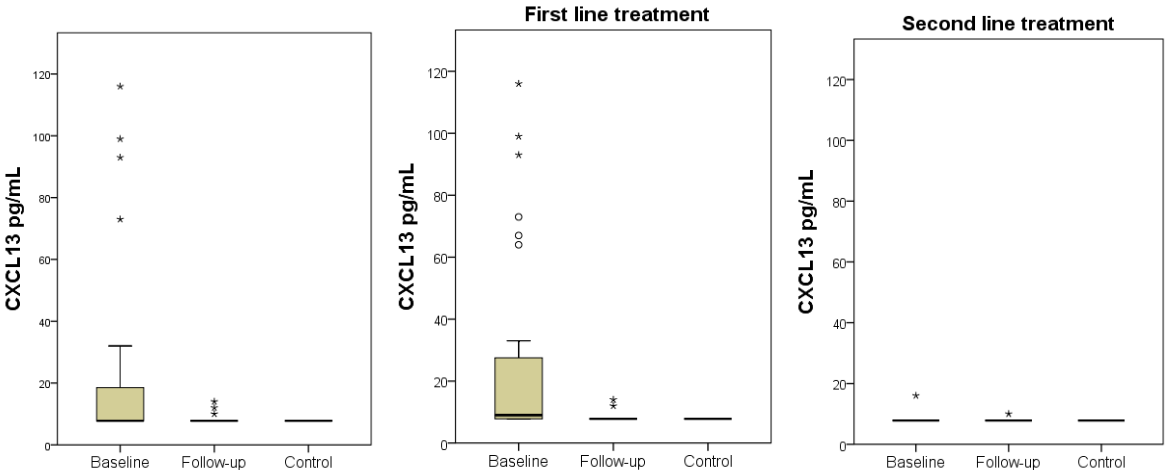


Figure 3.

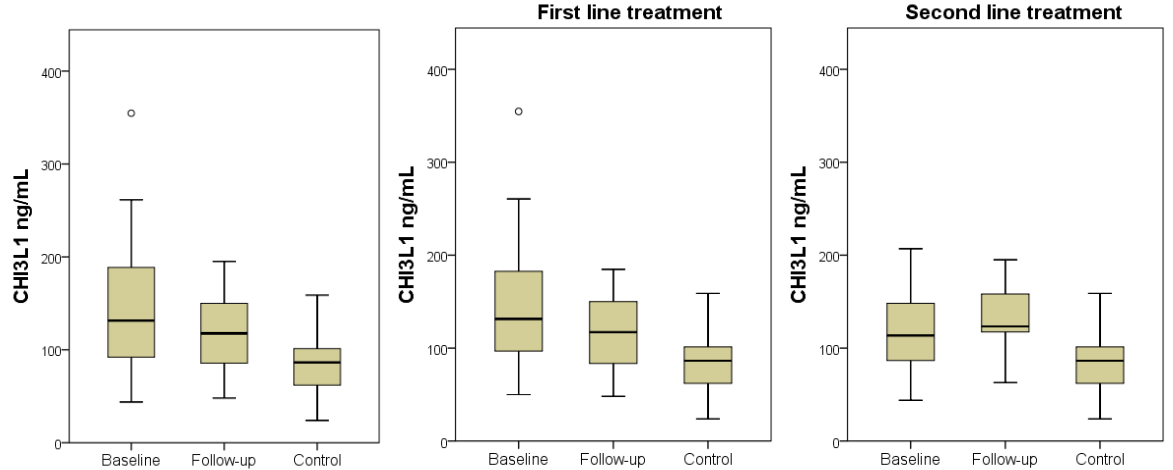


Figure legends

Figure 1. Neurofilament light (NFL) in cerebrospinal fluid before and after 4-12 months of fingolimod treatment (n=43). The NFL levels were compared with levels obtained in age-matched healthy controls (n=39). The box indicates the interquartile range (IQR), bar indicates the median, and whiskers indicate the 95% CI. Extreme values are marked with open dots ($\pm 1.5 \times \text{IQR}$) or with asterisks ($\pm 3 \times \text{IQR}$).

Figure 2. Levels of CXCL13 in cerebrospinal fluid before and after 4-12 months of fingolimod treatment (n=43). The CXCL13 levels were compared with levels obtained in age-matched healthy controls (n=39). The box indicates the interquartile range (IQR), bar indicates the median, and whiskers indicate the 95% CI. Extreme values are marked with open dots ($\pm 1.5 \times \text{IQR}$) or with asterisks ($\pm 3 \times \text{IQR}$).

Figure 3. Levels of CHI3L1 in cerebrospinal fluid before and after 4-12 months of fingolimod treatment (n=43). The CHI3L1 levels were compared with levels obtained in age-matched healthy controls (n=39). The box indicates the interquartile range (IQR), bar indicates the median, and whiskers indicate the 95% CI. Extreme values are marked with open dots ($\pm 1.5 \times \text{IQR}$) or with asterisks ($\pm 3 \times \text{IQR}$).

Tables

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

		Patients (n=43)	Controls (n=39)
Gender, Men/Women, no		16/27	25/14
Mean age, years (range)		39,65 (19-59)	33,59 (21-56)
Median/Mean EDSS (range)		2,5/2,63 (0-6,5)	NA
DMT, no	No previous treatment	3	NA
	First line treatment		
	Interferon beta	19	NA
	Glatiramer acetat	5	NA
	Teriflunomide	2	NA
	Second line treatment		
	Natalizumab	14	NA

DMT=disease modifying therapy

Biomarkers as measures of treatment efficacy

Table 2. Mean concentrations of CSF biomarkers before and after treatment of fingolimod in relapsing-remitting multiple sclerosis

	First line treatment (n=26)		Second line treatment (n=14)		HC (n=39)
	Baseline	Follow-up	Baseline	Follow-up	
NFL (pg/mL)	1 468 (SD 2 513) ^a	668 (SD 545) ^{a,b}	776 (1 466)	578 (350) ^a	364 (254)
CXCL13 (pg/mL)	25.8 (31.6) ^a	8.3 (1.5) ^{a,b}	8.4 (2.2)	7.8 (0)	7.8 (0)
CHI3L1(ng/mL)	160 (67) ^a	125 (41) ^{a,b}	111 (49)	107 (35) ^a	85 (31)
GFAP (pg/mL)	571 (240)	554 (185)	553 (206)	549 (166)	465 (210)
MCP1 (pg/mL)	398 (173)	381 (139)	406 (102)	375 (98)	375 (101)
NGRN (pg/mL)	164 (75) ^a	172 (94) ^a	226 (111)	193 (105)	241 (155)
CHIT1 (nkat/L)	1.95 (1.76) ^a	1.66 (1.58) ^{a,b}	0.81 (1.12) ^a	1.19 (1.45) ^a	0.37 (0.28)

HC=healthy controls; 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. HC; ^b p<0.05 before vs after fingolimod treatment; () standard deviation

Biomarkers as measures of treatment efficacy

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

Follow-up period	up to 6 months	more than 6 months
Number of Patients	22	21
Gender F//M	13//9	14//7
Age, mean (range)	40,91 (23-57)	38,33 (19-59)
1st line//2 nd line//n previous treatment	13//6//3	13//8//0
Relapse within 3m before switch, n (percent)	4 (18,2 %)	2 (9,5 %)
Relapse within 3w before switch, n (percent)	3 (13,6 %)	1 (4,8 %)
Valid MRI scan prior to fingolimod, n	21	20
Patients with Gd+ lesions, n (percent)	6 (27,3 %)	5 (23,8 %)
Patients with new T2 lesions, n (percent)	17 (77,3 %)	15 (71,4 %)
Valid MRI scan after fingolimod, n	20	18
Patients with Gd+ lesions, n (percent)	0 (0 %)	2 (9,5 %)
Patients with new T2 lesions, n (percent)	7 (31,8 %)	5 (23,8 %)

F=female, M=male, EDSS=Expanded Disability Status Scale, Gd+=gadolinium enhancing, MRI=magnetic resonance imaging

Biomarkers as measures of treatment efficacy

Table 4. Influence from previous first line and second line treatment on clinical and MRI outcome prior and after fingolimod treatment of relapsing-remitting multiple sclerosis.

	First line treatment	Second line treatment
Number of Patients	26	14
Gender F//M	18//8	8//6
Age, mean (range)	37,23 (19-55)	43,86 (23-59)
EDSS prior to fingolimod mean//median	2,31//2,5	3,14//3,25
EDSS after fingolimod mean//median	2,15//2,5	3,18//3,0
Relapse within 3m before switch, n (percent)	5 (19,2 %)	0
Relapse within 3w before switch, n (percent)	3 (11,5 %)	0
Relapse after switch, n (percent)	0	0
Valid MRI scan prior to fingolimod, n	24	14
Patients with Gd+ lesions, n (percent)	10 (38,5 %)	1 (7,1 %)
Patients with new T2 lesions, n (percent)	23 (88,5 %)	7 (50 %)
Valid MRI scan after fingolimod, n	22	13
Patients with Gd+ lesions, n (percent)	1 (3,8 %)	1 (7,1 %)
Patients with new T2 lesions, n (percent)	8 (30,8 %)	4 (28,6 %)

F=female, M=male, EDSS=Expanded Disability Status Scale, Gd+=gadolinium enhancing, MRI=magnetic resonance imaging