Evaluating biomarkers of neuronal degeneration and neuroinflammation in CSF of patients with multiple sclerosis – osteopontin as a potential marker of clinical severity

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Abstract

Biomarkers capable of predicting the clinical course and the rate of disease progression in multiple sclerosis are currently unavailable. Our objective was to examine if the levels of proteins associated with axonal and neuronal degeneration (Tau, p-Tau and β-amyloid$_{1-42}$) and T-cell-mediated autoimmunity (osteopontin) are altered in the cerebrospinal fluid (CSF) of MS patients, and to assess their potential in reflecting the clinical severity and predicting the progression and clinical evolution of early MS. The CSF samples collected from patients presenting with different clinical forms of MS were evaluated by enzyme-linked immunosorbent assays. The patients were regularly followed-up and their clinical status was re-evaluated 5 years after sampling. The results demonstrated that while CSF levels of Tau, p-Tau and β-amyloid$_{1-42}$ did not differ between MS and Control groups, the levels of osteopontin were significantly elevated in MS patients. This increase was associated with the presence of a relapse and correlated with clinical severity, which findings were independent of age and blood-CSF barrier function. However, none of the examined protein levels differed significantly between groups with different clinical evolution and no positive correlations with

clinical progression could be detected. We conclude that Tau, p-Tau and β-amyloid$_{1-42}$ are inappropriate as biomarkers in MS. This is the first report on CSF osteopontin as an independent marker of clinical severity in definite MS.

Key words: biomarkers, cerebrospinal fluid, ELISA, multiple sclerosis, beta-amyloid, tau, osteopontin.

1. Introduction

Multiple sclerosis (MS) is a chronic, progressive central nervous system (CNS) disorder, characterized by inflammation, demyelination, axonal damage and eventually neurodegeneration. MS affects women approximately 2-3 times as often as men. The course of the disease can vary in a broad range and finding a proper way to predict disease progression is in focus of biomarker research [1]. 85% of the patients has a clinical course of relapsing-remitting form (RR) characterized by recurrent acute exacerbations, which tend to leave more and more residual symptoms and lead to permanent disability after 20–40 years of disease progression, eventually resulting in death due to severe complications. A portion of patients remains in clinically isolated syndrome (CIS) for years without a subsequent relapse. Primary progressive MS (PP) presents with continuous progression and with a lack of remissions. This subtype represents ~10% of all cases; however, the pathomechanism of this form is suggested to be distinct, involving remarkable oligodendroglia degeneration [2,3].

There are no currently available biomarkers that can predict disease progression and future clinical manifestation of the disease at the time of its first presentation [1]. Identifying proper markers with relevant clinical predictive value is eagerly awaited, as such tools would help clinicians determine which patients need more intensive therapeutic approaches early in their clinical course. It can be hypothesised that proteins released into the cerebrospinal fluid (CSF) during axonal and neuronal injury can be useful as biomarkers in reflecting disease severity and/or predicting the clinical outcome of early MS patients. The main axonal candidates include human microtubule-associated protein Tau, a phosphoprotein that is involved in the polymerization and stabilization of axons and has essential roles in intraneuronal transport processes [4].

Accordingly, a number of small studies have been published examining the CSF levels of Tau in MS, which however demonstrated rather contradictory results [5-10]. Some of these studies also examined the CSF levels of hyperphosphorylated Tau (p-Tau) and β-amyloid$_{1-42}$ as markers of potentially altered protein processing in association with neuronal degeneration. The findings of such studies were similarly contradictory [7-10]. Osteopontin (OPN), an inflammatory protein associated with the preferential differentiation of T helper 17 (Th17) lymphocytes, has recently gained attention as a potentially useful biomarker in the CSF of MS patients [11-14]. OPN has also been implicated in neurodegeneration [15,16]; however, its roles are not yet fully elucidated.

In the present study, our objective was to examine if the levels of Tau, p-Tau, β-amyloid$_{1-42}$ and OPN are indeed altered in the CSF of MS patients from our Caucasian population, with additional focus on whether these proteins could serve as biomarkers in reflecting the clinical severity of MS and/or predicting the progression and future clinical manifestation of the disease at its first presentation.

2. Patients, materials and methods

We evaluated human CSF samples collected from patients presenting with different clinical forms of MS (n = 74; female/male ratio = 1.64; median age = 35.2 (interquartile range = 18.3)). Age-matched non-inflammatory control samples were collected from patients whose differential diagnostic procedure necessitated a lumbar sampling (mostly with the aim of excluding subarachnoidal hemorrhage) but the result of which did not reveal any abnormalities in the CNS (n = 30; female/male ratio = 1; median age = 36.3 (interquartile range = 19.4)). The lumbar punctures (LPs) were performed between 1999 and 2011. Written informed consent was obtained from all subjects and the study was

approved by the local Ethical Committee at the University of Szeged. The demographic, clinical and laboratory parameters of the (sub)cohorts are summarized in Supplementary table.

Samples of patients retrospectively meeting the 2010 McDonald criteria [17] for definite relapsing-remitting MS at the time of LP were classified as RR (n = 32). Samples from patients during or soon after the first symptomatic appearance of their disease were classified to the CIS group (n = 32). Samples of patients who were punctured at their first symptomatic appearance and subsequently proved to have a PP course were also included in the study (n = 10). The patients were untreated at the time of sampling, and for patients experiencing a relapse LP was performed prior to the administration of corticosteroid therapy. All patients underwent regular clinical and radiological follow-up. Based on the follow-up results, the group of patients initially classified as CIS were subdivided according to their clinical and MRI evolution within 5 years after LP as non-converters (n = 10) and converters to definite RR MS (n = 12). The Expanded Disability Status Scale (EDSS) score was evaluated for each patient at the time of LP and during the clinical check-ups. To provide standardised clinical measures, EDSS scores were comprehensively revised by a trained MS investigator (K.B.) who was blind to the experimental findings.

Following the LP, the CSF samples were centrifuged in 8.000 rpm for 10 minutes. The supernatants were stored in sterile polypropylene tubes in –80ºC until use, distributed into aliquots to avoid repeated freeze-thaw cycles. We used commercially available sandwich enzyme-linked immunosorbent assay (ELISA) kits for the quantitative detection of the examined proteins (Innogenetics N.V., Gent, Belgium for Tau, p-Tau and β-amyloid$_{1-42}$; R&D Systems Quantikine, Minneapolis, MN, USA for OPN). The assays were performed according to the manufacturers' instruction. Samples were diluted 1:50 for OPN analysis. All samples and standards were run in duplicates. The optical density values were

detected at 450/560 nm with a plate reader (Awareness Technology Inc, Palm City, FL, USA) and the respective concentrations were read from the standard curves fitted by Sigmaplot 10.0 software (Systat Software Inc., Richmond, CA, USA). The limit of detection of the assays was 87, 15, 87 and 550 pg/ml for Tau, p-Tau, β-amyloid$_{1-42}$ and OPN, respectively. Values were accepted if the respective coefficients of variation were less than 15%. Exclusions were required only for h-Tau, p-Tau and β-amyloid$_{1-42}$ (9, 6 and 2 values, respectively). Due to limited availability of aliquots, we could not allocate the same number of samples for all four proteins analyzed. The number of samples used for the final analyses is summarized in Table 1. The CSF total protein concentration determined by a direct colorimetric assay, the white blood cell count, as well as the albumin quotient, Link index and the percentage of intrathecal synthesis assessed by automated nephelometric analyses were performed in our diagnostic laboratory and were available in the clinical documentation. Our laboratory is approved for the diagnostic analyses of Tau, p-Tau and β-amyloid$_{1-42}$ by the applied kits, for which we could use samples running in the 'Alzheimer's Association QC program for CSF biomarkers' as quality control samples to rule out analytical bias.

The statistical analysis of the data was performed by SPSS Statistics 17.0 (SPSS Inc., Chicago, IL, USA). The normality of data was assessed by Shapiro-Wilk test. Due to the non-Gaussian distribution of the obtained data in the MS cohort, we used non-parametric tests (Mann-Whitney U, Kruskal-Wallis) for all examined parameters, except for β-amyloid$_{1-42}$, where the distribution was normal and parametric tests (Student's t, one-way ANOVA) were used for the comparative analyses accordingly. A $p < 0.05$ value was regarded as significant, following Bonferroni correction in multiple group comparisons. The crosstabulation analyses of EDSS were done by using the Fisher's exact test. The

correlation analysis within the Control group was performed by the use of parametric Pearson's correlation, whereas non-parametric Spearman's correlation was used for the analysis within the MS cohort, considering a correlation significant when $p < 0.05$ (two-tailed, uncorrected).

3. Results

3.1. The analysis of CSF Tau, p-Tau and β-amyloid$_{1-42}$ concentrations

The quantitative analysis revealed no significant differences between Control and MS groups in the measured protein concentrations (Supplementary fig., Table 2). There were no statistically significant differences between groups divided by the clinical stage at the time of the LP (CIS vs RR; Table 2). The subgroup analysis revealed a significant elevation of Tau ($p = 0.029$) and a non-significant tendentious elevation of p-Tau ($p = 0.082$) in RR samples collected during relapse compared to those during remission (Table 2). With regard to the putative value of these proteins to predict a PP disease course or a conversion to RR within 5 years, we found no significant differences in protein concentrations between different disease evolution patterns of patients sampled at first clinical manifestation (non-converters, converters and PP; Table 3).

3.2. The analysis of CSF osteopontin concentrations

The comparative analysis of CSF samples revealed a significant increase in the levels of OPN in MS patients compared to Control subjects ($p = 0.017$; Supplementary fig., Table 2). There was no significant difference between CIS and RR patients at the time of LP (Table 2). The subgroup analysis revealed that CSF concentrations of OPN were significantly higher in RR samples taken during relapse compared to those during remission ($p = 0.010$; Table 2). The variance analysis of disease evolution subgroups found no significant difference between non-converter, converter and PP groups (Table 3).

3.3. Analysis of correlations within the measured and available laboratory parameters

The CSF concentrations of Tau and p-Tau strongly and positively correlated with each other in both the Control and MS subgroups ($p < 0.001$ for all respective correlations). The concentrations of CSF OPN correlated with both Tau and p-Tau, but only in the MS group (OPN vs Tau: $p = 0.002$, Spearman's coefficient = 0.446, n = 48; OPN vs p-Tau: $p = 0.006$, Spearman's coefficient = 0.402, n = 46). In the Control population, the CSF concentration of OPN correlated with the age at LP ($p = 0.038$, Pearson's coefficient = 0.478, n = 19). No other examined protein levels correlated with age (data not shown). None of the examined proteins showed correlation with the total protein content, albumin quotient, intrathecal IgG synthesis and the Link index in the MS cohort (data not shown).

3.4. Correlations with clinical severity and progression

The CSF levels of OPN significantly correlated with the EDSS at LP within the whole MS cohort ($p = 0.008$, Spearman's coefficient = 0.371, n = 50; Fig. 1), whereas Tau only had a tendency to correlate ($p = 0.066$, Spearman's coefficient = 0.221, n = 70). The subcohort analysis revealed that these two parameters best correlated with EDSS at LP in the RR group (OPN: $p = 0.001$, Spearman's coefficient = 0.719, n = 17; Tau: $p = 0.002$, Spearman's coefficient = 0.530, n = 31), whereas among CIS patients such correlations were not observed. The correlation between EDSS and OPN existed also within the subgroup of MS (CIS and RR) patients sampled during a relapse ($p = 0.026$, Spearman's coefficient = 0.405, n = 30). The CSF concentrations of p-Tau and β-amyloid$_{1-42}$ did not correlate with EDSS at sampling. None of the examined protein concentrations correlated with clinical progression measured by EDSS 5 years after sampling (neither in the whole MS cohort, nor in subgroups), and none of them correlated with the first inter-attack interval (median = 18.5 months, interquartile range = 43.0 months) in CIS patients converting to RR within 5 years ($p > 0.05$).

4. Discussion

Despite the numerous studies aimed to identify biomarkers capable of predicting the prognosis of MS patients, currently no such molecules are available. Since the levels of Tau in the CSF might reflect the extent of axonal injury in the CNS, and p-Tau and β-amyloid$_{1-42}$ have been implicated in the neurodegenerative aspect of MS, in the past years, a number of groups aimed to assess whether there is a relationship

between the CSF proteomic status of these proteins and the presence and clinical severity of MS. Notably, however, the results were contradictory between the studies.

Indeed, while a number of publications reported increased CSF levels of Tau in MS [5,6,18-22], many other groups found no alteration [7-9,23-28], or detected elevation only in severe cases [10] or during relapse [29]. Our study concords with those reporting no overall difference between MS and control groups, but revealing associations with increased clinical severity and the presence of a relapse. Indeed, the higher levels observed in RR patients under relapse suggest that CSF Tau levels may somewhat reflect the activity of the disease, in accordance with some previous reports [18,29]. Our observation that EDSS at LP positively correlated with Tau in RR is similarly interesting; however, since most patients had a Tau level within control range, these appear to have little clinical relevance. A recent paper demonstrated that patients in secondary progressive (SP) phase had lower CSF Tau levels than those in RR phase, and correspondingly, Tau negatively correlated with clinical severity, reflecting the degree of parenchymal brain loss [30]. Not including an SP subgroup, we could no revisit this observation (not found by others [5,6,28]).

Few prior publications assessed the predictive/prognostic value of Tau levels by clinical follow-up. A group found higher CSF Tau to be an independent predictor for the next relapse presenting in a shorter time [24]; and reported a positive correlation with progression index [24]. In contrast, others reported CSF Tau having poor values to predict conversion from CIS to RR; though, they found Tau useful in improving the

prognostic sensitivity of MRI Barkhof criteria when assessed in combination with other markers of axonal degeneration [22]. Other publications, in accordance with our findings, reported no predictive/prognostic value of CSF Tau [6,28].

The rationale for examining p-Tau as a potential marker in MS was based on pathological studies reporting p-Tau accumulation within the brains of progressive MS patients [31-33]. This rationale is further supported by findings demonstrating that p-Tau accumulates within the brainstem of rats with acute experimental autoimmune encephalitis (EAE) [34], and also in the spinal cord of mice in a chronic model, correlating with neurodegeneration [32]. However, to our knowledge, no ELISA studies on CSF p-Tau could detect significant difference between MS and control groups [7,8,10], and also the latest published study (with no control group involved) [30] recorded p-Tau levels in MS within a range matching those seen in control population. Accordingly, our study (which is to our knowledge a comparative study of CSF p-Tau in MS with the highest number of subjects involved) demonstrated no significant difference between Control and MS groups. This indicates that even if Tau hyperphosphorylation is present in the pathogenesis of MS, the process is either not prominent enough to be manifested detectably in the CSF, or results in the predominant formation of insoluble Tau aggregates (as seen in neuropathological studies [31,32]), which cannot penetrate into the CSF. Considering that the level of p-Tau does not differ significantly between different MS subgroups and does not predict disease progression, we conclude that p-Tau is inappropriate as a biomarker in MS.

The available data on the roles of β-amyloid$_{1-42}$ in MS is rather little and highly controversial. Indeed, CSF levels of β-amyloid$_{1-42}$ have already been found decreased [9], elevated [7] and unchanged [8] compared to controls. In our study, enrolling the highest number of patients among such studies, β-amyloid$_{1-42}$ was clearly similar in MS and Control groups. Moreover, β-amyloid$_{1-42}$ was the only CSF parameter in which the distribution of the data in the MS cohort remained normal, suggesting that β-amyloid$_{1-42}$ is not involved in MS, and would be of no clinical use as a biomarker.

Emerging experimental data support that OPN has pathogenic roles in MS. In a relapsing-remitting EAE model, administration of OPN induced recurrent relapses, worsened the paralysis and evoked neurological deficits [35]. In contrast, OPN-deficient mice are resistant to EAE [36,37], and the severity of EAE can also be drastically attenuated by anti-OPN antibody treatment [38]. It appears that OPN has a critical role in promoting the survival of activated T cells [35] and enhancing the differentiation of Th17 cells, which are assumed to be the main effectors of CNS autoimmunity. Consistently, interferon-beta decreased the number OPN+ and Th17 cells in the spinal cord of EAE mice [39].

The possible role of OPN as a biomarker in MS has recently emerged and OPN has consistently been reported elevated in the CSF in MS patients compared to non-inflammatory controls [11-14]. Moreover, the level of OPN transcripts had previously been found elevated in human MS brain plaques and in the CNS of EAE animals [36]. However, CSF OPN appears to be a non-specific marker, as its levels are indistinguishably elevated in other CNS inflammatory conditions as well [40].

In a self-controlled comparison, CSF OPN was shown to be decreased in remission in CIS and RR patients compared to the first values measured during a relapse [13]. Our study – enrolling MS patients both under relapse and during remission – confirms and extends these previous observations, as we report that CSF OPN was elevated in our MS cohort, was similar in CIS and RR patients, but was elevated in RR patients in relapse compared to those in remission.

The present study is the first to report a clear significant correlation between CSF OPN levels and the clinical status (EDSS) at the time of LP in MS ($p = 0.008$), in particular in the RR subcohort ($p = 0.001$). The analysis of routine laboratory parameters further showed that the CSF total protein content and the albumin quotient did not correlate with CSF OPN levels in MS patients ($p = 0.13$ and $p = 0.24$, respectively), demonstrating that the observations related to OPN were independent of the integrity of the blood-CSF barrier and reflected a CNS-specific pathology. Considering the possibility that the correlation of OPN levels with EDSS may only reflect the difference in OPN between remission and relapse (later being generally associated with higher EDSS), a subgroup analysis on samples collected under relapse was performed revealing a statistically weaker ($p = 0.024$) but still marked correlation (Spearman's coefficient = 0.405, n = 30). These results collectively suggest that CSF OPN might be useful as a surrogate marker of clinical severity. Although previous studies did not find significant correlation between EDSS and CSF OPN levels in RR MS patients (a correlation was detected only in the PP subgroup [13]), it is of note that one of them studied RR patients who were under immunomodulatory therapy at the time of sampling [40], another group enrolled patients who were all sampled during a relapse (or already in a progressive phase) [13], and two other groups sampled patients only under remission [11,14], which may have resulted in narrower spectra of clinical severity compared to our cohort (EDSS ranged 0–7).

The findings that the levels of OPN positively correlated with Tau (observed also by others [13]) and p-Tau in all subgroups of our MS cohort support the rationale of monitoring axonal proteins as biomarkers in MS, despite the fact that Tau and p-Tau themselves appear to be inappropriate. This is also in correspondence with the recently reported correlation between the CSF levels of OPN and neurofilament light chain in progressive MS patients [41]. In line with prior observations [13], CSF OPN levels in control individuals (but not in MS) weakly correlated with age, but did not correlate with cell count and intrathecal IgG synthesis in MS.

To our knowledge, the present study was the first to address the clinical usefulness of OPN as a biomarker for predicting the prognosis and clinical evolution of MS. Despite the demonstrated associations of OPN with MS pathology, the CSF levels of OPN in our study cohort failed to predict the clinical evolution of patients with first manifestation during a 5-year follow-up period. Correspondingly, OPN levels in MS patients correlated neither with EDSS 5 years after sampling, nor with the first inter-attack interval in converters. A notable limitation of our study, however, was the relatively low number of subjects per group in the analysis of first-presenting MS patients divided upon their clinical evolution, which necessitates further investigation and additional studies examining CSF OPN levels from this respect.

5. Conclusions

Previous studies assessing the levels of Tau, p-Tau and β-amyloid$_{1-42}$ in the CSF of MS patients provided contradictory results. Besides revisiting the concepts of these studies, our study aimed to assess the clinical usefulness of these candidates as well as that of the recently

emerged potential marker of CNS autoimmunity, OPN. Our results demonstrated that Tau, p-Tau and β-amyloid$_{1-42}$ levels did not differ significantly between Control and MS groups, and had no predictive value for disease progression. On the other hand, CSF levels of OPN were clearly elevated in MS patients, which elevation was associated with increased clinical severity and the presence of a relapse, and appeared to be independent of the integrity of the blood-CSF barrier. CSF OPN levels, however, also failed to predict disease progression.

We conclude that while Tau, p-Tau and β-amyloid$_{1-42}$ appear to lack clinical relevance in MS, CSF OPN might serve as a surrogate marker for current clinical severity and disease activity. Further studies specifically powered for the long-term follow-up of early MS patients are needed to permit conclusion on the clinical usefulness of measuring the CSF levels of OPN in MS.

Conflicts of interest

The authors report no conflicts of interest.

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Fig. 1. **Correlation between the CSF concentrations of osteopontin and EDSS in MS patients.**
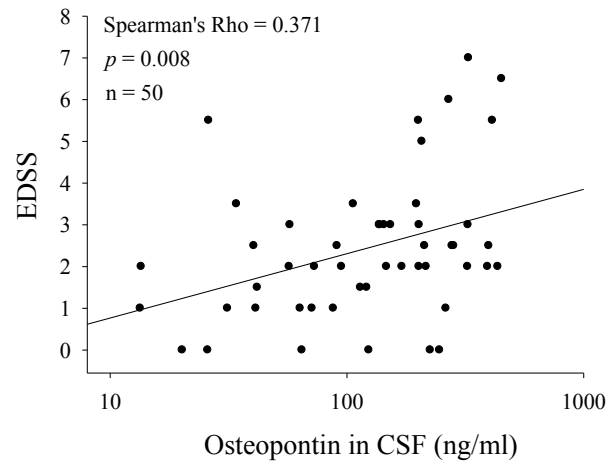
The scatter plot demonstrates significant positive correlation between osteopontin levels and disability at the time of sampling.

Abbreviations: CSF, cerebrospinal fluid; EDSS, expanded disability status scale.

Supplementary figure. **The CSF concentrations of Tau, p-Tau, β-amyloid$_{1-42}$ and osteopontin in control subjects and MS patient.**
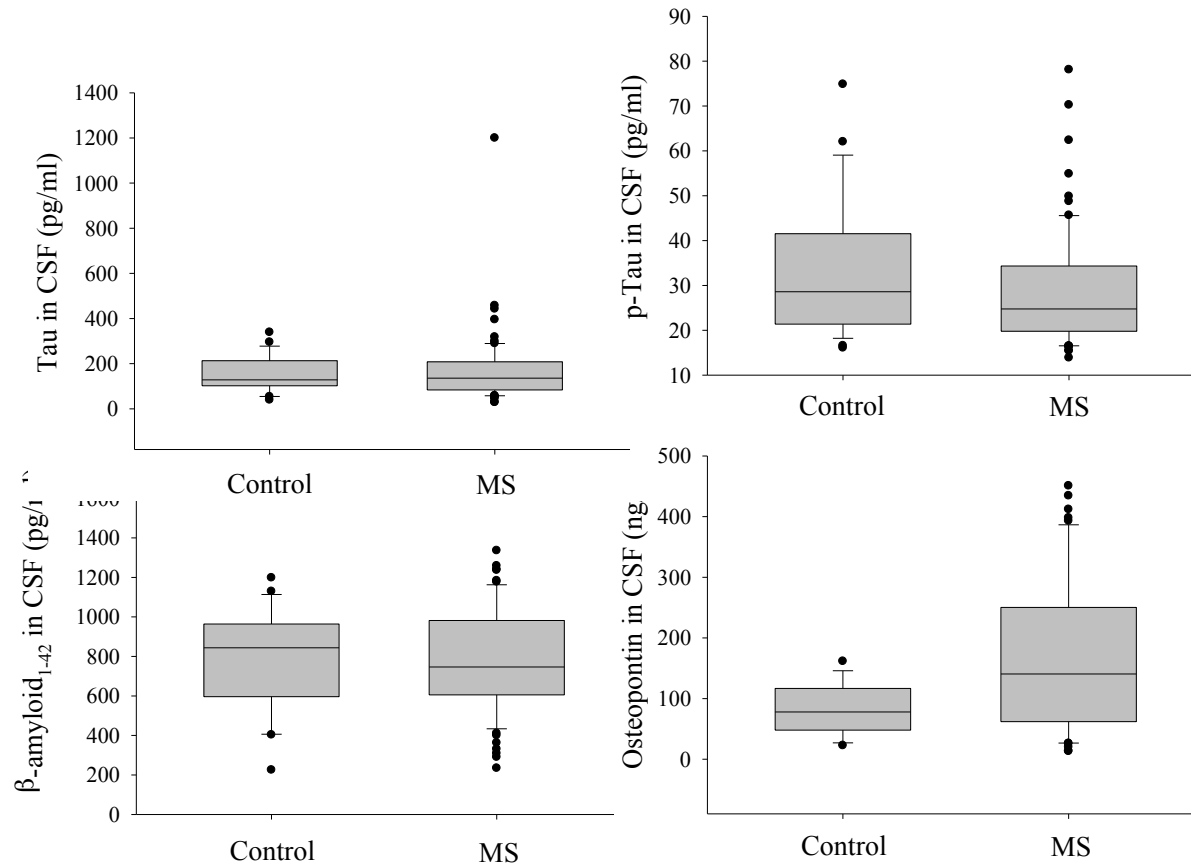
The box plots demonstrate significantly increased osteopontin levels in MS compared to Control subject, and no significant alterations in Tau, p-Tau and β-amyloid$_{1-42}$ levels.

Table 1.   Number of samples analyzed per group in the respective comparisons.

	Number of samples analyzed for			
	Tau	p-Tau	β-amyloid$_{1\text{-}42}$	Osteopontin
Control vs MS	25 vs 70	28 vs 70	28 vs 74	19 vs 50
CIS vs RR	29 vs 31	30 vs 30	32 vs 32	23 vs 17
Relapse vs Remission in RR	24 vs 7	24 vs 6	25 vs 7	11 vs 6
Non-converter vs Converter vs PP	8 vs 12 vs 10	9 vs 11 vs 10	10 vs 12 vs 10	9 vs 11 vs 10

The limited number of aliquots available in the biobank and the exclusion of values having a CV > 15% resulted in different number of samples analyzed per comparison. Due to the low range (pg/ml) and controversial literature related to Tau, p-Tau and β-amyloid$_{1\text{-}42}$ we allocated more samples for their analysis than for osteopontin (ng/ml).

Abbreviations: CIS, clinically isolated syndrome; CV, coefficient of variation; PP, primary progressive; RR, relapsing-remitting form.

Table 2.   Analysis of CSF Tau, p-Tau, β-amyloid$_{1-42}$ and osteopontin concentrations in cohorts and subcohorts.

		Control vs MS			CIS vs RR			Relapse vs Remission in RRMS		
		Control	MS	p	CIS	RR	p	Relapse	Remission	p
Tau	pg/ml	128.6 (110.9)	136.1 (124.5)	0.946	137.4 (112.8)	134.8 (138.6)	0.684	141.5 (115.3)	72.5 (73.3)	0.029*
p-Tau	pg/ml	28.6 (20.17)	24.7 (14.6)	0.162	31.4 (21.9)	23.8 (13.9)	0.115	28.2 (11.2)	17.8 (10.5)	0.082
β-amyloid$_{1-42}$	pg/ml	843.4 (367.6)	746.5 (376.2)	0.936	784.3 (474.2)	764.1 (243.2)	0.555	818.5 (247.9)	720.8 (269.1)	0.413
Osteopontin	ng/ml	77.9 (68.8)	140.6 (188.4)	0.017*	123.9 (144.4)	147.1 (248.5)	0.503	213.1 (210.9)	48.0 (106.9)	0.010*

The results demonstrate increased osteopontin concentrations in MS compared to Control, and increased h-Tau and osteopontin concentrations in relapse compared to remission in definitive RRMS. Values are presented in median (interquartile range). Asterisks denote significance.

Abbreviations: CIS, clinically isolated syndrome; MS, multiple sclerosis; RR, relapsing-remitting form.

Table 3.   **Analysis of CSF Tau, p-Tau, β-amyloid$_{1-42}$ and osteopontin concentrations in patients with first clinical appearance classified upon the evolution of their disease.**

		Disease evolution during the 5-year follow-up			
		non-converter	converter	PP	p
Tau	pg/ml	136.1 (65.0)	112.5 (133.6)	138.8 (155.9)	0.984
p-Tau	pg/ml	28.8 (20.9)	31.7 (27.9)	21.5 (8.6)	0.245
β-amyloid$_{1-42}$	pg/ml	631.0 (530.3)	677.6 (586.9)	690.9 (530.3)	0.927
Osteopontin	ng/ml	137.2 (233.3)	121.4 (174.5)	202.3 (226.8)	0.401

The results demonstrate no significant prognostic value of the examined proteins to predict a PP disease course or a conversion to RR within 5 years. Values are presented in median (interquartile range).

Abbreviations: PP, primary progressive; RR, relapsing-remitting form.

Supplementary table.   **Demographic, laboratory and clinical characteristics of the subject cohorts in the respective comparisons.**

		Control vs MS			CIS vs RR			Relapse vs Remission in RRMS		
		Control	MS	*p*	CIS	RR	*p*	Relapse	Remission	*p*
No. of subjects		30	74	-	32	32	-	25	7	-
Female/male		15/15	46/28	-	21/11	19/13	-	16/9	3/4	-
Age at LP	year	36.3 (19.4)	35.2 (18.3)	-	30.3 (16.7)	34.1 (14.0)	-	34.7 (15.7)	33.4 (13.4)	-
Relapse/remission		-	61/13	-	26/6	25/7	-	-	-	-
EDSS at LP		-	2.0 (1.5)	-	2.0 (1.8)	2.0 (1.4)	0.365	2.0 (2.25)	1.0 (1.0)	0.014
OGP		-	71/74	-	32/32	29/32	-	23/25	6/7	-
Cell count	M/l	1.0 (1.0)	4.0 (6.25)	< 0.0001	5.0 (7.25)	4.0 (7.0)	0.531	4.0 (13.0)	4.0 (6.0)	0.562
Total protein	mg/l	345.5 (156.3)	409.5 (157.3)	0.005	385.0 (142.0)	411.0 (138.8)	0.757	419.0 (163.5)	336.0 (131.0)	0.191
Albumin quotient	$\times 10^3$	4.1 (2.0)	5.1 (2.5)	0.094	4.8 (2.6)	5.1 (2.0)	0.840	5.3 (2.9)	4.4 (1.6)	0.324
Link index		0.5 (0.1)	0.9 (0.7)	< 0.0001	0.8 (0.5)	0.9 (0.9)	0.432	0.9 (0.8)	1.0 (1.0)	0.420
Intrathecal synthesis	%	0.0 (0.0)	18.5 (49.5)	< 0.0001	12.5 (38.3)	21.5 (56.5)	0.379	34.0 (62.0)	19.0 (54.5)	0.370

Abbreviations: CIS, clinically isolated syndrome; EDSS, expanded disability status scale; LP, lumbar puncture; MS, multiple sclerosis; OGP, oligoclonal gammopathy; RR, relapsing-remitting form. Calculated values are presented in median (interquartile range).