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Domperidone-induced elevation of serum prolactin levels and immune response in multiple sclerosis

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ABSTRACT

Increasing systemic prolactin levels improves remyelination and neuronal survival in animal models of Multiple Sclerosis (MS), but it has been suggested that this therapeutic strategy may also increase inflammatory responses, and potentially harm patients. We analyzed serum prolactin and cytokine, chemokine and growth factor levels in sera from MS patients enrolled in two clinical trials who were treated with domperidone, a generic drug that increases systemic prolactin levels. In patients treated with domperidone, molecule levels changed little during follow up, while prolactin levels increased several-fold. We found no significant association between prolactin levels and radiological or clinical outcome.

1. Introduction

Multiple Sclerosis (MS) is an autoimmune inflammatory and neurodegenerative disease of the central nervous system with unclear cause (Reich et al., 2018; Thompson et al., 2018). Most patients with MS have a relapsing-remitting disease course, in which the disease is driven by focal inflammatory lesions, which can manifest as clinical relapses and gadolinium enhancing lesions on brain MRI, and result in permanent disability. Some patients with RRMS go on to develop secondary progressive MS (SPMS) later in their disease course, in which disability progression is steady and unrelenting and occurs independently of relapses. Hormonal factors have been implicated in RRMS as well as in SPMS (Ysrraelit and Correale, 2019). The most well-known investigation of hormonal factors in MS is the classical PRIMS study, which showed that the risk of relapses decreases during pregnancy, and increases in the three months following delivery in women with RRMS (Confavreux et al., 1998). This phenomenon is believed to be caused by fluctuations in sex hormones during pregnancy and the puerperium (Ysrraelit and Correale, 2019).

One hormone that is of particular interest in MS is prolactin, a protein hormone produced in the pituitary gland. Prolactin's most prominent function is its role in triggering lactation in women after delivery. Basic research in animal models of MS suggests that increasing systemic prolactin levels may improve remyelination (Gregg et al., 2007; Zhornitsky et al., 2013). Other research in an animal model of neurodegeneration suggests that increasing prolactin levels may also have a neuroprotective effect (Farooq et al., 2011). Both of these effects of prolactin make it interesting for exploration as a treatment in RRMS and SPMS, and we are currently conducting two clinical trials (clinicaltrials.gov identifiers NCT02493049 and NCT02308137) in which we use the strategy of increasing systemic prolactin levels in patients with RRMS and SPMS with the antiemetic drug domperidone (Bernini et al., 1988; Brouwers et al., 1980; Brown et al., 1981) in order to harness these possible remyelinating and neuroprotective effects.

However, the association of prolactin levels and disease activity in human MS is unclear, and there is the possibility that increasing prolactin levels may increase MS disease activity. While most studies in humans found no association between prolactin levels and MS disease activity (Harirchian et al., 2006; Heesen et al., 2002; Markianos et al., 2010; Safarinejad, 2008; Vieira Borba and Shoenfeld, 2019), case reports suggest a possible association between hyperprolactinemia and increased disease activity (Kira et al., 1991; Yamasaki et al., 2000), and even with the number of MRI lesions (Then Bergh et al., 2006) and the SPMS disease course (Da Costa et al., 2011). Given our interest in prolactin as a therapeutic option in RRMS and SPMS, we investigated the effect of increasing prolactin levels on the production of serum

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Table 1
Characteristics of the control, SPMS and RRMS groups.

	SPMS Study		RRMS Study	
	Control	SPMS	RRMS Treatment	RRMS Control
n	16	30	12	5
Age (mean, SD)	54.56, 7.11	51.87, 6.90	41.2, 10.6	38.4, 7.0
Sex: f/m	11/5	23/7	11/1	3/2
Average serum prolactin at screening [mg/L] (mean, SD)	–	10.5, 5.82	13.4, 6.1	12.0[a], 4.2
Average serum prolactin during FU [mg/L] (mean, SD)	–	88.7, 91.2	129.8, 130.18	13.0, 4.5
Progression of disability at 12 months (yes/no)	–	8/22	–	–
Patients with Gad+ lesions at 16 weeks (n)	–	–	4	2

[a] Screening prolactin level was missing in one participant from the control RRMS; subsequent prolactin levels at weeks 6 and 16 did not increase beyond normal.

cytokines and on clinical and imaging outcome measures in the participants of two clinical trials.

2. Materials and methods

2.1. Participant groups

2.1.1. Domperidone in secondary progressive multiple sclerosis trial

The 'Domperidone in Secondary Progressive Multiple Sclerosis' trial (clinicaltrials.gov identifier NCT02308137) enrolls patients with SPMS (Lublin et al., 2014) aged 18–60 years, with advanced disability (EDSS 4.0 to 6.5 inclusive and Timed 25 Foot Walk (T25FW) time at screening of 9 s or more). Study participants are treated with 40 mg of domperidone per day for one year to raise serum prolactin levels. The primary outcome measure is the presence or absence of worsening by 20% or more on the T25FW at 12 months compared to baseline. Serum prolactin levels are measured at screening, 1 month, 6 months and 12 months. An interim analysis of this trial was performed after 30 patients had finished the trial. The characteristics and outcomes of trial participants are shown in Table 1.

2.1.2. Control group

In order to compare cytokine levels of people with SPMS and normal controls, we collected serum samples from 16 volunteers, and an attempt was made to match age and sex distribution to the participants of the SPMS trial group. These volunteers did not receive domperidone. Their characteristics are shown in Table 1.

2.1.3. Domperidone in relapsing-remitting multiple sclerosis trial

The 'Pilot Trial of Domperidone in Relapsing-Remitting Multiple Sclerosis' (clinicaltrials.gov identifier NCT02493049) enrolls patients with RRMS (Lublin et al., 2014) aged 18 to 60 years, who are on disease modifying treatment (DMT), and have a gadolinium enhancing lesion on a treatment monitoring MRI. Study participants are randomized in a 2:1 ratio to either receive add-on domperidone (30 mg per day) or no add-on treatment for 16 weeks. Study participants are then followed for an observation period without add-on domperidone for another 16 weeks. Contrast-enhanced cranial MRI scans are performed at screening, 16 weeks and 32 weeks, and serum prolactin levels are measured at screening, 6 weeks and 16 weeks. At the time of this analysis 17 patients (12 in the treatment group, 5 in the control group) had been enrolled and had finished the 16 weeks follow up of the study. The characteristics and outcomes of trial participants are shown in Table 1.

Ethical approval for the clinical trials, the serum collection in these trials and the SPMS control group, and the analysis presented in this manuscript was obtained from the University of Calgary Research Ethics Board.

2.2. Prolactin and cytokine, chemokine and growth hormone analyses

Prolactin levels were determined by Calgary Laboratory Services (Calgary, Canada). In our analyses, we used the average of the prolactin level at 1, 6 and 12 months for the SPMS group, and the average of the prolactin levels at 6 and 16 weeks for the RRMS group. Cytokine analyses were performed by Eve Technologies (Calgary, Canada) with the Human Cytokine 42-Plex Discovery Assay that measures serum levels of 42 molecules. These are the cytokines interferon (IFN)alpha2, IFNgamma, interleukin (IL)-1alpha, IL-1beta, IL-1ra, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-15, IL-17A, IL-18, granulocyte-colony stimulating factor (G-CSF), granulocyte macrophage (GM)-CSF, transforming growth factor (TGF)alpha, tumor necrosis factor (TNF)alpha and TNFbeta; the growth factors epidermal growth factor (EGF), fibroblast growth factor (FGF-2), vascular endothelial growth factor (VEGF)-A, platelet-derived growth factor (PDGF)-AA and AB/BB, and Fms-related tyrosine kinase 3 ligand (Flt-3 L); and the chemokines IL-8, eotaxin-1, Fractalkine, GRO(alpha), inducible protein (IP)-10, macrophage chemoattractant protein (MCP)-1, MCP-3, macrophage-derived chemokine (MDC), macrophage inhibitory protein (MIP)-1alpha, MIP-1beta, RANTES and sCD40L.

2.3. Statistical analyses

We investigated differences in cytokine, chemokine and growth factor levels between healthy volunteers and SPMS at screening and between SPMS at screening and the active, treated RRMS treatment group at screening using independent samples Student's *t*-test.

In order to investigate whether increasing prolactin levels over the course of the studies was associated with changes in cytokine, chemokine or growth factor profiles, we compared SPMS at screening and SPMS at 12 months, and the domperidone-treated RRMS participants at screening and at 16 weeks with a paired samples Student's t-test.

To investigate the association of rising prolactin levels with clinical or MRI outcomes, we used logistic regression analyses. For the SPMS group, we used significant worsening on the T25FW as the outcome variable, and for the RRMS group we used gadolinium enhancing lesions at 16 weeks as the outcome variable. In both models, we designated sex, age and the average prolactin level during follow-up as the predictor variables.

We used the R statistical software package version 3.5.1 for Windows (R Development Core Team, 2017) for all statistical analyses. Statistical significance was taken to be at the two-tailed 0.05 level. Because of the large number of comparisons in the cytokine analyses, we used a Bonferroni correction of the significance level, correcting for the fact that we are performing 42 tests (Haynes, 2013). The significance level for the cytokine analysis was adjusted to $0.05/42 = 0.0012$.

3. Results

3.1. Study participant groups

The baseline and follow up characteristics of the groups of study

participants is given in Table 1. The control group included 16 healthy volunteers who did not receive domperidone, while the SPMS group had 30 participants who all received domperidone treatment. None of the SPMS patients were using DMT. The RRMS group included 17 participants (12 on domperidone treatment and 5 control subjects receiving no domperidone), all of whom were using DMT (9 glatiramer acetate, 4 fingolimod, 3 dimethylfumarate, and 1 interferon beta-1a) and had gadolinium-enhancing MRI lesions. Baseline prolactin levels in the SPMS and RRMS groups were within normal limits (normal range from 0 to 25 μg/L for women and 0–15 μg/L for men). Mean prolactin levels increased very significantly with domperidone treatment during follow up, with an increase in the SPMS group from a mean serum prolactin level of 10.5 μg/L at screening to 88.7 μg/L during follow up, and an increase in the RRMS treatment group from 13.4 μg/L at screening to 129.8 μg/L during follow up (the RRMS control group did not change significantly from 12.0 μg/L at screening to 13.0 μg/L during follow up). In the SPMS group 8 of 30 patients had significant worsening of disability at 12 months. In the RRMS group, 6 of 17 patients had gadolinium enhancing lesions on cranial MRI at 16 weeks (4 of 12 in the domperidone group, and 2 of 5 in the control group, Table 1).

3.2. Cytokine, chemokine and growth factor analyses

The results of the cytokine, chemokine and growth factor analyses at baseline are shown in Table 2. In almost all analyses, molecule levels in some of the samples were undetectable. The number of samples available for each analysis is given in the table. There were no significant differences in any of the investigated cytokines between the control and the SPMS group. Levels of the molecules FGF-2, IL-17A, IL-8, and IL-18 were significantly higher in SPMS compared to RRMS. However, screening and follow up levels did not differ for any of the investigated molecules in either the SPMS or the RRMS group (Table 2).

3.3. Prolactin levels and outcome measures

The results of the logistic regression analyses are shown in Table 3. The association of the predictor variables ‘average prolactin level during follow up’, ‘sex’ and ‘age’ with the outcomes are expressed as odds ratios with their 95% confidence intervals. Confidence intervals not including 1.0 suggest a significant association.

In the SPMS group, none of the predictor variables was associated with the outcome ‘significant worsening of disability at 12 months’, the odds ratio for the predictor variable ‘average prolactin level during follow up’ was 0.973 (95% confidence interval of 0.939 to 1.000, $p = .07$). Similarly, in the RRMS group, none of the predictor variables was associated with the outcome ‘gadolinium enhancing lesions at 16 weeks’, the odds ratio for the predictor variable ‘average prolactin level during follow up’ was 0.981 (95% confidence interval of 0.950 to 1.000, $p = .14$). There was no significant association of prolactin levels during follow up and either significant worsening of disability in the SPMS, or the presence of gadolinium enhancing lesions on the cranial MRI scan at 16 weeks in the active, treated RRMS group.

4. Discussion

While the elevation of estrogen during pregnancy in MS is thought to reduce the risk of relapses through its anti-inflammatory activity at high concentrations (Itoh et al., 2017), the role of prolactin has been controversial. On the one hand, prolactin can have pro-remyelinating and neuroprotective benefits as described earlier (Farooq et al., 2011; Gregg et al., 2007; Vieira Borba and Shoenfeld, 2019; Zhornitsky et al., 2013), while on the other hand, it is thought to have pro-inflammatory effects that may exacerbate MS (Da Costa et al., 2011; Then Bergh et al., 2006). Our trials of domperidone in MS, with serum prolactin levels elevated to and beyond post-partum levels, provided the opportunity to determine whether prolactin is pro-inflammatory and potentially harmful in patients with MS.

In both of our trials, prolactin levels increased several-fold during treatment with domperidone. This very significant increase in systemic prolactin levels, however, was not associated with significant changes in the cytokine, growth factor and chemokine profiles in either RRMS or SPMS, as the production of the investigated molecules did not change over the course of the studies. This finding is in keeping with the existing literature on breastfeeding in MS. Prolactin levels are increased during breastfeeding, and studies to date either show a beneficial effect of breastfeeding on MS disease activity (Hellwig et al., 2015; Langer-Gould et al., 2017, 2009), or fail to find such an effect (Airas et al., 2010; Portaccio et al., 2011), but none suggests that breastfeeding and its associated state of increased systemic prolactin would increase disease activity.

Compared to RRMS, SPMS patients had increased levels of the growth factor FGF-2 and the cytokines IL-17A, IL-18 and IL-8. While the comparison in this study between RRMS and SPMS is made difficult by the difference in age between these groups, our results are in keeping with the literature on these molecules in MS. One study including 20 normal control subjects, 40 RRMS and 30 SPMS patients found FGF-2 levels elevated in the serum and CSF of patients with progressing SPMS compared to normal controls and compared to patients with stable RRMS.(Sarchielli et al., 2008) Similarly, in another study including 20 healthy control subjects, 21 people with RRMS and 18 people with SPMS, IL-18 production by anti-CD3/CD28 stimulated peripheral blood monocytes was found elevated in both RRMS and SPMS compared to healthy controls, and the IL-18 level correlated with disease duration in SPMS (Karni et al., 2002). This suggests that IL-18 may be a marker of long-standing SPMS. The fact that IL-17A and IL-8 are lower in our group of DMT-treated RRMS patients may be a treatment effect. IL-17 is an important pro-inflammatory cytokine, and serum IL-17 levels are generally increased in patients with RRMS (Li et al., 2017) and SPMS (Tang et al., 2015). A large study in DMT treated ($n = 152$) and treatment naïve (n-68) RRMS patients showed significantly lower serum IL-17A levels in DMT-treated patients compared to treatment naïve individuals (Trenova et al., 2017). A similar treatment effect was seen in a study of 20 patients with RRMS, which showed reduced serum IL-8 levels and reduced IL-8 secretion from PBMC of DMT-treated patients compared to before the start of DMT treatment (Lund et al., 2004).

There are some limitations to our study. We are analyzing interim data from two clinical trials, which translates into a limited sample size. Furthermore, while gadolinium enhancing lesions are a generally seen as a close approximation of inflammatory activity in the brain, the outcome measures of T25FW we use in the SPMS cohort is a rather indirect and general functional measure that is somewhat removed from the pathobiology of SPMS. The analyses are further limited through missing data points in the analyses, because in almost all of the cytokine, chemokine and growth factor analyses the levels of the molecule in question could not be determined in one or multiple samples. However, we believe that these limitations are mitigated by the fact that we were able to analyze a large number of cytokines, chemokines and growth factors, and to investigate the association of these molecules with clinical and radiological outcomes.

Importantly, our cytokine, chemokine and growth factor results are aligned with the clinical outcomes in our trials: increased prolactin levels were neither associated with worsening disability in SPMS, nor with the development of gadolinium enhancing lesions in RRMS. Taken together, our analyses suggest that increasing prolactin levels as a therapeutic strategy in active, treated RRMS and SPMS is not associated with increased disease activity or with the production of pro-inflammatory cytokine profiles.

Table 2
Cytokine levels in the control, SPMS and RRMS groups. The numbers shown are cytokine levels in pg/mL, standard deviation, and number of participants in each analysis. The *p* values of the significance tests are shown unadjusted, p values below the Bonferroni corrected 0.0012 level are marked in bold font.

Molecule [pg/mL]	Control	SPMS screening	SPMS 12 months	RRMS treatment group screening	RRMS treatment group 16 weeks	p Control vs. SPMS screening[a]	p RRMS treatment group screening vs. SPMS screening[a]	p SPMS screening vs SPMS 12 months[b]	p RRMS treatment group screening vs RRMS treatment group 16 weeks [b]
EGF (mean, SD, n)	164.5, 158.4, 15	48.5, 37.2, 28	55.5, 52.4, 28	123.9, 73.2, 10	209.0, 116.7, 10	0.01	0.01	0.19	0.03
FGF-2 (mean, SD, n)	141.1, 102.6, 16	174.3, 138.2, 29	169.8, 144,4, 29	54.7, 28.3, 10	65.9, 36.8, 10	0.36	**0.0002**	0.8	0.26
Eotaxin- 1 (mean, SD, n)	105.9, 63.0, 16	108.5, 59.6, 30	119.3, 59.7, 30	84.2, 41.9, 10	97.7, 37.7, 10	0.89	0.17	0.12	0.08
TGF-alpha (mean, SD, n)	24.5, 51.5, 13	18.6, 41.9, 26	15.8, 33.8, 26	5.0, 4.7, 10	4.5, 3.8, 10	0.72	0.11	0.49	0.54
G-CSF (mean, SD, n)	22.7, 43.4, 13	57.4, 114.1, 30	41.1, 72.4, 30	16.6, 11.6, 3	15.7, 12.1, 5	0.16	0.07	0.38	0.91
FLT-3 L (mean, SD, n)	86.4, 57.6, 6	75.7, 95.5, 19	84.4, 78.8, 18	5.2, 4.4, 3	22.4, 18.9, 5	0.74	0.004	0.73	0.01
GM-CSF (mean, SD, n)	36.7, 37.9, 15	114.0, 203.7, 30	122.8, 219.1, 30	1.9, 1.2, 10	9.6, 16.5, 10	0.05	0.005	0.70	0.16
Fractalkine (mean, SD, n)	106.5, 124.0, 12	177.7, 254,8, 21	202.9, 299.5, 21	16.8, 15.4, 6	31.5, 38.9, 6	0.29	0.009	0.40	0.42
IFN-alpha-2 (mean, SD, n)	35.6, 56.8, 14	46.7, 68.4, 28	51.3, 90.7, 28	8.8, 19.5, 7	21.0, 25.9, 7	0.58	0.02	0.71	0.24
IFN-gamma (mean, SD, n)	103.8, 270.9, 13	87.1, 132.7, 20	79.6, 148.3, 20	5.6, 6.5, 8	7.2, 13.2, 10	0.83	0.01	0.75	0.31
GRO-alpha (mean, SD, n)	1087.4, 520.4, 16	1316.9, 654.1, 30	1322.1, 697.5, 30	1350.0, 887.6, 10	1045.0, 577.8, 10	0.20	0.91	0.96	0.14
IL-10 (mean, SD, n)	5.8, 10.0, 9	4.8, 8.6, 21	4.0, 7.0, 21	1.0, 0.9, 9	1.8, 2.0, 7	0.79	0.06	0.21	0.36
MCP-3 (mean, SD, n)	122.6, 102.6, 9	103.1, 91.9, 14	95.8, 91.3, 14	66.1, 54.6, 3	129.7, 113.6, 4	0.65	0.40	0.41	0.35
IL-12 P40 (mean, SD, n)	70.4, 141.6, 6	122.7, 188.5, 11	132.2, 226.8, 11	3.7, 4.3, 5	6.4, 7.6, 6	0.53	0.06	0.76	0.21
MDC (mean, SD, n)	1050.0, 1140.5, 16	1423.1, 956.2, 29	1477.5, 1283.6, 29	785.3, 307.9, 10	922.0, 372.1, 10	0.28	0.003	0.73	0.15
IL-12 P70 (mean, SD, n)	137.5, 304.0, 11	119.5, 148.9, 15	145.0, 254.2, 15	11.5, 5.1, 3	13.6, 11.1, 5	0.86	0.01	0.56	0.11
PDGF-AA (mean, SD, n)	2047.5, 1180.4, 16	5202.6, 10,750.7, 25	2520.4, 1109.6, 25	1598.0, 551.9, 10	1575.0, 491.1, 10	0.16	0.11	0.21	0.65
IL-13 (mean, SD, n)	45.2, 42.4, 10	52.4, 77.8, 13	37.4, 48.5, 13	14.3, 9.8, 3	37.0, 30.3, 4	0.78	0.11	0.25	0.14
PDGF-BB (mean, SD, n)	6690.0, 3964.7, 16	8895.0, 4567.0, 30	7684.0, 4586.4, 30	7405.0, 4089.0, 10	6250.0, 2240.0, 10	0.09	0.35	0.04	0.04
IL-15 (mean, SD, n)	7.6, 10.6, 10	15.7, 24.2, 18	16.6, 24.1, 18	1.0, 1.0, 7	1.0, 2.9, 10	0.24	0.02	0.71	0.22
sCD40L (mean, SD, n)	2994.0, 2701.0, 16	2205.5, 1258.3, 30	1995.6, 1284.4, 30	1872.0, 589.3, 10	1970.0, 519.6, 10	0.28	0.27	0.34	0.58
IL-17A (mean, SD, n)	98.6, 208.0, 13	110.6, 119.4, 21	98.3, 137.2, 21	7.4, 7.8, 10	11.2, 12.4, 9	0.85	**0.0007**	0.51	0.16
IL-1 RA (mean, SD, n)	111.6, 146.2, 13	140.2, 170.7, 21	151.1, 220.6, 21	36.3, 26.5, 9	74.0, 92.4, 9	0.60	0.01	0.54	0.25
IL-1a (mean, SD, n)	253.7, 417.2, 12	184.1, 385.9, 21	120.4, 197.1, 21	51.0, 61.9, 5	26.4, 32.7, 9	0.64	0.15	0.29	0.65
IL-9 (mean, SD, n)	1.5, 3.7, 16	5.7, 15.7, 30	5.7, 18.0, 30	0.4, 0.6, 2	0.8, 0.6, 3	0.18	0.08	0.95	n/a
IL-1b (mean, SD, n)	28.2, 52.6, 7	11.2, 14.6, 12	12.48, 15.4, 12	1.5, 0.8, 4	2.5, 2.2, 6	0.43	0.04	0.47	0.14
IL-2 (mean, SD, n)	5.1, 11.5, 12	9.8, 14.8, 17	13.9, 22.3, 17	1.1, 1.3, 9	2.6, 3.0, 7	0.34	0.03	0.14	0.39
IL-3 (mean, SD, n)	1.0, 2.0, 16	0.9, 2.3, 30	0.9, 3.1, 30	0.4, n/a, 1	1.0, n/a, 1	0.88	n/a	0.83	n/a
IL-4 (mean, SD, n)	117.2, 407.1, 13	21.88, 46.5, 21	15.6, 32.4, 21	5.3, 4.9, 10	9.6, 10.5, 10	0.42	0.13	0.52	0.19
IL-5 (mean, SD, n)	3.2, 4.9, 12	7.6, 17.3, 14	9.9, 27.9, 14	0.2, 0.2, 10	0.5, 0.4, 10	0.37	0.14	0.43	0.1

(*continued on next page*)

Table 2 (*continued*)

Molecule [pg/mL]	Control	SPMS screening	SPMS 12 months	RRMS treatment group screening	RRMS treatment group 16 weeks	p Control vs. SPMS screening[a]	p RRMS treatment group screening vs. SPMS screening[a]	p SPMS screening vs SPMS 12 months[b]	p RRMS treatment group screening vs RRMS treatment group 16 weeks [b]
IL-6 (mean, SD, n)	14.1, 28.2, 11	11.5, 16.0, 21	9.5, 19.6, 21	3.9, 4.5, 4	13.7, 18.9, 5	0.78	0.08	0.50	0.12
IL-7 (mean, SD, n)	6.9, 18.8, 14	4.2, 1.0, 28	3.6, 4.0, 28	3.6, 4.3, 8	3.2, 3.0, 8	0.60	0.71	0.31	0.57
IL-8 (mean, SD, n)	48.4, 62.0, 16	38.6, 38.9, 28	35.1, 45.9, 28	8.6, 5.1, 10	15.4, 12.5, 10	0.57	**0.0004**	0.53	0.12
IP-10 (mean, SD, n)	101.8, 70.9, 16	107.9, 46.1, 30	101.5, 49.8, 30	103.9, 48.3, 10	111.7, 59.3, 10	0.76	0.82	0.32	0.68
MCP-1 (mean, SD, n)	339.0, 135.5, 16	467.3, 209.0, 30	432.3, 195.8, 30	302.1, 120.7, 10	308.3, 87.5, 10	0.02	0.004	0.24	0.84
MIP-1a (mean, SD, n)	29.7, 29.8, 14	26.2, 23.1, 21	24.7, 23.4, 21	9.9, 4.5, 5	10.7, 8.1, 8	0.71	0.006	0.59	0.92
MIP-1b (mean, SD, n)	96.9, 139.6, 15	83.8, 89.9, 28	82.0, 91.5, 28	44.1, 23.5, 10	42.9, 20.0, 10	0.75	0.04	0.86	0.86
RANTES (mean, SD, n)	661.2, 449.9, 16	408.3, 298.6, 27	389.3, 340.7, 27	609.9, 355.1, 10	551.6, 472.9, 10	0.06	0.13	0.58	0.34
TNF-alpha (mean, SD, n)	25.8, 16.7, 16	24.2, 20.8, 30	23.3, 25.5, 30	16.6, 8.4, 10	17.0, 6.0, 10	0.55	0.11	0.80	0.75
TNF-beta (mean, SD, n)	320.6, 359.4, 8	396.8, 515.0, 11	448.1, 793.3, 11	73.6, 97.9, 3	227.0, 264.8, 4	0.71	0.07	0.59	0.24
VEGF-A (mean, SD, n)	183.0, 224.9, 14	179.3, 127.6, 26	168.8, 132.1, 26	74.7, 79.0, 10	104.9, 81.7, 9	0.96	0.006	0.50	0.06
IL-18 (mean, SD, n)	156.0, 122.4, 16	217.1, 94.9, 28	189.6, 119.8, 28	119.2, 53.2, 9	131.0, 84.8, 10	0.10	**0.0006**	0.09	0.04

[a] Student's t-test.
[b] Student's t-test for repeated measures.

Table 3
Results of the logistic regression models in the SPMS and RRMS groups.

	Odds ratio	*p*-value
SPMS, Outcome: Significant worsening of the T25FW at 12 months		
Average prolactin level during FU[a]	0.973 (0.939 to 1.000)	0.07
Sex:		0.24
Female	1.0 (reference)	
Male	0.208 (0.011 to 2.423)	
Age[b]	0.876 (0.742 to 1.004)	0.07
RRMS, Outcome: Gadolinium enhancing lesions at 16 weeks		
Average prolactin level during FU[a]	0.981 (0.950 to 1.000)	0.14
Sex:		
Female	1.0 (reference)	0.43
Male	0.290 (0.008 to 5.753)	
Age[b]	0.916 (0.767 to 1.042)	0.23

[a] Per unit increase.
[b] Per year increase.

Declarations of interests

None.

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