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Clinical tria

Increased CCL18 plasma levels are associated with neurodegenerative MRI outcomes in multiple sclerosis patients

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ABSTRACT

Background: Chemokine ligands and co-stimulatory factors are involved in macrophage activation and differentiation processes that could contribute to multiple sclerosis (MS) pathogenesis.
Objective: To investigate associations of C-C motif Ligand 18 (CCL18), C-C motif ligand 5 (CCL5) and soluble Cluster of Differentiation 86 (sCD86) with clinical and MRI measures in MS patients.
Methods: Plasma levels of CCL18, CCL5 and sCD86 were evaluated in 138 MS patients (85 relapsing-remitting, RR-MS; 53 progressive, P-MS), and in 42 age- and sex-matched healthy individuals (HI). All subjects underwent standardized 3T MRI and clinical examinations. Linear regression analysis of MRI outcomes as dependent variables was performed with age, gender, having P-MS, and plasma proteins as predictor variables.
Results: Higher CCL18 plasma levels were found in P-MS (median = 51.5, IQR = 41.0–63.6 ng/mL) compared to RR-MS (median = 43.0, IQR = 29.1–55.0 ng/mL, $p = 0.014$) and to HI (median = 41.3, IQR = 30.9–54.1 ng/mL, $p = 0.009$). Disease-modifying treatments altered CCL5 ($p = 0.036$) and sCD86 ($p < 0.001$) levels. Higher CCL18 levels were associated with increased lateral ventricular volume ($p = 0.006$) and T2 lesion volume (LV) ($p = 0.034$), and decreased grey matter ($p = 0.006$), thalamic ($p = 0.007$) and cortical ($p = 0.01$) volumes.
Conclusions: Our results provide evidence that higher CCL18 plasma levels are associated with more severe inflammatory and neurodegenerative brain MRI outcomes in MS.

1. Introduction

Multiple sclerosis (MS) is a chronic autoimmune disorder, characterized by immune-mediated inflammation, demyelination and axonal damage in the central nervous system (CNS) (Dendrou et al., 2015).

The formation of MS plaques begins with activation of myelin reactive T lymphocytes by antigen presenting cells, leading to multifocal immune cell infiltration into the CNS. The immunoreactive processes are sustained by activated T lymphocytes that secrete cytokines and chemokines. These molecules regulate recruitment and migration of lymphocytes and monocytes/macrophages to the damaged CNS regions and promote their differentiation (Minagar and Alexander, 2003; Vogel et al., 2014). Macrophage infiltration is associated with more severe tissue destruction and the balance between macrophage subpopulations is important for formation, progression and regression of MS plaques (Boven et al., 2006; Kuhlmann et al., 2017; Vogel et al., 2013; Zrzavy et al., 2017). The pro-inflammatory classically activated or type I macrophages (CAM or M1) have been observed predominantly in initial stages of active MS lesions, while the anti-inflammatory alterna-

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tively activated or type II macrophages (AAM or M2) might be induced through re-polarization after myelin ingestion (Porcheray et al., 2005; Zrzavy et al., 2017).

The C-C motif ligand 18 (CCL18 - also called Pulmonary and Activation-Regulated Chemokine, PARC, or Alternative Macrophage Activation-Associated CC-chemokine, AMAC-1) is the most highly expressed chemokine in several human chronic inflammatory diseases (Schraufstatter et al., 2012). It is synthesized by monocytes and dendritic cells upon infection or inflammation to attract T cells and is known for being a specific marker of M2 macrophages (Kodelja et al., 1998; Schraufstatter et al., 2012; Tarique et al., 2015).

The chemokine C-C motif ligand 5 (CCL5 or regulated upon activation normal T-cell expressed and secreted, RANTES) is a marker of M1 (Tarique et al., 2015). CCL5 has been detected in actively demyelinating MS lesions (Simpson et al., 1998). Moreover, a genetic polymorphism of CCL5, able to influence the chemokine levels, was associated with a worse progression of MS disability (high-producer allele) or reduced risk of severe axonal loss (low-producer allele) (van Veen et al., 2007). However, several studies investigated CCL5 serum and cerebrospinal fluid (CSF) levels in MS patients with discordant results (Bartosik-Psujek and Stelmasiak, 2005; Mori et al., 2016; Rentzos et al., 2010; Szczucinski and Losy, 2011). The monomeric/oligomer states of chemokines, both CCL5 and CCL18, influence their half-life in blood thus enabling their detection and modulate their functions through the activation of their cognate receptors (von Hundelshausen et al., 2017).

CD86 is a membrane protein member of the immunoglobulin superfamily expressed by antigen-presenting cells, which positively or negatively regulates T-cell activation, depending on differential receptor binding (Alegre et al., 2001). CD86 is involved in initial co-stimulatory signaling in the immune response and the soluble form (sCD86), produced either by shedding or by alternative mRNA splicing, is detectable in plasma could be a marker of non-activated monocytes (M0) (Jeannin et al., 2000).

Given their important roles in macrophage function, CCL18, CCL5 and sCD86 could potentially modulate MS disease progression. However, the association of their levels in plasma with inflammatory and neurodegenerative magnetic resonance imaging (MRI) outcomes has not been systematically investigated. In this study, we used lesion volumes (LV) as MRI indicators of brain inflammation, and global and regional brain atrophy as neurodegenerative MRI biomarkers. We investigated the association between CCL18, CCL5 and sCD86 plasma levels with clinical and MRI outcomes in MS patients.

2. Materials and methods

2.1. Study population

The study population included subjects who participated in the case-control study of cardiovascular, environmental and genetic risk factors for disease progression in patients with MS (CEG-MS study; IRB ID: MODCR00000352) (Ziliotto et al., 2018; Zivadinov et al., 2016c).

All subjects underwent neurological and MRI examinations and provided blood samples. The data collected included demographic and clinical information. Body mass index (BMI) was derived from the subject's height and weight. The Expanded Disability Status Scale (EDSS) was assessed in MS patients.

The study protocol was approved by the local Institutional Review Board and all participants gave their written informed consent.

2.2. Inclusion and exclusion criteria

Subjects with the following characteristics were included: i) having MS according to the revised McDonald criteria (Polman et al., 2011), ii) having relapsing-remitting (RR-MS) or progressive (P-MS) course or being a healthy individual (HI), iii) having an MRI scan at the 3T scanner using the standardized MRI protocol, iv) age between 18–75 years and v) physical/neurologic examination within 30 days from the standardized MRI study protocol.

The exclusion criteria consisted of i) presence of relapse and corticosteroid treatment within the 30 days preceding study entry due to the pseudoatrophy effect on brain volume measurement, ii) pre-existing medical conditions known to be associated with brain pathology (e.g., neurodegenerative disorders, cerebrovascular disease, positive history of alcohol abuse, etc.) and iii) pregnancy.

2.3. MRI acquisition and image analysis

Subjects were examined on a General Electric (GE) 3T Signa Excite HD 12.0 scanner (Milwaukee, WI) using an eight-channel head and neck coil. We acquired 2D T2/PD-weighted images (WI), fluid-attenuated inversion recovery (FLAIR), spin-echo T1-WI with and without gadolinium contrast, and a 3D high resolution T1-WI. 2D sequences were acquired using a 256×192 matrix and $256\times192 mm^2$ FOV, resulting in a nominal in-plane resolution of $1\times1\ mm^2$. 48 gap-less 3 mm thick slices were acquired for whole-brain coverage. Sequence-specific parameters were: dual FSE proton density and T2-WI (TE1/TE2/TR = 9 ms/98 ms/5300 ms; echo-train length = 14), 4:31 min long; FLAIR (TE/TI/TR = 120 ms/2100 ms/8500 ms; flip angle = 90°; echo-train length = 24), 4:16 min long; and spin-echo T1-WI (TE/TR = 16 ms/600 ms), 4:07 min long. In addition, a 3D high resolution T1WI fast spoiled gradient echo sequence with a magnetization-prepared inversion recovery pulse was acquired (TE/TI/TR = 2.8 ms/900 ms/5.9 ms, flip angle = 10°), 4:39 min long, with 184 slices of 1 mm thickness, resulting in isotropic resolution.

MRI analysts were blinded to the subject's physical and neurologic condition. T2- and T1 lesion volume (LV) were assessed using a semi-automated edge detection contouring/thresholding technique previously reported (Zivadinov et al., 2012). Normalized whole brain, gray matter (GM), white matter (WM) and cortical volumes were obtained using SIENAX software (version 2.6) (Smith et al., 2002), as previously reported (Zivadinov et al., 2012). Deep GM and thalamic volumes were calculated using FIRST (Patenaude et al., 2011), and subsequently normalized using the SIENAX-derived scaling factor, as previously reported (Zivadinov et al., 2012). Prior to tissue segmentation, lesion filling was utilized to reduce the impact of T1 hypointensities (Gelineau-Morel et al., 2012).

2.4. Assays for CCL18, CCL5 and sCD86

EDTA plasma samples for CCL18, CCL5 and sCD86 investigation were obtained at the visit. Analysts were blinded to the clinical status of samples.

CCL18 levels were assayed using Luminex Screening Assays magnetic bead kits (Luminex R&D Systems Inc., Minneapolis, MN, USA) whereas CCL5 levels were measured using Milliplex™ magnetic bead kits (human neurodegenerative disease panel 3, HNDG3MAG-36 K, Merck Millipore, Germany). Data were acquired using the Luminex® 100 system and analyzed using Bioplex Manager Software version 6.0 (both from Biorad Laboratories, Hercules, CA). Concentrations were calculated according each standard curve generated for the specific target and expressed as ng/mL.

sCD86 levels were measured using ELISA kits (ab45921, Abcam, United Kingdom) following the manufacturer's instructions. CD86 levels were expressed in U/mL. The inter-assay coefficient of variations for CCL18, CCL5 and sCD86 were 3.2%, 4.7% and 3.2%, respectively.

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CCL18 levels were not assessed for 1 MS patient and 2 HI, because the values were outside the range of the standard curve.

2.5. Statistical analysis

All statistical analyses were performed using IBM® SPSS® Statistics version 24 software (IBM Corp. Armonk, NY, USA) and figures were produced by Graphpad prism version 6.01 (GraphPad Softwere, Inc. La Jolla, CA, USA). 46 secondary-progressive and 7 primary-progressive MS patients were categorized as progressive MS (P-MS).

The Kolmogorov–Smirnov test was used to test the normal distribution of the data. The Fisher's exact test was used to compare differences in categorical variables and Student's *t*-test was used to compare age and brain volume measurements between total MS and HI groups. Spearman's rank correlation was used to assess associations among the protein levels, and with demographic characteristics, disability status and disease duration.

Comparisons of CCL18, CCL5 and sCD86 levels between HI, RR-MS and P-MS were conducted with Kruskal-Wallis test followed by the Mann-Whitney U test. The same tests were used to assess whether CCL18, CCL5 and sCD86 levels were significantly different between patients receiving interferon-beta (IFN-b), glatiramer acetate (GA), other or no current disease-modifying treatments (DMTs). Multiple regression analysis was used for the following dependent variables: T2 and T1 lesions volume (T2-LV, T1 LV), normalized brain volume (NBV), normalized cortical volume (NCV), lateral ventricular volume (LVV), deep grey matter (DGM) and thalamic volume. Age, gender, having P-MS, and protein of interest were used as predictor variables. BMI was included as predictor variable in regression analysis of MRI measures with CCL18, because of its established influence on CCL18. A conservative p-value ≤ 0.01 was used for significance assessment given the multiple testing involved. A p-value ≤ 0.05 was considered a trend.

3. Results

3.1. Demographic and clinical characteristics

The study included 138 total MS patients (85 RR-MS, 53 P-MS) and 42 HI. The demographic and clinical characteristics of the study sample are summarized in Table 1. The demographic characteristics of MS and HI groups were similar. The majority of MS patients were on DMT. As expected, brain MRI measures (Table 2) were significantly different between the MS and HI groups.

3.2. CCL18, CCL5 and sCD86 levels in plasma

The levels of CCL18, CCL5 and sCD86 in the MS and HI groups are summarized in Fig. 1.

Differences between groups were present only for CCL18 levels (p = 0.015, Kruskal-Wallis test). CCL18 levels were higher in P-MS (median = 51.5, IQR = 41.0–63.6 ng/mL) compared to RR-MS (median = 43.0, IQR = 29.1–55.0 ng/mL, p = 0.014, Mann-Whitney U test) and to HI (median = 41.3, IQR = 30.9–54.1 ng/mL, p = 0.009). No significant differences in CCL5 and sCD86 levels were observed between RR-MS, P-MS and HI groups (Fig. 1).

3.3. Clinical associations of CCL18, CCL5 and sCD86

Plasma levels of CCL18, CCL5 and sCD86 were not associated with EDSS or disease duration.

Differences among DMT subgroups (interferon-beta, IFN-b; glatiramer acetate, GA; other or no DMTs; Fig. 2) were present for CCL5 ($p = 0.036$, Kruskal-Wallis test) and sCD86 ($p < 0.001$).

CCL5 levels were lower in MS treated with other DMT (median = 54.7, IQR = 34.4–88.0 ng/mL) compared to IFN-b (median = 90.6, IQR = 54.0–147.5 ng/mL, $p = 0.005$, Mann-Whitney U test) and to no DMTs (median = 98.6, IQR = 40.1–168.9 ng/mL, $p = 0.02$).

sCD86 levels were higher in MS treated with IFN-b (median = 348.2, IQR = 288.0–44.9 U/mL) compared to GA (median = 278.0, IQR = 240.0–334.3 U/mL, $p < 0.001$, Mann-Whitney U test), to other (median = 263.5, IQR = 241.4–291.3 U/mL, $p < 0.001$) and none DMTs (median = 282.9, IQR = 248.3–346.3 U/mL, $p = 0.007$).

3.4. Association of MRI measures with CCL18, CCL5 and sCD86 levels

The associations of CCL18, CCL5 and sCD86 with MRI measures, assessed within MS and HI groups, are reported in Tables 3 and 4.

CCL18 levels were associated with increased LVV and T2-LV, and with decreased DGM and thalamic volumes. The regression analysis results suggested that a 1 ng/mL increase in CCL18 corresponded to an increase of 0.24 mL in LVV (p = 0.006) and of 0.13 mL in T2-LV (p = 0.034). For each 1 ng/mL increase in CCL18, the DGM volume de-

Table 1
Demographic and clinical characteristics of the cohort.

	All MS	RR-MS	P-MS	HI
Sample size, n	138	85	53	42
Female, n (%)	100 (72.5)	60 (70.6)	40 (75.5)	31 (73.8)
Age, years	54.3 ± 10.8	50.1 ± 10.7	60.9 ± 7.2	51.0 ± 14.3
BMI	27.6 ± 6.0	27.9 ± 6.4	27.2 ± 5.5	26.1 ± 5.5
Age onset in years	32.9 ± 9.5	32.6 ± 9.1	33.3 ± 10.1	–
Disease duration, years	21.1 ± 10.6	17.0 ± 8.8	27.6 ± 10.0	–
EDSS, median (IQR)	3.5 (2–6)	2 (1.5–3.5)	6 (4–6.5)	–
Annual relapse rate	0.2 (0.4)	0.2 (0.4)	0.1 (0.3)	–
DMT status, *n* (%)				–
Interferon-beta	45 (32.6)	30 (35.3)	15 (28.3)	
Glatiramer acetate	42 (30.4)	23 (27.1)	19 (35.9)	
Natalizumab	5 (3.6)	4 (4.7)	1 (1.9)	
Other DMT*	19 (13.8)	13 (15.3)	6 (11.3)	
No DMT	27 (19.6)	15 (17.6)	12 (22.6)	

MS: Multiple Sclerosis; RR-MS: Relapsing Remitting Multiple Sclerosis; P-MS: Progressive Multiple Sclerosis; HI: Healthy Individuals; BMI: body mass index; EDSS: Expanded Disability Status Scale; IQR: interquartile range; SD: standard deviation; *n*: number; DMT: disease-modifying treatment.
*Other DMTs included intravenous immunoglobulin, mitoxantrone and methotrexate.
All continuous variables (age and disease duration) are mean ± standard deviation. For the ordinal EDSS, the median (interquartile range) is given.
Descriptive analysis between MS and HI were performed using Fisher's exact test and Student *t*-test.

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Table 2
MRI characteristics of the cohort.

	All MS	RR-MS	P-MS	HI	MS vs. HI *p*-value
T2-LV, ml	15.8 (19.0)	11.8 (15.9)	22.2 (21.9)	0.2 (0.6)	< 0.001
T1-LV, ml	2.9 (6.2)	2.0 (4.6)	4.4 (8.1)	0.0 (0.0)	< 0.001
NBV, ml	1438 (92.1)	1469 (82.4)	1387 (85.2)	1528 (97.9)	< 0.001
NCV, ml	591 (48.6)	606 (44.8)	567 (44.8)	630 (53.3)	< 0.001
LVV, ml	55.1 (27.0)	50.7 (25.2)	62.3 (28.5)	32.2 (14.5)	< 0.001
DGM volume, ml	53.6 (7.1)	55.5 (6.5)	50.4 (6.9)	60.5 (46.4)	< 0.001
Thalamic volume, ml	17.7 (2.5)	18.4 (2.3)	16.5 (2.4)	20.3 (1.9)	< 0.001

MS: Multiple Sclerosis; RR-MS: Relapsing Remitting Multiple Sclerosis; P-MS: Progressive Multiple Sclerosis; HI: Healthy Individuals; LV: lesion volume; NBV: normalized brain volume; NCV: normalized cortical volume; LVV: lateral ventricular volume; DGM: deep grey matter n: number.
Lesion and brain volumes are expressed in milliliters.
P-values represent MS and HI group comparisons and were derived using Student *t*-test.

creased by 0.062 mL ($p = 0.006$) and the thalamic volume decreased by 0.02 mL ($p = 0.007$).

CCL5 and sCD86 were not associated with the any of assessed MRI measures.

4. Discussion

Taking into account the role of macrophage infiltration in tissue destruction, and the role of CCL18, CCL5 and sCD86 in macrophage activation, we hypothesized that the expression of these proteins could be associated with disease stage or progression. Quantitative measurements of brain atrophy have been shown to be the most robust correlates and long-term predictors of both cognitive and clinical disability (Zivadinov et al., 2016d).

In this study, which investigated CCL18, CCL5 and sCD86 plasma levels in MS patients, CCL18 levels were found to be associated with inflammatory and neurodegenerative brain MRI outcomes, thus supporting a role for CCL18 in the progression of MS. In fact, higher CCL18 levels were found in P-MS compared to RR-MS and to HI, and multiple and coherent correlations with MRI measurements were observed. In particular, higher CCL18 levels were associated with increased T2-LV and LVV, and with decreased NCV, DGM, and thalamic volumes.

We report for the first time evidence that plasma CCL18 concentration might reflect the undergoing disease progression within CNS. Despite the positive association between CCL18 levels and MRI measurements, no association was found between CCL18 levels with EDSS and disease duration.

We investigated for the first time the effects of DMTs on CCL18 levels, in particular IFN-b and GA which are commonly used first line therapies in MS, and we did not detect any significant modification. Hence, the observed CCL18 correlation with MRI measurements could depend on disease progression mechanisms rather than relating to DMTs.

In light of the distribution of CCL18 plasma levels within the RR-MS, P-MS and HI groups, the range of variability limits the utility of CCL18 levels as diagnostic or prognostic biomarker. However, changes in plasma CCL18 have not been previously investigated in MS and our study provides insight into the pathways altered by dysimmune pathological mechanisms in MS. Indeed, CCL18 is known to be involved in chemotaxis of immune cells and exerts regulatory effects on them (Chenivesse and Tsicopoulos, 2018). Higher CCL18 levels in P-MS compared to RR-MS seem to corroborate the idea that more severe brain injury, defined as increase in lesions volumes and more advanced GM and central atrophy (Zivadinov et al., 2016b), is associated with increased levels of this chemokine.

Intriguingly, CCL18 is evolutionary present only in primates (Schraufstatter et al., 2012), which could have implication for human

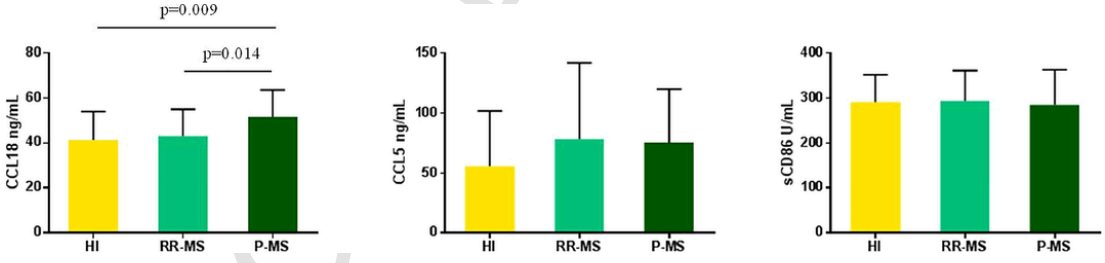

Fig. 1. CCL18, CCL5 and sCD86 levels in healthy individuals (HI), relapsing-remitting multiple sclerosis (RR-MS) and progressive MS (P-MS). The p-values from a Mann–Whitney U test are provided for comparisons between groups where Kruskal-Wallis test resulted significant. The error bars indicate the interquartile range. CCL18: C-C motif ligand 18; CCL5: C-C motif ligand 5; sCD86: soluble cluster of differentiation 86.

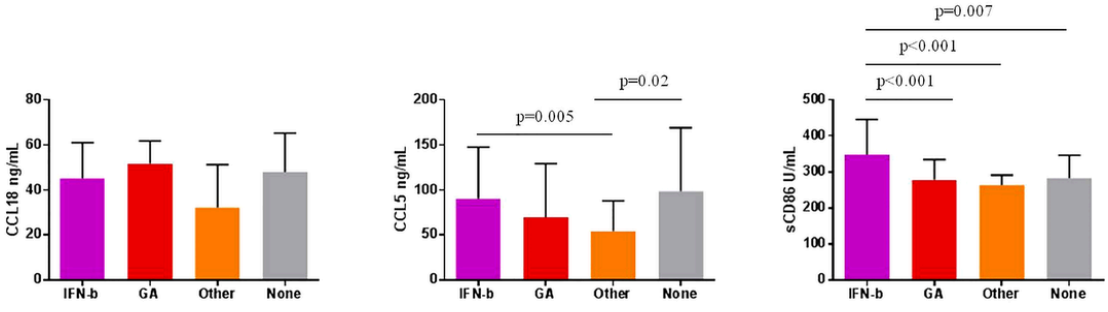

Fig. 2. CCL18, CCL5 and sCD86 levels in multiple sclerosis cohort according to the disease-modifying treatment. The p-values from a Mann–Whitney U test are provided for comparisons between groups where Kruskal-Wallis test resulted significant. The error bars indicate interquartile range. CCL18: C-C motif ligand 18; CCL5: C-C motif ligand 5; sCD86: soluble cluster of differentiation 86; GA: Glatiramer acetate; IFN-b: Interferon-beta; None: no disease-modifying therapy; Other: other disease-modifying therapy.

Table 3
Association of CCL18, CCL5 and sCD86 with MRI characteristics of the MS cohort.

	CCL18		CCL5		sCD86	
	r_p	P	r_p	P	r_p	P
T2-LV	**0.188**	**0.034**	0.028	0.75	−0.031	0.72
T1-LV	0.100	0.27	0.002	0.98	0.010	0.91
NBV	−0.151	0.093	0.098	0.27	−0.017	0.85
NCV	**−0.230**	**0.010**	0.096	0.28	0.022	0.80
LVV	**0.246**	**0.006**	−0.035	0.70	−0.027	0.76
DGM volume	**−0.247**	**0.006**	0.104	0.24	0.073	0.41
Thalamic volume	**−0.239**	**0.007**	0.111	0.21	0.059	0.50

CCL18: C-C motif ligand 18; CCL5: C-C motif ligand 5; sCD86: soluble cluster of differentiation 86; LV: lesion volume; NBV: normalized brain volume; NCV: normalized cortical volume; LVV: lateral ventricular volume; DGM: deep grey matter.
Partial correlation (r_p) and P value from regression analysis are shown. Multiple regression model: each MRI characteristic was used as the dependent variable while gender, age, being P-MS and the protein of interest as predictor variables. Additionally, BMI was included as predictor variables in the multiple regression model with CCL18.

Table 4
Association of CCL18, CCL5 and sCD86 with MRI characteristics of the HI cohort.

	CCL18		CCL5		sCD86	
	r_p	P	r_p	P	r_p	P
T2-LV	−0.13	0.46	−0.037	0.82	−0.10	0.54
NBV	−0.015	0.93	−0.067	0.68	0.13	0.42
NCV	−0.084	0.64	−0.14	0.40	0.020	0.90
LVV	0.22	0.21	−0.25	0.13	−0.22	0.18
DGM volume	−0.17	0.33	−0.21	0.16	−0.029	0.86
Thalamic volume	−0.14	0.44	−0.056	0.74	0.15	0.35

CCL18: C-C motif ligand 18; CCL5: C-C motif ligand 5; sCD86: soluble cluster of differentiation 86; LV: lesion volume; NBV: normalized brain volume; NCV: normalized cortical volume; LVV: lateral ventricular volume; DGM: deep grey matter.
Partial correlation (r_p) and P value from regression analysis are shown. Multiple regression model: each MRI characteristic was used as dependent variable while gender, age and the protein of interest were predictor variables. Additionally, BMI was included as predictor variables in the multiple regression model with CCL18.

brain disease. Furthermore, a recent study identified CCL18 as a top-3 upregulated gene in the rim of chronic active MS lesions, where foamy, myelin-accumulating macrophages are abundant. These findings would also indicate that demyelinating sites around chronic active lesions are indeed expanding in time (Hendrickx et al., 2017). In agreement, chronic active plaques are typically associated with P-MS and, as neurodegeneration in P-MS continues, preexisting chronic plaques may increase in size, resulting in slowly expanding, "smoldering" plaques (Frischer et al., 2015; Zeydan and Kantarci, 2018). Our in vivo results, using lesion volumes as MRI indicators of brain inflammation, and global and regional brain atrophy as neurodegenerative MRI outcomes, would support this model. Nevertheless, it is difficult to reconcile the potential anti-inflammatory role of CCL18 in relation to its expression by M2, with the worsening of clinical and MRI outcomes associated to increased CCL18 levels in patients.

Interestingly, CCL18 is involved in the lipid uptake and its levels were extremely elevated, between one and two orders of magnitude, in plasma of Gaucher disease patients, in whom a genetic deficiency in lysosomal glucocerebrosidase activity leads all macrophages to accumulate specific lipids (Boot et al., 2004). In cancer, infiltration of tumor-associated macrophages, and their CCL18 expression, correlate with serum infection titers of Epstein-Barr virus (EBV) (Huang et al., 2017), an environmental risk factor in MS patients. In previous studies we suggested that higher levels of EBV antibodies are associated with increased MRI lesion activity and greater brain atrophy, particularly of the GM (Zivadinov et al., 2016a). Further studies are needed to investigate the hypothesis of mechanistic association of EBV with CCL18 in MS progression.

Similarly to previous studies (Bartosik-Psujek and Stelmasiak, 2005; Szczucinski and Losy, 2011), we observed that CCL5 levels in plasma did not differ significantly in stable RR-MS group compared to controls. Additionally, stable RR compared to P-MS did not show differences in CCL5 levels (Rentzos et al., 2010). The increased levels detectable during relapse in MS patients as compared to stable RR-patients or controls (Bartosik-Psujek and Stelmasiak, 2005; Rentzos et al., 2010; Szczucinski and Losy, 2011), which could depict the ongoing inflammatory state, cannot be investigated in our cohort of MS patients, which did not include those in relapse. It is worth noting that our data, obtained in a larger cohort of patients, display ample variability in levels of each clinical and treatment groups, despite low inter-assay and intra-assay variations. Nevertheless, we detected significant DMTs related variations albeit not associated to GA treatment in accordance with previous data (Losy et al., 2005). We did not detect a trend for lower CCL5 levels after IFN-b treatment compared to none DMT (Iarlori et al., 2000).

Taking into account the DMT-related variations that we observed, these treatments could substantially contribute to produce heterogeneity in CCL5 levels.

A recent study, which investigated CCL5 levels in CSF of a patients' cohort comparable to ours, reported an association with the presence of gadolinium-enhanced brain MRI lesions (Mori et al., 2016). Our attempt to correlate peripheral CCL5 levels with MRI measures of lesion volumes and brain atrophy in a large cohort of MS patients, failed to find an association.

Higher sCD86 levels were reported in autoimmune disorders such as systemic lupus erythematosus and rheumatoid arthritis (Hock et al., 2006; Wong et al., 2005). The current study, which for the first time evaluated sCD86 plasma levels in MS, did not detect differences between MS clinical subgroups and with HI. Noteworthy, significantly increased sCD86 levels were detected in relation to disease treatment with IFN-b. Accordingly, it is known that INF-b upregulates CD86 on monocytes of MS patients, who positively responded to the treatment (Wiesemann et al., 2008). We infer that INF-b treatment could also induce CD86 mRNA alternative splicing/protein shedding, leading to the release of the evaluated soluble form (Jeannin et al., 2000). We did not

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detect any significant correlation of sCD86 with MRI measures. However, we have not investigated sCD86 plasma levels in the early stage of MS, as a potential marker of predisposition to the disease onset (Jeannin et al., 2000).

Our study presents some limitations. First, we studied peripheral plasma chemokines levels as indirect measure of macrophages-mediated expression, even if we did not investigate the number of circulating antigen-presenting cell populations. Second, the assay that we used for quantifying the chemokine concentration does not distinguish the different forms (e.g., homodimers, heterodimers and oligomers) that are known to induce different pathways according to the activated receptor (von Hundelshausen et al., 2017).

In conclusion, our results provide evidence that higher CCL18 plasma levels are associated with more severe inflammatory and neurodegenerative brain MRI outcomes in MS. Data support further investigation of plasma CCL18 levels in MS patients in association to disease progression, as well as functional and inhibition studies of CCL18, aimed at providing new insights into pathogenic mechanisms of MS.

Ethics approval and consent to participate

The study protocol was approved by the local Institutional Review Boards of University of Buffalo, USA (CEG-MS study; IRB ID: MOD-CR00000352) and of University/Hospital of Ferrara, Italy (IRB ID: 170,585). All participants gave their written informed consent.

Conflict of interest

Nicole Ziliotto, Francesco Bernardi, Dejan Jakimovski, Marcello Baroni, Niels Bergsland, Deepa P. Ramasamy, Paolo Zamboni and Giovanna Marchetti have no conflicts of interests.

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