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Relationship of microglia-associated pro-inflammatory cytokines to clinical and radiological parameters in patients with multiple sclerosis - a single-centre study in a Polish population

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Abstract

Introduction:

Multiple sclerosis (MS) is a demyelinating disease of the central nervous system with a chronic course. Available data point to an autoimmune basis for MS. It is currently suggested that microglia-associated cytokines have a role that is especially relevant.

Study objective:

To determine the association between microglia cytokines: C-C motif chemokine ligand 2/ Monocyte Chemoattractant Protein-1 (CCL2/MCP-1), Interferon gamma (IFN-), Interleukin-1 (IL-1), Interleukin-6 (IL-6), Interleukin-18 (IL-18) and clinical and radiological parameters of MS patients.

Material and methods

The study involved 96 patients with MS diagnosed according to the 2017 McDonald's criteria. The control group consisted of 73 healthy participants. Patients in the study group negated other immunological conditions.

Results

CCL2/MCP-1 levels were significantly increased in the study group. CCL/2MCP-1 levels were significantly higher in the other MS group compared to Relapse-remitting multiple sclerosis (RRMS). A significant positive correlation was identified between CCL2 (MCP-1) and disease duration. IFN-g levels were significantly higher in the control group. IFN-g concentrations exhibited a trend toward higher levels in the Expanded Disability Status Scale (EDSS) ≥ 4 score group. Significantly higher IFN-g levels were found in patients without active lesions on Magnetic resonance imaging (MRI). IL-18 levels were significantly increased in patients with no or single T2-weighted lesions.

Conclusions

The inverse correlation between IL-18 levels and MRI lesions suggests that this cytokine may serve as a predictive marker for monitoring the course of MS. IFN-g is a cytokine with an important role in the development of inflammation in MS. Higher plasma levels of CCL2 (MCP-1) with disease duration suggest progressive immune activation.

1. Introduction

Multiple sclerosis (MS) is a chronic disorder of the central nervous system (CNS) with a presumed autoimmune basis [1]. It is an inflammatory disease characterised by progressive neurodegeneration [1, 2]. There are currently an estimated 2.8 million people worldwide with the disease, which amounts to 35.9 patients per 100,000 people. Annually, there are 2.1 new cases of MS per 100,000 people [3]. MS is therefore the most common demyelinating disease, and, at present, this is an incurable condition [4, 5]. Fundamental to the pathogenesis of MS are disturbances in the control and balance of the immune response [6]. B lymphocytes, whose role includes the production of autoantibodies and cytokines, are involved in the pathophysiology of this disease [1, 7]. B cells also mediate the effects exerted by antigen-presenting cells. The result is the activation of T cells, which is thought to be a key element in the pathomechanism of the disease [7, 8]. This results in infiltration of the brain and spinal cord by inflammatory cells. A dual role for microglia has been demonstrated in the pathogenesis of MS, where it exerts both beneficial and negative effects [2]. As inflammation occurs in the CNS, as is the case in MS pathogenesis, microglia also have secretory and modulatory functions [2, 9–13]. Soluble factors via which microglia participate in negative effects in MS pathogenesis include interferon gamma (IFN-), tumour necrosis factor alpha (TNF-), reactive oxygen species, interleukin 1β (IL-1β), IL-6, IL-18, IL-12 and IL-23, but also C-C motif chemokines ligands (CCLs) [2, 10, 11]. Thus, microglia are involved in pathological processes in both white matter and grey matter [2, 14]. This aspect is all the more important because currently existing disease-modifying therapies (DMTs) only reduce white matter loss but have limited properties to significantly reduce or prevent grey matter neurodegeneration [2, 14]. Current disease-modifying treatments have only a limited effect on this process. Due to this, determining the relationship between plasma levels of microglia-associated pro-inflammatory cytokines and clinical status and radiological findings could provide important data on the pathogenesis of MS, as well as contributing to the development of new treatments for the condition.

The aim of this study is to determine the relationship between the levels of pro-inflammatory microglia cytokines and the clinical and radiological status of patients with MS.

2. Methodology

Participants provided informed consent to participate in the study. The study group included 96 patients with MS diagnosed according to the 2017 McDonald's criteria. Participants in the study group negated malignancies and other immunological diseases. The control group included 73 participants. These subjects negated the presence of neurological conditions, immunological diseases and malignancies.

The study lasted from April 1st, 2023, to June 1st, 2024. It was approved by the Bioethics Committee Medical University of Silesia in Katowice, Poland in accordance with the Declaration of Helsinki, BNW/NWN/0052/KB1/132/I/22/23 (March 23rd, 2023). All participants have been given a consent for using clinical results we obtained to publish.

Serum concentrations of CCL2/MCP-1, IL-1 beta/IL-1F2, IL-6, IFN-gamma and IL-18/IL-1F4 were determined in both control and study participants using Human Magnetic Luminex® Assays (R&D

SYSTEMS, biotechne).

Each drug was classified into a larger pharmacological category using the second level of the Anatomical Therapeutic Chemical (ATC) classification system (ATC2), which identifies the main therapeutic group of each substance (L01 - anticancer agents, L03 - immunostimulants, L04 - immunosuppressants). This was intended to group the drugs consistently.

These ATC2 categories were used to organise the active substances into clinically meaningful groups relevant to immune modulation, particularly in the treatment of multiple sclerosis. No further subclassifications using more granular levels of the ATC system (such as ATC4) were performed. According to the ATC2 classification, the L01 category covered Cladribine and Ofatumumab, the L03 category included glatiramer acetate, interferon beta 1a and 1b, while the L04 category listed dimethyl fumarate, fingolimod, ocrelizumab, ozanimod, siponimod, teriflunomide.

For the EDSS score, medical justification for level grouping was used [15]. Scores of 2.0-2.5 were classified as “Minimal/Mild”, 3.0-3.5 as “Moderate” and 4.0 or higher as “Significant”.

This categorisation was intended to ensure both clinical consistency but also comparability between groups, where each group represented approximately one-third of the study group.

The significance level was taken as p = 0.05, while p-values showing statistically significant findings are in bold.

Statistical analysis calculations were performed using R software (R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/), version 4.3.2.

3. Results

3.1 Baseline characteristics

There were 169 participants in the study, with 56.8% (N = 96) being the study group and 43.2% (N = 73) being the control group. Gender data were available for 152 participants, with 61.2% (n = 93). The median age of participants was 34 years (IQR: 24–46), while the mean age was 36.35 years (SD = 14.45). It should be noted that participants in the study group were significantly older (median age = 43 years) than those in the control group (median age = 22.5 years).

Marker levels were assessed in up to 160 individuals. For CCL2/MCP-1, the mean was 442.6 pg/ml (SD = 184.71), and the median was 418.65 pg/ml (IQR: 311.55–551.83). IL-1b had a median concentration of 0.52 pg/ml (IQR: 0.31–2.04) and a mean of 2.85 pg/ml (SD = 6.28), reflecting a right-skewed distribution. IFN-g and IL-18 also showed skewed distributions, with IL-18 presenting the highest median concentration of 792.22 pg/ml (IQR: 640.75–928.1). Detailed information on cytokine concentrations in the subgroups as well as for baseline characteristics is presented in Table 1.

Table 1
Baseline characteristics

Variable	Parameter	Overall (N = 169)	Study (N = 96)	Control (N = 73)
Group	Study	56.8% (N = 96)	-	-
	Control	43.2% (N = 73)	-	-
	Missing data	N = 0	-	-
Gender	Male	38.8% (N = 59)	33.8% (N = 27)	44.4% (N = 32)
	Female	61.2% (N = 93)	66.2% (N = 53)	55.6% (N = 40)
	Missing data	N = 17	N = 16	N = 1
Age (years)	N	127	81	46
	Mean (SD)	36.35 (14.45)	44.42 (11.97)	22.13 (2.47)
	Median (Q1-Q3)	34 (24–46)	43 (35–52)	22.5 (20–24)
	Range	18–72	19–72	18–27
	Missing data	N = 42	N = 15	N = 27
CCL2/MCP-1 [pg/ml]	N	160	96	64
	Mean (SD)	442.6 (184.71)	472.02 (207.67)	398.48 (133.33)
	Median (Q1-Q3)	418.65 (311.55–551.83)	429.71 (331.41–600.03)	395.64 (288.96–504.04)
	Range	2.88–1233.31	2.88–1233.31	127.53–681.29
	Missing data	N = 9	N = 0	N = 9
IL-1b [pg/ml]	N	160	96	64
	Mean (SD)	2.85 (6.28)	2.49 (6.2)	3.4 (6.41)
	Median (Q1-Q3)	0.52 (0.31–2.04)	0.41 (0.28–1.46)	0.73 (0.49–3.32)
	Range	0.08–51.3	0.19–51.3	0.08–32.95
	Missing data	N = 9	N = 0	N = 9

Variable	Parameter	Overall (N = 169)	Study (N = 96)	Control (N = 73)
IFN-g [pg/ml]	N	160	96	64
	Mean (SD)	2.95 (4.64)	2.63 (4.67)	3.42 (4.58)
	Median (Q1-Q3)	1.55 (0.87–3.14)	1.2 (0.8–2.58)	1.77 (1.1–3.57)
	Range	0.06–41.68	0.11–41.68	0.06–27.73
	Missing data	N = 9	N = 0	N = 9
IL-18 [pg/ml]	N	160	96	64
	Mean (SD)	811.32 (270.56)	786.89 (266.89)	847.96 (273.97)
	Median (Q1-Q3)	792.22 (640.75–928.1)	772.73 (630.54–913.25)	826.12 (670.03–1036.67)
	Range	54.42–1800.93	54.42–1800.93	89.06–1773.45
	Missing data	N = 9	N = 0	N = 9
Body mass (kg)	N	72	72	-
	Mean (SD)	71.78 (13.36)	71.78 (13.36)	-
	Median (Q1-Q3)	69.5 (62–81.25)	69.5 (62–81.25)	-
	Range	50–104	50–104	-
	Missing data	N = 97	N = 24	-
Height (cm)	N	71	71	-
	Mean (SD)	170.31 (8.98)	170.31 (8.98)	-
	Median (Q1-Q3)	170 (162.5–176)	170 (162.5–176)	-
	Range	155–191	155–191	-
	Missing data	N = 98	N = 25	-
Body mass index (BMI) [kg/m^2]	N	71	71	-
	Mean (SD)	24.75 (3.95)	24.75 (3.95)	-
	Median (Q1-Q3)	24.03 (21.93–26.19)	24.03 (21.93–26.19)	-

Variable	Parameter	Overall (N = 169)	Study (N = 96)	Control (N = 73)
	Range	17.78–34.06	17.78–34.06	-
	Missing data	N = 98	N = 25	-
MS type	PPMS	1.5% (N = 1)	1.5% (N = 1)	-
	RRMS	87.9% (N = 58)	87.9% (N = 58)	-
	SPMS	10.6% (N = 7)	10.6% (N = 7)	-
	Missing data	N = 103	N = 30	-
MS type - category	RRMS	87.9% (N = 58)	87.9% (N = 58)	-
	Other	12.1% (N = 8)	12.1% (N = 8)	-
	Missing data	N = 103	N = 30	-
Disease duration (years)	N	80	80	-
	Mean (SD)	8.79 (7.58)	8.79 (7.58)	-
	Median (Q1-Q3)	7 (2.88–12)	7 (2.88–12)	-
	Range	0.58–37	0.58–37	-
	Missing data	N = 89	N = 16	-
Disease duration (years) - category	Less than 3	25% (N = 20)	25% (N = 20)	-
	(3–7)	23.8% (N = 19)	23.8% (N = 19)	-
	(7–12)	22.5% (N = 18)	22.5% (N = 18)	-
	At least 12	28.7% (N = 23)	28.7% (N = 23)	-
	Missing data	N = 89	N = 16	-
Time from the first symptoms to the diagnose (years)	N	72	72	-
	Mean (SD)	4.2 (6.02)	4.2 (6.02)	-
	Median (Q1-Q3)	2 (0.5–5.25)	2 (0.5–5.25)	-
	Range	0–26	0–26	-
	Missing data	N = 97	N = 24	-

Variable	Parameter	Overall (N = 169)	Study (N = 96)	Control (N = 73)
Time from the first symptoms to the diagnose (years) - category	Less than 0.5	23.6% (N = 17)	23.6% (N = 17)	-
	(0.5-2)	22.2% (N = 16)	22.2% (N = 16)	-
	(2-5.5)	29.2% (N = 21)	29.2% (N = 21)	-
	At least 5.5	25% (N = 18)	25% (N = 18)	-
	Missing data	N = 97	N = 24	-
Age of MS diagnose (years)	N	80	80	-
	Mean (SD)	35.86 (9.91)	35.86 (9.91)	-
	Median (Q1-Q3)	34 (29.5–43.25)	34 (29.5–43.25)	-
	Range	17–65	17–65	-
	Missing data	N = 89	N = 16	-
Age of MS diagnose (years) - category	Less than 30	25% (N = 20)	25% (N = 20)	-
	(30–35)	27.5% (N = 22)	27.5% (N = 22)	-
	(35–44)	22.5% (N = 18)	22.5% (N = 18)	-
	At least 44	25% (N = 20)	25% (N = 20)	-
	Missing data	N = 89	N = 16	-
Number of relapses (last year)	N	78	78	-
	Mean (SD)	0.23 (0.51)	0.23 (0.51)	-
	Median (Q1-Q3)	0 (0–0)	0 (0–0)	-
	Range	0–2	0–2	-
	Missing data	N = 91	N = 18	-
Relapses in last year	Any	19.2% (N = 15)	19.2% (N = 15)	-
	None	80.8% (N = 63)	80.8% (N = 63)	-
	Missing data	N = 91	N = 18	-

Variable	Parameter	Overall (N = 169)	Study (N = 96)	Control (N = 73)
EDSS score	2	14.1% (N = 11)	14.1% (N = 11)	-
	2.5	20.5% (N = 16)	20.5% (N = 16)	-
	3	28.2% (N = 22)	28.2% (N = 22)	-
	3.5	6.4% (N = 5)	6.4% (N = 5)	-
	4	11.5% (N = 9)	11.5% (N = 9)	-
	4.5	6.4% (N = 5)	6.4% (N = 5)	-
	5	2.6% (N = 2)	2.6% (N = 2)	-
	5.5	5.1% (N = 4)	5.1% (N = 4)	-
	6	3.8% (N = 3)	3.8% (N = 3)	-
	6.5	1.3% (N = 1)	1.3% (N = 1)	-
	Missing data	N = 91	N = 18	-
EDSS score - categorization	2-2.5	34.6% (N = 27)	34.6% (N = 27)	-
	3-3.5	34.6% (N = 27)	34.6% (N = 27)	-
	>=4	30.8% (N = 24)	30.8% (N = 24)	-
	Missing data	N = 91	N = 18	-
Amount of MRI pathologic lesions (T2) - category	Numerous	81.3% (N = 61)	81.3% (N = 61)	-
	Single	17.3% (N = 13)	17.3% (N = 13)	-
	None	1.3% (N = 1)	1.3% (N = 1)	-
	Missing data	N = 94	N = 21	-
Amount of MRI pathologic lesions (T2) - categorization	Numerous	81.3% (N = 61)	81.3% (N = 61)	-
	Single or none	18.7% (N = 14)	18.7% (N = 14)	-
	Missing data	N = 94	N = 21	-

Variable	Parameter	Overall (N = 169)	Study (N = 96)	Control (N = 73)
Active lesion - category	Yes	45.9% (N = 17)	45.9% (N = 17)	-
	No	54.1% (N = 20)	54.1% (N = 20)	-
	Missing data	N = 132	N = 59	-
Hyperintensive - category	Yes	63.6% (N = 14)	63.6% (N = 14)	-
	No	36.4% (N = 8)	36.4% (N = 8)	-
	Missing data	N = 147	N = 74	-
Nonactive - category	Yes	98% (N = 50)	98% (N = 50)	-
	No	2% (N = 1)	2% (N = 1)	-
	Missing data	N = 118	N = 45	-
Number of lesions in Gd+ - category	Numerous	43.3% (N = 29)	43.3% (N = 29)	-
	Single	7.5% (N = 5)	7.5% (N = 5)	-
	None	49.3% (N = 33)	49.3% (N = 33)	-
	Missing data	N = 102	N = 29	-
Number of lesions in Gd+ - categorization	Numerous	43.3% (N = 29)	43.3% (N = 29)	-
	Single or none	56.7% (N = 38)	56.7% (N = 38)	-
	Missing data	N = 102	N = 29	-
Active substance	Cladribine	1.2% (N = 1)	1.2% (N = 1)	-
	Dimethyl fumarate	37% (N = 30)	37% (N = 30)	-
	Fingolimod	6.2% (N = 5)	6.2% (N = 5)	-
	Glatiramer acetate	2.5% (N = 2)	2.5% (N = 2)	-
	Interferon beta 1a	2.5% (N = 2)	2.5% (N = 2)	-
	Interferon beta 1b	6.2% (N = 5)	6.2% (N = 5)	-

Variable	Parameter	Overall (N = 169)	Study (N = 96)	Control (N = 73)
	Ocrelizumab	11.1% (N = 9)	11.1% (N = 9)	-
	Ofatumumab	19.8% (N = 16)	19.8% (N = 16)	-
	Ozanimod	1.2% (N = 1)	1.2% (N = 1)	-
	Siponimod	8.6% (N = 7)	8.6% (N = 7)	-
	Teriflunomide	3.7% (N = 3)	3.7% (N = 3)	-
	Missing data	N = 88	N = 15	-
ATC2	L01	21% (N = 17)	21% (N = 17)	-
	L03	11.1% (N = 9)	11.1% (N = 9)	-
	L04	67.9% (N = 55)	67.9% (N = 55)	-
	Missing data	N = 88	N = 15	-
White blood cells (WBC) [x10^3/µl]	N	75	75	-
	Mean (SD)	5.85 (1.87)	5.85 (1.87)	-
	Median (Q1-Q3)	5.41 (4.46–6.8)	5.41 (4.46–6.8)	-
	Range	3.01–11.8	3.01–11.8	-
	Missing data	N = 94	N = 21	-
Red blood cells (RBC) [mln/µl]	N	76	76	-
	Mean (SD)	4.77 (1.25)	4.77 (1.25)	-
	Median (Q1-Q3)	4.6 (4.39–4.99)	4.6 (4.39–4.99)	-
	Range	3.73–15	3.73–15	-
	Missing data	N = 93	N = 20	-
Hemoglobin (HG) [g/dl]	N	76	76	-
	Mean (SD)	13.94 (1.33)	13.94 (1.33)	-
	Median (Q1-Q3)	14 (13.1–14.7)	14 (13.1–14.7)	-
	Range	9.6–17.1	9.6–17.1	-
	Missing data	N = 93	N = 20	-

Variable	Parameter	Overall (N = 169)	Study (N = 96)	Control (N = 73)
Platelets (PLT) [x10^3/µl]	N	76	76	-
	Mean (SD)	256.2 (63.31)	256.2 (63.31)	-
	Median (Q1-Q3)	244.5 (218.25–281.5)	244.5 (218.25–281.5)	-
	Range	75–455	75–455	-
	Missing data	N = 93	N = 20	-
Thyroid-stimulating hormone (TSH) [mIU/l]	N	41	41	-
	Mean (SD)	1.8 (1.09)	1.8 (1.09)	-
	Median (Q1-Q3)	1.57 (0.93–2.27)	1.57 (0.93–2.27)	-
	Range	0.01–4.46	0.01–4.46	-
	Missing data	N = 128	N = 55	-
Aminotransferase Alanina (AlAT) [U/l]	N	74	74	-
	Mean (SD)	23.93 (11.24)	23.93 (11.24)	-
	Median (Q1-Q3)	20.25 (15.4–32.6)	20.25 (15.4–32.6)	-
	Range	7.3–55.3	7.3–55.3	-
	Missing data	N = 95	N = 22	-
Aspartate aminotransferase (AspAT) [U/l]	N	75	75	-
	Mean (SD)	22.92 (8.96)	22.92 (8.96)	-
	Median (Q1-Q3)	20 (17.7–24.75)	20 (17.7–24.75)	-
	Range	12.2–70.08	12.2–70.08	-
	Missing data	N = 94	N = 21	-
Creatinine [µmol/l]	N	75	75	-
	Mean (SD)	69.62 (13.14)	69.62 (13.14)	-
	Median (Q1-Q3)	68 (61–78.84)	68 (61–78.84)	-
	Range	46–122	46–122	-
	Missing data	N = 94	N = 21	-

Variable	Parameter	Overall (N = 169)	Study (N = 96)	Control (N = 73)
CRP [mg/l]	N	14	14	-
	Mean (SD)	1.58 (1.78)	1.58 (1.78)	-
	Median (Q1-Q3)	1.07 (0.46–1.64)	1.07 (0.46–1.64)	-
	Range	0.2–6.35	0.2–6.35	-
	Missing data	N = 155	N = 82	-
Lymphocyte percent	N	72	72	-
	Mean (SD)	26.01 (10.44)	26.01 (10.44)	-
	Median (Q1-Q3)	25.85 (17.68–33.6)	25.85 (17.68–33.6)	-
	Range	3.3–49.6	3.3–49.6	-
	Missing data	N = 97	N = 24	-
Lymphocytes [x10^3/µl]	N	75	75	-
	Mean (SD)	1.54 (0.76)	1.54 (0.76)	-
	Median (Q1-Q3)	1.35 (0.92–2.04)	1.35 (0.92–2.04)	-
	Range	0.32–3.9	0.32–3.9	-
	Missing data	N = 94	N = 21	-

3.2 Parameter Comparison Between Study Groups

Statistically significant differences in the comparison between the study group (N = 96) and the control group (N = 64) were shown for the following cytokines:

CCL2/MCP-1 concentrations were significantly higher in the study group (median: 429.71 pg/ml) than in controls (median: 395.64 pg/ml; $p = 0.0377$, $r = 0.164$), though not confirmed by the Wald test ($p = 0.705$).

IL-1b levels were significantly elevated in the control group (median: 0.73 pg/ml) versus the study group (median: 0.41 pg/ml; $p < 0.001$, $r = 0.263$), with a non-significant Wald test ($p = 0.472$).

IFN-g was also significantly higher in controls (median: 1.77 pg/ml) than in the study group (median: 1.2 pg/ml; $p = 0.0121$, $r = 0.198$), though not confirmed in the Wald test ($p = 0.384$). Other markers (IL-4, IL-13, IL-18) did not show statistically significant differences ($p > 0.05$).

3.3 Parameter Comparison Within Study Group

MS type – category

CCL2/MCP-1 was notably elevated in the "Other" MS group (median: 644.99 pg/ml) versus relapse-remitting multiple sclerosis (RRMS) (median: 417.33 pg/ml), with statistical significance (p = 0.0103, effect size = 0.316), though not confirmed by the Wald test (p = 0.418).

Other markers, including IL-1b, IFN-g and IL-18, did not show statistically significant differences between RRMS and other MS subtypes (p > 0.05).

EDSS score – categorisation

IFN-g concentrations exhibited a trend toward higher levels in the EDSS ≥ 4 group (median: 1.50 pg/ml) relative to the 3–3.5 group (median: 0.82 pg/ml) (Fig. 1). Although this trend did not reach significance in the unadjusted analysis (p = 0.0533, effect size = 0.001), it became statistically significant in the adjusted model (Wald test p = 0.046, R^2 = 0.158) (Fig. 2).

No statistically significant differences were observed for the other cytokines across EDSS categories in either unadjusted or adjusted analyses (all p > 0.05).

Amount of MRI pathologic lesions (T2) – categorisation

Among the cytokines assessed in relation to the amount of MRI T2 lesions (N = 96), one marker showed a significant difference:

IL-18 levels were significantly higher in patients with single or no T2 lesions (median: 933.6 pg/ml) compared to those with numerous lesions (median: 748.57 pg/ml), with p = 0.0194 in the unadjusted analysis (effect size = 0.859) (Fig. 3). This result was confirmed after adjustment, with the estimated marginal mean (emmeans) of IL-18 for the "Numerous" group at 742.68 pg/ml (95% CI: 673.28–812.07), and for the "Single or none" group at 959.09 pg/ml (95% CI: 812.20–1105.98). The adjusted difference was statistically significant: − 216.41 pg/ml (95% CI: − 379.42 to − 53.40; Wald test p = 0.008, R^2 = 0.148), indicating a robust association between lower lesion burden and higher IL-18 levels (Fig. 4).

Other cytokines showed no statistically significant differences between lesion burden groups in both unadjusted and adjusted analyses (all p > 0.05).

Active lesion – category

Among the cytokines assessed in relation to the presence of active MRI lesions (N = 96), only one marker showed a statistically significant difference:

IFN-g levels were significantly lower in patients with active lesions (median: 0.82 pg/ml) compared to those without active lesions (median: 1.57 pg/ml), with p = 0.0302 in the unadjusted non-parametric analysis (effect size = 0.356) (Fig. 5). This finding was confirmed in the adjusted analysis, where the estimated marginal mean (emmeans) for the "Active lesions" group was 0.918 pg/ml (95% CI: 0.623–

1.351), and for the "No active lesions" group it was 1.962 pg/ml (95% CI: 1.402–2.743). The adjusted difference was 0.468 pg/ml (95% CI: 0.276–0.794), indicating significantly higher IFN-g levels in patients without active lesions (Wald test p = 0.003, R^2 = 0.426) (Fig. 6).

Other cytokines did not show statistically significant differences between the two groups in either unadjusted or adjusted analyses (all p > 0.05).

Hyperintensive – category

No statistically significant associations were observed between the analyzed cytokines and the hyperintensity occurrence.

Number of lesions in Gd+ - categorisation

Similarly, no statistically significant associations were observed between the analyzed cytokines and the number of lesions in Gd+.

ATC2

CCL2/MCP-1 levels differed significantly between ATC2 groups (Kruskal-Wallis p = 0.0264, effect size = 0.001). Post-hoc Dunn's tests revealed significantly higher MCP-1 levels in the L03 group compared to L01 (p = 0.0225) and L01 to L04 (p = 0.0368), but no significant difference between L03 and L04 (p = 0.4384) (Table 2) (Fig. 7). This difference was not statistically significant in the adjusted model (Wald p = 0.719, R^2 = 0.037).

Other markers did not show statistically significant differences between ATC2 groups in either unadjusted or adjusted analyses (all p > 0.05).

Table 2
Dunn post-hoc tests – variable CCL2/MCP-1.

Variable	Comparison	p-value
CCL2/MCP-1 (pg/ml)	L01 - L03	**0.0225**
	L01 - L04	**0.0368**
	L03 - L04	0.4384

Other categories

There were no statistically significant differences in disease duration, time from symptom onset to diagnosis, age at diagnosis, or the presence of recent relapses for any of the cytokines assessed.

Correlation analysis Within Study Group

Spearman correlation analysis was conducted to evaluate the association between selected cytokines and clinical, biochemical and haematological parameters.

IL-1b was most strongly correlated with IL-6 ($r = 0.534$, $p < 0.001$), followed by IL-6 and IFN-g ($r = 0.526$, $p < 0.001$).

A significant positive correlation was identified between CCL2 (MCP-1) and disease duration ($r = 0.271$, $p = 0.015$), which may reflect progressive immune activation over time.

Cytokine levels were also correlated with haematological parameters. IL-6, IL-1b, and IFN-g demonstrated weak but statistically significant positive correlations with total white blood cell (WBC) count ($r = 0.262$, $p = 0.023$; $r = 0.243$, $p = 0.036$; and $r = 0.231$, $p = 0.046$, respectively).

No statistically significant correlations were found between cytokine levels and age, height, weight, or most anthropometric measures, apart from a weak but significant correlation between IL-6 and BMI ($r = 0.245$, $p = 0.039$).

4. Discussion

According to available information, the study we conducted is one of the first aimed at comprehensively assessing the mutual relationships between the level of pro-inflammatory microglia-associated cytokines in the course of MS and the presence of lesions in neuroimaging studies. This highlights the significant innovative nature of this study.

Considering the fact that diagnoses of this disease are increasingly being made in various geographic regions of the world (more prevalent in Europe and in Northern America - >100 per 100.000 people, less frequent in sub-Saharan Africa and East Asia – only 2 per 100.000 people), as well as the lack of possibilities for its effective cure, the search for new treatment methods or ways to monitor MS seems particularly relevant [4]. These therapies could potentially reduce not only white matter loss, but also grey matter neurodegeneration, which is crucial for disability in MS [14].

In some countries, including those in Europe, women have three times the incidence of men, however, this is only true for the relapsing-remitting form of MS (RRMS) [4, 7]. In the comparison with ratio of members in our study, it is about 2:1 (66.2% of females and 33.8% of males), which does not much differ to the general trend.

It is important to emphasize the aspect of microglia-associated cytokines participation in the processes of neurodegeneration in MS. Demyelination, affecting both grey and white matter, can be reversed by, among other things, remyelination, in which microglia also participate through secreted cytokines [2, 14]. Neurodegeneration of the grey matter, on the other hand, is irreversible, occurs very early in the course of MS and is largely responsible for permanent disability [2].

Areas of CNS demyelination are also formed [9]. Initially, the inflammatory process, stimulated by pro-inflammatory cytokines predominates, while later the degenerative process takes over. There is neuronal, axonal and synaptic damage, as well as destruction of glial cells [9]. Resident macrophages in the CNS, which are called microglia, have been shown to play a significant role in these pathological processes [2, 7]. In a way, microglia behave as a controller that monitors the processes of the microenvironment and the proper functioning of neuronal cells, dendrites and axons [2]. In addition, these cells phagocytose necrotic cells and their parts [2]. The nature of microglia is highly plastic and, depending on the situation, can exert different, often opposing effects [2].

Our results indicate that IFN-γ concentrations are elevated in patients with a higher degree of disability as reflected by the EDSS score. However, given the lack of significance in the primary (unadjusted) test and the non-significant pairwise comparisons using estimated marginal means (mmeans), this result should be interpreted with caution. Nevertheless, in the study by Trenova et al., IFN-γ concentrations, as in our study, were positively correlated with a higher score on the EDSS scale, but only in specific clinical situations, such as during therapy with interferon beta or during a relapse [16].

In 1987, a study was conducted in which patients with MS were given a trial of therapy with IFN-γ [17]. The consequence of this trial appeared to be an exacerbation of disease symptoms in less than half of the patients. This was due to an increase in the expression of MHC class II monocytes, thus indicating a deleterious adverse effect of this cytokine in MS [17]. These results agree with those obtained from our studies.

One therapeutic strategy contributing to delaying MS progression may be to restore the balance between the pro-inflammatory and immunomodulatory actions of IFN-γ. This may be served by reducing the concentration of this cytokine and other pro-inflammatory molecules [17, 18, 19]. It is noteworthy that disruption of this balance is one of the proven causes underlying the development of the disease [20, 21]. Our study also showed higher IFN-γ levels in patients without active lesions on brain MRI. Thus, the observation from a randomized trial in a group of patients with primary-progressive multiple sclerosis (PPMS), according to which the effect of neutralizing antibodies to IFN-γ significantly slows the development of disability, seems interesting. This was confirmed both by MRI studies, by showing fewer active lesions, and by the shift in the cytokine profile of activated blood cells found in biochemical analysis [22, 23]. It caused, among other things, a reduction in the synthesis of IL-1β, TNF-α and IFN-γ. These results suggest that IFN-γ inhibition may represent a promising new therapeutic strategy, especially in the treatment of PPMS [22, 23].

Thus, the findings in this manuscript confirm current knowledge of IFN-γ as a molecule with pro-inflammatory effects. Moreover, in agreement with this is another phenomenon we observed. Namely, a positive correlation between white blood cell counts and IFN-γ levels, as well as two other microglia-associated cytokines that aggravate MS, specifically IL-1b and IL-6 [24, 25].

In terms of IL-18, we have shown that the level of this cytokine is significantly higher in patients with a lower number of T2-dependent lesions on MRI. This finding is supported by results from a paper by Losy

et al. [26]. This study reported significantly higher levels of IL-18 in both CSF and serum among participants who were also characterized by the presence of active CNS lesions visualized by MRI with gadolinium enhancement [27, 28]. Based on this, the authors concluded that IL-18 has a significant effect relative to MS immunopathogenesis, especially in the active phases of the disease [26–29].

Taking into account the fact that these lesions shown by MRI are indicative of disease activity in the course of MS, and IL-18 has a proven inflammation-promoting effect, it is reasonable to assume that this cytokine may in the future serve as a predictive marker for monitoring MS.

Our study also showed a moderate positive correlation between IL1b and IL-6, as well as IL-6 and INF-g. The cytokines IL-1 and IL-6 show a pro-inflammatory character [29–34]. The correlation of IL-6 with IFN-, which is a cytokine that promotes the development of MS and worsens prognosis in later stages of the disease, seems understandable [29–32].

CCL2 is seen as a molecule with pro-inflammatory effects [35–38]. The interaction of CCL2 with the CCR2 receptor results in the attraction of monocytes, macrophages, dendritic cells, natural-killer (NK) cells and T lymphocytes to the CNS [35–37]. As a result of the activation of the aforementioned cells, pro-inflammatory molecules are released: IL-1β, IL-6 and TNF-α, exerting destructive effects on the myelin sheaths of neurons [37, 38]. Moreover, it has been shown that one of the causes of neuronal cell inflammation and subsequent degeneration may be the effect that CCL2 has toward microglia activation [39, 40]. Previously published reports have revealed that CCL2 levels in the cerebrospinal fluid (CSF) are elevated in patients, suffering from MS and show a correlation with a more severe disease course [41]. This does not differ with our results, where we showed a significant positive correlation between CCL2/MCP-1 and disease duration, which may reflect progressive immune activation over time [41–43]. This may lay the groundwork for potential exploration of CCL2 use in clinical practice, including as a marker.

5. Summary

The aim of this study was to assess the relationship of pro-inflammatory microglia cytokines with clinical and radiological parameters of MS patients.

IL-18 levels were significantly increased in patients with single or non-T2-weighted lesions on MRI, suggesting a possible inverse association with lesion burden.

No significant differences in cytokine profiles were observed with respect to disease duration, time from symptom onset to diagnosis, age at diagnosis, or the presence of recent relapses. Additionally, correlations between cytokine levels and radiological or clinical metrics were generally weak, with only a few statistically significant associations.

The results suggest that IFN- levels may be higher in patients with higher levels of disability, but this should be interpreted with caution. It was also shown that IFN- levels were higher in patients without

active brain MRI lesions. These findings suggest the action of IFN-□ as a cytokine with important effects on the development of inflammation in the context of MS.

Higher plasma levels of CCL2/MCP-1 with disease duration suggest progressive immune activation. This suggests the possibility of using this molecule as a marker of MS.

Moreover, a positive correlation was obtained between white blood cell count and IFN-□ levels, as well as IL-1b and IL-6, which are microglia-associated cytokines already known to have a negative prognostic effect in MS.

It seems that the findings obtained may provide an interesting starting point for further studies aimed at further clarifying the role of the indicated molecules, as well as establishing and developing methods to exploit their properties.

Abbreviations

MS
Multiple sclerosis
CCL2/MCP
1-C-C motif chemokine ligand 2/ Monocyte Chemoattractant Protein-1
IFN
□-Interferon gamma
IL
1-Interleukin-1
IL
6-Interleukin-6
IL
18-Interleukin-18
IL
12-Interleukin 12
IL
23-Interleukin 23

Declarations

Ethics approval and consent to participate:

• A study was approved by the Bioethics Committee Medical University of Silesia in Katowice, Poland in accordance with the Declaration of Helsinki, BNW/NWN/0052/KB1/132/I/22/23 (March 23rd, 2023). All participants provided informed consent to participate in the study.

Consent for publication:

• All participants have been given an agreement for using clinical results we obtained to publish.

Competing interests:

• We declare that the authors have no competing interests as defined by BMC, or other interests that might be perceived to influence the results and/or discussion reported in this paper.

Conflicts of Interest:

• The authors declare no conflicts of interest.

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Author Contribution

Conceptualization: H.M., Data curation: H.M., Formal analysis: H.M., A.S., Funding acquisition: H.M., Investigation: H.M., A.S., Methodology: H.M., Project administration: H.M., M.A.-S., Resources: H.M., Software: H.M., Supervision: M.A.-S., Validation: H.M., Visualization: H.M., Writing – original draft: H.M., A.S., Writing – review and editing: H.M., M.A.-S.

Data Availability

All data supporting the findings of this study are available within the paper and its Supplementary Information.

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Figures

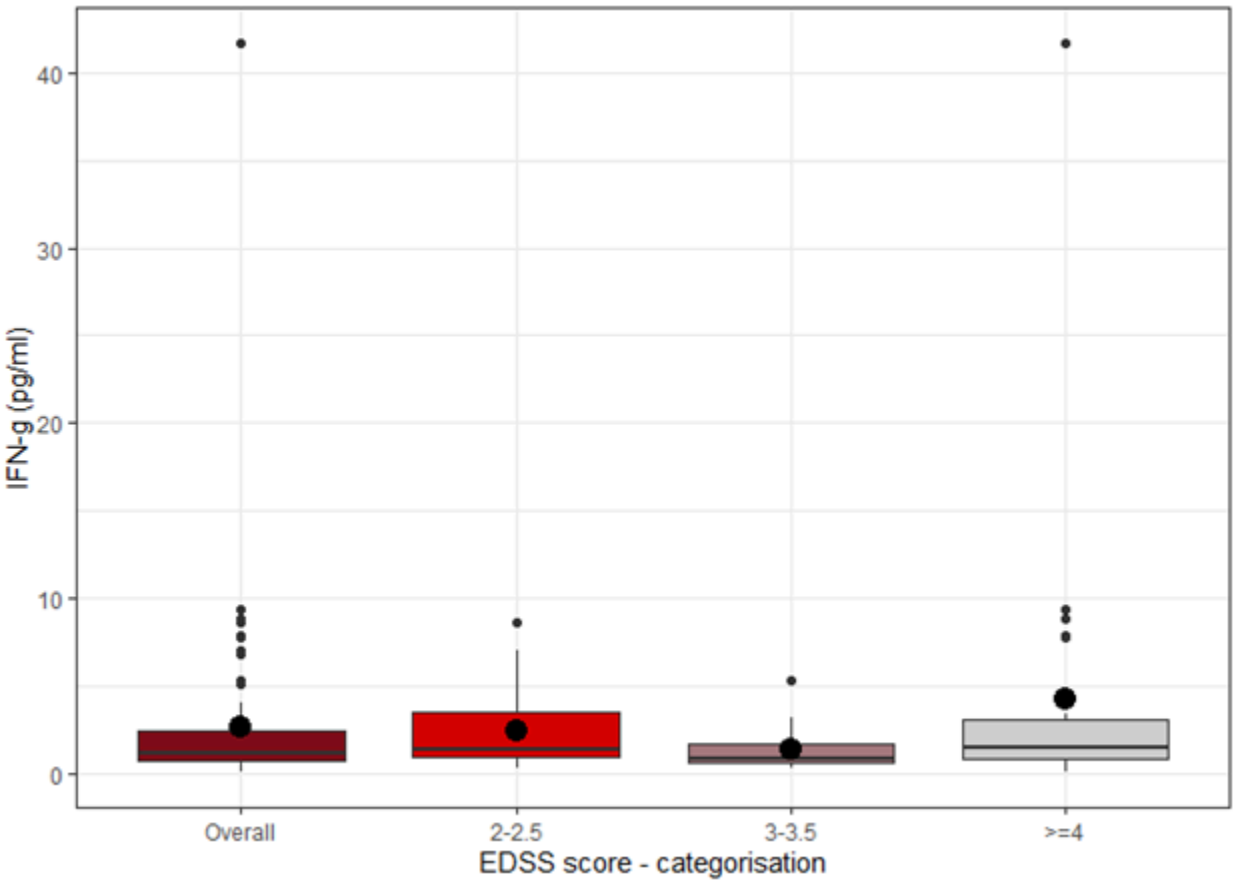


Figure 1

Dependency between variables: IFN-g (pg/ml) and EDSS score - categorisation in study group (“Overall”: N = 96; “2-2.5”: N = 27; “3-3.5”: N = 27; “>=4”: N = 24; median: 1.2 pg/ml; p = 0.004; effect size r = 0.227; Wald test)

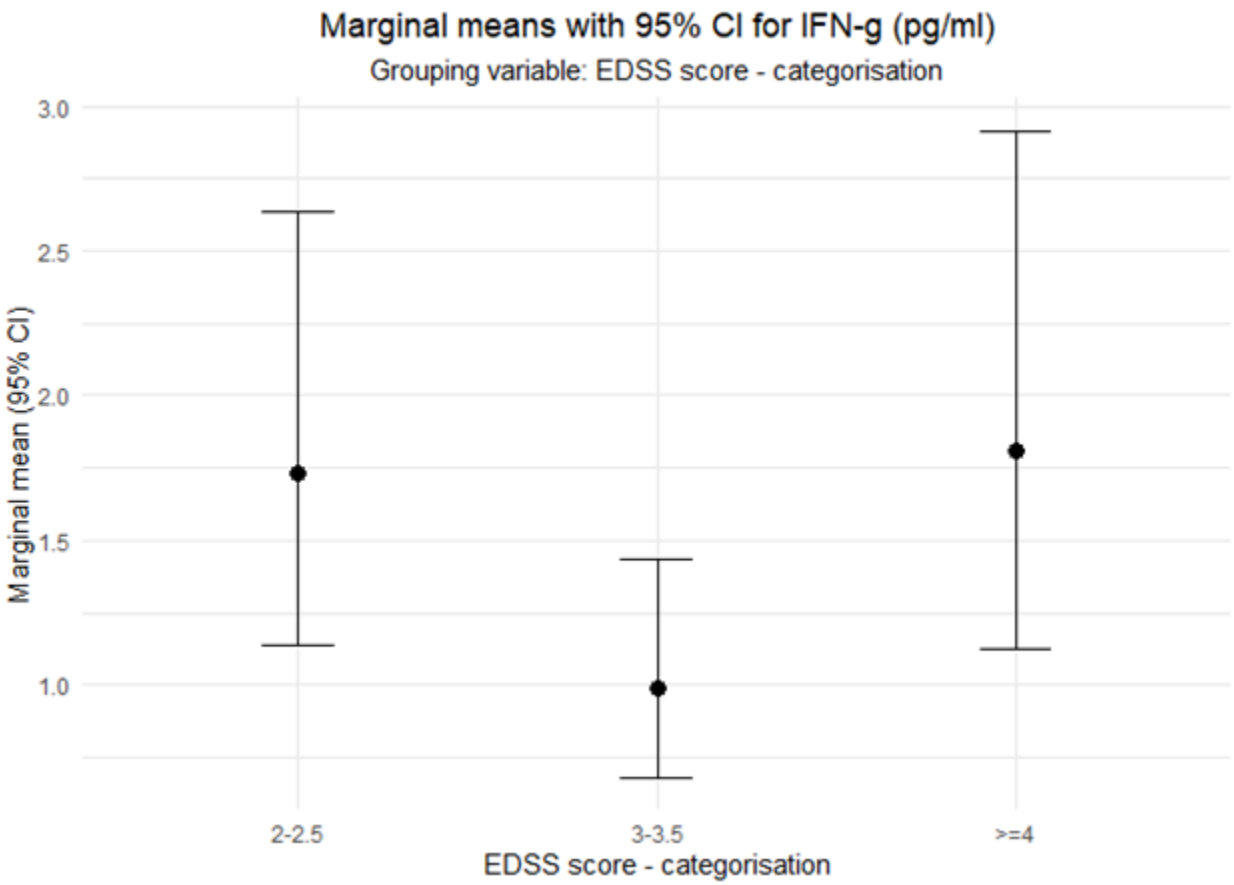


Figure 2

Marginal means for IFN-g (pg/ml) by EDSS score – categorisation (“Overall”: N = 96; “2-2.5”: N = 27; “3-3.5”: N = 27; “>=4”: N = 24; median: 1.2 pg/ml; p = 0.004; effect size r = 0.227; Wald test)

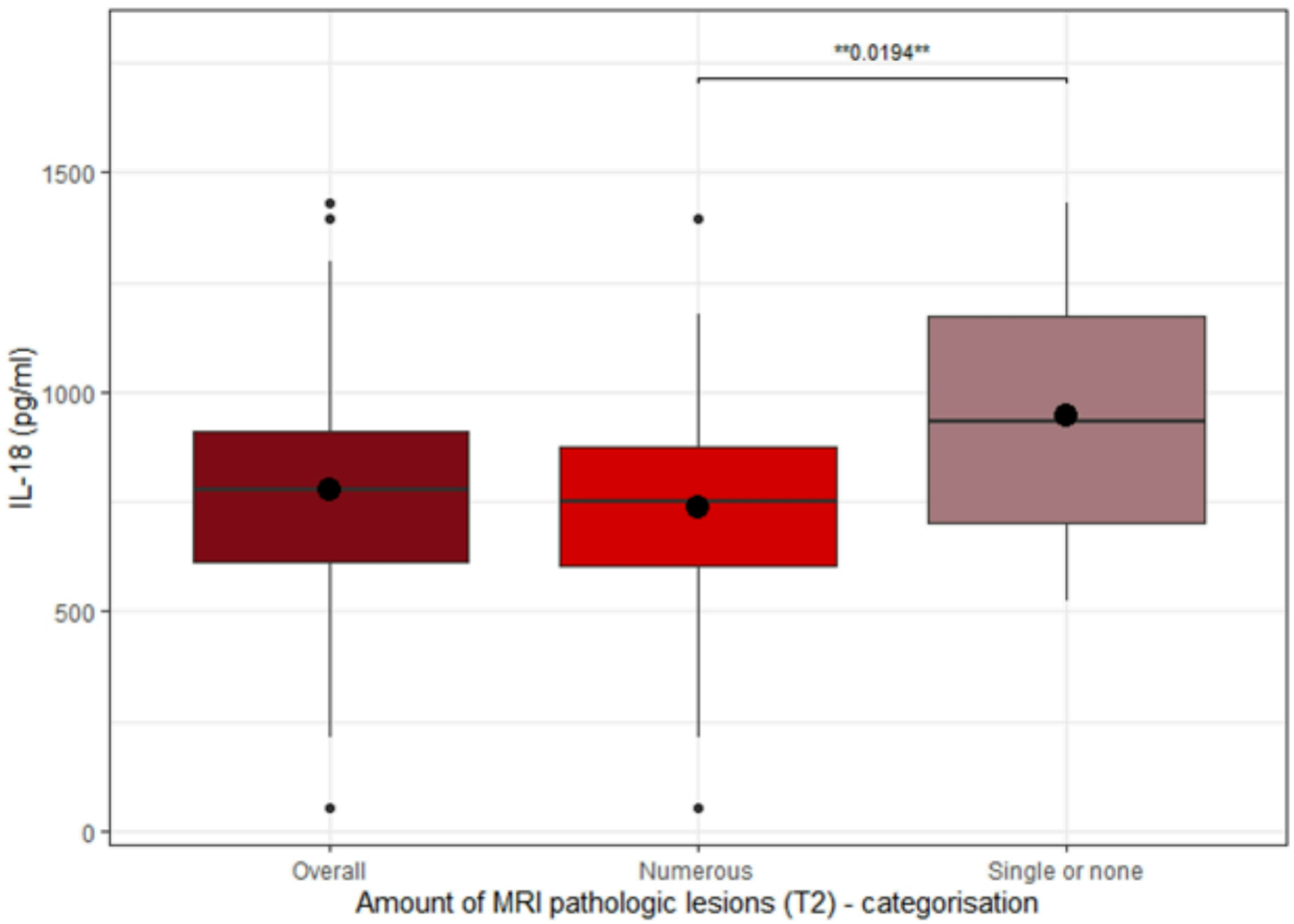


Figure 3

Dependency between variables: IL-18 (pg/ml) and Amount of MRI pathologic lesions (T2) - categorisation in study group (N = 96; “Numerous”: N = 61; “Single or none”: N = 14; median: 772.73 pg/ml; p = 0.0194; effect size r = 0.859; Wald test p = 0.008)

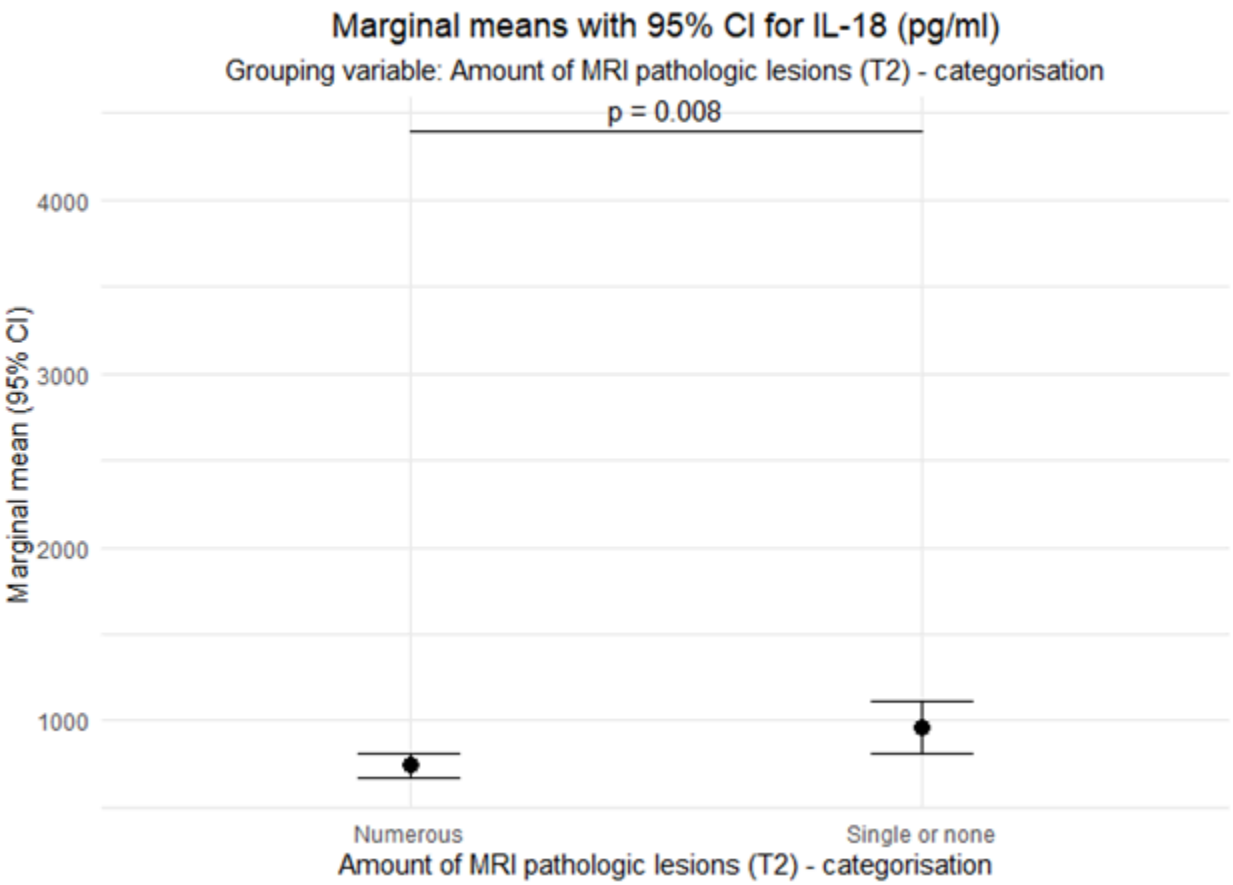


Figure 4

Marginal means for IL-18 (pg/ml) by Amount of MRI pathologic lesions (T2) - categorisation (N = 96; “Numerous”: N = 61; “Single or none”: N = 14; median: 772.73 pg/ml; p = 0.0194; effect size r = 0.859; Wald test p = 0.008)

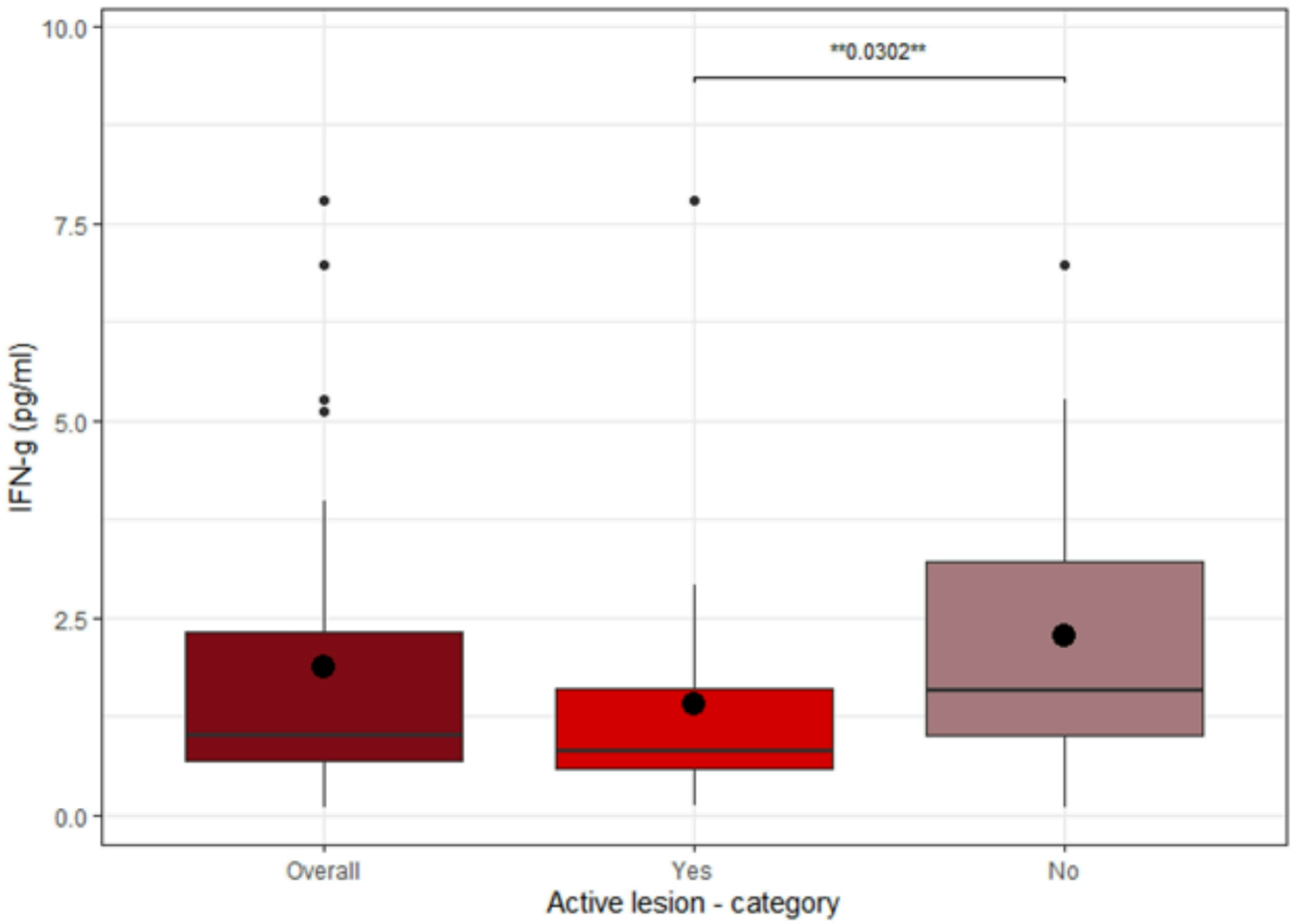


Figure 5

Dependency between variables: IFN-g (pg/ml) and Active lesion – category in study group (N = 96; “Yes”: N = 17; “No”: N = 20; median = 1.2 pg/ml; p = 0.302; r effect size = 0.356; U Mann – Whitney test)

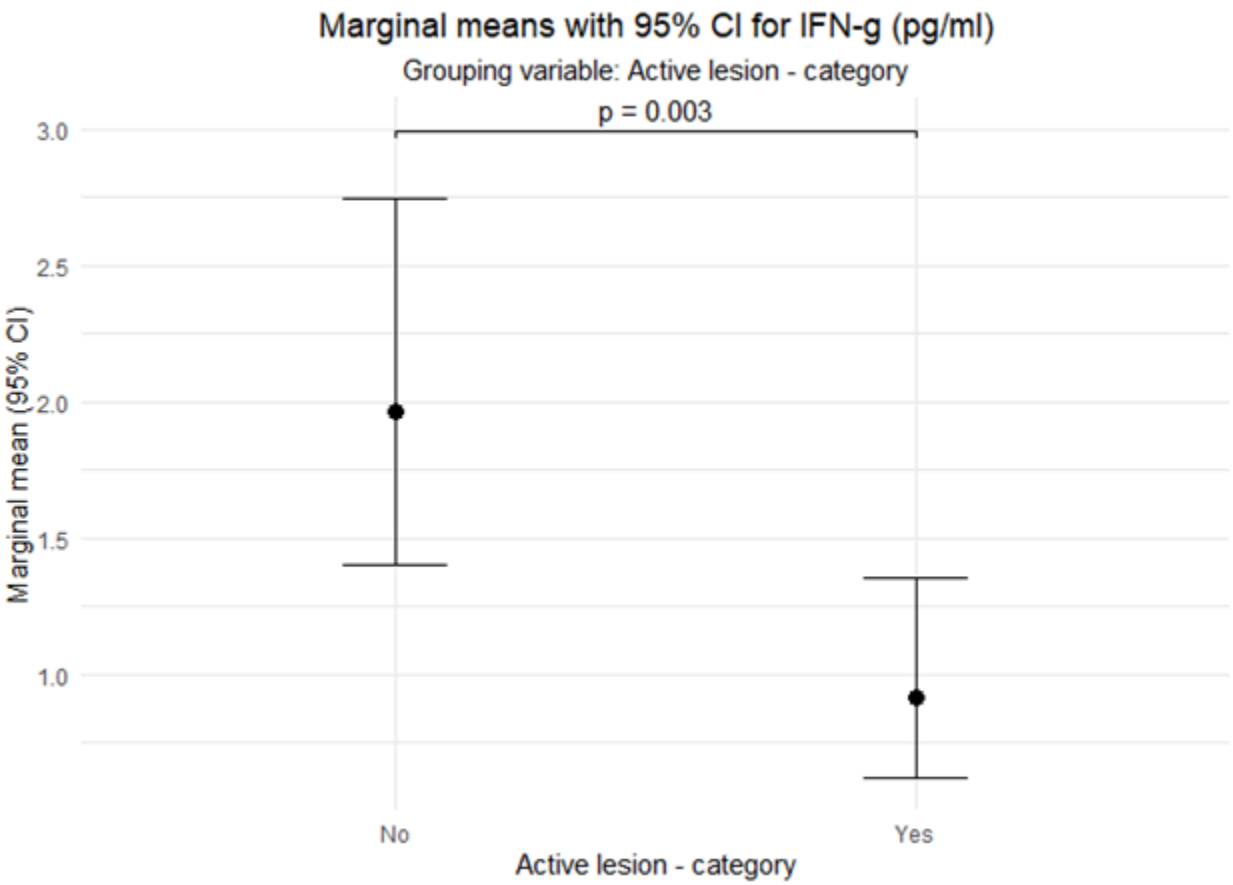


Figure 6

Marginal means for IFN-g (pg/ml) by Active lesion – category (N = 96; “Yes”: N = 17; “No”: N = 20; median = 1.2 pg/ml; p = 0.302; r effect size = 0.356; U Mann - Whitney test)

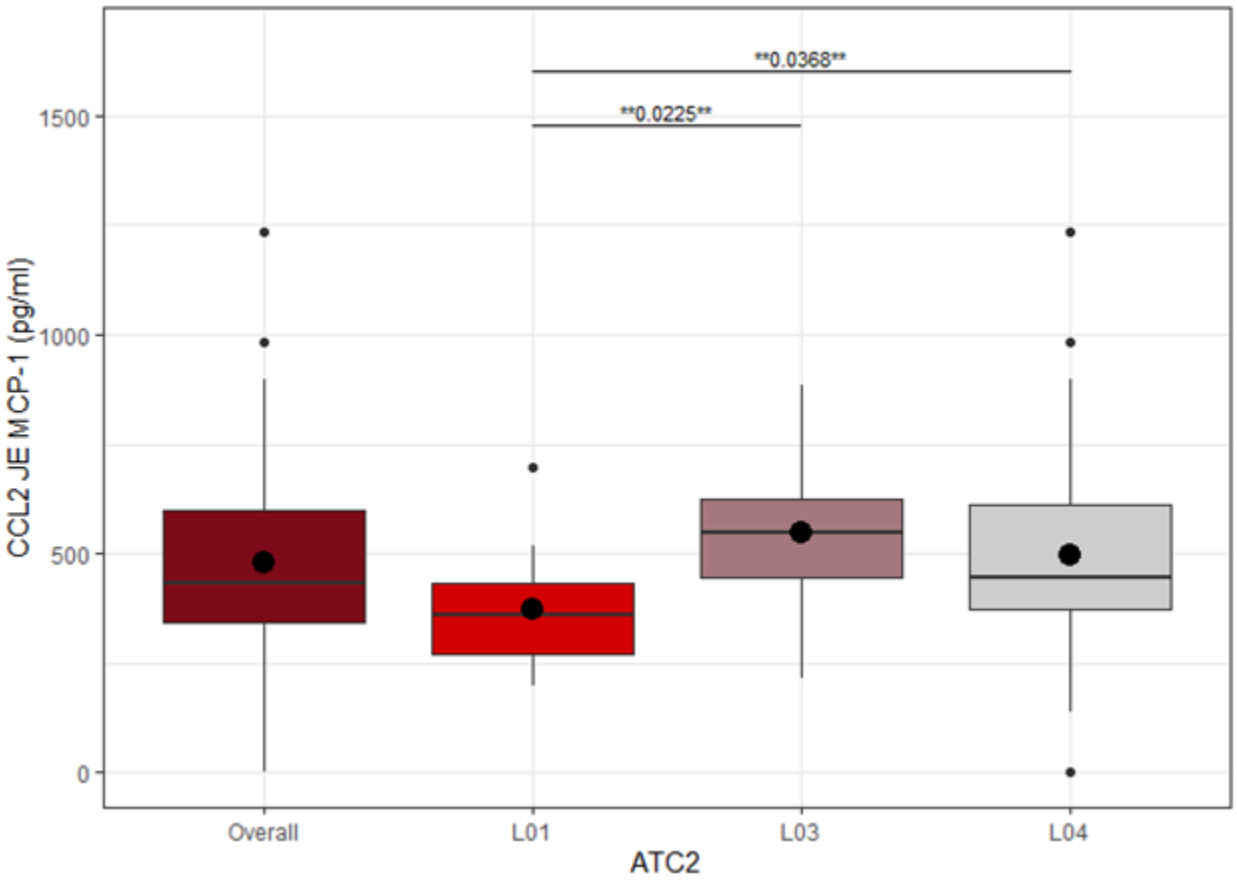


Figure 7

Dependency between variables: CCL2/MCP-1 (pg/ml) and ATC2 in study group (N = 96; L01: N = L03: N = 9; L04: N = 55; median: 429.71 pg/ml; p = 0.026; effect size r = 0.001; Kruskal – Wallis test)