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Serum Lymphocyte-Associated Cytokine Concentrations Change More Rapidly over Time in Multiple System Atrophy Compared to Parkinson Disease

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Abstract

Objective—Chronic inflammatory processes contribute to the eventual death of motor neurons and the development of symptoms in both idiopathic Parkinson disease (PD) and multiple system atrophy (MSA). Given the faster rate of progression and more severe symptoms associated with MSA, we hypothesized that markers of inflammation would be more evident in the peripheral blood of MSA than PD patients, and that evidence of this inflammation might assist early diagnosis of MSA versus PD.

Methods—We performed multiplex analysis to determine the concentrations of 37 immune-associated cytokines and chemokines isolated from the plasma of patients with PD ($n = 25$) and MSA ($n = 14$) and compared our results to those of age-matched controls ($n = 15$). We then applied a mixed-effect multiple regression model to determine if the concentration of cytokines in the plasma of patients with PD and MSA changed significantly over time.

Results—Patients with MSA had a trend towards overall lower levels of immune-associated cytokines, while serum cytokine levels were increased in patients with PD. Statistically adjusted comparisons of overall changes in cytokine concentrations between the PD and MSA groups revealed higher concentrations of T-cell-associated cytokines TNFβ and IL-7 in PD. Comparison of samples taken over time revealed significantly faster rates of change in 4 different cytokine concentrations (IL-4, IL-15, IL-2, and IL-9) in patients with MSA versus patients with PD.

Conclusions—Our results suggest that single measurements of plasma concentrations of inflammation-associated cytokines cannot be used to distinguish disease states. However, measurements made over time may correlate with pathogenesis. The significant changes in T-cell-associated cytokines may shed light on immune mechanisms that contribute to PD and MSA disease progression.

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Keywords

Cytokines; Chemokines; Neuroinflammation; Parkinsonism

Introduction

Multiple system atrophy (MSA) is a rare adult-onset neurodegenerative disease characterized by motor impairment and autonomic failure. MSA, like the more common parkinsonism, idiopathic Parkinson disease (PD), is pathologically characterized as an α-synucleinopathy. Even when the cardinal motor features of PD are present, it is estimated that 10–20% of idiopathic PD and 25–50% of other parkinsonisms, including MSA cases, are misdiagnosed, leading to inappropriate treatment and delays in disease-modulating therapies [1]. While patients with well-managed idiopathic PD can live with their disease for over 20 years, neurodegeneration in patients with MSA progresses very rapidly, with mean survival after disease onset between 6 and 9 years. Despite these significant changes in the rate of disease progression, a true diagnosis of MSA versus PD can only be made following autopsy. Thus, clinicians rely almost exclusively on observational clinical assessments over time to determine treatment, as differential diagnostic imaging or other biomarkers of specific symptomatic phases are not currently available.

The pathologic mechanisms by which idiopathic PD and MSA develop and progress are unknown, but it is likely that a chronic neuroinflammatory process is involved in both disease states [2–5]. Hence, there has been considerable interest in identifying inflammatory cytokines in the peripheral blood that might be used as biomarkers to predict disease progression. Patients with MSA were found to have an increase in proinflammatory TNFα in serum [6]. Other evidence shows increased peripheral inflammatory cytokines, including IL-6, TNFα, and IL-1β in the serum of patients with PD [7]. Epidemiologic evidence also indicated that increased serum levels of IL-6 were associated with a greater risk of PD development [8], though the results of subsequent studies were variable regarding significant increases or associations of IL-6 with symptoms [9–12]. Importantly, disease progression is likely influenced by multiple cytokines acting over time, and a recent longitudinal study suggests that a cluster of proinflammatory mediators can be linked to disease progression [13].

The pathogenesis of both MSA and PD is likely to include a progression of adaptive immune mediators stemming from the initial inflammatory response. Data from an animal model of MSA indicate that T-cell cytokines are increased in the midbrain and brainstem [14]. In PD, there is evidence for CD4+ and CD8+ T-cell involvement in neuroinflammation in animal models and autopsied brain tissue [2]. A potential role for B cells is suggested by the presence of anti-α-synuclein antibodies in the serum of patients with parkinsonian syndromes [15]. Though systemic changes in lymphocyte populations have been reported in the blood of PD patients [16, 17], clear evidence for the usefulness of tracking changes in B- and T-cell-related cytokines as markers for disease progression has yet to emerge.

Given the more rapid progression of MSA, we hypothesized (1) that patients with this disease would demonstrate increased immune-associated cytokines in the peripheral blood

compared to patients with PD, and (2) that cytokine concentrations would increase over time in patients with MSA and PD.

Patients and Methods

Patient Visits

All subjects provided informed consent and were enrolled in a study approved by our Institutional Review Board and in accordance with the Declaration of Helsinki (ClinicalTrials.gov identifier: NCT00817726). Subjects with a diagnosis of PD or MSA and aged-matched controls (±3 years) were enrolled from the University of Texas Health Science Center-Houston (UTHSC-H) Movement Disorders Clinic, the Memorial Hermann Hospital Sleep Disorders Clinic, or the community between August 2009 and September 2014. PD diagnosis was based on the United Kingdom Brain Bank criteria; subjects with parkinsonian symptoms due to vascular parkinsonism, or medicine/toxin-induced parkinsonism were excluded. MSA diagnosis was based on the second consensus statement by the American Academy of Neurology. Controls were defined by the absence of a personal history of REM sleep behavior disorder, PD, or other neurodegenerative diseases. Any potential subject with an unstable medical or psychiatric condition or renal/liver failure was excluded from participation. All clinical measures, rating scales, and blood samplings were taken in the conventionally defined off-medication state (patients hold levodopa-containing medications at least 12 h prior to each visit). Diagnoses were confirmed by a movement disorder specialist, and, to exclude advanced disease, we used a Hoehn-Yahr disability scale with a cutoff of ≤ 3 in the off-medication state. For longitudinal data analysis, serum samples were collected from patients and controls at follow-up visits approximately every 6 months for up to 2 years. Patient characteristics are listed in Table 1.

Multiplex Analysis

Serum samples were collected from a peripheral vein at early morning visits every 6 months over the course of 2 years and stored at −80 ° C until use. To determine the levels of cytokines in plasma, we used a Millipore Multiplex MAP® 37-plex human cytokine/chemokine panel (EMD Millipore, Billerica, MA, USA). Briefly, 25 μL of serum were added in duplicate wells to a 96-well magnetic plate, and the protocol followed according to the manufacturer's instructions. Plates were analyzed on a Luminex® 200™ equipped with xPONENT® 3.1 software (Luminex, Austin, TX, USA). Serum sample cytokine concentrations (pg/mL) were extrapolated from standard curves generated from the kit reagents via nonlinear regression analysis using GraphPad Prism for Windows version 5.00 (Graph Pad Software, San Diego, CA, USA). Lower limits for each of the analytes were determined using the manufacturer-supplied minimum detectable concentration as calculated by Statelier® Immunoassay Analysis software (Brendan Technologies, Carlsbad, CA, USA) and ranged from 0.5 to 26.3 pg/mL.

Statistical Analyses

Cytokine values collected from each patient at the first visit were grouped according to disease state as depicted in Table 2. As the data were not normally distributed, a log-rank

test was used for analysis of statistical significance between each patient group, with $p \leq 0.05$ considered significant.

To compare the changes in cytokine concentration over time, we applied a linear mixed-effect model [18] which allowed us to use all follow-up data collected from each patient while taking into account the fact that repeated measures on the same individual are correlated with one another. The analyses were adjusted for covariates associated with disease prevalence and progression (age, sex, and Montreal Cognitive Assessment and olfaction scores). Age is associated with movement disorder progression [19], and gender has been demonstrated to have a significant effect on cytokine concentrations [20]. Cognitive and olfaction scores are used as defining parameters of disease [21]. Therefore, our model was also normalized for the effects of these variables in order to prevent confounding of the results. The generated results are expressed as regression $y = \beta_0 + \beta_1 x + \mu + \varepsilon$, where β_0 is the intercept, β_1 is the slope, μ is a random effect, and ε is the error. We then tested whether significant differences exist in cytokines between PD and MSA groups via investigation of the regression coefficients. Pairwise comparisons were performed on MSA versus control (see online suppl. Table 1, www.karger.com/doi/10.1159/000460297) Data were analyzed using R version 3.2.1.

Results

Serum Cytokine Concentrations Measured at the First Visit

Serum samples from control patients and patients clinically diagnosed with PD or MSA were analyzed for 37 different immune-associated cytokines and chemokines. Subjects with a diagnosis of PD at enrollment were severely microsmic or anosmic and in the early stages of disease (Braak stage ≤ 3). Patients diagnosed with MSA were all ambulatory at the start of the study (Table 1).

We compared the average cytokine concentrations in plasma sampled at the first clinic visit to determine if any of our panel of immune-associated cytokines were significantly increased in the serum of patients with PD or MSA compared to age-matched controls. Serum from patients with PD showed an overall trend towards increased cytokine concentrations (Table 2), including significant increases in TNFα, CCL2, and CXCL10. In contrast, the median concentrations of cytokines measured in the serum of patients with MSA were similar or less than concentrations in the serum of age-matched controls. Patients with MSA had significantly less GM-CSF, CCL7, and IL-17 (Table 2).

Changes in Plasma Cytokine Concentrations over Time in Patients with PD versus Patients with MSA

Comparisons of measurements of cytokine concentrations at the first visit are limited by both the small size of our sample and by the extreme interpatient variability in cytokine concentrations in samples isolated at one time point. The question of whether plasma cytokine concentrations can reflect progression and development of movement disorders is more pertinent. To answer this, we collected patient and control plasma at repeated clinic visits every 6 months for up to 2 years and measured cytokine concentrations. As repeated

measures from each individual were correlated with one another, we used a mixed-effect model to analyze changes in cytokine concentrations over time. We then performed a pairwise analysis to determine if there were significant differences in cytokine concentrations between the PD and the MSA group.

Comparison of the differences in cytokine concentrations via a pairwise comparison between PD and MSA (the PD: β column in online suppl. Table 1) again demonstrated that there was a trend towards increased cytokines in patients with PD compared to MSA. The concentrations of TNFβ and IL-7 were significantly increased in the PD compared with the MSA group. In comparison, CCL11 was significantly increased in patients with MSA in relation to patients with PD (Fig. 1 a).

We applied our model to compare the rate of changes over time of cytokine concentrations measured over repeated visits (the time: β columns in online suppl. Table 1). The average rate of changes (pg cytokine/unit time) in MSA patients was nearly 3 times greater than the average rate of change in PD patients (0.327 vs. 0.099). The rates of change in several lymphocyte-associated cytokines had the most significant changes between the groups. The concentration of IL-4 increased over time in both PD and MSA patients, though change/time was significantly greater in the patients with MSA (Fig. 1 b). In contrast, secretion of IL-2, IL-9, and IL-15 decreased over time in patients with PD, but was significantly increased in patients with MSA (Fig. 1 b).

Discussion

Multiple attempts to determine the usefulness of cytokine secretion as a marker for the severity and progression of movement disorders have been performed, with varying and often contradictory results. Animal models cannot provide definitive answers. Likewise, single measurements of cytokine levels in the cerebral spinal fluid of patients do not correlate with PD motor symptoms [10], and repeated measures are complicated by the invasive nature of spinal fluid collection. In contrast, collection of peripheral blood is relatively noninvasive and easily accomplished, and thus it may be used for repeated measures of changes in immune profiles over time. Recent literature also supports the concept that peripheral blood reflects the disease process due to blood-brain barrier disruption in PD and MSA [22]. Our results provide evidence that changes in immune markers can be measured over time in the peripheral blood of patients with movement disorders and suggest that repeated measures of several different cytokines should show predictable specific changes. To our knowledge, this is the first longitudinal study to compare the patterns of specific cytokine expression in subjects diagnosed with different movement disorders.

Given the more rapid progression of MSA, we hypothesized that overall cytokine concentrations in the peripheral blood would be increased in patients with this disorder compared to patients with PD. Unexpectedly, the median cytokine concentrations measured at baseline visits together with the β values calculated in our mixed-effect model suggest that peripheral immune cytokine concentrations are, in fact, lower in patients with early MSA. PD has a long prodromal period associated with nonmotor symptoms (e.g., reduced

olfaction, REM sleep behavior disorder, and depression). The trend towards greater cytokine concentrations in patients with PD may reflect pathological changes accrued over this prodromal period. In contrast, prodromal MSA is not well defined, perhaps because the timeline to progression to motor symptoms is compressed. Our finding that rates of change in serum cytokine secretion over time were faster in patients with MSA than PD are likely indicative of this faster disease progression.

Our findings of significant differences in cytokine concentrations and rates of change also provide insights into underlying differences in the pathogenesis of these diseases. One such difference might be reflected in the significant increase in CCL11 (eotaxin) in the serum of patients with MSA compared to PD. Patients with MSA are more likely to manifest cognitive disorders, early dementia, and mood disorders than patients with PD [23], and increased peripheral secretion of CCL11 is associated with mood disorders and severe depression [24]. Furthermore, a direct role for CCL11 in the impairment of hippocampal function in aging has recently been described [25]. Therefore, it is possible that CCL11 might contribute to the development of nonmotor symptoms that accompany MSA.

The significant increases in IL-4, IL-2, IL-15, and IL-9 in the serum of patients with MSA also point to a role for T lymphocytes in the pathogenesis of this disease. Secretion of IL-15 and IL-2 are associated with the development of CD8+ T lymphocytes. Both CD8+ and CD4+ T lymphocytes are found in the brains of postmortem PD patients and in mouse models of PD; however, only CD4+ cells appear to be necessary for dopaminergic cell death [2]. In contrast, CD8+ T lymphocytes are thought to be the primary contributors to oligodendrocyte death and demyelination in multiple sclerosis [26]. Importantly, the pathogenesis of MSA is characterized by death of oligodendrocytes and demyelination, which do not occur in idiopathic PD. An increase in CD8+ T-lymphocyte-associated cytokines, then, might be indicative of a role for CD8+ cells in oligodendrocyte pathology that contributes to MSA progression [26]. Interestingly, recent evidence also points to a contributing role for “Th9” CD4+ T lymphocytes in the pathogenesis of multiple sclerosis [27]. Given that the rates of IL-9 and IL-4 secretion are also significantly increased in MSA patients, we cannot discount the possibility that a Th9 subpopulation might also contribute to disease progression.

The overall significant increase in TNFβ (also known as lymphotoxin α) and IL-7 in the serum of patients with PD compared to MSA was an unexpected finding. The presence of TNFβ together with IL-7 is required for lymph node and Peyer’s patch development, and increased concentrations of both of these cytokines may contribute to the formation of tertiary lymphoid organs in chronic inflammatory diseases [28, 29]. Of note, tertiary lymphoid organs are found in meninges of patients with multiple sclerosis and in mice with experimental autoimmune encephalitis [30]. Thus, it is intriguing to speculate that chronic inflammatory conditions associated with idiopathic PD may also drive the development of tertiary lymphoid organs, and these structures might provide a local source of activated T and B cells that contribute to the spread of brain pathology.

Together, our findings strongly suggest that the measurement of changes in lymphocyte-associated cytokine secretion over multiple patient visits have the potential to identify

immune profiles that – paired with clinical measures – could provide clear differentiation between MSA and PD. Expansion of these investigations may also prove useful in identifying a panel of cytokines that may associate with specific subtypes of MSA or PD, further advancing the concept of precision medicine based on immune responses.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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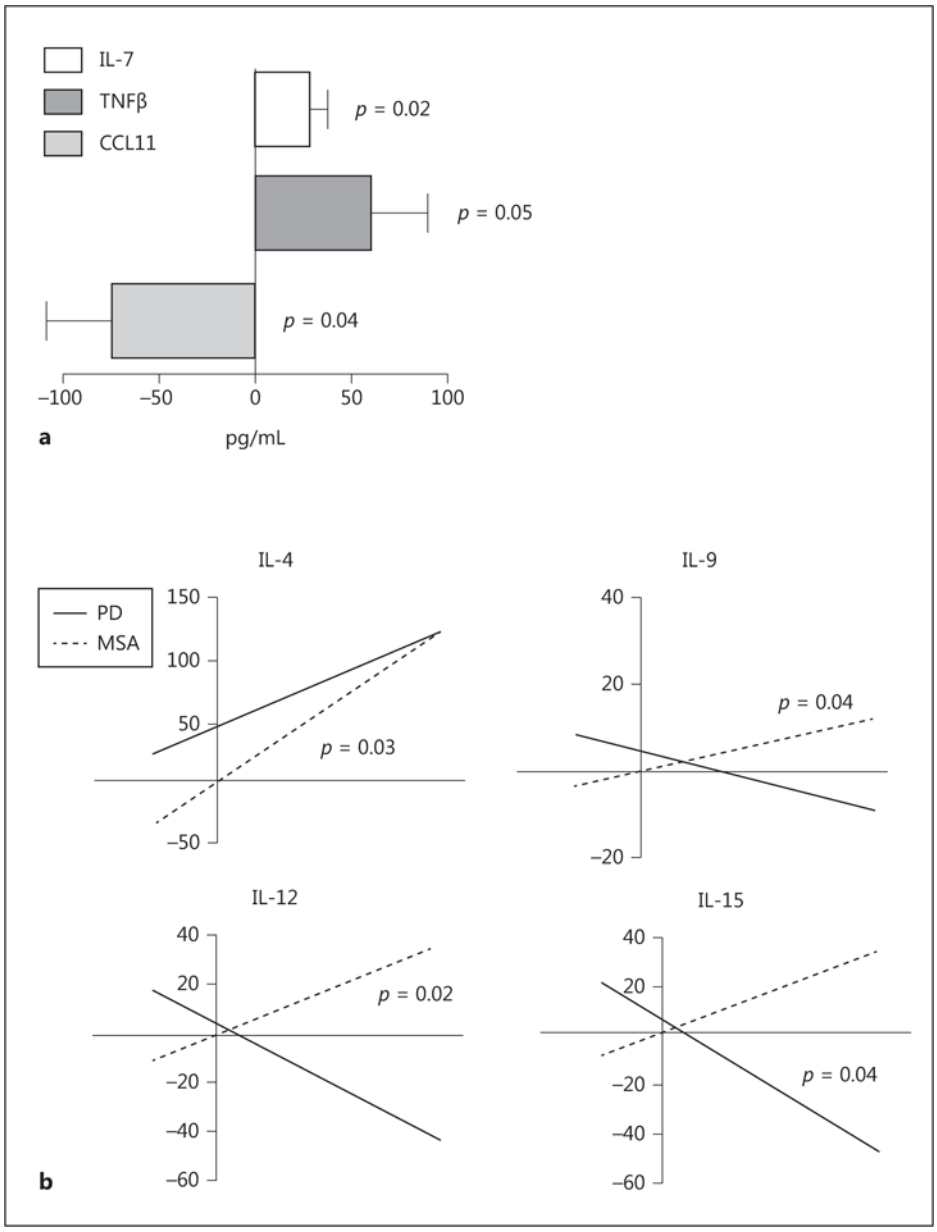

Fig. 1.

Significant changes in cytokine secretion between PD and MSA. A linear mixed-effect model was used to normalize for the effects of covariates associated with movement disorder disease progression (age, sex, and Montreal Cognitive Assessment and olfaction scores). The generated results were expressed as regression coefficient and intercept ($y = \beta_0 + \beta_1 x$) where β_0 is the intercept, and β_1 is the slope. Pairwise analysis comparison determined significance differences between slopes and intercepts of PD and MSA groups. **a** Representation of significant differences in intercepts between PD and MSA groups. A negative value indicates that this concentration was greater in MSA, while a positive value was increased in PD. **b** Representation of significant changes in cytokine secretion/unit time.

x values of –50 and 180 were used to generate a graphic representation of the unit change in cytokine concentration/unit time in PD (solid line) and MSA (dashed line) groups.

Table 1

Demographics of the patients with multiple system atrophy (MSA) and Parkinson disease (PD) and controls

	Controls	PD	MSA
Subjects, *n*	15	24	14
Female:male ratio	5:10	8:16	3:11
Age, years	56.8 ± 9.2	64.2 ± 8.6	60.4 ± 8.3
Mean body mass index	27.3	26	29.9
Duration of disease, years	0	5.2 ± 3.3	4.9 ± 2.4
Race, *n*			
Caucasian	13	18	12
Hispanic	1	2	2
Asian	0	4	0
African-American	1	0	0
Levodopa equivalent dose, mg	0	588.4 ± 339.5	365.6 ± 343.3
Montreal Cognitive Assessment score	28.7 ± 2.1	28 ± 2.2	24.3 ± 3.3
Olfaction score	35.5 ± 5.1	19.6 ± 4.9	29.6 ± 7.6
Unified PD rating scale, total scores	3.2 ± 3.6	29.1 ± 14.8	54.2 ± 19.5
Unified PD rating scale, motor scores	0.5 ± 1.0	18.5 ± 10.6	31.4 ± 13.9
Hoehn-Yahr staging	0	1.6 ± 0.8	3.3 ± 1.1
Tremor-dominant/akinetic-rigid *, *n*	–	13/11	–
Samples collected, *n*/patient	2.6 ± 1.5	2.6 ± 1.1	3.1 ± 1.3
Months followed from the first visit	16.6 ± 7.1	14.0 ± 8.6	16.9 ± 7.8

Means ± SD and numbers are shown.

*Subtype was determined using the Schiess et al. criteria [31].

Table 2

Median plasma concentrations of cytokines at the first visit (pg/mL)

Analyte	Controls	PD	MSA
Growth factors[b]			
EGF	28.4 (12.1 – 44.6)	27.5 (18.3 – 48.2)	27.6 (20.8 – 51.9)
FGF-2	31.7 (26.7 – 53.0)	29.1 (7.6 – 64.3)	34.5 (29.4 – 43.8)
Flt-3L	15.3 (5.40 – 33.7)	8.2 (5.4 – 51.1)	5.40 (5.40 – 5.5)
G-CSF	46.0 (28.0 – 62.5)	36.8 (1.8 – 82.0)	**22.5 (10.1 – 58.5)**[a,b]
GM-CSF	10.6 (7.50 – 16.6)	7.50 (7.50 – 26.6)	10.3 (7.50 – 16.8)
IL-3	0.70 (0.70 – 1.30)	0.70 (0.70 – 0.70)	0.70 (0.70 – 0.70
IL-7	2.57 (1.40 – 7.31)	2.18 (1.40 – 22.1)	1.40 (1.40 – 9.19)
VEGF	102 (81.2 – 279)	171 (111 – 261)	112 (62.0 – 157)
Inflammation			
IFNα2	23.5 (12.6 – 34.9)	2.9 (2.90 – 15.6)	11.6 (2.9 – 31.7)
IL-6	0.90 (0.90 – 53.3)	0.90 (0.90 – 37.4)	0.90 (0.90 – 0.90)
IL-1α	9.40 (9.40 – 111)	50.5 (9.40 – 76.4)	9.40 (9.40 – 37.2)
IL-1β	0.80 (0.80 – 3.74)	0.80 (0.80 – 4.33)	0.80 (0.80 – 2.37)
IL-1RA	13.3 (8.30 – 32.1)	51.6 (8.30 – 186)	11.2 (8.30 – 21.1)
TGFα	0.82 (0.80 – 5.09)	0.93 (0.80 – 12.0)	1.64 (1.08 – 1.86)
TNFα	4.14 (2.54 – 9.75)	**9.70 (6.12 – 16.2)**[c]	8.67 (4.39 – 12.9)
TNFβ	2.60 (1.50 – 21.0)	1.50 (1.50 – 36.7)	1.50 (1.50 – 28.6)
Chemotaxis			
CCL2 (MCP-1)	431 (268 – 559)	**543 (400 – 752)**[c]	501 (396 – 749)
CCL3 (MIP-1α)	8.62 (6.07 – 9.81)	9.57 (5.39 – 14.6)	7.47 (4.67 – 10.7)
CCL4 (MIP-1β)	27.1 (16.8 – 36.1)	21.9 (5.42 – 35.4)	26.8 (9.91 – 40.8)
CCL7 (MCP-3)	16.0 (5.48 – 23.9)	4.34 (3.80 – 53.1)	**6.72 (3.80 – 21.5)**[b]
CCL11 (eotaxin)	78.8 (67.3 – 139)	136 (89.3 – 209)	147 (79.2 – 215)
CCL22 (MDC)	1,131 (996 – 1537)	1,005 (829 – 1370)	1,224 (960 – 1427)
CXCL1 (GRO)	643 (535 – 1001)	700 (553 – 857)	537 (408 – 973)
CXCL10 (IP-10)	248 (186 – 330)	**346 (288 – 401)**[c]	312 (286 – 402)
CX3CL1 (fractalkine)	49.3 (22.7 – 120)	35.2 (22.7 – 148)	22.7 (22.7 – 41.2)
IL-8	12.0 (10.0 – 16.2)	16.3 (7.21 – 22.1)	12.3 (4.72 – 16.7)
Lymphocytes			
IFNγ	7.55 (3.57 – 37.7)	10.95 (0.80 – 28.5)	5.21 (0.80 – 6.38)
IL-2	1.04 (1.00 – 12.5)	2.57 (1.00 – 16.4)	1.00 (1.00 – 2.43)
IL-4	4.50 (4.50 – 54.4)	4.50 (4.50 – 33.8)	4.50 (4.50 – 4.5)
IL-5	0.50 (0.50 – 3.32)	2.30 (0.50 – 6.09)	1.79 (0.50 – 3.15)
IL-9	1.24 (1.20 – 3.26)	2.58 (1.20 – 8.09)	1.20 (1.20 – 1.24)
IL-10	2.61 (1.10 – 7.20)	8.20 (1.10 – 32.6)	1.10 (1.10 – 2.51)
IL-12 (p40)	7.40 (7.40 – 35.4)	22.3 (7.40 – 60.8)	7.40 (7.40 – 7.40)

Analyte	Controls	PD	MSA
IL-12 (p70)	2.61 (1.08 – 5.66)	3.07 (0.60 – 8.22)	1.19 (0.60 – 2.61)
IL-13	4.70 (1.30 – 20.2)	1.30 (1.30 – 55.5)	1.3 (1.30 – 3.50)
IL-15	6.33 (1.73 – 14.8)	6.24 (1.20 – 12.0)	2.36 (1.20 – 6.01)
IL-17	3.89 (1.90 – 13.9)	5.35 (0.70 – 11.2)	**2.18 (0.70 – 2.51)**[b]

Plasma cytokine concentrations (pg/mL) were determined using a Millipore Multiplex MAP® 37-plex human chemokine/cytokine kit (EMD Millipore). Median values (25 – 75% interquartile ranges) are reported for serum isolated from age-matched controls ($n = 15$) or patients with Parkinson disease (PD; $n = 25$) or multiple system atrophy (MSA; $n = 14$). Analytes are grouped by their most well-known biological effects.

[a] Mann Whitney test.

[b] Significant decrease vs. control ($p \leq 0.04$).

[c] Significant increase vs. control ($p \leq 0.05$).