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Mood disturbances in newly diagnosed Parkinson's Disease patients reflect intrathecal inflammation

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ABSTRACT

In Parkinson's disease (PD), neuroinflammation may be involved in the pathogenesis of mood disorders, contributing to the clinical heterogeneity of the disease.

The cerebrospinal fluid (CSF) levels of interleukin (IL)-1β, IL-2, IL-6, IL-7, IL-8, IL-9, IL-12, IL-17, interferon (IFN)γ, macrophage inflammatory protein 1-alpha (MIP-1a), MIP-1b, granulocyte colony stimulating factor (GCSF), eotaxin, tumor necrosis factor (TNF), and monocyte chemoattractant protein 1 (MCP-1), were assessed in 45 newly diagnosed and untreated PD patients and in 44 control patients. Spearman's correlations were used to explore possible associations between CSF cytokines and clinical variables including mood. Benjamini-Hochberg (B–H) correction for multiple comparisons was applied. Linear regression was used to test significant associations correcting for other clinical variables.

In PD patients, higher CSF concentrations of the inflammatory molecules IL-6, IL-9, IFNγ, and GCSF were found (all B–H corrected $p < 0.02$). Significant associations were found between BDI-II and the levels of IL-6 (Beta = 0.438; 95%CI 1.313–5.889; $p = 0.003$) and IL-8 (Beta = 0.471; 95%CI 0.185–0.743; $p = 0.002$). Positive associations were also observed between STAI-Y state and both IL-6 (Beta = 0.452; 95%CI 1.649–7.366; $p = 0.003$), and IL-12 (Beta = 0.417; 95%CI 2.238–13.379; $p = 0.007$), and between STAI-Y trait and IL-2 (Beta = 0.354; 95%CI 1.923–14.796; $p = 0.012$), IL-6 (Beta = 0.362; 95%CI 0.990–6.734; $p = 0.01$), IL-8 (Beta = 0.341; 95%CI 0.076–0.796; $p = 0.019$), IL-12 (Beta = 0.328; 95%CI 0.975–12.135; $p = 0.023$), and IL-17 (Beta = 0.334; 95CI 0.315–4.455; $p = 0.025$).

An inflammatory CSF milieu may be associated with depression and anxiety in the early phases of PD, supporting a role of neuroinflammation in the pathogenesis of mood disturbances.

1. Introduction

Parkinson's disease (PD) is a common degenerative disorder of the central nervous system (CNS). Originally described as a movement disorder characterized by resting tremor, bradykinesia and rigidity, PD is now recognized as a heterogeneous disease which also includes non-motor symptoms such as mood, and cognitive disturbances, autonomic dysfunction, pain and sensory deficits [1]. Depression and anxiety are frequent in PD patients and can be observed since the earliest phases of the disease, often preceding the onset of motor manifestations [2]. Mood disorders have gained increased attention as they can critically contribute to the overall disability and to quality of life (QoL) deterioration [2], and understanding the mechanisms underlying these symptoms is important to develop effective treatments.

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It has been evidenced that neuroinflammation plays a crucial role in the pathogenesis of different neurodegenerative conditions [3]. Experimental studies in PD showed that chronic low-grade inflammation is involved in disease onset and progression, and is associated with mitochondrial dysfunction and increased oxidative stress [4]. Notably, higher levels of several proinflammatory cytokines have been found in brain samples and in the cerebrospinal fluid (CSF) of patients with PD [5].

Peripheral and central inflammation have been implicated in the pathogenesis of mood disorders in several clinical conditions. Increased expression of inflammatory molecules has been associated with higher levels of anxiety and depression in patients with autoimmune diseases such as multiple sclerosis [6]. In addition, a role of inflammatory mediators has been demonstrated in psychiatric disorders, including major depression and bipolar disorder [7].

Previous studies in patients with PD reported that higher expression of peripheral and CSF inflammatory cytokines may be associated with non-motor symptoms and cognitive deficits [8]. Although some association between CSF inflammatory biomarkers such as c-reactive protein (CRP), and depression in patients with PD has been previously reported [8], the role of central inflammation in mood alterations in PD is still unclear.

In this cross-sectional study, we first compared the CSF levels of a set of proinflammatory cytokines and chemokines in a group of newly diagnosed drug-naïve PD patients and in a group of control patients. In addition, in PD patients we explored the associations between CSF inflammatory molecules and clinical characteristics, including depression and anxiety.

2. Materials and methods

2.1. Study population and study design

A group of 45 consecutive PD patients and 44 control patients admitted to the Unit of Neurology (IRCCS Neuromed) between 2017 and 2020 were enrolled. The diagnosis of PD was made by a movement disorders specialist, according to the UK PD society Brain Bank Clinical Diagnostic Criteria [9]. Exclusion criteria for both groups were concomitant autoimmune diseases, any acute or chronic inflammatory disease, anti-inflammatory or antidepressant co-medication. We included PD patients with normal cognitive function as evaluated by Montreal Cognitive Assessment (MoCA) scores adjusted for age and education according to recent normative dataset for Italian population. The control group included 44 patients undergoing lumbar puncture (LP) for diagnostic purposes and diagnosed with vascular leukoencephalopathy (N = 25 patients), metabolic and hereditary polyneuropathies (N = 5), normal pressure hydrocephalus (N = 4), functional neurological disorder (N = 4), spondylotic myelopathy (N = 3), migraine (N = 1), papilledema (N = 1), spastic paraparesis (N = 1). The local ethics committee approved the study (CE numbers 06/17 and 11/17) and written informed consent was obtained from all subjects.

At the time of diagnosis, patients underwent clinical and neuropsychological assessment and LP, as described below. A brain magnetic resonance examination was also performed to exclude other abnormalities and secondary parkinsonism.

2.2. Clinical and neuropsychological assessment

Demographic and clinical characteristics were collected at the time of diagnosis by a movement disorder specialist. Presence of metabolic comorbidities such as diabetes mellitus was recorded. BMI was calculated as weight (kg)/height (m^2), and patients were classified as "normal" (BMI 18.5–24.9), "overweight" (BMI 25–29.9), and "obese" (BMI>30) [10].

Disease stage was evaluated using the Hoehn and Yahr scale (H&Y). Disease duration was defined as the time interval between symptoms onset and diagnosis. Motor symptoms were assessed using the Unified Parkinson's Disease Rating Scale part III (UPDRS-III). We have calculated the average global tremor score and the mean score for postural instability/gait difficulties (PIGD). Patients were classified as either "Tremor" or "PIGD" as in Jankovic et al., 1990 [11].

Cognitive screening was performed in all PD patients by MoCA test performed by a trained neuropsychologist. Depression was assessed using the Beck Depression Inventory-Second Edition (BDI-II). Anxiety was assessed with State-Trait Anxiety Inventory-form Y (STAI-Y). Patients-reported QoL was evaluated using Parkinson's Disease Questionnaire-8 (PDQ-8). A higher total score reflects a lower health-related QoL.

2.3. Samples collection and cytokines measurement

CSF samples were collected by LP performed between 9:00 a.m. and 11:00 a.m. after overnight fasting. CSF was sampled into polypropylene tubes. The first 2–3 mL were used for routine testing. 4 mL were centrifuged at +4 °C at 2000g for 10 min to remove cells and debris. The CSF supernatants were immediately stored at −80 °C until the analyses of cytokines, avoiding freeze/thaw cycles. CSF cytokine analysis was performed periodically, once a sufficient number of samples was reached, generally every 4–6 months. Operators blinded to the diagnosis performed the measurements. The concentrations of a set of cytokines, interleukin (IL)-1β, IL-2, IL-6, IL-7, IL-8, IL-9, IL-12, IL-17, macrophage inflammatory protein 1-alpha (MIP-1a), MIP-1beta (MIP-1b), granulocyte colony stimulating factor (GCSF), eotaxin, interferon (IFN)γ, tumor necrosis factor (TNF), monocyte chemoattractant protein 1 (MCP-1), were assessed using a Bio-Plex multiplex assay (Bio-Rad Laboratories, Hercules, CA, USA) according to the manufacturer's instructions. CSF cytokines concentrations were analyzed according to a standard curve generated for each target and expressed as pg/ml.

2.4. Statistical analysis

Kolmogorov–Smirnov test was used to assess the normality distribution of analyzed variables. Data were presented as median and interquartile range (IQR). Categorical variables were presented as number (n). Differences in continuous variables between two groups were analyzed using non-parametric Mann–Whitney test. A p value $\leq$ 0.05 was considered statistically significant. Spearman's non-parametric correlation was used to evaluate possible correlations between CSF cytokines and demographic clinical characteristics, and the results were plotted using the corrplot package (v0.84) running under R (v3.6.1) in RStudio (1.1.456). When comparing CSF cytokines in PD and control patients, and when exploring correlations between clinical characteristics and CSF cytokines, Benjamini-Hochberg (B–H) procedure was used to decrease the false discovery rate and avoid Type I errors (false positives). Linear regression was used to further verify the significant associations between CSF cytokines and clinical scales, considering also other clinical variables (age, sex, disease duration, UPDRS-III). All analyses were performed using IBM SPSS Statistics for Windows (IBM Corp.). The data that support the findings of this study are available from the corresponding author upon reasonable request.

3. Results

3.1. CSF inflammatory molecules in PD and control patients

The study cohort included 45 PD patients and 44 control patients. The demographic and clinical characteristics of PD and control patients are reported in Table 1. No significant differences were found in age and sex distribution between the two groups (p = 0.389 and p = 0.9, respectively).

We compared the CSF levels of a set of inflammatory cytokines and chemokines in PD and control patients. CSF concentrations of

Table 1
Demographic and clinical characteristics of PD and control patients.

		PD patients	Control patients	p
N		45	44	
Sex, F	N (%)	19 (42.2)	18 (40.9)	0.9*
Age at diagnosis, years	Median (IQR)	60 (54.3–66.4)	58.2 (52.1–64.7)	0.389§
Diabetes Mellitus	N (%)	6 (13.6)	4 (9.1)	0.526*
BMI, group	N (%)	Normal: 19 (42.2) Overweight: 17 (37.8) Obese: 9 (20)	Normal: 29 (65.9) Overweight: 8 (18.2) Obese: 7 (15.9)	0.062*
Disease duration, months	Median (IQR)	12 (8–18)	-	
H&Y	Median (IQR)	1 (1–2)	-	
UPDRS-III	Median (IQR)	16 (12.5–21)	-	
MoCA	Median (IQR)	22.34 (20.17–24.36)	-	
BDI-II	Median (IQR)	13 (6–17)	-	
STAI-Y state/ trait	Median (IQR)	44 (39–50)/42 (33–49)	-	
PDQ-8	Median (IQR)	8 (4–11)	-	

*Pearson's Chi-square p; §Mann-Whitney p.
Abbreviations: BDI-II, Beck depression inventory-second edition; BMI, body mass index; H&Y, Hoehn and Yahr Scale; MoCA, Montreal Cognitive Assessment; PD, Parkinson's disease; PDQ-8, 8-item Parkinson's disease questionnaire; STAI-Y, state-trait anxiety inventory-form Y; UPDRS-III, motor subscore of the Unified Parkinson's Disease Rating Scale.

inflammatory cytokines in the two groups are shown in Table 2. Higher CSF concentrations of the proinflammatory molecules IL-6 (B–H p = 0.0187), IL-9 (B–H p = 0.00075), IFNγ (B–H p = 0.0187); GCSF (B–H p = 0.0075) were found in PD patients compared with control patients (Fig. 1).

Cytokine levels showed a high positively skewed (e.g. skewness coefficient: IL-6 = 1.42, IFNγ = 2.43, GCSF = 3.70, IL-9 = 3.45) and leptokurtic distribution (e.g. *k*: IFNγ = 8.155, GCSF = 16.125, IL-9 = 13.710). As evidenced in Fig. 1, outlier values are present in both the PD and control group. We repeated the analyses for IL-6, IFNγ, GCSF, and IL-9, after excluding outlier values. Significant differences were confirmed in the two groups in the CSF levels of IL-6 (Mann-Whitney p = 0.001), IFNγ (Mann-Whitney p = 0.014), GCSF (Mann-Whitney p = 0.009), and IL-9 (Mann-Whitney p = 0.001).

Table 2
CSF concentrations of inflammatory cytokines in PD and control patients.

Cytokine	PD patients	Control patients	B–H p
IL-1β	0.04 (0–0.9)	0.005 (0–0.05)	0.195
IL-2	0 (0–0.07)	0 (0–0)	0.48
IL-6	0.48 (0–1.20)	0.005 (0–0.41)	**0.01875**
IL-7	0.23 (0–2.92)	0 (0–0.61)	0.051
IL-8	20.85 (16.9–27.93)	20.125 (16.86–24.28)	0.618
IL-9	2.75 (1.25–6.40)	1.3 (0.62–1.99)	**0.00075**
IL-12	0.07 (0–0.57)	0.02 (0–0.39)	0.5175
IL-17	0.09 (0–0.9)	0.115 (0–0.63)	0.896
IFNγ	3.51 (2.335–5.65)	2.36 (1.39–3.37)	**0.01875**
MIP-1a	0.42 (0.29–0.54)	0.41 (0.26–0.57)	0.896
MIP-1b	3.67 (2.35–4.88)	3.115 (2.50–4.12)	0.603
GCSF	14.82 (11.20–22.28)	9.71 (5.59–15.05)	**0.00075**
Eotaxin	0.89 (0.6–1.19)	0.87 (0.57–1.37)	0.896
TNF	1.71 (0.59–2.72)	1.61 (0.245–3.15)	0.896
MCP-1	166.27 (124.7–213.02)	158.29 (128.69–198.59)	0.705

CSF cytokines concentrations are expressed in pg/ml. Median and IQR are shown.
Abbreviations: B–H, Benjamini-Hochberg; CSF, cerebrospinal fluid; GCSF, granulocyte colony-stimulating factor; IFN, interferon; IL, interleukin; MCP-1, monocyte chemoattractant protein 1; MIP, macrophage inflammatory protein; PD, Parkinson's disease; TNF, tumor necrosis factor.

3.2. CSF inflammation and demographic/clinical characteristics in PD and control patients

We explored the association between CSF cytokines and demographic characteristics in PD and control patients. In the control group, no significant association was found between CSF molecules and sex (all B–H p > 0.05) and no significant correlations were found with age at LP (all B–H p > 0.05).

In PD patients, positive correlations were found between age at diagnosis and the CSF concentrations of MIP-1a (Spearman's rho = 0.472; p = 0.001, B–H p = 0.015, N = 45) (Supplementary Table 1). Moreover, an association emerged between sex and GCSF levels, although not significant after controlling for multiple comparisons (F, median: 19.21 pg/ml; M, median: 12.89 pg/ml; p = 0.024; B–H p > 0.05) (Supplementary Table 2). Finally, no significant associations emerged in PD patients between CSF molecules and either the presence of diabetes mellitus or BMI group (all p > 0.05).

We explored the association between CSF inflammation and clinical characteristics in PD patients (Fig. 2 **A**) (Supplementary Table 1). A negative trend was observed between disease duration and the CSF concentrations of IL-17 (Spearman's rho = −0.381; p = 0.010, N = 45), IL-7 (Spearman's rho = −0.317; p = 0.034, N = 45), and IL-9 (Spearman's rho = −0.367; p = 0.013, N = 45), although not significant after controlling for multiple comparisons (all B–H p > 0.05). No significant correlations were found between the CSF molecules analyzed and either H&Y stage or motor symptom's severity assessed with the UPDRS-III (all p > 0.05). In addition, no significant associations were found between disease phenotype (tremor dominant vs PIGD) and CSF inflammatory molecules (all p > 0.05). Finally, no significant correlations were found between MoCA score and CSF molecules (all p > 0.05).

3.3. CSF inflammation and mood in PD patients

We explored in PD patients the association between CSF inflammation and mood disorders at the time of diagnosis. Significant correlations were observed between the CSF concentrations of different molecules and BDI-II and STAI-Y scores (Fig. 2 **A-B**; Supplementary Table 3).

Positive correlations were found between BDI-II score and the CSF levels of IL-6 (Spearman's rho = 0.514, p = 0.0003, B–H p = 0.0015, N = 45), and IL-8 (Spearman's rho = 0.421, p = 0.004, B–H p = 0.03 N = 45). Notably, the positive correlation between IL-6 and BDI-II scores was significant after removing the 3 outliers identified in Fig. 1 (Spearman's rho = 0.512, p = 0.001, N = 42). Linear regression confirmed a significant association between BDI-II and these two cytokines also considering the effect of other clinical variables (age, sex, disease duration and UPDRS-III): IL-6 (Beta = 0.438; 95%CI 1.313–5.889; p = 0.003), IL-8 (Beta = 0.471; 95%CI 0.185–0.743; p = 0.002).

Significant correlations were found between measures of state and trait anxiety and different CSF inflammatory molecules. STAI-Y state positively correlated with IL-6 (Spearman's rho = 0.525, p = 0.0002, B–H p = 0.015, N = 45), and IL-12 (Spearman's rho = 0.405, p = 0.006, B–H p = 0.045, N = 45). Linear regression, considering the effect of other clinical variables (age, sex, disease duration and UPDRS-III), confirmed a significant association between STAI-Y state and both IL-6 (Beta = 0.452; 95%CI 1.649–7.366; p = 0.003), and IL-12 (Beta = 0.417; 95%CI 2.238–13.379; p = 0.007). STAI-Y trait score positively correlated with the CSF levels of various inflammatory molecules: IL-2 (Spearman's rho = 0.392, p = 0.008, B–H p = 0.027), IL-6 (Spearman's rho = 0.428, p = 0.003, B–H p = 0.027), IL-8 (Spearman's rho = 0.401, p = 0.006, B–H p = 0.027), IL-12 (Spearman's rho = 0.383, p = 0.009, B–H p = 0.027), IL-17 (Spearman's rho = 0.374, p = 0.011, B–H

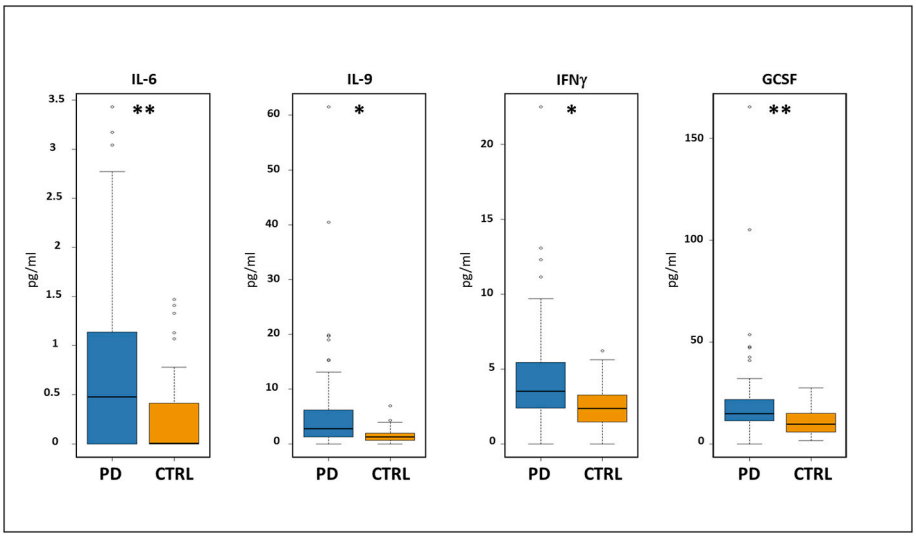

Fig. 1. CSF concentrations of IL-6, IL-9, IFNγ and GCSF in PD and control patients
Fig. 1 legend. Boxplot of CSF concentrations of IL-6, IL-9, IFNγ and GCSF in PD and control patients. The circles represent outlier patients. *Mann-Whitney B–H adjusted $p < 0.05$, ** Mann-Whitney B–H adjusted $p < 0.01$. **Abbreviations**: B–H, Benjamini-Hochberg; CTRL, control; CSF, cerebrospinal fluid; GCSF, granulocyte colony-stimulating factor; IFN, interferon; IL, interleukin; PD, Parkinson's disease.

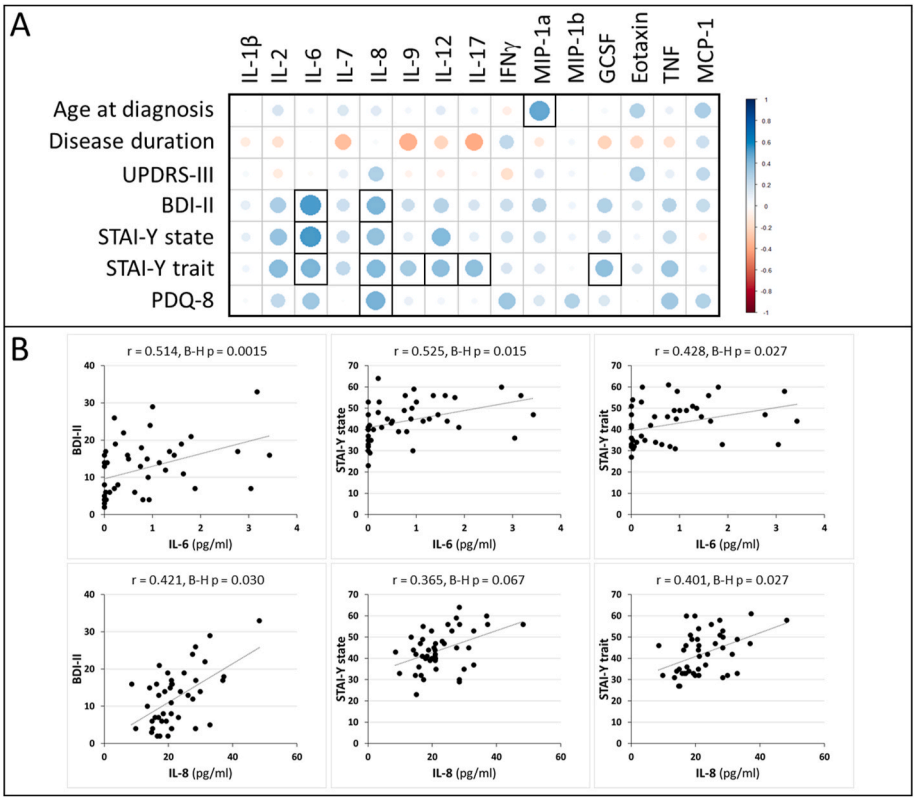

Fig. 2. CSF cytokines and clinical characteristics in PD patients
Fig. 2 legend. A: Correlation matrix heatmap of clinical characteristics and CSF cytokines in PD patients. Blue dots represent positive correlations, and red dots are negative Spearman's rho correlations. **B:** Correlations between CSF cytokines and mood and QoL evaluation. Spearman's rho and B–H adjusted p are shown. **Abbreviations**: BDI-II, Beck depression inventory-second edition; B–H, Benjamini-Hochberg; CSF, cerebrospinal fluid; GCSF, granulocyte colony-stimulating factor; IFN, interferon; IL, interleukin; MCP-1, monocyte chemoattractant protein 1; MIP, macrophage inflammatory protein; PD, Parkinson's disease; PDQ-8, 8-item Parkinson's disease questionnaire; QoL, quality of life; STAI-Y, state-trait anxiety inventory-form Y; TNF, tumor necrosis factor; UPDRS-III, unified Parkinson's disease rating scale-part III. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

p = 0.027), and GCSF (Spearman's rho = 0.378, p = 0.01, B–H p = 0.027). Linear regressions confirmed significant associations between STAI-Y trait and IL-2 (Beta = 0.354; 95%CI 1.923–14.796; p = 0.012), IL-6 (Beta = 0.362; 95%CI 0.990–6.734; p = 0.01), IL-8 (Beta = 0.341; 95%CI 0.076–0.796; p = 0.019), IL-12 (Beta = 0.328; 95%CI 0.975–12.135; p = 0.023), IL-17 (Beta = 0.334; 95%CI 0.315–4.455; p

= 0.025). Also excluding the outliers identified in Fig. 1, the positive correlations between IL-6 and both STAI-Y state (Spearman's rho = 0.564, p = 0.0001, N = 42), and trait scores (Spearman's Rho = 0.455, p = 0.002, N = 42), were significant.

Finally, significant positive correlations were found between PDQ-8 score and the CSF levels of IL-8 (Spearman's rho = 0.431; p = 0.003, B–H p = 0.045, N = 45). Linear regression confirmed a significant association between IL-8 and PDQ-8 also considering the effect of other clinical variables (age, sex, disease duration and UPDRS-III) (Beta = 0.60 95%CI 0.114–0.484; p = 0.002).

3.4. CSF inflammation and mood in male and female PD patients

We analyzed the correlations between CSF inflammatory molecules and clinical characteristics separately in male and female PD patients (Supplementary Fig. 1). The clinical characteristics of male and female PD patients are shown in Supplementary Table 4. The levels of depression and anxiety measured by the BDI-II and STAI-Y scores did not differ between male and female subjects, conversely, PDQ8 scores were significantly higher in female patients. Given the limited number of patients and the exploratory nature of this analysis, we did not apply corrections for multiple comparisons.

In male PD patients, age at diagnosis positively correlated with MIP-1a (Spearman's rho = 0.463, p = 0.017, N = 26) and MCP-1 (Spearman's rho = 0.554, p = 0.003, N = 26). Disease duration negatively correlated with IL-9 (Spearman's rho = −0.458, p = 0.019, N = 26), IL-17 (Spearman's rho = −0.412, p = 0.036, N = 26), GCSF (Spearman's rho = −0.471, p = 0.015, N = 26), and eotaxin (Spearman's rho = −0.464, p = 0.017, N = 26). No significant correlations were found between CSF inflammatory molecules and UPDRS-III. Furthermore, in male PD patients, the CSF levels of IL-6 and IL-8 positively correlated with BDI-II (IL-6: Spearman's rho = 0.545, p = 0.004, N = 26; IL-8: Spearman's rho = 0.456, p = 0.019, N = 26), STAI-Y state (IL-6: Spearman's rho = 0.615, p = 0.001, N = 26; IL-8: Spearman's rho = 0.425, p = 0.031, N = 26), STAI-Y trait (IL-6: Spearman's rho = 0.509, p = 0.008, N = 26; IL-8: Spearman's rho = 0.519, p = 0.007, N = 26), and PDQ-8 (IL-6: Spearman's rho = 0.560, p = 0.003, N = 26; IL-8: Spearman's rho = 0.465, p = 0.017, N = 26).

In female PD patients, age at diagnosis positively correlated with IL-17 (Spearman's rho = 0.486, p = 0.035, N = 19), MIP-1a (Spearman's rho = 0.457, p = 0.049, N = 19). Furthermore, IL-17 negatively correlated with disease duration (Spearman's rho = −0.558, p = 0.013, N = 19). A positive correlation was found between the CSF levels of eotaxin and UPDRS-III at diagnosis in female PD patients (Spearman's rho = 0.683, p = 0.001, N = 19). Finally, differently from male PD patients, positive correlations were found between BDI-II and IL-9 (Spearman's rho = 0.480, p = 0.038, N = 19), GCSF (Spearman's rho = 0.769, p = 0.0001, N = 19), and TNF (Spearman's rho = 0.504, p = 0.028, N = 19). STAI-Y state correlated with IL-7 (Spearman's rho = 0.479, p = 0.038, N = 19), IL-12 (Spearman's rho = 0.596, p = 0.007, N = 19), and GCSF (Spearman's rho = 0.745, p = 0.002, N = 19). Moreover, STAI-Y trait correlated with IL-7 (Spearman's rho = 0.513, p = 0.025, N = 19), IL-9 (Spearman's rho = 0.527, p = 0.02, N = 19), GCSF (Spearman's rho = 0.634, p = 0.004, N = 19), and TNF (Spearman's rho = 0.541, p = 0.017, N = 19). No significant correlations were found between PDQ-8 and CSF cytokines in female patients.

4. Discussion

In recent years, experimental studies have consistently highlighted a role of inflammation in the pathogenesis of PD. Activation of the innate and adaptive immune response [12], and increased expression of inflammatory molecules in various biological fluids and tissues have been detected in animal models and in patients with PD [13]. Altered expression of various inflammatory mediators has been reported in blood and CSF samples collected from PD patients [14]. However, it has been suggested that central and peripheral inflammatory markers may be independently regulated in PD, and CSF molecules showed stronger associations with biomarkers of neurodegeneration [14]. It has been proposed that alpha-synuclein aggregates may play a specific role in activating microglial cells and triggering neuroinflammation [15]. Accordingly, in patients with PD microglial activation has been evidenced along with increased CSF levels of inflammatory mediators expressed by microglial cells [16]. Previous studies analyzing inflammatory cytokines in the CSF of patients with PD have reported elevated levels of several proinflammatory molecules, including IL-1β, IL-6, IL-2, TNF, and MCP-1, compared with controls [17]. However, there is considerable variability between studies [8,17].

In the present study, the association between CSF levels of a set of inflammatory molecules and clinical characteristics, including mood disorders, has been investigated in a group of newly diagnosed and untreated PD patients. We first compared CSF levels of inflammatory molecules between patients with PD and a control group of patient. We found that the levels of the inflammatory molecules IL-6, IL-9, IFNγ and GCSF were significantly higher in PD patients.

IL-6 is a pleiotropic inflammatory cytokine produced by several cell types, including activated lymphocytes, microglial cells, and astrocytes. This molecule plays a major role in the activation of immune responses and has been implicated in the pathogenesis of several neuroinflammatory disorders [18]. Previous studies have reported higher CSF levels of IL-6 in PD patients compared with controls [8,19]. In addition, increased CSF expression of IL-6 has been associated with worse motor symptoms and altered iron metabolism in PD patients [8,19], further suggesting a role for this cytokine in disease progression. IFNγ is another proinflammatory mediator released by different immune cells, including lymphocytes and macrophages, that has been implicated in several neuroinflammatory and neurodegenerative disorders, including PD [20]. Increased levels of IFNγ have been reported in the midbrain of PD patients, along with co-expression with alpha-synuclein [21]. Furthermore, IFNγ has been associated with neurotoxic effects on dopaminergic neurons in animal models of PD [22]. Only a few studies with small sample sizes have analyzed the expression of this molecule in the CSF of PD patients and controls and found no differences [23]. Finally, we found higher CSF levels of IL-9 and GCSF in PD patients compared with controls. The CSF expression of these two cytokines in patients with PD has been less investigated. The levels of IL-9 are increased in autoimmune diseases such as systemic lupus erythematosus and psoriasis [24]. This cytokine has been associated with T helper 2 responses [24]. Only one small study, involving 10 PD patients, reported lower CSF concentrations of IL-9 in PD patients compared with controls [20]. GCSF is a hematopoietic factor that regulates cell proliferation and survival and plays an important role in modulating inflammatory responses. However, this molecule and its receptors are also expressed by neurons and glial cells [25]. The role of GCSF in PD is still poorly defined, and there is evidence that this molecule may have protective effects by reducing the production of other inflammatory mediators [25].

Our results, obtained in a cohort of newly diagnosed and untreated PD patients, are in line with previous studies and support the notion that low-grade CSF inflammation may be present from the earliest stages of the disease.

When investigating the associations between CSF molecules and clinical characteristics of PD patients, a significant positive correlation was found between MIP-1a levels and age at diagnosis. MIP-1a is a proinflammatory mediator released by monocytes/macrophages and lymphocytes, that is involved in the chemotaxis and activation of various immune cells [26]. Aging has been associated with changes in the innate and adaptive immune responses, a process referred to as inflammaging [27]. Even in PD, aging has been associated with a chronic inflammatory state characterized by the overexpression of proinflammatory molecules [27]. We also observed a negative trend between the CSF levels of different inflammatory molecules and disease duration, which is not significant after correction for multiple

comparisons. Although some experimental data suggest that the activation of the immune response is more pronounced in the early stages of the disease [28], longitudinal studies evaluating the expression of CSF inflammatory mediators in PD patients are lacking, no significant correlations were found between CSF molecules and motor symptoms assessed by UPDRS-III. Notably, some association has been previously reported between motor symptoms severity and the CSF levels of inflammatory cytokines, particularly IL-6 and MCP-1 [19,29]. However, differences in the clinical characteristics of PD patients, and in particular the inclusion in our study of newly diagnosed patients with mild motor symptoms, may contribute to this result.

We then assessed in PD patients possible correlations between the CSF levels of inflammatory molecules and mood disturbances at the time of diagnosis. A link between inflammation and mood alterations has been clearly identified in different neuropsychiatric conditions. Proinflammatory mediators have been implicated in the pathophysiology of depression and anxiety through multiple mechanisms [30]. Overexpression of proinflammatory cytokines may promote dysregulation of the hypothalamic-pituitary-adrenal-axis, modification of the reuptake and availability of monoamine neurotransmitters, and reduction of tryptophan levels [7]. Furthermore, studies in animal models have shown that specific inflammatory mediators, including IL-1β, IL-6, IFNγ, and TNF, may directly alter neuronal activity in specific brain areas involved in mood regulation, such as the striatum [31]. Previous studies have reported some correlations between peripheral inflammatory molecules, including IL-6, IL-17, IFNγ, IL-10, and TNF, and depression in PD patients [17]. Notably, a longitudinal study showed that peripheral levels of IL-2 and IL-6 were associated with progression of non-motor symptoms in PD [32]. Conversely, correlations between CSF inflammation and mood disorders in PD patients have been less investigated, although a previous study reported an association between depression and CSF levels of CRP and MCP-1 in a group of patients with PD and PD dementia [8]. We found a significant association between CSF inflammatory molecules and the levels of depression and anxiety at the time of diagnosis. Notably, most of the correlations observed are weak (rho coefficients ranging from 0.3 to 0.52); however, these data are comparable with previous studies reporting positive correlations between CSF cytokines and mood scales in different neurological disorders [6,8]. Particularly, the CSF concentrations of IL-6 and IL-8 positively correlated with depression and anxiety levels. In addition, the CSF levels of these two molecules negatively correlated with QoL evaluated using the PDQ-8. These findings, obtained in a group of newly diagnosed and untreated PD patients, suggest that a proinflammatory CSF milieu may be associated with higher levels of depression and anxiety in the early phases of the disease.

Overall, our study showed higher CSF inflammation in PD patients at the time of diagnosis and an association between levels of inflammatory molecules and mood disturbances. In particular, the proinflammatory cytokine IL-6 may represent an important mediator, being both elevated in the CSF of PD patients at the time of diagnosis and associated with mood disorders. This finding is consistent with previous clinical and preclinical studies showing a role for this molecule in the pathogenesis of neurodegenerative diseases, including PD [3]. Moreover, IL-6 has been directly involved in the pathogenesis of mood disorders in patients with neuropsychiatric and autoimmune diseases, suggesting a that the role of this cytokine in mood alterations is not specific for PD [7,32,33]. Our findings therefore extend previously available data supporting a possible role of neuroinflammation in the pathogenesis of mood disturbances in the early phases of PD.

Previous studies showed that sex is an important factor affecting inflammatory responses, and differences in inflammatory profiles between male and female PD patients have been reported [34]. We found no significant differences in the CSF levels of inflammatory molecules between male and female PD patients at the time of diagnosis, although the concentrations of GCSF were slightly increased in female patients. In addition, we found that depression and anxiety were associated with different CSF cytokines in male and female PD patients. Although these data may suggest that differences in inflammatory CSF profile may contribute to clinical variability in the two sexes, these results should be considered with caution, and further studies are needed to better define this aspect.

In the present study we included only untreated PD patients in the early stages of the disease, with short disease duration and mild motor symptoms. Accordingly, it has been observed that differences in clinical characteristics of the study cohort, including age, disease stage, and treatment condition, may represent a significant source of variability [17]. However, the small sample size and the lack of follow-up data represent important limitations of the present study. Prospective studies evaluating depression and anxiety during the follow-up are needed to assess whether CSF inflammation may predict mood alterations during the course of the disease. In addition, important aspects including sleep disturbances, pain, and dysautonomia have not been specifically investigated, and further studies are required to better define the impact of CSF inflammation on non-motor symptoms in PD. Finally, we focused on a set of proinflammatory CSF molecules, however, it has been evidenced that also the expression of anti-inflammatory cytokines and chemokines is altered in PD [17,35]. Therefore, studies exploring a large set of both proinflammatory and anti-inflammatory molecules are important to better characterize the CSF inflammatory profile of PD.

In newly diagnosed PD patients, increased expression of specific proinflammatory cytokines is associated with higher levels of depression and anxiety, further strengthening the hypothesis that neuroinflammation may play a role in mood disorders in PD. Better understanding of pathogenesis of mood disorders may help to explain clinical heterogeneity in PD and is important to develop novel therapeutic strategies.

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Data availability statement

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

CRediT authorship contribution statement

Mario Stampanoni Bassi: Conceptualization, Formal analysis, Writing – original draft, Writing – review & editing. **Luana Gilio:** Conceptualization, Data curation, Writing – original draft, Writing – review & editing. **Giovanni Galifi:** Data curation, Writing – review & editing. **Fabio Buttari:** Data curation. **Ettore Dolcetti:** Data curation. **Antonio Bruno:** Data curation. **Lorena Belli:** Data curation. **Nicola Modugno:** Data curation. **Roberto Furlan:** Data curation. **Annamaria Finardi:** Data curation. **Georgia Mandolesi:** Data curation. **Alessandra Musella:** Data curation. **Diego Centonze:** Conceptualization, Funding acquisition, Writing – review & editing. **Enrica Olivola:** Conceptualization, Data curation, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: **Diego Centonze** is an Advisory Board member of Almirall, Bayer Schering, Biogen, GW Pharmaceuticals, Merck Serono, Novartis, Roche, Sanofi-Genzyme, and Teva and received honoraria for speaking or consultation fees from Almirall, Bayer Schering, Biogen, GW Pharmaceuticals, Merck Serono, Novartis, Roche, Sanofi-Genzyme, and Teva.

He is also the principal investigator in clinical trials for Bayer Schering, Biogen, Merck Serono, Mitsubishi, Novartis, Roche, Sanofi-Genzyme, and Teva. His preclinical and clinical research was supported by grants from Bayer Schering, Biogen Idec, Celgene, Merck Serono, Novartis, Roche, Sanofi- Genzyme and Teva.

The other authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parkreldis.2024.106071.

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