Cerebrospinal fluid inflammatory markers in Parkinson’s disease – Associations with depression, fatigue, and cognitive impairment

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ABSTRACT

Neuroinflammation may be involved in the pathophysiology of Parkinson’s disease (PD) and specifically in non-motor symptoms such as depression, fatigue and cognitive impairment. The aim of this study was to measure inflammatory markers in cerebrospinal fluid (CSF) samples from PD patients and a reference group, and to investigate correlations between non-motor symptoms and inflammation.

We quantified C-reactive protein (CRP), interleukin-6, tumor necrosis factor-alpha, eotaxin, interferon gamma-induced protein-10, monocyte chemotactic protein-1 (MCP-1), and macrophage inflammatory protein 1-β in CSF samples from PD patients (N = 87) and the reference group (N = 33). Sixteen of the PD patients had a dementia diagnosis (PDD). We assessed symptoms of fatigue, depression, anxiety and cognitive function using the Functional Assessment of Chronic Illness Therapy-Fatigue, the Hospital Anxiety and Depression Scale, and the Mini Mental State Examination, respectively.

There were no significant differences in mean levels of inflammatory markers between PD patients and the reference group. After controlling for age, gender and somatic illness, patients with PDD had significantly higher levels of CRP compared to non-demented PD patients (p = 0.032) and the reference group (p = 0.026). Increased levels of inflammatory markers in CSF were significantly associated with more severe symptoms of depression, anxiety, fatigue, and cognition in the entire PD group. After controlling for PD duration, age, gender, somatic illness and dementia diagnosis, high CRP levels were significantly associated with more severe symptoms of depression (p = 0.010) and fatigue (p = 0.008), and high MCP-1 levels were significantly associated with more severe symptoms of depression (p = 0.032).

Our results indicate that non-motor features of PD such as depression, fatigue, and cognitive impairment are associated with higher CSF levels of inflammatory markers.

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1. Introduction

Parkinson’s disease (PD) is one of the most common neurodegenerative diseases with causes still largely unknown. Results from pre-clinical and epidemiological studies suggest that neuroinflammation may play an important role in the death of dopaminergic neurons in the substantia nigra, which is the pathological hallmark of PD (Barnum and Tansey, 2010). Also, in vivo and post-mortem studies of PD patients have described microglial activation in the putamen, hippocampus and in frontol and temporal cortical re-
gions (Gerhard et al., 2006; Imamura et al., 2003). A few clinical studies have indeed shown that PD patients display significantly higher blood levels of pro-inflammatory (Dobbs et al., 1999; Lindqvist et al., 2012; Scalzo et al., 2010), although some studies have also reported negative results (Scalzo et al., 2011).

The few previous studies investigating inflammatory markers in the cerebrospinal fluid (CSF) of PD patients have been conducted on comparatively small numbers of subjects, and often without a healthy control group for comparison. Blum-Degen et al. reported significantly higher CSF, but not plasma, levels of IL-6 and IL-1-beta in 22 de novo PD patients compared to 12 patients with other neurological disorders (Blum-Degen et al., 1995). Mogi et al. found higher levels of TNF-alpha in CSF samples and post-mortem tissue from 15 parkinsonian patients compared to 16 controls (Mogi et al., 1994). Interestingly, neuroinflammation has not only been implicated in PD per se, but more specifically in the generation of non-motor symptoms (Barnum and Tansey, 2012). Non-motor symptoms such as depression, anxiety, fatigue, and cognitive decline are frequently occurring in PD (Aarsland et al., 2009) and may in fact be even stronger determinants of low quality of life and poor health status than motor symptoms (Hinnell et al., 2012; McKinlay et al., 2008; Soh et al., 2011). Serum (Lindqvist et al., 2012) and plasma (Menza et al., 2010) levels of cytokines have been found to correlate with symptoms of depression, fatigue, and cognitive difficulties in PD, but no previous studies have investigated inflammatory markers in CSF in relation to such symptoms.

To date, the pathophysiological mechanisms behind non-motor symptoms of PD are poorly understood, and current treatment options are in many cases inadequate (Aarsland et al., 2009; Weintraub et al., 2005). By investigating associations between inflammatory markers and non-motor symptoms we hope to gain further insight into this area, which in turn could open up the possibility of novel treatment options. In this study we set out to quantify inflammatory markers in CSF of patients with PD (n = 87) and a reference group (n = 33), all evaluated for fatigue, depression, anxiety and cognitive function. As opposed to measurements of immune markers in blood samples, this approach aims to link PD symptomatology directly to signs of neuroinflammation. To the best of our knowledge, this is the largest study measuring inflammatory markers in the CSF of PD patients and a reference group, and the first to investigate correlations between non-motor symptoms and neuroinflammation in PD patients. We hypothesized that PD patients would have higher mean levels of inflammatory markers than those in the reference group and that the highest levels would be observed in those with more severe symptoms of depression, anxiety, fatigue, and cognitive impairment.

2. Methods

2.1. Study participants

Eighty-seven PD patients were enrolled in this study between the years 2008 and 2012. Sixteen of these patients suffered from Dementia in PD (PDD). PD patients were recruited to the Skåne University Hospital in Lund, Sweden from neurological clinics in southern Sweden.

Thirty-three individuals comprising the reference group were mainly recruited by contacting spouses of PD patients but also by recruitment during public lectures. Twenty-five (76%) of the individuals comprising the reference group were spouses of patients, and two (6%) were blood relatives. None of the participants in the reference group had a neurological disease, suffered from dementia, or had any signs of ongoing depression.

None of the study participants were treated with NSAIDs or corticosteroids or had any acute or chronic inflammatory disease. The most common somatic disorders were: cardiovascular disease (PD patients N = 18 [21%]; Reference group N = 12 [36%]; asthma/allergies (PD patients N = 10 [12%]; Reference group N = 2 [6%]); osteoarthritis (PD patients N = 8 [9%]; Reference group N = 4 [12%]); and diabetes mellitus (PD patients N = 3 [3%]; Reference group N = 1 [%]). Demographic characteristics of non-demented PD patients, PDD patients and the reference group are given in Table 1. Mean age was significantly higher in the PDD group compared to the non-demented PD and the reference group (F(2,117) = 4.44, p = 0.014; PDD vs non-demented PD, p = 0.005; PDD vs the reference group p = 0.013). The gender distribution differed borderline significantly between the groups (Pearson’s X² = 5.7, p = 0.059), with a higher proportion of men in the PD groups. A significantly larger proportion of the PDD patients received antidepressants.

Table 1

Demographic characteristics and inflammatory markers of the reference group, non-demented PD patients, and PDD patients. Concentrations are given in pg/ml, except for CRP where concentrations are given in ng/ml. Raw values of inflammatory markers and clinical variables are presented.

|                   | Controls (n = 33) | Non-demented PD (n = 71) | PDD (n = 16) | P-value |
|-------------------|-------------------|--------------------------|-------------|---------|
| Sex               | f = 19 (58%), m = 14 | f = 27 (39%), m = 44 | f = 4 (22%), m = 12 | .059    |
| Age (years, mean ± SD) | 65.8 ± 8.8 | 64.1 ± 10.5 | 72.0 ± 5.8<sup>a</sup> | .014 |
| Illness duration (years, mean ± SD) | N/A | 6.4 ± 5.6 | 15.8 ± 6.5 | .00 |
| Hoehn and Yahr (mean ± SD) | 1.9 ± 0.8 | 3.1 ± 0.9 | .00 |
| Schwab and England (median, IQR) | 100, 100–100 | 90, 90–100<sup>b</sup> | 70, 63–80<sup>a</sup> | .00 |
| UPDRS motor score (median, IQR) | 0, 0–2 | 18, 10–25<sup>b</sup> | 32, 23–50<sup>a</sup> | .00 |
| FACIT-fatigue score (median, IQR) | 51, 50–52 | 43, 34–47<sup>b</sup> | 29, 14–40<sup>a</sup> | .00 |
| HADS depression score (median, IQR) | 0, 0–1 | 3, 1–5<sup>b</sup> | 8, 5–10<sup>a</sup> | .00 |
| HADS anxiety score (median, IQR) | 1, 0–3 | 4, 2–7<sup>b</sup> | 6, 4–8<sup>a</sup> | .00 |
| MMSE score (median, IQR) | 29, 28–30 | 29, 27–29 | 25, 21–27<sup>a</sup> | .00 |
| Eotaxin (median, IQR) | 22.0, 18.5–27.5 | 23.8, 20.3–30.4 | 23.0, 19.6–28.8 | .50 |
| MIP-1<sub>α</sub> (median, IQR) | 9.6, 5.4–17.5 | 7.9, 5.0–12.8 | 8.4, 5.0–12.7 | .56 |
| IP-10 (median, IQR) | 295, 204–412 | 302, 221–453 | 381, 234–488 | .54 |
| MCP-1 (median, IQR) | 583, 488–673 | 583, 509–687 | 637, 351–864 | .29 |
| IL-6 (median, IQR) | 0.8, 0.6–1.1 | 0.7, 0.5–1.1 | 1.0, 0.7–1.1 | .22 |
| TNF-alpha (median, IQR) | 0.07, 0.04–0.08 | 0.07, 0.03–0.10 | 0.06, 0.04–0.10 | .94 |
| CRP (median, IQR) | 19.7, 8.6–51.9 | 22.8, 8.2–51.7 | 57.1, 18.3–246.1<sup>b</sup> | .038 |

Abbreviations: UPDRS = unified Parkinson’s disease rating scale, FACIT = functional assessment of chronic illness therapy, HADS = hospital anxiety and depression scale, MMSE = mini mental state examinations, MCP = monocyte chemotactic protein, CRP = C-reactive protein, IL = interleukin, TNF = tumor necrosis factor, IP = interferon gamma-induced protein, MIP = macrophage inflammatory protein.

<sup>a</sup> Non-demented PD patients differed significantly from both non-demented PD patients and healthy controls (p < 0.05).

<sup>b</sup> Non-demented PD patients differed significantly from healthy controls (p < 0.05).
than the non-demented PD patients and those in the reference group (Pearson's $X^2 = 19.1, p < 0.001$).

2.2. Ethics statement

The Ethics Committee of Lund University approved this study. Study participants gave written informed consent to participate. The study was conducted in accordance with the provisions of the Helsinki Declaration.

2.3. Study procedures/assessments

All study participants underwent a general physical examination, routine blood screening and complete medical history was taken. All study participants were evaluated by a licensed and experienced medical doctor using the Unified Parkinson's Disease Rating Scale (UPDRS)-3 (Fahn et al., 1987), the Hoehn & Yahr scale (Hoehn and Yahr, 1967), and the Schwab & England scale (Schwab and England, 1969). PD diagnosis was verified according to the National Institute of Neurological Disorders and Stroke diagnostic criteria (Gebel et al., 1999). PDD was diagnosed according to the Clinical Diagnostic Criteria for Dementia Associated with PD (Emre et al., 2007).

All study participants were asked to complete Functional Assessment of Chronic Illness Therapy (FACIT)-Fatigue (Cella, 1997) and the Hospital Anxiety and Depression Scale (HADS) (Zigmond and Snith, 1983). Mini Mental State Examinations (MMSE) were performed by a trained clinician (Folstein et al., 1975).

HADS scores were missing for three individuals and FACIT fatigue scores for 9 individuals. Non-motor symptom scores of non-demented PD patients, PDD patients and the reference group are given in Table 1.

2.4. CSF sampling and biological assays

Lumbar punctures were carried out in the morning following the clinical evaluation. Approximately 20 ml CSF were collected between L3 and L4, L4 and L5, or L5 and S1. CSF was collected in polypropylene tubes and gently mixed to avoid gradient effects. All samples were centrifuged within 30 min at +4°C and stored at −80°C pending biochemical analysis.

C-reactive protein (CRP), interleukin-6 (IL-6), eosinax, tumor necrosis factor (TNF)-alpha, interferon gamma-induced protein-10 (IP-10), monocyte chemotactic protein-1 (MCP-1), and macrophage inflammatory protein-1β (MIP-1β) levels in CSF were quantified using multiplex electrochemiluminescence-based immunoassays (MesoScale Discovery, Gaithersburg, Maryland, USA) as per the manufacturer’s protocol with small modifications. Briefly, 10% bovine serum albumin (Fisher Scientific, Gothenburg, Sweden) was added to the blocking buffer and 50 μl of undiluted sample per replicate was used in the assay. For CRP, however, based on the data from the pilot experiment, 25 μl of samples was used. All the samples were measured in duplicates on the same day using the same reagents. Data was collected and analyzed using SECTOR Imager 6000 reader and Discovery Workbench™ Software (www.mesoscale.com). Detection limits of the assay were: CRP, 7 pg/ml; IL-6, 0.03 pg/ml; TNF-alpha, 0.04 pg/ml; Eotaxin 3.4 pg/ml; IP-10, 0.99 pg/ml; MIP-1β, 5.76 pg/ml; MCP-1, 0.17 pg/ml. Intra-assay CV was below 20% for all samples.

Semipur levels of CRP, TNF-alpha, and IL-6 have previously been published in the reference group using methods described elsewhere (Lindqvist et al., 2012).

2.5. Statistical analyses

The Statistical Package for the Social Sciences (SPSS) for Mac was used for statistical calculations. Pearson's $X^2$ was used to compare proportions. Student’s $t$-test and one-way analysis of variance (ANOVA) was used for group comparisons, controlling for covariates when appropriate (full factorial ANCOVA). Non-normally distributed variables were transformed into their natural logarithms prior to statistical analyses with parametric methods and in cases when log transformation did not result in normal distribution, non-parametric methods were used for univariate group comparisons (Kruskal–Wallis non-parametric ANOVA or Mann–Whitney U-test). Univariate associations between two continuous variables were analyzed using the Pearson’s $r$ (normally distributed variables) or Spearman’s Rho (skewed variables and/or ordinal data). To further explore significant correlations between levels of inflammatory markers and symptom severity, hierarchical multiple regressions were carried out, controlling for appropriate covariates as described below. The respective non-motor symptom score was entered as dependent variable in each model. Total somatic illness, age, gender, PDD diagnosis (except in the case of MMSE scores), and PD duration were entered into the first block as independent variables, and the inflammatory marker of interest was subsequently entered into the second block. There was one outlier on FACIT-fatigue scores, two outliers on HADS depression scores, three outliers on HADS anxiety scores, and nine outliers on MMSE scores. Hierarchical multiple regressions were carried out both with and without outliers, and results from both analyses are given in the results section. All tests were 2-tailed with an alpha = 0.05, $P$-values between 0.05 and 0.1 were considered trends.

2.6. Rationale for selecting covariates

Based on our hypotheses that more severe somatic co-morbidity could potentially influence both inflammatory markers and non-motor symptom severity, “total somatic illness” was selected a priori as a covariate. This was a continuous variable calculated as a composite score of all of the most common somatic co-morbidities for each individual. The following variables correlated significantly (or near-significantly) with non-motor symptoms and/or inflammatory markers in bivariate analyses: Age, gender, PD duration in years, and PDD diagnosis. Thus, these variables were also entered as covariates in all analyses investigating associations between severity of non-motor symptoms and levels of inflammatory markers. PDD diagnosis was not controlled for in analyses between inflammatory markers and cognitive performance. Variables UPDRS-3 score, Schwab & England score, and PD duration were all highly inter-correlated (all Spearman’s $r > 0.7$, $p < 0.001$), seemingly sharing variance. Since PD duration was normally distributed after log-transformation (as opposed to UPDRS-score and Schwab & England score) we used this variable as a proxy for “general burden of illness”.

3. Results

3.1. Demographics

Demographic and clinical characteristics of the reference group, non-demented PD patients, and PDD patients are given in Table 1. In the entire group, age was significantly correlated with MCP-1 (Spearman’s $r = 0.37, p < 0.001$), IP-10 (Spearman’s $r = 0.23, p = 0.01$) and CRP (Pearson’s $r = 0.30, p = 0.001$). Moreover, men had significantly higher mean levels of eosinax ($t(117) = -2.7, p = 0.009$), and MCP-1 ($t(117) = -2.7, p = 0.02$). Total
somatic illness correlated negatively with eotaxin levels (Spearman’s Rho = –0.19, p = .04) and positively with CRP levels (Spearman’s Rho = 0.18, p = .059). UDORs motor score correlated positively with CRP (Spearman’s Rho = 0.21, p = 0.023) and MCP-1 (Spearman’s Rho = 0.16, p = .08). In the PD group, illness duration was correlated with CRP (Pearson’s r = 0.21, p = 0.054). PD staging, according to the Hoehn & Yahr scale correlated positively with CRP (Spearman’s Rho = 0.28, p = .0011) and MIP-1 beta (Spearman’s Rho = 0.18, p = .092). Status as a de novo PD patient had no significant impact on any of the measured biomarkers (data not shown).

3.2. Group comparisons, CSF inflammatory markers

There were no significant differences (or trends) in mean or median scores for any of the measured inflammatory markers between PD patients (n = 87) and the reference group (n = 33) (Student T-tests, Mann Whitney U tests, ANCOVA’s, all non significant (NS)).

Mean CRP levels were significantly higher in the PDD group compared to the non-demented PD patients and the reference group (F(2,112) = 3.36, p = 0.038; PDD patients vs non-demented PD patients, p = 0.018; PDD patients vs the reference group, p = 0.017). There were no significant differences in mean or median levels for any of the other cytokines between PD patients without dementia (n = 71), PDD patients (n = 16) and the reference group (n = 33) (One-way ANOVA’s and Kruskal Wallis non-parametric ANOVA’s, NS (Table 1). After controlling for the effects of age, gender, and total somatic illness, PD patients displayed significantly higher mean levels of CRP compared to the reference group (p = 0.026) and the non-demented PD patients (p = 0.032). There were no significant differences in any of the other inflammatory markers between PD patients, non-demented PD patients and the reference group (data not shown). After excluding two of the individuals in the reference group whom were blood relatives to PD patients, the p-values in all analyses were similar.

3.3. Associations between inflammatory markers and non-motor symptoms in PD patients

Table 2 summarizes all univariate correlations between severity of non-motor symptoms and CSF inflammatory markers in all PD patients. Spearman’s Rho was used, correlation coefficients are given. Since FACIT is an inverse rating scale, a negative correlation herein indicates that the patients with higher levels of inflammatory markers display more severe fatigue.

Table 2

| CRP | IL-6 | TNF-alpha | IL-10 | MIP-1beta | IP-10 |
|-----|------|-----------|-------|-----------|-------|
| .33b | .12 | -.32b | - .09 | - .27b |
| .03  | -.03 | -.06 | -.11 | -.09 |
| -.01  | -.06 | -.04 | -.01 |
| -.02 | -.10 | .00 | -.05 |
| .34b | -.09 | -.30b | -.13 |
| .05 | -.07 | .06 | -.04 |
| .12 | .22b | -.32b | -.03 |

a p < 0.05.  
b p < 0.01.

In order to further explore the significant correlations, we carried out hierarchical multiple regressions, controlling for total somatic illness, PD duration, age, gender, and PDD diagnosis. FACIT score as dependent variable: CRP (β = −0.29, p = 0.008), IP-10 (β = −0.25, p = 0.015), but not MCP-1 (β = −0.16, p = 0.19) remaining significantly associated with FACIT scores. After excluding one outlier on FACIT-fatigue, CRP (β = −0.22, p = 0.035), but not IP-10 (β = −0.17, p = 0.087) or MCP-1 (β = −0.13, p = 0.26) remained significantly associated with FACIT-fatigue scores.

HADS depression score as dependent variable: CRP (β = 0.28, p = 0.010) and MCP-1 (β = 0.26, p = 0.032) remained significantly associated with high HADS depression scores. After excluding two HADS depression outliers both CRP (β = 0.21, p = 0.041) and MCP-1 (β = 0.27, p = 0.013) were significantly associated with HADS depression scores.

HADS anxiety score as dependent variable: IP-10 was not significantly associated with HADS anxiety scores either before (β = 0.18, p = 0.10) or after (β = 0.13, p = 0.26) excluding four outliers.

MMSE score as dependent variable, IL-6 was not significantly associated with MMSE scores either before (β = 0.18, p = 0.01) or after (β = 0.13, p = 0.26) excluding nine outliers.

In Table 2, FACIT-fatigue score correlated significantly and negatively with CRP (Spearman’s Rho = −0.32 p = 0.004), IP-10 (Spearman’s Rho = −0.32, p = 0.004), and MCP-1 (Spearman’s Rho = −0.30 p = 0.006). Since FACIT is an inverse rating scale, a negative correlation herein indicates that the patients with higher levels of inflammatory markers display more severe fatigue.
3.4. Correlations between inflammatory markers in serum and CSF

We have previously measured serum levels of CRP, TNF-alpha, and IL-6 in this sample of PD patients and in the reference group (Lindqvist et al., 2012). We here found strong correlations between CRP in CSF and CRP in blood in PD patients and in the reference group (Spearman’s Rho = 0.57, p < 0.001, N = 115). There was a trend for a significant correlation between TNF-alpha in serum and CSF (Spearman’s Rho = 0.17, p = 0.063, N = 119), but for IL-6, the serum/CSF correlation was not statistically significant (Spearman’s Rho = 0.13, p = 0.16, N = 116).

4. Discussion

We here show, for the first time, significant associations between high levels of pro-inflammatory markers in CSF and severity of fatigue, depression, anxiety and cognitive impairment in individuals with PD. Degree of neuroinflammation was significantly associated with more severe depression, fatigue, and cognitive impairment even after controlling for appropriate confounders such as age, gender, somatic illness and, when appropriate, dementia diagnosis, and PD duration. We have previously reported associations between peripheral inflammatory markers and severity of depression and fatigue in this cohort of PD patients (Lindqvist et al., 2012). We now demonstrate that these inflammatory changes are present also in CSF. Given the limited permeability of the blood brain barrier (BBB) for cytokines (Schippers et al., 2005), these findings are important as they show that relatively higher levels of inflammatory markers, in direct contact with the brain parenchyma, are associated with more severe symptoms of depression and fatigue, as well as cognitive impairment. Interestingly, higher levels of inflammatory markers were found in those PD patients with dementia and more severe symptoms of depression and fatigue although we did not see this pattern in the PD group as a whole.

Our findings are mainly in line with two previous PD studies reporting associations between pro-inflammatory cytokines in serum and symptoms of depression, fatigue, and cognitive impairment (Lindqvist et al., 2012; Menza et al., 2010). Although we did not see any significant differences in inflammatory markers between the PD group as a whole and the reference group, a few earlier small-scale studies have reported higher CSF levels of pro-inflammatory cytokines in PD patients compared to individuals with other neurological disorders (Blum-Degen et al., 1995; Mogi et al., 1996), and neurologically healthy controls (Mogi et al., 1994). In line with our findings, however, a larger and more recent study reported no differences in CSF levels of inflammatory markers Flt3 ligand and fractalkine between PD patients and healthy controls (Shi et al., 2011). In addition to differences in sample sizes and control group, other possible reasons for the divergent results described above may be due to differences in sample preparation and handling in the different studies. Several of the individuals comprising the reference group were spouses of the PD patients, and two were blood relatives. One may speculate that this may have contributed to our failure to reject the null hypothesis, i.e. some of the individuals in the reference group may share an inflammatory endophenotype with some of the PD patients, or may have been subjected to caregiver stress that could potentially have influenced their cytokine levels. The individuals in the reference group, however, displayed none, or extremely mild, symptoms of depression, anxiety, and fatigue which suggest that they were not likely to be under severe stress at the time of the CSF sampling. Moreover, one may argue that the inclusion of spouses in the reference group would be an advantage, since they share the same environment as the patients, and any significant differences between the two groups may therefore be considered more disease specific. It is also noteworthy that a larger proportion of the reference group had cardiovascular disease, which may have increased cytokine values. We did, however, control for somatic illness in our analyses and this did not change the main results.

None of the above-mentioned studies attempted to distinguish PD patients based on depressive or cognitive symptoms, nor investigate correlations between inflammatory markers and non-motor symptoms. Previous clinical and population-based studies have linked increased inflammation to depression, fatigue and cognitive impairment in other neurodegenerative disorders. Worse cognitive performance or cognitive decline has been associated with higher blood or CSF levels of inflammatory markers in patients with vascular dementia (Wada-Isoe et al., 2004) and Alzheimer’s disease (AD) (Holmes et al., 2003). There is also some evidence from longitudinal studies that high levels of inflammatory markers may indicate increased future risk for developing vascular dementia and progress from mild cognitive impairment to AD (Schmidt et al., 2002; Westin et al., 2012). Based on these and other studies, a “cytokine model for cognitive function” has been suggested (McAfoose and Baune, 2009). In line with this hypothesis, we found that PDD patients had higher mean levels of CSF-CRP than those comprising the reference group and non-demented PD patients, and this difference did remain significant after controlling for age, gender, and somatic illness. In depressed, non-parkinsonian patients, higher blood levels of pro-inflammatory cytokines are commonly seen (Dowlati et al., 2010), and high CSF levels of pro-inflammatory cytokine IL-6 have been associated with more severe depressive symptoms in suicidal patients (Lindqvist et al., 2009). Moreover, in individuals with leukemia and multiple sclerosis, symptoms of fatigue have consistently been associated with higher levels of inflammatory markers in CSF and in the periphery (Heesen et al., 2006; Meyers et al., 2005). In all, our findings that more pronounced fatigue, depression, and cognitive impairment is linked to higher levels of CSF inflammatory markers further strengthens the hypothesis that these symptoms are associated with neuroinflammatory mechanisms, regardless of diagnosis.

Higher cytokine levels in PD patients with more severe non-motor symptoms may be due to an increased peripheral production of inflammatory markers crossing the BBB (Schippers et al., 2005), or augmented secretion by microglia and astrocytes within the central nervous system (CNS) (Hanisch and Kettenmann, 2007; McG-
er and McGeer, 1995). CRP was the inflammatory marker that showed the strongest association with non-motor symptoms in our material; high CRP levels were significantly associated with more severe symptoms of fatigue and depression, as well as a dementia diagnosis, even after controlling for potential confounders. CRP has traditionally been viewed as one of the acute phase reactants but studies have shown that CRP may also have direct biological activities on vascular cells and monocytes/macrophages (Yeh, 2005). CRP is mainly synthesized in the liver, however results from post-mortem studies on patients with AD and intracerebral hemorrhage suggest that CRP can be produced from within the brain by neurons and glial cells (Di Napoli et al., 2012; Yasojima et al., 2000). Interestingly, a recent animal study found that microglial cultures produce CRP, suggesting that microglial cells may be the source of CRP in the central nervous system (Juma et al., 2011). We here found a strong correlation between CSF and plasma CRP levels, providing important evidence that certain inflammatory markers in the blood mirror inflammation in the CNS. However, it is noteworthy that we did not find any correlation between IL-6 in blood and CSF. This has also been shown in previous studies (Lindqvist et al., 2009). Thus, potential future peripheral biomarkers for CNS inflammation in PD should be carefully selected based on their ability to cross the BBB and communicate with the periphery.

Traditionally, psychiatric symptoms in PD have been thought to be the result of either a reaction to the disability caused by PD or a pathological mechanism associated with the neurodegenerative process in PD. In this study we focused on neuroinflammation as a pathophysiological mechanism potentially underlying the development of such symptoms. To what extent a casual relationship between neuroinflammation and depression can be inferred has not been fully determined, but results from animal and clinical studies do suggest such a relationship (Capuron and Miller, 2004; Frenois et al., 2007). In the regression analyses conducted in the present study, we found that higher CRP levels in CSF were significantly associated with severity of depression and fatigue, and high MCP-1 levels were significantly associated with severity of depression. These associations remained significant even after taking into account potential confounders such as PD duration, as a putative indicator of disability or general burden of disease, and somatic illness. Thus, our results indicate that neuroinflammation may be independently associated depression and fatigue in PD. In non-PD patients with major depression, treatment with anti-inflammatory components like the TNF-α antagonist infliximab may reduce depression in treatment-resistant depression patients with increased baseline inflammatory status (Raison et al., 2013). Interestingly, the effects of infliximab on depressive symptoms may be mediated by transcriptional regulation of for instance genes related to activation of the immune system (Mehta et al., 2013). If cytokine antagonism could be useful for treatment of depression in PD patients as well, remains to be investigated.

The potential roles of cytokines and chemokines in the generation of non-motor symptoms of PD are not yet completely understood. On the one hand they promote immune activation, e.g. via microglial activation and induction of leukocyte chemotaxis (Tansey et al., 2007). In this capacity, chemokines and cytokines are closely associated with chronic neuroinflammation, potentially leading to neuropsychiatric manifestations. Conversely, cytokines and chemokines may have important functions as neuromodulators under normal conditions (Vitkovic et al., 2000). There are many different potential mechanisms by which pro-inflammatory cytokines may generate symptoms of depression, fatigue, and cognitive impairment. Cytokines may have to direct effects on monoaminergic neurotransmission (Dunn, 2006), the Hypothalamus–Pituitary–Adrenal axis (Berczi et al., 2009), and the kynurenine pathway of tryptophan degradation (Dantzker et al., 2008), all mechanisms that have been implicated in the pathophysiology of depressive symptoms (Bao et al., 2007; Erhardt et al., 2013; Hirschfeld, 2000).

To summarize, this is the first study to show that severity of non-motor symptoms of PD are correlated with degree of neuroinflammation, as indicated by high levels of pro-inflammatory markers in CSF. These results suggest that non-motor symptoms of PD may be associated with neuroinflammatory mechanisms. If our results are reproduced by future studies it introduces the intriguing possibility that anti-inflammatory medications may be used to treat such symptoms specifically.

5. Conflict of interest statement

All authors declare no conflicts of interest.

6. Funding/financial disclosures

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