Journal of Neuroimmunology 403 (2025) 578610

Contents lists available at ScienceDirect

Journal of Neuroimmunology

journal homepage: www.elsevier.com/locate/jneuroim

Full Length Article

Sex-specific immune-biological profiles in Parkinson's disease

Roberta Bovenzi [a], Matteo Conti [a], Clara Simonetta [a], Jacopo Bissacco [a], Davide Mascioli [a], Maria Mancini [a], Veronica Buttarazzi [a], Federica Veltri [a], Giulia Maria Sancesario [b], Silvio Bagetta [a], Francesca D'Amaro [a], Massimo Pieri [c,d], Rocco Cerroni [a,e], Claudio Liguori [a,e], Valerio Chiurchiù [f,g], Mariangela Pierantozzi [a,e], Alessandro Stefani [a,e,h], Nicola Biagio Mercuri [a,e,i], Tommaso Schirinzi [a,e,*]

[a] Department of Systems Medicine, University of Rome Tor Vergata, Rome, Italy
[b] Clinical Neurochemistry Unit and Biobank, IRCCS Fondazione Santa Lucia, European Centre for Brain Research, Rome, Italy
[c] Department of Experimental Medicine, University of Rome "Tor Vergata", Rome, Italy
[d] Department of Clinical Biochemistry, Tor Vergata University Hospital, Rome, Italy
[e] Neurology Unit, University Hospital of Rome Tor Vergata, Rome, Italy
[f] Institute of Translational Pharmacology, National Research Council, Rome, Italy
[g] Laboratory of Resolution of Neuroinflammation, Santa Lucia Foundation IRCCS, Rome, Italy
[h] UOSD Parkinson Centre, Tor Vergata University Hospital, Rome, Italy
[i] Laboratory of Experimental Neuroscience, Santa Lucia Foundation IRCCS, Rome, Italy

ARTICLE INFO

Keywords:
Parkinson's disease
Gender
Sex hormones
Inflammation

ABSTRACT

Depending on age, both the risk and characteristics of Parkinson's disease (PD) differ between the sexes. The immune system might have a role; however, human-based evidence remains scarce. Here, we investigated the relationship between peripheral immune cellular composition and the clinical-biological sexual dimorphism of PD. The leukocyte population count (neutrophils, lymphocytes, monocytes, eosinophils, and basophils), the neutrophil-to-lymphocyte ratio (NLR), and the monocytes-to-lymphocytes ratio (MLR) were collected and compared in 117 PD patients and 86 controls (CTLs), and then related to blood levels of sex hormones, CSF markers of neurodegeneration (α-synuclein, amyloid-β-42, amyloid-β-40, total tau, and phosphorylated-181-tau), and clinical features in male and female PD patients. Finally, a cluster analysis based on the three main leukocyte populations (neutrophils, lymphocytes, monocytes) was performed for the entire PD cohort. Male PD patients had lower lymphocyte counts and higher NLR than male CTLs. Females with PD had lower monocyte counts, NLR, and MLR than males with PD. Lymphocyte counts correlated with cognition in male, but not female, PD patients. Finally, two clusters of peripheral immune cellular composition were identified: the "high peripheral inflammation" one, mostly comprising male patients, with worse clinical features and greater central α-synuclein burden, and the "low peripheral inflammation cluster", which mainly comprised female patients, with milder clinical features and lower central synucleinopathy. In conclusion, the peripheral immune pattern entails sex-specific clinical-biological profiles in PD. Moreover, systemic inflammation clusters with sex, sexual hormones, clinical features, and central synucleinopathy in PD, supporting the relevance of immunity in sexual dimorphism of the disease.

1. Introduction

Parkinson's disease (PD) is characterized by profound heterogeneity. Although the PD-related neuropathology hallmarks, namely the widespread intraneuronal accumulation of α-synuclein (α-syn) containing Lewy bodies (LBs) and the loss of dopaminergic neurons of Substantia Nigra pars compacta, are well defined, the clinical-pathological correlations vary at an individual level due to the complexity of pathogenic mechanisms (Kalia and Lang, 2015).

In this regard, biological sex is a main determinant of PD presentation. PD is more prevalent in males, with the male-to-female ratio progressively increasing with age (Moisan et al., 2016), and its clinical

* Corresponding author at: Unit of Neurology, Department of Systems Medicine, University of Rome Tor Vergata, Via Montpellier, 00133 Rome, Italy.
E-mail address: t.schirinzi@yahoo.com (T. Schirinzi).

https://doi.org/10.1016/j.jneuroim.2025.578610
Received 10 February 2025; Received in revised form 29 March 2025; Accepted 4 April 2025
Available online 5 April 2025

presentation exhibits a distinct sex dimorphism (Bovenzi et al., 2024a), suggesting that several factors influence aging and neurodegeneration differently in males and females (Bovenzi et al., 2024b, 2023).

Compelling evidence suggests that sex hormones likely play a substantial role (Bovenzi et al., 2023; Conti et al., 2025). Similarly, neuroinflammation and the immune system, two major drivers in PD pathophysiology (Madetko et al., 2022; Massaro Cenere et al., 2024), might exhibit different activity between males and females, contributing to the sex specificity of the disease (Ferreira et al., 2023; Lopez-Lee et al., 2022; Sciarra et al., 2023).

Existing studies have mostly addressed the relationship between sex or immunity and clinical-biological aspects of PD separately, never providing a unifying approach.

Here, we combined different biomarkers series to directly examine the interactions among these three axes in patients and further dissect the pathophysiological bases of sexual dimorphism in PD.

In particular, we collected both leukocyte count and the ratios among leukocyte populations – the Neutrophils-to-lymphocytes ratio (NLR) and the Monocyte-to-lymphocyte ratio (MLR) – as markers of the systemic inflammatory response (Bissacco et al., 2024; Conti et al., 2024; Grillo et al., 2023; Rosina et al., 2024), as well as CSF neurodegeneration biomarkers (α-syn, amyloid, and tau proteins), and the sex hormone levels in a wide cohort of deeply phenotyped male and female PD patients. We then performed a group analysis to assess sex-specific differences in the clinical-biological pattern and a cluster analysis to investigate how inflammatory factors grouped in determining the composite profiles of the disease.

2. Materials and methods

2.1. Study population

This study involved 117 PD patients and 86 controls (CTLs), enrolled at the Neurology Unit of Tor Vergata University Hospital (Rome, Italy) between 2020 and 2024.

PD patients were diagnosed by movement disorder specialists following the 2015 MDS Criteria. Controls were subjects with other non-neurodegenerative and non-inflammatory conditions receiving lumbar puncture for diagnostic purposes (e.g., headache, functional disorders, or PNS diseases). Exclusion criteria for both PD patients and CTLs were main acute/chronic internal/inflammatory/infectious diseases potentially affecting blood counts, recent vaccination (<3 months), immunosuppressant/immunomodulant therapies, other neurodegenerative diseases and/or movement disorders, a history of gynecological/prostate malignancy, and use of hormone therapies.

For all participants, demographic, clinical, and anthropometric data were recorded. PD patients were assessed in the "ON" state (under the effect of dopaminergic therapy) within one week from fluids sampling. Motor severity was evaluated using the MDS Unified Parkinson's Disease Rating Scale Part II, III, and IV (MDS-UPDRS II, III, and IV) (Fahn, S; Elton, 1987) and the Hoehn and Yahr scale (H&Y). Cognitive function was assessed using the mini-mental state examination (MMSE) adjusted for age and educational level (Folstein et al., 1975), and the Montreal cognitive assessment (MoCA) (Nasreddine et al., 2005). Non-motor symptoms were evaluated using the Non-motor Symptoms Scale (NMSS) (Chaudhuri et al., 2007). For all patients, the personal levodopa equivalent daily dose (LEDD, mg/day) was calculated using the conventional formula (Schade et al., 2020).

2.2. Sex hormones assay

Serum sex hormone levels, including estradiol (E2), total testosterone (TT), and gonadotropins (luteinizing hormone - LH, follicular stimulating hormone - FSH), were measured in $n = 112$ PD patients and $n = 34$ CTLs, as previously described (Bovenzi et al., 2023). All blood samples were taken between 8 and 10 a.m. In fertile females ($n = 9$ PD patients and n = 1 HC), sex hormone levels were measured during the follicular phase of their menstrual cycle (days 3 to 9).

2.3. CSF biomarkers assay

CSF was withdrawn via lumbar puncture. The levels of amyloid-β-42 (Aβ42), amyloid-β-40 (Aβ40), total tau (t-tau), phosphorylated-181-tau (p-tau), and total α-syn were measured in $n = 109$ PD patients and $n = 60$ CTLs, as previously described (Bovenzi et al., 2023). The Aβ42/p-tau and the Aβ42/Aβ40 ratios were calculated for all subjects.

2.4. Peripheral immune cell count

Blood was withdrawn within 30 min of the lumbar puncture in a fasting state from all PD patients and $n = 61$ CTLs. It was analyzed in the local lab by using an automated hematological analyzer (Dasit-Sysmex, Milan, Italy).

Leukocyte counts (neutrophils, lymphocytes, monocytes, eosinophils, and basophils) were recorded for all subjects. The neutrophil, lymphocyte, and monocyte counts were used to calculate their ratios and to generate the NLR and the MLR.

2.5. Statistical analysis

The Kolmogorov-Smirnov test was used to preliminarily assess the normal distribution of data. If variables were non-normally distributed, they were Log10-transformed. Qualitative variables between groups were compared using the Chi-square test. Clinical variables between male and female PD patients were compared using an independent two-sample *t*-test and the one-way-ANCOVA test, adjusted for age and disease duration.

Biological variables between male and female PD patients and male and female CTLs were compared using one-way ANOVA with post-hoc Tukey correction and then validated through an ANCOVA test adjusted for age.

Associations between sex hormone levels, inflammatory markers, clinical parameters, and CSF biomarkers were examined using simple Pearson correlations and partial correlations, with age and disease duration as covariates.

The false discovery rate (FDR) method was applied to correct for multiple comparisons and control for type I errors.

Cluster analysis, using the K-means algorithm was employed (Ikotun et al., 2023), was performed to divide the PD cohort into distinct groups based on peripheral immune pathways. Neutrophil, lymphocyte, and monocyte counts were used as variables in the cluster analysis. The K-means method iteratively assigned each data point to one of the K clusters by minimizing the variance within each cluster, aiming to find the optimal centroids. K values considered for the analysis ranged from 2 to 10. A maximum number of 100 was allowed. The silhouette index (Rousseeuw, 1987) was used to evaluate the stability and quality of these clusters. A silhouette score close to 1 indicates well-separated and cohesive clusters, while a score near 0 suggests overlapping clusters. Silhouette scores were repeated 15 times for each K cluster, and the mean values were considered. A Chi-square test and a two-sample *t*-test were used to compare the qualitative and quantitative variables of the clusters found.

3. Results

The demographic, cellular, and biochemical data of the study population are summarized in Table 1, while clinical data are presented in Table 2.

The male-to-female ratio was similar between PD patients and CTLs (M/F = 74/43 and 46/30 respectively, $\chi^2 = 0.15$, $p = 0.703$). Age was similar across the four groups. The main clinical motor and non-motor features, as well as dopaminergic requirements, did not differ between

Table 1
Main demographic, clinical, and biological data of the study population.

	PD Males ($n = 74$)	PD Females ($n = 43$)	Male CTLs ($n = 46$)	Females CTLs ($n = 30$)	F	p-Value
Demographic data	**N = 74**	**N = 43**	**N = 46**	**N = 30**		
Age (years)	62.88 ± 9.70	62.42 ± 9.41	63.50 ± 8.92	64.17 ± 8.60	1.25	$p = 0.86$
Peripheral immune cells	**N = 74**	**N = 43**	***N* = 37**	***N* = 24**		
Leukocytes (10^3/ μL)	6.29 ± 1.27	5.70 ± 1.746	6.65 ± 0.26	6.46 ± 1.80	3.06	**$p = 0.030$**
Neutrophils (10^3/ μL)	3.88 ± 1.06	3.19 ± 1.09	3.88 ± 1.41	3.70 ± 1.18	3.69	**p = 0.013**
Lymphocytes (10^3/ μL)	1.80 ± 0.43	1.81 ± 0.50	2.31 ± 0.66	2.00 ± 0.63	8.98	**$p < 0.001$**
Monocytes (10^3/ μL)	0.50 ± 0.13	0.38 ± 0.12	0.55 ± 0.14	0.48 ± 0.16	11.39	**$p < 0.001$**
Eosinophils (10^3/ μL)	0.18 ± 0.09	0.12 ± 0.06	0.16 ± 0.09	0.19 ± 0.19	2.82	**p = 0.004**
Basophils (10^3/ μL)	0.04 ± 0.02	0.04 ± 0.02	0.04 ± 0.02	0.03 ± 0.02	0.67	$p = 0.57$
NLR	2.29 ± 0.89	1.85 ± 0.73	1.71 ± 0.46	1.96 ± 0.55	6.20	**p = 0.001**
MLR	0.27 ± 0.08	0.24 ± 0.09	0.25 ± 0.09	0.25 ± 0.06	7.05	**p < 0.001**
Sex hormones	***N* = 70**	***N* = 42**	***N* = 16**	***N* = 18**		
E2 (pg/ml)	25.37 ± 15.72	31.67 ± 66.10	15.38 ± 10.71	14.67 ± 15.42	1.23	$p = 0.300$
TT (ng/dl)	481.75 ± 152.81	26.63 ± 14.67	355.97 ± 124.51	21.10 11.08	168.89	**p < 0.001**
FSH (mIU/ml)	5.23 ± 4.10	56.69 ± 26.95	11.43 ± 11.28	60.87 ± 24.54	104.14	**p < 0.001**
LH (mIU/ml)	3.47 ± 2.35	20.89 ± 9.34	4.78 ± 2.88	20.32 ± 9.47	87.49	**p < 0.001**
CSF biomarkers	***N* = 71**	***N* = 38**	***N* = 40**	***N* = 20**		
α-syn (pg/ml)	776.50 ± 296.74	1081.58 ± 335.69	1051.01 ± 391.06	1238.54 ± 466.77	36.02	**p = 0.001**
Aβ42 (pg/ml)	946.04 ± 353.20	1037.79 ± 416.85	894.24 ± 347.36	1203.65 ± 495.34	3.36	**p = 0.020**
Aβ40 (pg/ml)	6415.24 ± 3012.64	6659.55 ± 1875.07	7336.87 ± 3078.65	7901.67 ± 2772.83	1.15	$p = 0.33$
Aβ42/Aβ40 ratio	0.16 ± 0.08	0.16 ± 0.08	0.16 ± 0.04	0.16 ± 0.04	0.12	$p = 0.89$
t-tau (pg/ml)	248.48 ± 131.27	246.50 ± 126.13	234.52 ± 125.18	284.79 ± 150.49	0.66	$p = 0.58$
p-tau (pg/ml)	29.17 ± 17.89	32.67 ± 25.20	30.84 ± 15.11	36.20 ± 17.01	0.80	$p = 0.49$
Aβ42/p-tau ratio	42.67 ± 34.19	40.74 ± 20.87	35.04 ± 22.97	37.08 ± 14.32	0.73	$p = 0.54$

NLR, neutrophil-to-lymphocyte ratio; MLR, monocytes-to-lymphocyte ratio; E2, estradiol; TT, total testosterone; FSH, follicle-stimulating hormone; LH luteinizing hormone; CSF, cerebrospinal fluid; α-syn, total α-synuclein; Aβ42, Amyloid-β-42; Aβ40, Amyloid-β-40; t-tau, total tau; p-tau, 181-phosphorylated tau. F and p-values are obtained by one-way ANOVA between the four groups. Significant results are marked in bold.

Table 2
Differences in demographic and clinical data between male and female PD patients.

	PD Males (n = 74)	PD Females (n = 43)	p-Value
Age (years)	62.88 ± 9.70	62.4 ± 9.41	$p = 0.80$
Disease duration (years)	2.86 ± 2.88	3.52 ± 3.00	$p = 0.25$
AAO (years)	60.72 ± 10.57	58.51 ± 9.62	$p = 0.26$
BMI (kg/m^2)	26.14 ± 3.15	26.50 ± 4.29	$p = 0.65$
H&Y	2.05 ± 0.55	2.20 ± 0.42	$p = 0.13$
MDS UPDRS II	7.98 ± 5.65	9.55 ± 6.15	$p = 0.18$
MDS UPDRS III	28.73 ± 10.47	30.88 ± 13.74	$p = 0.35$
MDS UPDRS IV	0.80 ± 2.00	1.55 ± 3.69	$p = 0.17$
NMSS	37.42 ± 28.68	41.36 ± 28.34	$p = 0.48$
MMSE	28.12 ± 2.20	28.40 ± 2.24	$p = 0.53$
MoCA	25.85 ± 3.93	25.85 ± 2.60	$p = 0.11$
LEDD (mg/day)	245.65 ± 377.04	326.87 ± 364.07	$p = 0.59$

AAO, age at onset; BMI, body mass index; H&Y, Hoehn & Yahr Scale; MDS-UPDRS, Movement Disorder Society Unified Parkinson's Disease Rating Scale; NMSS, Non Motor Symptoms Scale; MMSE, Mini Mental State Examination; MoCA, Montreal Cognitive Assessment Scale; LEDD, levodopa equivalent daily dose.

male and female PD patients.

3.1. Cellular and biochemical differences between groups

A significant difference emerged between groups in all the assessed peripheral immune cells (Table 1).

As shown in Fig. 1, Panel A, the post-hoc analysis showed that males with PD had lower lymphocyte counts than male CTLs ($p < 0.001$). No significant differences emerged in the lymphocyte count between females with PD and female CTLs.

Interestingly, the NLR value was higher in male PD patients than in male CTLs ($p = 0.001$) and female PD patients ($p = 0.013$). However, no intersex differences emerged in the CTLs group (Fig. 1, Panel B).

Furthermore, females with PD had a lower monocyte count than males with PD ($p < 0.001$), and both female ($p = 0.026$) and male CTLs ($p < 0.001$), while no intersex differences emerged in the monocyte count in CTLs (see Fig. 1, Panel C).

Of note, as shown in Fig. 1, Panel D, females with PD had significantly lower MLR than males with PD ($p < 0.001$), while no differences emerged between male and female CTLs.

Additionally, a significant difference emerged between groups in TT and gonadotropin levels, while E2 levels did not differ between the four groups (Table 1). As expected, the post-hoc analysis showed that TT levels were significantly higher in males than females, whereas gonadotropin levels were significantly higher in females compared to males, both in PD patients and CTLs.

Moreover, TT levels were significantly higher in male PD patients compared to male CTLs ($p < 0.001$), even in a model using age as a covariate ($F = 8.56$, $p = 0.004$).

Finally, as for CSF biomarkers, a significant difference emerged in α-syn and Aβ42 levels (Table 1), and the post-hoc analysis showed that male CTLs had significantly lower Aβ42 levels than female CTLs ($p = 0.020$), whereas no differences were found between male and female PD patients. In terms of α-syn, male PD patients had significantly lower levels than female PD patients ($p = 0.022$), even in a model using age and disease duration as covariates ($F = 9.53$, $p = 0.003$).

Male PD patients also had significantly lower α-syn levels than their sex-matched CTLs ($p = 0.005$), while no differences were found between female PD patients and CTLs.

3.2. Correlation analyses in male PD patients

Although peripheral immune cells did not correlate with age, lymphocytes negatively correlated with MoCA scores ($r = -0.390$, $p = 0.014$), independently of age and disease duration (Fig. 2, Panel A).

Both FSH ($r = 0.372, p = 0.001$) and LH ($0.427, p < 0.001$) correlated with age, whereas no correlations were found between age and other sex hormones.

No correlations were found between sex hormones and peripheral

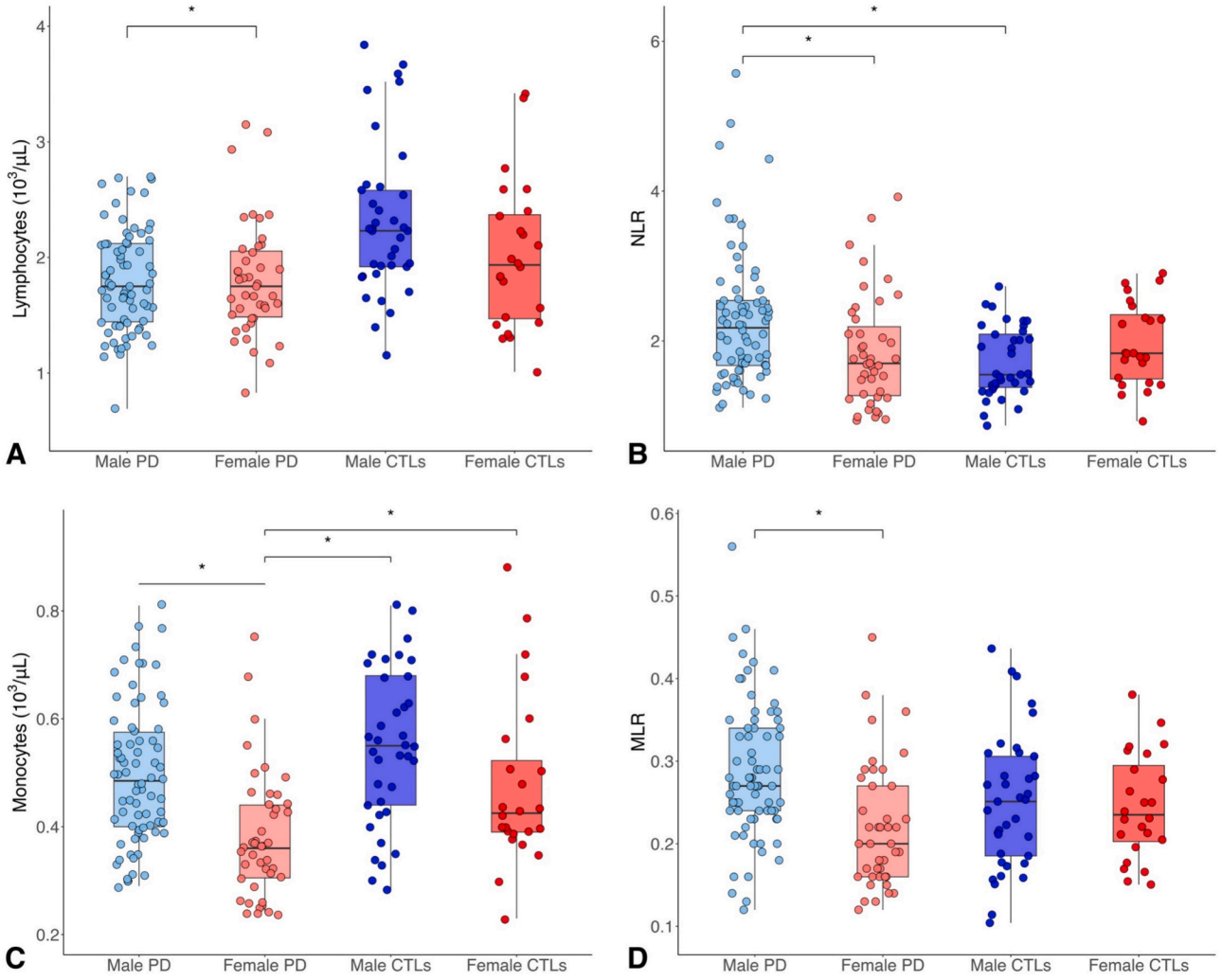

Fig. 1. The figure shows the main quantitative differences in peripheral immune parameters: lymphocytes count (A), monocytes count (C), NLR (B), and MLR (D) between male and female Parkinson's disease patients (PD) and controls (CTLs) using one-way ANOVA with post-hoc Tukey correction. Cell counts are expressed as cells/μL. *Significant differences between groups.

immune cells.

Simple correlations were found between E2 levels and H&Y, MDS-UPDRS part III, and MDS-UPDRS part IV scores ($r = -0.242$, $p = 0.043$, $r = -0.288$, $p = 0.019$, and $r = -2.44$, $p = 0.050$, respectively). However, these findings were lost in the model adjusted for age and disease duration.

Both FSH and LH levels negatively correlated with MoCA scores ($r = -0.268$, $p = 0.034$ and $r = -0.298$, $p = 0.015$); however, these findings were also lost in the model using age and disease duration as covariates.

No correlations between TT and clinical scores, or between any sex hormone level and BMI, were found.

3.3. Correlation analyses in female PD patients

Similarly to what we observed in males, peripheral immune cells did not correlate with age.

No correlations between peripheral immune changes and clinical features were found (Fig. 2, Panel B). However, age negatively correlated with E2 ($r = -0.614$, $p < 0.001$) and positively correlated with FSH and LH levels ($r = 0.491$, $p = 0.001$ and $r = 0.366$, $p = 0.017$, respectively).

No correlations between sex hormones, clinical features, or peripheral immune changes were found.

3.4. Cluster analysis in the PD cohort based on peripheral immune pathway

At this point, we performed a K-means algorithm to divide the PD cohort into distinct clusters based on neutrophil, lymphocyte, and monocyte counts (Fig. 3, Panel A), the three most frequent leukocyte populations, to establish whether peripheral immunity patterns may entail specific clinical-biological profiles. We identified two groups with optimal clustering at K = 2, achieving a mean silhouette index of 0.66 (Fig. 3, Panel B). The centroid of Cluster 1 (*"high peripheral inflammation"*) presented the following coordinates: neutrophils 4.74, lymphocytes 1.87, monocytes 0.49, while the coordinates of the centroid of Cluster 2 (*"low peripheral inflammation"*) were: neutrophils 2.88, lymphocytes 1.75, monocytes 0.43.

The main differences between the two Clusters in demographic, clinical, and biochemical data are reported in Table 3.

4. Discussion

This study aimed to investigate the interplay between the peripheral immune cellular composition, sex hormones, and central markers of neurodegeneration in a broad cohort of PD patients, in order to identify sex-specific immune-biological signatures that drive the peculiar sex dimorphism of the disease.

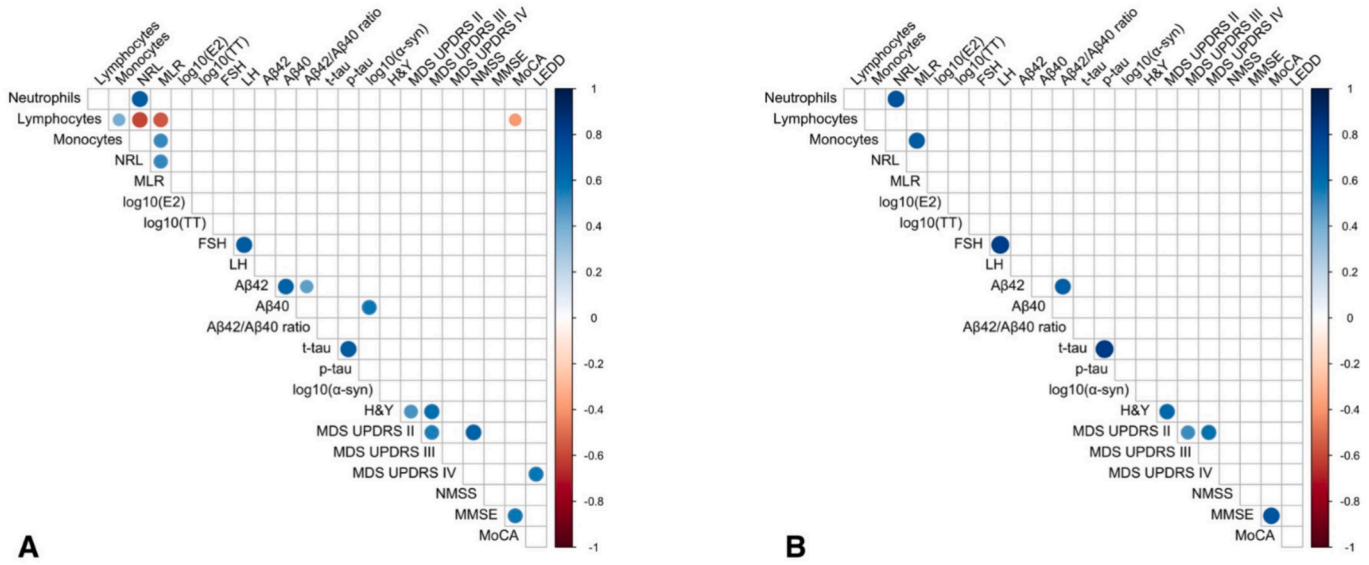

Fig. 2. Correlation matrix showing the significant positive (blue) and negative (red) partial Pearson correlation coefficients (rho) between the main biological and clinical data in male PD patients (Panel A) and female PD patients (Panel B). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

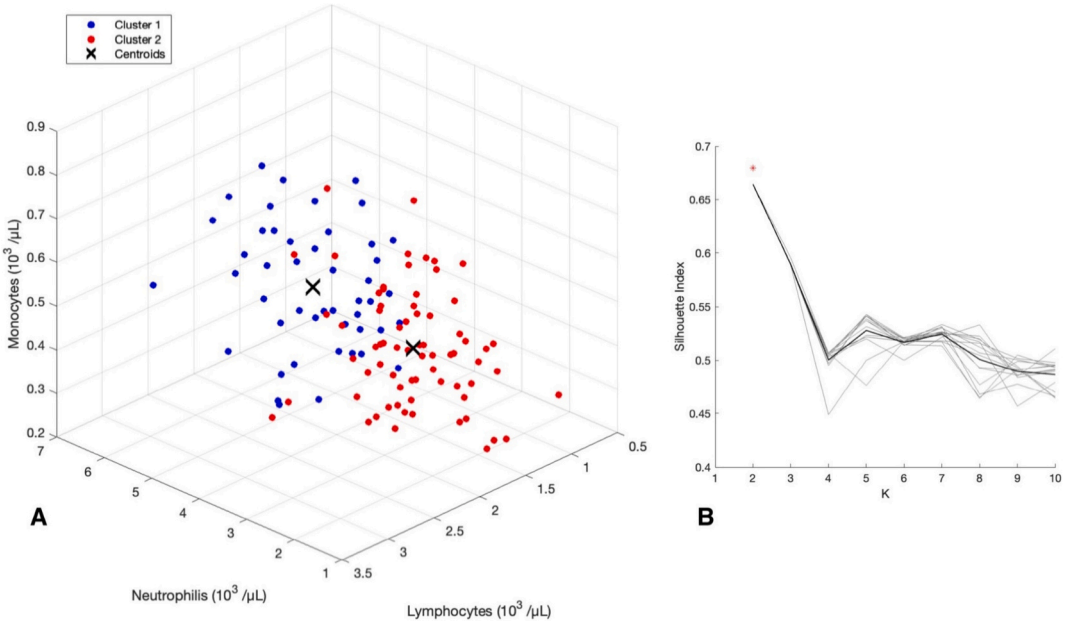

Fig. 3. The figure shows the two clusters identified in the Parkinson's disease cohort using a K-means algorithm based on neutrophil, lymphocyte, and monocyte counts (Panel A). The two groups (Cluster 1, "high peripheral inflammation" and Cluster 2, "low peripheral inflammation", had an optimal clustering at K = 2, achieving a mean silhouette index of 0.66 (Panel B).

First, we found a rearrangement of leukocyte populations between males and females, indicative of a sex-dependent differentiated peripheral inflammatory response. Indeed, while no significant intersex differences in peripheral immune parameters were found in the control group, PD patients demonstrated distinct immune activation signatures based on sex. Notably, male PD patients showed lower lymphocyte counts and a higher NLR than their sex-matched controls, whereas no differences in these parameters were found between female PD and sex-matched controls. On the contrary, we found that females with PD had significantly lower monocyte counts, NLR, and MLR than their male counterparts.

These results align with preclinical and clinical evidence suggesting a sexually dimorphic central and peripheral immune response in PD (Konstantin Nissen et al., 2022; Lauritsen and Romero-Ramos, 2023; Nissen et al., 2021, 2019), especially regarding the macrophagic and monocytic populations (Ferreira et al., 2023; Nissen et al., 2021, 2022; Villa et al., 2018), which may be relevant with regards to the different risk and progression of PD encountered between the two sexes (Nissen et al., 2019).

Along with the monocytic response, the reduction in lymphocytic count and the increase in NLR are well-described markers of both peripheral immune activation and disease severity in PD, possibly reflecting the central migration of lymphocytes participating in the neurodegenerative process (Bissacco et al., 2024; Grillo et al., 2023; Lindestam Arlehamn et al., 2020). Here, we found that only males with PD presented lower lymphocytes and higher NLR than controls.

Table 3
Differences in demographic, clinical, and biological data between Cluster 1 and Cluster 2.

	Cluster 1 *"High peripheral inflammation"* (*n* = 57)	Cluster 2 *"Low peripheral inflammation"* (n = 60)	p-Value
Demographic and clinical data			
Sex (M/F)	43/14	31/29	**p = 0.003**
Age (years)	62.47 ± 9.05	62.93 ± 10.10	p = 0.80
Disease duration (years)	3.31 ± 2.95	2.91 ± 2.93	*p* = 0.47
AAO (years)	59.74 ± 9.64	60.07 ± 10.88	p = 0.86
H&Y	2.05 ± 0.55	2.16 ± 0.47	p = 0.25
MDS UPDRS II	8.09 ± 5.44	9.02 ± 6.26	*p* = 0.41
MDS UPDRS III	31.05 ± 11.50	26.57 ± 10.15	***p* = 0.031**
MDS UPDRS IV	1.07 ± 2.89	1.09 ± 2.67	*p* = 0.98
NMSS	37.86 ± 26.50	39.93 ± 30.59	p = 0.70
MMSE	28.11 ± 2.66	28.34 ± 1.67	p = 0.59
MoCA	24.06 ± 4.31	25.62 ± 2.25	***p* = 0.023**
LEDD (mg/day)	271.79 ± 361.60	264.56 ± 368.93	*p* = 0.92
Peripheral immune cell Ratios			
NLR	2.56 ± 0.87	1.72 ± 0.62	**p < 0.001**
MLR	0.27 ± 0.09	0.25 ± 0.08	*p* = 0.32
Sex hormones			
E2 (pg/ml)	26.16 ± 29.12	29.25 ± 51.96	p = 0.70
TT (ng/dl)	362.97 ± 226.72	265.20 ± 267.46	***p* = 0.040**
FSH (mIU/ml)	16.84 ± 25.53	32.80 ± 32.20	**p = 0.004**
LH (mIU/ml)	7.20 ± 8.66	12.64 ± 11.19	**p = 0.005**
CSF biomarkers			
α-syn (pg/ml)	771.58 ± 259.03	951.47 ± 332.33	***p* = 0.039**
Aβ42 (pg/ml)	1018.35 ± 420.07	941.24 ± 332.99	*p* = 0.29
Aβ40 (pg/ml)	6689.87 ± 3048.24	6322.38 ± 2243.52	p = 0.54
Aβ42/Aβ40 ratio	0.17 ± 0.08	0.15 ± 0.07	p = 0.41
t-tau (pg/ml)	244.48 ± 107.83	250.81 ± 146.44	p = 0.80
p-tau (pg/ml)	28.41 ± 14.07	32.19 ± 25.25	*p* = 0.34
Aβ42/p-tau ratio	40.07 ± 15.51	43.75 ± 39.05	p = 0.53

AAO, age at onset; BMI, body mass index; H&Y, Hoehn & Yahr Scale; MDS-UPDRS, Movement Disorder Society Unified Parkinson's Disease Rating Scale; NMSS, Non Motor Symptoms Scale; MMSE, Mini Mental State Examination; MoCA, Montreal Cognitive Assessment Scale; LEDD, levodopa equivalent daily dose; NLR, neutrophil-to-lymphocyte ratio; MLR, monocytes-to-lymphocyte ratio; E2, estradiol; TT, total testosterone; FSH, follicle-stimulating hormone; LH luteinizing hormone; CSF, cerebrospinal fluid; α-syn, total α-synuclein; Aβ42, Amyloid-β-42; Aβ40, Amyloid-β-40; t-tau, total tau; p-tau, 181-phosphorylated tau. Significant results are marked in bold.

Furthermore, the lymphocyte count was correlated with cognitive performance at MoCA scores exclusively in the male population, confirming the highly relevant role of immune changes in cognition (Konstantin Nissen et al., 2022; Nissen et al., 2021), and suggesting a predominant role in the sex-specific vulnerability of males to cognitive impairment (Caminiti et al., 2025). On the other hand, peripheral immune changes in females with PD did not grossly differ from sex-matched healthy controls and did not correlate with any clinical features or central markers of neurodegeneration, unlike males, possibly indicating a more contained peripheral immune response in this group.

We then investigated whether such sex-specific rearrangement of the leukocyte population could be linked to circulating sex hormone levels. We found no specific relationships between sex-dimorphic peripheral immune changes and steroidal sex hormones, either estradiol or total testosterone.

As the main finding of this study, we then performed an unbiased cluster analysis of the most abundant leukocyte populations (neutrophils, lymphocytes, and monocytes) in the PD cohort, finding two main clusters of the peripheral inflammatory response, which were tightly related to the two sexes. Indeed, female PD patients mainly matched the "low peripheral inflammation" Cluster, whereas males mostly reflected the "high peripheral inflammation" Cluster. The two clusters, identified by different signatures of the peripheral leukocytic immune composition, not only differed in the male-to-female ratio but also showed distinct clinical and biological features. Notably, patients in the "high peripheral inflammation" Cluster generally had higher testosterone and lower gonadotropin levels, worse motor and cognitive scores, as shown by higher MDS-UPDRS part III and lower MoCA scores, and a higher load of central synucleinopathy, indicating a more severe underlying neurodegenerative process. On the other hand, the second Cluster, which shaped a "low peripheral inflammation" profile, was characterized by lower motor and cognitive impairment and a more contained central synuclein burden.

The inflammatory activation is closely and bi-directionally linked to the central and peripheral synucleinopathy that characterizes PD neuropathology. Misfolded α-syn triggers inflammatory changes, and the inflammatory response promotes prion-like mechanisms involved in the misfolding cascade (Di Lazzaro et al., 2024). Therefore, the association between a distinct peripheral immune cellular composition and greater central synucleinopathy encountered in Cluster 1, mostly comprising male patients, would indicate a more widespread neurodegenerative process in this group of patients, consistent with a more rapid motor and cognitive progression over time encountered among males (Picillo et al., 2022).

Together, these findings provide direct human-based support on the intimate relationship between differential motor and cognitive trajectories in males and females (Picillo et al., 2022), sex-dimorphic immune response (Lucero et al., 2024), and neuropathological burden in PD.

This study has some limitations. First, the cross-sectional design could not allow an observatory evaluation of clinical and biological features over time. Second, we did not perform a deep immunophenotyping of immune cell subsets, nor did we evaluate other peripheral inflammatory mediators (i.e., cytokines or lipid mediators (Chiurchiù et al., 2022; Stampanoni Bassi et al., 2024)). Third, we could not exclude the potential confounding effect of the dopaminergic therapy on clinical and biological parameters or sex hormone production, albeit no gross correlations between levodopa requirements and biological data were found. Finally, we did not investigate specific motor or non-motor PD features, such as gastrointestinal dysfunction, which has been tightly linked to the NLR (Grillo et al., 2023, 2022).

5. Conclusions

In conclusion, despite the need for more studies, possibly contemplating the evaluation of other peripheral and central immune markers and/or lymphocytic subpopulations and a larger number of patients, to confirm and extend these results, this study demonstrates how variations in peripheral immune cellular composition might entail distinct clinical-biological profiles with substantial sex segregation. Specifically, a stronger immune activation was mostly noticed in males, in association with higher testosterone and lower gonadotropin levels, greater motor and cognitive impairment, and heavier central synucleinopathy. Conversely, a milder inflammatory response was mostly observed in females with a lesser clinical-pathological burden.

CRediT authorship contribution statement

Roberta Bovenzi: Writing – original draft. **Matteo Conti:** Validation, Methodology, Investigation, Formal analysis, Data curation. **Clara Simonetta:** Visualization, Investigation, Data curation. **Jacopo Bissacco:** Visualization, Investigation, Data curation. **Davide Mascioli:**

Visualization, Investigation, Data curation. **Maria Mancini:** Visualization, Investigation, Data curation. **Veronica Buttarazzi:** Visualization, Investigation, Data curation. **Federica Veltri:** Visualization, Investigation, Data curation. **Giulia Maria Sancesario:** Visualization, Investigation, Data curation. **Silvio Bagetta:** Visualization, Investigation, Data curation. **Francesca D'Amaro:** Visualization, Investigation, Data curation. **Massimo Pieri:** Visualization, Investigation, Data curation. **Rocco Cerroni:** Visualization, Resources. **Claudio Liguori:** Writing – review & editing, Validation, Resources, Funding acquisition. **Valerio Chiurchiù:** Writing – review & editing, Validation, Resources, Funding acquisition. **Mariangela Pierantozzi:** Writing – review & editing, Validation, Resources, Funding acquisition. **Alessandro Stefani:** Writing – review & editing, Validation, Resources, Funding acquisition. **Nicola Biagio Mercuri:** Writing – review & editing, Validation, Resources, Funding acquisition. **Tommaso Schirinzi:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization.

Ethics approval and consent to participate

The study was conducted in agreement with the principles of the Helsinki declarations. The study was approved by the Ethics Committee of the University of Tor Vergata (protocol number 0026092/2017). Informed consent was obtained from each participant in this study.

Funding

The research leading to these results has received funding from the European Union (NextGenerationEU) through the Italian Ministry of University and Research (MUR) under PNRR–M4C2-I1.3 Project PE_00000019 "Heal Italia" to TS; trough the MUR under PNRR project MNESYS (PE0000006) to NMB, MP, CL, and AS; through the Ministry of Health project PNRR-MAD-2022-12376556 to NMB, VC and RB. The views and opinions expressed are those of the authors only and do not necessarily reflect those of the European Union or the European Commission. This work was also supported by the MUR PRIN 20222FYK9R to TS.

Declaration of competing interest

None.

Acknowledgments

None.

Data availability

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

References

Bissacco, J., Simonetta, C., Mascioli, D., Zenuni, H., Bovenzi, R., Grillo, P., Di Giuliano, F., Stefani, A., Mercuri, N.B., Schirinzi, T., 2024. Peripheral immunity changes are associated with neurodegeneration and worse clinical outcome in idiopathic normal pressure hydrocephalus. Eur. J. Neurol. 31. https://doi.org/10.1111/ene.16179.

Bovenzi, R., Sancesario, G.M., Conti, M., Grillo, P., Cerroni, R., Bissacco, J., Forti, P., Giannella, E., Pieri, M., Minosse, S., Ferrazzoli, V., Pucci, N., Laudazi, M., Floris, R., Garaci, F., Pierantozzi, M., Stefani, A., Mercuri, N.B., Picchi, E., Di Giuliano, F., Schirinzi, T., 2023. Sex hormones differentially contribute to Parkinson's disease in males: a multimodal biomarker study. Eur. J. Neurol. https://doi.org/10.1111/ene.15801.

Bovenzi, R., Conti, M., De Franco, V., Pierantozzi, M., Schirinzi, T., Cerroni, R., Stefani, A., Mercuri, N.B., Liguori, C., 2024a. Sex differences in Parkinson's disease-related non motor symptoms: a focus on sleep problems. Acta Neurol. Belg. https://doi.org/10.1007/s13760-024-02535-8.

Bovenzi, R., Schirinzi, T., Conti, M., Sancesario, G.M., Zenuni, H., Simonetta, C., Bissacco, J., Mascioli, D., Pieri, M., Cerroni, R., Stefani, A., Mercuri, N.B., Pierantozzi, M., 2024b. A biological characterization of patients with postmenopausal Parkinson's disease. J. Neurol. https://doi.org/10.1007/s00415-024-12258-8.

Caminiti, S.P., Avenali, M., Galli, A., Malito, R., Cuconato, G., Galandra, C., Calabrese, R., Pilotto, A., Padovani, A., Blandini, F., Perani, D., Tassorelli, C., Valente, E.M., 2025. Male sex accelerates cognitive decline in GBA1 Parkinson's disease. NPJ Parkinsons Dis. 11, 41. https://doi.org/10.1038/s41531-025-00883-7.

Chaudhuri, K.R., Martinez-Martin, P., Brown, R.G., Sethi, K., Stocchi, F., Odin, P., Ondo, W., Abe, K., MacPhee, G., MacMahon, D., Barone, P., Rabey, M., Forbes, A., Breen, K., Tluk, S., Naidu, Y., Olanow, W., Williams, A.J., Thomas, S., Rye, D., Tsuboi, Y., Hand, A., Schapira, A.H.V., 2007. The metric properties of a novel non-motor symptoms scale for Parkinson's disease: results from an international pilot study. Mov. Disord. https://doi.org/10.1002/mds.21596.

Chiurchiù, V., Tiberi, M., Matteocci, A., Fazio, F., Siffeti, H., Saracini, S., Mercuri, N.B., Sancesario, G., 2022. Lipidomics of bioactive lipids in Alzheimer's and Parkinson's diseases: where are we? Int. J. Mol. Sci. https://doi.org/10.3390/ijms23116235.

Conti, M., Cirillo, F., Maio, S., Fernandes, M., Bovenzi, R., Placidi, F., Izzi, F., Mercuri, N. B., Liguori, C., 2024. Increased neutrophil to lymphocyte ratio as a possible marker to detect neuroinflammation in patients with narcolepsy type 1. J. Clin. Sleep Med. https://doi.org/10.5664/jcsm.11368.

Conti, M., Bovenzi, R., Pierantozzi, M., Simonetta, C., Ferrari, V., Bissacco, J., Cerroni, R., Liguori, C., Di Giuliano, F., Mercuri, N.B., Schirinzi, T., Stefani, A., 2025. Sex hormones shape EEG-based functional connectivity in early-stage Parkinson's disease patients. Neuroimage Clin. 45, 103721. https://doi.org/10.1016/j.nicl.2024.103721.

Di Lazzaro, G., Picca, A., Boldrini, S., Bove, F., Marzetti, E., Petracca, M., Piano, C., Bentivoglio, A.R., Calabresi, P., 2024. Differential profiles of serum cytokines in Parkinson's disease according to disease duration. Neurobiol. Dis. 190. https://doi.org/10.1016/j.nbd.2023.106371.

Fahn, S., Elton, R.M. of the U.D.Committee, 1987. The Unified Parkinson's Disease Rating Scale. Recent Developments in Parkinson's Disease, Vol 2.

Ferreira, S.A., Li, C., Klæstrup, I.H., Vitic, Z., Rasmussen, R.K., Kirkegaard, A., Toft, G.U., Betzer, C., Svendsen, P., Jensen, P.H., Luo, Y., Etzerodt, A., Moestrup, S.K., Romero-Ramos, M., 2023. Sex-dimorphic neuroprotective effect of CD163 in an α-synuclein mouse model of Parkinson's disease. NPJ Parkinsons Dis. 9. https://doi.org/10.1038/s41531-023-00606-w.

Folstein, M.F., Folstein, S.E., McHugh, P.R., 1975. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J. Psychiatr. Res. https://doi.org/10.1016/0022-3956(75)90026-6.

Grillo, P., Sancesario, G.M., Mascioli, D., Geusa, L., Zenuni, H., Giannella, E., Della Morte, D., Mercuri, N.B., Schirinzi, T., 2022. Constipation distinguishes different clinical-biochemical patterns in de novo Parkinson's disease. Parkinsonism Relat. Disord. 102, 64–67. https://doi.org/10.1016/j.parkreldis.2022.08.001.

Grillo, P., Sancesario, G.M., Bovenzi, R., Zenuni, H., Bissacco, J., Mascioli, D., Simonetta, C., Forti, P., Degoli, G.R., Pieri, M., Chiurchiù, V., Stefani, A., Mercuri, N. B., Schirinzi, T., 2023. Neutrophil-to-lymphocyte ratio and lymphocyte count reflect alterations in central neurodegeneration-associated proteins and clinical severity in Parkinson disease patients. Parkinsonism Relat. Disord. 112. https://doi.org/10.1016/j.parkreldis.2023.105480.

Ikotun, A.M., Ezugwu, A.E., Abualigah, L., Abuhaija, B., Heming, J., 2023. K-means clustering algorithms: a comprehensive review, variants analysis, and advances in the era of big data. Inf. Sci. (N Y) 622. https://doi.org/10.1016/j.ins.2022.11.139.

Kalia, LV, Lang, AE, 2015 Aug 29. Parkinson's disease. Lancet 386 (9996), 896–912. https://doi.org/10.1016/S0140-6736(14)61393-3. Epub 2015 Apr 19. PMID: 25904081.

Konstantin Nissen, S., Farmen, K., Carstensen, M., Schulte, C., Goldeck, D., Brockmann, K., Romero-Ramos, M., 2022. Changes in CD163+, CD11b+, and CCR2 + peripheral monocytes relate to Parkinson's disease and cognition. Brain Behav. Immun. 101. https://doi.org/10.1016/j.bbi.2022.01.005.

Lauritsen, J., Romero-Ramos, M., 2023. The systemic immune response in Parkinson's disease: focus on the peripheral immune component. Trends Neurosci. https://doi.org/10.1016/j.tins.2023.07.005.

Lindestam Arlehamn, C.S., Dhanwani, R., Pham, J., Kuan, R., Frazier, A., Rezende Dutra, J., Phillips, E., Mallal, S., Roederer, M., Marder, K.S., Amara, A.W., Standaert, D.G., Goldman, J.G., Litvan, I., Peters, B., Sulzer, D., Sette, A., 2020. A-Synuclein-specific T cell reactivity is associated with preclinical and early Parkinson's disease. Nat. Commun. 11. https://doi.org/10.1038/s41467-020-15626-w.

Lopez-Lee, C., Kodama, L., Gan, L., 2022. Sex differences in neurodegeneration: the role of the immune system in humans. Biol. Psychiatry. https://doi.org/10.1016/j.biopsych.2021.01.002.

Lucero, J., Gurnani, A., Weinberg, J., Shih, L.C., 2024. Neutrophil-to-lymphocyte ratio and longitudinal cognitive performance in Parkinson's disease. Ann. Clin. Transl. Neurol. https://doi.org/10.1002/acn3.52144.

Madetko, N., Migda, B., Alster, P., Turski, P., Koziorowski, D., Friedman, A., 2022. Platelet-to-lymphocyte ratio and neutrophil-to-lymphocyte ratio may reflect differences in PD and MSA-P neuroinflammation patterns. Neurol. Neurochir. Pol. 56. https://doi.org/10.5603/PJNNS.a2022.0014.

Massaro Cenere, M., Tiberi, M., Paldino, E., D'Addario, S.L., Federici, M., Giacomet, C., Cutuli, D., Matteocci, A., Cossa, F., Zarrilli, B., Casadei, N., Ledonne, A., Petrosini, L., Berretta, N., Fusco, F.R., Chiurchiù, V., Mercuri, N.B., 2024. Systemic inflammation accelerates neurodegeneration in a rat model of Parkinson's disease overexpressing human alpha synuclein. NPJ Parkinsons Dis. 10, 213. https://doi.org/10.1038/s41531-024-00824-w.

Moisan, F., Kab, S., Mohamed, F., Canonico, M., Le Guern, M., Quintin, C., Carcaillon, L., Nicolau, J., Duport, N., Singh-Manoux, A., Boussac-Zarebska, M., Elbaz, A., 2016. Parkinson disease male-to-female ratios increase with age: French nationwide study

and meta-analysis. J. Neurol. Neurosurg. Psychiatry. https://doi.org/10.1136/jnnp-2015-312283.

Nasreddine, Z.S., Phillips, N.A., Bédirian, V., Charbonneau, S., Whitehead, V., Collin, I., Cummings, J.L., Chertkow, H., 2005. The Montreal cognitive assessment, MoCA: a brief screening tool for mild cognitive impairment. J. Am. Geriatr. Soc. https://doi.org/10.1111/j.1532-5415.2005.53221.x.

Nissen, S.K., Shrivastava, K., Schulte, C., Otzen, D.E., Goldeck, D., Berg, D., Møller, H.J., Maetzler, W., Romero-Ramos, M., 2019. Alterations in blood monocyte functions in Parkinson's disease. Mov. Disord. 34. https://doi.org/10.1002/mds.27815.

Nissen, S.K., Ferreira, S.A., Nielsen, M.C., Schulte, C., Shrivastava, K., Hennig, D., Etzerodt, A., Graversen, J.H., Berg, D., Maetzler, W., Panhelainen, A., Møller, H.J., Brockmann, K., Romero-Ramos, M., 2021. Soluble CD163 changes indicate monocyte association with cognitive deficits in Parkinson's disease. Mov. Disord. 36. https://doi.org/10.1002/mds.28424.

Konstantin Nissen, S., Farmen, K., Carstensen, M., Schulte, C., Goldeck, D., Brockmann, K., Romero-Ramos, M., 2022 Mar. Changes in CD163+, CD11b+, and CCR2+ peripheral monocytes relate to Parkinson's disease and cognition. Brain Behav Immun. 101, 182–193. https://doi.org/10.1016/j.bbi.2022.01.005. Epub 2022 Jan 10. PMID: 35026420.

Picillo, M., Lafontant, D.E., Bressman, S., Caspell-Garcia, C., Coffey, C., Cho, H.R., Burghardt, E.L., Dahodwala, N., Saunders-Pullman, R., Tanner, C.M., Amara, A.W., 2022. Sex-related longitudinal change of motor, non-motor, and biological features in early Parkinson's disease. J. Parkinsons Dis. 12. https://doi.org/10.3233/JPD-212892.

Rosina, M., Veltri, F., Nesci, V., Bissacco, J., Bovenzi, R., Mascioli, D., Simonetta, C., Zenuni, H., Maftei, D., Marano, M., Pierantozzi, M., Stefani, A., Chiurchiù, V., Longone, P., Valle, C., Mercuri, N.B., Ferri, A., Schirinzi, T., 2024. Immunometabolic signature and tauopathy markers in blood cells of progressive supranuclear palsy. Mov. Disord. https://doi.org/10.1002/mds.30009.

Rousseeuw, P.J., 1987. Silhouettes: a graphical aid to the interpretation and validation of cluster analysis. J. Comput. Appl. Math. 20. https://doi.org/10.1016/0377-0427(87)90125-7.

Schade, S., Mollenhauer, B., Trenkwalder, C., 2020. Levodopa equivalent dose conversion factors: an updated proposal including opicapone and safinamide. Mov. Disord. Clin. Pract. https://doi.org/10.1002/mdc3.12921.

Sciarra, F., Campolo, F., Franceschini, E., Carlomagno, F., Venneri, M.A., 2023. Gender-specific impact of sex hormones on the immune system. Int. J. Mol. Sci. https://doi.org/10.3390/ijms24076302.

Stampanoni Bassi, M., Gilio, L., Galifi, G., Buttari, F., Dolcetti, E., Bruno, A., Belli, L., Modugno, N., Furlan, R., Finardi, A., Mandolesi, G., Musella, A., Centonze, D., Olivola, E., 2024. Mood disturbances in newly diagnosed Parkinson's disease patients reflect intrathecal inflammation. Parkinsonism Relat. Disord. 122. https://doi.org/10.1016/j.parkreldis.2024.106071.

Villa, A., Gelosa, P., Castiglioni, L., Cimino, M., Rizzi, N., Pepe, G., Lolli, F., Marcello, E., Sironi, L., Vegeto, E., Maggi, A., 2018. Sex-specific features of microglia from adult mice. Cell Rep. 23. https://doi.org/10.1016/j.celrep.2018.05.048.