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ORIGINAL ARTICLE

Fecal Calprotectin in Parkinson's Disease and Multiple System Atrophy

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ABSTRACT

Objective Converging evidence suggests that intestinal inflammation is involved in the pathogenesis of neurodegenerative diseases. Previous studies on fecal calprotectin in Parkinson's disease (PD) were limited by small sample sizes, and literature regarding intestinal inflammation in multiple system atrophy (MSA) is very scarce. We investigated the levels of fecal calprotectin, a marker of intestinal inflammation, in PD and MSA.

Methods We recruited 169 subjects (71 PD, 38 MSA, and 60 age-similar nonneurological controls). Clinico-demographic data were collected. PD and MSA were subtyped and the severity assessed using the MDS-UPDRS and UMSARS, respectively. Fecal calprotectin and blood immune markers were analyzed.

Results Compared to controls (median: 35.7 [IQR: 114.2] μg/g), fecal calprotectin was significantly elevated in PD (median: 95.6 [IQR: 162.1] μg/g, $p = 0.003$) and even higher in MSA (median: 129.5 [IQR: 373.8] μg/g, $p = 0.002$). A significant interaction effect with age was observed; between-group differences were significant only in older subjects (i.e., ≥ 61 years) and became more apparent with increasing age. A total of 28.9% of MSA and 18.3% of PD patients had highly abnormal fecal calprotectin levels (≥ 250 μg/g); however, this difference was only significant for MSA compared to controls. Fecal calprotectin correlated moderately with selected blood immune markers in PD, but not with clinical features of PD or MSA.

Conclusions Elevated fecal calprotectin suggests a role for intestinal inflammation in PD and MSA. A more complete understanding of gut immune alterations could open up new avenues of research and treatment for these debilitating diseases.

Keywords Fecal calprotectin; Intestinal inflammation; Multiple system atrophy; Parkinson's disease.

Parkinson's disease (PD) and multiple system atrophy (MSA) are neurodegenerative disorders that are pathologically characterized by the accumulation of insoluble α-synuclein protein in the nervous system, mainly in neurons in the case of PD and glial cells in MSA. Clinically, in addition to having overlapping motor features, patients with PD and MSA commonly experience gastrointestinal (GI) symptoms such as constipation, and pathological α-synuclein has also been found within the enteric ner-

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vous system in both conditions.[1] Therefore, there has been a growing recognition of the importance of the "gut-brain axis" in the pathogenesis of these disorders, especially in PD,[2,3] including the dysregulation of immune responses in the gut and brain.[4]

Studies have demonstrated significantly altered gut microbiome compositions in patients with PD and MSA compared to controls.[3,5-8] In a PD mouse model study, gut microbes were shown to promote neuroinflammatory responses, leading to increased motor disability and α-synuclein deposition in the brain.[9] Interestingly, several colonic biopsy and fecal studies found evidence of intestinal inflammation in PD, somewhat akin to the changes seen in inflammatory bowel disease (IBD).[10] Devos et al.[10] reported increased mRNA expression of the main proinflammatory cytokines (tumor necrosis factor alpha [TNF-α], interferon gamma [IFN-γ], interleukin 6 [IL-6], and interleukin 1 beta [IL-1β]) in colonic biopsies from 19 PD patients; subsequently, Houser et al.[11] reported elevated levels of IL-1α, 1β, CXCL8, and C-reactive protein in the stool of PD patients. Other studies have shown an epidemiological and genetic link between IBD and PD.[12] Meanwhile, the literature regarding intestinal inflammation in MSA remains very scarce, involving a total of only 12 patients from two studies, which had conflicting results.[12,13] Specifically, Engen et al.[13] performed immunohistochemistry studies of the colonic sigmoid mucosa from 6 MSA patients and found increased lamina propria expression of the endotoxin-related inflammation marker Toll-like receptor 4 (TLR4). In contrast, Rolli-Derkinderen et al.[12] found increased mRNA expression of proinflammatory cytokines in colonic biopsies only in PD (n = 29) but not MSA (n = 6) patients.

Calprotectin is a protein released predominantly from neutrophils during an inflammatory response, with a lesser contribution from monocytes and macrophages.[14] It accounts for approximately 60% of the cytosolic protein in neutrophils and has a direct antimicrobial effect.[14] It is found in various body fluids in proportion to the severity of any existing inflammation; in the healthy state, its concentration in feces is approximately ×6 that of its concentration in plasma.[14] In bowel inflammation, calprotectin is released into the gut lumen by inflammatory cells infiltrating the mucosa. As a noninvasive biomarker of intestinal inflammation, fecal calprotectin is currently widely used for diagnosis and treatment decision-making in IBD.[14,15] Recently, it has emerged as a potentially useful research marker for intestinal inflammation in PD,[16] with three cross-sectional case-control studies (n = 34–55 patients) reporting higher levels in PD patients than in controls.[16-18] To our knowledge, there are no published studies on fecal calprotectin in MSA.

In this study, we aimed to validate the findings of elevated fecal calprotectin in a larger cohort of PD patients and to compare this with MSA patients and nonneurological controls. We also aimed to investigate the correlations between fecal calprotectin and the clinical phenotype, including standardized and validated measures of clinical disease severity in PD and MSA.

MATERIALS & METHODS

Subjects

This study was approved by the medical research ethics committees of the University of Malaya Medical Centre (UMMC; ID No.: 201837-6089) and University Kebangsaan Malaysia Medical Centre (UKMMC; ID No.: FF-2019-258), and conducted in accordance with the Declaration of Helsinki. Consecutive patients with PD and MSA attending neurology clinics were recruited by movement disorder neurologists (SYL, AHT, and NMI), together with spousal controls without neurological disorders. Written informed consent was obtained from all subjects. "Clinically probable" PD and MSA were diagnosed according to consensus criteria.[3,5] The exclusion criteria were antibiotic use within the preceding 3 months, probiotic use within the preceding month, a history of colorectal disease or major abdominal/pelvic surgery, long-term care residence, tube feeding, or an inability to complete the study assessments. Additional exclusion criteria for patients were antiparkinsonian medication initiation within the preceding 3 months and adjustment within the preceding month.

Clinical evaluation

Demographic data were collected. Clinical evaluations included body mass index (BMI), assessment of constipation and cognitive function using the Patient Assessment of Constipation-Symptoms (PAC-SYM), and the Montreal Cognitive Assessment (MoCA). The PD severity was evaluated using the International Parkinson and Movement Disorder Society-Unified PD Rating Scale (MDS-UPDRS) during the ON medication state, while MSA severity was evaluated using the Unified MSA Rating Scale (UMSARS).

Fecal sample collection and calprotectin analysis

Subjects were provided a written protocol for optimal fecal collection (how to avoid sample contamination) and transportation methods. Fecal samples were collected at home by the subjects in sterile containers and were immediately placed on ice. Upon arrival at UMMC or UKMMC, the samples were aliquoted and immediately frozen at -20°C (in most cases, this occurred within 1–3 hours of collection). Fecal calprotectin was measured using an enzyme-linked immunosorbent assay (ELISA; EUROIMMUN Medizinische Labordiagnostika AG; Lubeck, Germany) following the manufacturer's instructions.

Peripheral blood counts and immunological evaluations

Blood samples were obtained between 8.00–10.00 am after an overnight fast in a subset of subjects (24 PD, 18 MSA, and 56 controls) and immediately sent to the UMMC laboratory for full blood count analysis. The remaining samples were centrifuged within 2 hours of collection, and the extracted serum was immediately stored at -80°C until further analysis. Serum levels of cytokines and their precursors (TNFα, IFNγ, IL-1b, IL-4, IL-6, and IL-17, nuclear factor-kappa-light-chain-enhancer of activated B cells [NF-κb] and caspase-1) were measured using double-antibody sandwich ELISA (Sunred Biological Technology, Shanghai, China).

Statistical analysis

Analyses were performed using SPSS version 21 (IBM Corp., Armonk, NY, USA). Descriptive data for the outcomes were expressed as the mean ± standard deviation (for normally distributed data) or median with interquartile range (nonnormally distributed data). The Shapiro-Wilk test was used to determine the normality of the data before comparisons were made. Categorical data were analyzed using the chi-square test, while continuous data were analyzed using one-way analysis of variance (ANOVA) with Tukey's honestly significant difference (HSD) test, the Kruskal-Wallis test or the Mann-Whitney test. Generalized linear models were used to investigate the effects of age and constipation severity on between-group differences in fecal calprotectin. Correlation analyses were conducted using Spearman's rank correlation coefficient test. Two-sided p values < 0.05 were considered to indicate statistical significance. Graphics were generated using the ggplot2 R package in R (version 3.6.3; R Development Core Team, Vienna, Austria).

RESULTS

The demographic and clinical characteristics of the subjects are summarized in Table 1. The PD patients were significantly older than the MSA patients and controls. The MSA and PD patients had significantly worse constipation severity than the controls. Two patients with PD were taking fiber supplements.

Between-group differences in fecal calprotectin

Compared to the controls (median: 35.7 [IQR: 114.2] μg/g of stool), fecal calprotectin levels were significantly elevated in PD (median: 95.6 [IQR: 162.1] μg/g, $p = 0.003$) and even higher in MSA (median: 129.5 [IQR: 373.8] μg/g, $p = 0.002$) (Figure 1). There was no significant difference in fecal calprotectin levels between PD and MSA patients ($p = 0.405$). Using a cutoff value of ≥ 250 μg/g,[15] 28.9% of MSA patients and 18.3% of PD patients had abnormal fecal calprotectin levels, which were significantly higher than that of controls (8.3%, $p = 0.029$); *post hoc* analyses revealed that this difference was significant for MSA vs. controls ($p = 0.007$) but not between PD vs. controls ($p = 0.098$) or between PD vs. MSA ($p = 0.202$) (Figure 1).

As fecal calprotectin has been reported to be higher in older people,[14] we examined the effects of age on the between-group differences in fecal calprotectin. We found significant interaction effects between age and disease status in the calprotectin comparisons between PD vs. controls and between MSA vs. controls. These interaction effects are depicted in scatter plots (Figure 2A and B), where the differences in fecal calprotectin levels between PD and controls and between MSA and controls are only apparent in older subjects and become more apparent with increasing age. When the subjects were dichotomized using an age cutoff of 60 years, there were significant differences in fecal calprotectin levels between PD (median: 108.0 [IQR: 185.5] μg/g, $p = 0.002$) and controls (median: 39.0 [IQR: 113.2] μg/g), and between MSA (median: 150.4 [IQR: 351.9] μg/g, $p = 0.011$) and controls, in those aged ≥ 61 years (Figure 2C and D). However, there were no significant between-group differences for subjects ≤ 60 years.

Next, we proceeded to analyze constipation severity as a covariate using generalized linear models. Between-group differences in fecal calprotectin levels remained significant for the comparison between MSA vs. controls ($p < 0.001$) and were borderline significant for the comparison between PD vs. controls ($p = 0.085$). Notably, constipation severity did not have a significant effect on fecal calprotectin in either model.

Correlations with clinical variables and immunological markers

In the control cohort, fecal calprotectin levels did not correlate with age, sex, smoking status, BMI, or constipation severity (Supplementary Table 1 in the online-only Data Supplement). Fecal calprotectin correlated with age in PD patients with a small effect size ($r_s = 0.252$, $p = 0.034$). In the PD and MSA subgroups, fecal calprotectin did not correlate with sex, smoking status, disease duration, age of onset, constipation severity, BMI, levodopa-equivalent daily dosage, PD motor severity (MDS-UPDRS part III), severity of motor response complications (MDS-UPDRS part IV), MSA severity (total of UMSARS Parts I and II), or cognitive function (Supplementary Tables 2 and 3 in the online-only Data Supplement). In PD, there was no significant difference in fecal calprotectin levels between the tremor-dominant vs. postural instability-gait disorder subtypes (median: 87.7 [IQR: 187.4] vs. 96.9 [IQR: 161.2] μg/g, $p = 0.913$). In MSA, fecal calprotectin levels were nonsignificantly higher in the parkinsonian vs. cerebellar-predominant subtype (median: 153.1 [IQR: 414.9] vs.

Table 1. Demographics and clinical characteristics of the recruited subjects

	PD (*n* = 71)	MSA (*n* = 38)	Controls (*n* = 60)	*p*-value
Age, yr	67.8 ± 7.3	62.7 ± 7.8	64.3 ± 6.8	0.001*†
Sex, %				
Male	69.0	36.8	50.0	0.004*‡
Ethnicity, %				0.048*‡
Malay	9.9	7.9	3.3	
Chinese	74.6	92.1	88.3	
Indian	15.5	0.0	8.3	
Smoking status, %				0.233‡
Current smoker	2.8	2.6	3.3	
Past smoker	32.4	21.1	16.7	
Never smoked	64.8	76.3	80.0	
Body mass index, kg/m^2	23.6 ± 3.9	21.8 ± 5.0	24.9 ± 4.2	0.003*†
Comorbidities, %				
Diabetes mellitus	16.9	5.4	20.0	0.141‡
Hypertension	31.0	16.2	43.9	0.019‡
Ischemic heart disease	11.3	13.2	14.0	0.891‡
Stroke	2.8	8.1	1.8	0.244‡
Cancer	2.8	10.8	1.8	0.076‡
Gastrointestinal symptoms				
PAC-SYM score (0–36)	5.5 [6]	8.0 [10.0]	1.0 [4.0]	< 0.001*§
Less than 3 bowel movements per week, %	90.1	63.2	20.0	< 0.001*‡
Cognitive function				
MoCA score (0–30)	25.0 [7.0]	24.5 [9.0]	27.0 [4]	< 0.001*§
Disease duration, onset & LEDD				
Disease duration, yr	10.0 [10.0]	3.75 [4.0]		< 0.001*§
Age of onset, yr	57.9 ± 10.0	59.1 ± 8.1		0.535†
LEDD, mg/d	600.0 [475.0]	300.0 [431.0]		< 0.001*§
PD severity and subtype				
Motor subtype, %		-	-	
Tremor dominant	19.4	-	-	
Indeterminate	11.9	-	-	
PIGD	68.7	-	-	
MDS-UPDRS part III	30.0 ± 13.3	-	-	
MDS-UPDRS total	60.7 ± 21.9	-	-	
Hoehn & Yahr	2.0 [1.0]	-	-	
With motor response complications‖, %	26.1	-	-	
MSA severity and subtype				
MSA subtype, %	-		-	
MSA-P (predominant parkinsonism)	-	57.9	-	
MSA-C (predominant cerebellar)	-	34.2	-	
MSA-PC (mixed)	-	7.9	-	
UMSARS (total of parts I and II)	-	57.2 ± 18.3	-	
Hoehn & Yahr	-	4.0 [1.0]	-	

Normally distributed data are presented as the mean ± standard deviation, while nonnormally distributed data are presented as the median [interquartile range]. *significant difference between groups; †analyzed using one-way analysis of variance (ANOVA); ‡analyzed using chi-square test; §analyzed using Kruskal-Wallis test; ‖score of MDS-UPDRS part IV of one or more. PD, Parkinson's disease; MSA, multiple system atrophy; LEDD, levodopa equivalent daily dose; MDS-UPDRS, International Parkinson and Movement Disorder Society-Unified Parkinson's Disease Rating Scale; UMSARS, Unified Multiple System Atrophy Rating Scale; MoCA, Montreal Cognitive Assessment; PAC-SYM, Patient Assessment of Constipation Symptoms; PIGD, postural instability gait disorder.

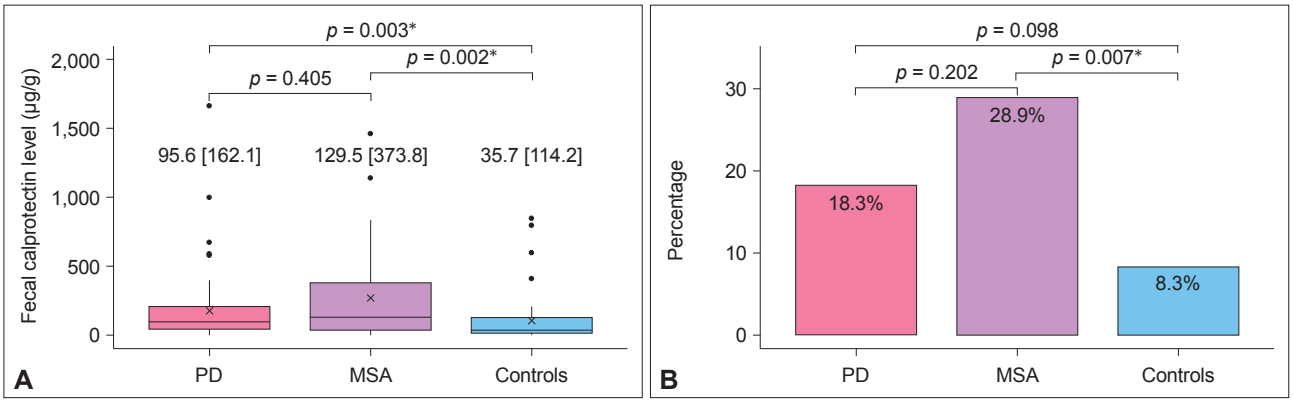

Figure 1. Fecal calprotectin levels in PD, MSA and controls. A: Between-group comparisons were analyzed using the Mann–Whitney U test. Data are expressed as median [interquartile range]. B: Percentage of subjects with highly abnormal calprotectin levels (> 250 μg/g). Between-group comparisons were analyzed using the chi-square test. *denotes statistical significance. PD, Parkinson's disease; MSA, multiple system atrophy.

85.6 [IQR: 168.0], $p = 0.219$).

In PD, fecal calprotectin levels correlated positively with blood monocyte ($r_s = 0.417$, $p = 0.048$) and eosinophil ($r_s = 0.542$, $p = 0.008$) counts and negatively with IL-17 ($r_s = -0.443$, $p = 0.027$) (Supplementary Table 4 in the online-only Data Supplement). In MSA, no correlations were found between fecal calprotectin levels and any of the blood markers (Supplementary Table 4 in the online-only Data Supplement).

DISCUSSION

Fecal calprotectin was found to be significantly elevated in PD and MSA compared with age-similar nonneurological controls, with no significant difference between PD and MSA. Notably, we found a significant interaction effect with age in our analyses; between-group differences in fecal calprotectin were only significant in older subjects (i.e., ≥ 61 years), and they became more apparent with increasing age. More than a quarter of MSA patients and almost one-fifth of PD patients had highly abnormal fecal calprotectin levels in the range typically seen in patients with active IBD (≥ 250 μg/g).[15] The difference vs. controls in this comparison was significant only for MSA but not PD. To our knowledge, increased fecal calprotectin has not previously been reported in MSA and is thus a novel finding in our study. Taken together with previous work,[10,11,13] our observations support the notion that intestinal inflammation is present in PD and MSA, and age-associated inflammation may further aggravate this process. However, fecal calprotectin did not correlate with the clinical subtype or with the severity of motor and nonmotor features, including GI symptoms and cognitive function, in PD and MSA.

Our observation of elevated fecal calprotectin in PD is in line with the findings of previous studies.[16-18] An overview comparison shows that the fecal calprotectin elevation in PD (means/medians of 54.5, 87.1 and 176.5 μg/g in the different studies) and MSA (129.5 μg/g in this study) was generally less pronounced than that seen in active IBD, likely indicating low-grade or subclinical intestinal inflammation.[12] In IBD, fecal calprotectin levels exceeding 250 μg/g provide better specificity than thresholds of 100 μg/g or 50 μg/g in differentiating active IBD from remission.[15] In one study, ulcerative colitis patients in clinical and endoscopic remission who showed biopsy evidence of active histologic inflammation had a median fecal calprotectin level of 278 μg/g,[19] and some authors have suggested fecal calprotectin levels in the range of 50 to 250 or 300 μg/g indicate low-grade intestinal inflammation.[20] However, caution is needed when making comparisons among studies due to issues with the different fecal calprotectin assays, which is discussed further below. Although no studies have evaluated fecal calprotectin in MSA, one small study ($n = 6$) demonstrated increased expression of TLR4, an endotoxin-related inflammation marker, in the colonic lamina propria of MSA patients.[13]

The occurrence of intestinal inflammation in PD and MSA might be related to alterations in the colonic microbiome and metabolome reported in these disorders.[3,5-8] One distinct finding in PD microbiome studies is a reduced abundance of short-chain fatty acid (SCFA)-producing bacteria, and fecal metabolite studies demonstrated reduced fecal SCFA levels in PD patients.[3,21] Interestingly, fecal SCFA levels have also been reported to be reduced in MSA.[5] SCFAs play an important role in microbiota-gut-brain crosstalk through the regulation of inflammatory cascades, blood-brain barrier integrity and neuronal survival.[3,22] SCFAs are major energy substrates for the colonic epithelium, and reduced levels may lead to disruption of gut barrier integrity and intestinal inflammation.[3,22] More recently, a case-control study reported increased fecal calprotectin together with reduced fecal SCFA levels in PD; however, there were no significant correlations between the two parameters.[16] Small intestinal inflamma-

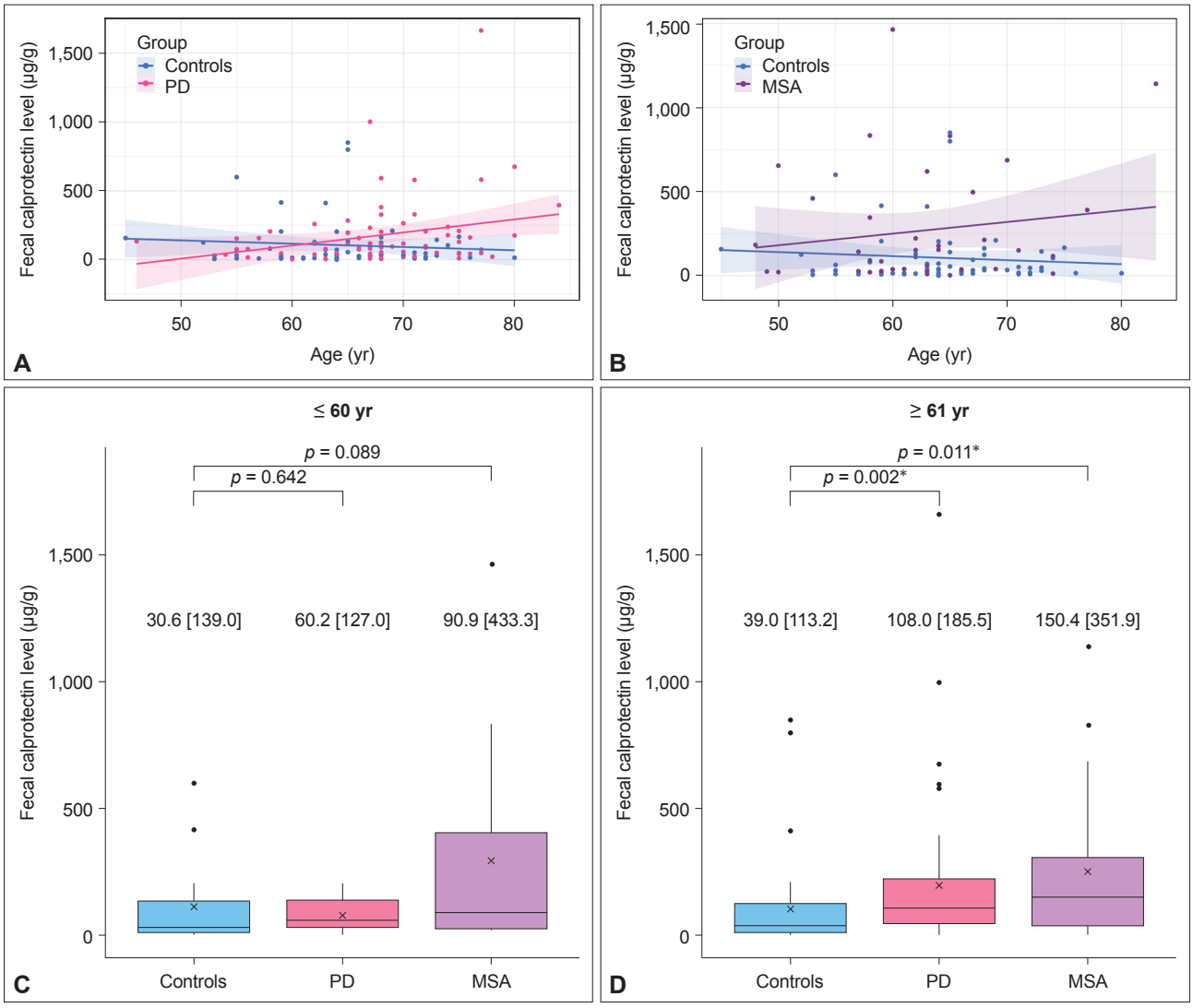

Figure 2. Fecal calprotectin levels in PD, MSA and controls, according to age. A: Scatterplot of fecal calprotectin levels (μg/g) in PD vs. controls against age. B: Scatterplot of fecal calprotectin levels (μg/g) in MSA vs. controls against age. C and D: Comparison of fecal calprotectin levels (μg/g) between PD, MSA and controls in two age groups, 60 years and below and 61 years and above. Between-group comparisons were analyzed using the Mann–Whitney U test. Data are expressed as median [interquartile range]. *denotes statistical significance. PD, Parkinson's disease; MSA, multiple system atrophy.

tion can also elevate fecal calprotectin,[20] and there is evidence implicating small bowel involvement in PD.[23] Epidemiological studies also highlight the relevance of enteric inflammation in PD pathogenesis, with recent studies showing an elevated risk of incident PD among IBD patients.[24]

Consistent with previous studies in PD, we did not find any correlations between fecal calprotectin level and sex,[17] PD duration,[18] Hoehn and Yahr score,[17] MDS-UPDRS score,[18] PD motor subtype,[17] levodopa-equivalent daily dosage,[17,18] or constipation severity.[17] Additionally, we did not find any correlation between fecal calprotectin and cognitive function in PD. Only one study found fecal calprotectin to be inversely linked to symptoms of irritable bowel syndrome in PD, the implications of which are currently unclear.[16] Likewise, fecal calprotectin did not correlate with clinical features in MSA patients (cerebellar vs. parkinsonian-predominant subtype, severity as assessed by the total UMSARS Parts I–II, or cognitive function). One possible reason for the overall lack of clinical correlations could be a "ceiling" effect in terms of the intestinal inflammation being relatively low grade in these conditions. Perhaps more importantly, disease manifestations and mechanisms are now understood to be highly heterogeneous, especially in PD, and there is likely to be a variable degree of the contribution of gut-related processes to the pathogenesis of PD and MSA. For example, a "bottom-up" body-first sequence of disease involvement is proposed to occur in some PD patients, but a "top-down" brain-first process takes place in

others[25] (in whom intestinal inflammation could be anticipated to play a lesser role). This idea is consistent with previous findings that not all, but only a subset, of PD patients demonstrate an "enteric pro-inflammatory profile."[10,12]

In our study, we found a larger and more significant difference between MSA vs. controls compared to PD vs. controls. There was also a nonsignificant trend of calprotectin levels being higher in MSA patients than in PD patients. Unlike in PD, where pathogenetic processes both within and outside the brain are well recognized, MSA pathology has hitherto been considered to primarily involve the central rather than peripheral nervous system.[26] Inflammatory changes in the brain, including microglial activation, have been observed in MSA for almost two decades.[27] Studies of peripheral inflammation, as detected by serum inflammatory markers, have produced conflicting results, with earlier work (n = 14 MSA patients) reporting elevations, particularly TNF-α, although no increase was found in a later study involving 27 patients.[26] There were also suggestions that variants in genes associated with gut inflammation, such as nucleotide-binding oligomerization domain protein 2 (NOD2)[28] and leucine-rich repeat kinase 2 (LRRK2),[29] may contribute to MSA susceptibility. Indeed, a large multinational effort recently identified substantial polygenic overlap between MSA and IBD.[30] Taken together, these results support the relevance of the gut-brain axis and gut inflammation in MSA, although additional studies are necessary to more precisely discern their role in disease pathophysiology.

We believe an important point that has not been sufficiently highlighted in previous work on gut-related issues in neurodegenerative disorders is the effect of aging,[3,31] which shares many overlapping biological features with disease states, including impaired proteasomal/lysosomal and mitochondrial function, oxidative stress, and inflammation.[32] Similar to PD[3] and MSA,[5] aging has also been associated with gut dysbiosis and with multiple contributing factors, such as impaired intestinal barrier and motility, gut immunosenescence, lifestyle changes (e.g., diet, living conditions, physical activity), and health status (e.g., comorbidities, medications, frailty).[33] Many of these factors interact and modulate each other and converge on chronic low-grade inflammation ("inflammaging").[33] Few studies have specifically investigated the influence of aging on fecal calprotectin levels in healthy adults. A small study from South Korea (n = 45) found that the oldest subjects (≥ 70 years) had a mean fecal calprotectin level ×10 higher than that in the ≤ 50 years group (160.3 vs. 15.88 μg/g, $p < 0.001$), with an intermediate level (mean 35.46 μg/g) seen in the intermediate (51−69 years)-age group.[34] In our study, elevated fecal calprotectin levels in PD and MSA were observed primarily in older patients, with the differences becoming more pronounced with increasing age. Thus, it appears that the superimposition of PD and MSA may further aggravate aging-related changes in the gut.

In a subset of subjects with available blood analyses, fecal calprotectin levels showed few correlations with blood indices, consistent with the findings in another PD case-control study that investigated similar markers.[16] Interestingly, however, moderate-sized correlations were observed between fecal calprotectin levels in PD and blood monocytes, eosinophils and IL-17, suggesting that gut inflammatory changes (and/or dysbiosis, as reported by Lin et al.[35]) could potentially be reflected systemically in blood parameters. In our study, fecal calprotectin in PD correlated inversely with serum IL-17, which at first glance may seem surprising, since IL-17 is generally regarded as "proinflammatory."[36] However, IL-17 activity has intriguingly been associated with dual effects, e.g., increased in the gut mucosa of IBD patients, yet its inhibition induced or worsened colitis in human and animal studies.[37] Notably, the combined use of fecal and blood biomarkers has shown superiority to the use of fecal markers alone in detecting endoscopically active IBD.[38] Additional studies are required to understand the systemic effects of intestinal inflammation and dysbiosis and the utility of combining fecal and blood biomarkers in PD and MSA.

Our study has some limitations. The cross-sectional design provides a "snapshot" of differences, and whether elevated fecal calprotectin has a causative role in the development of PD and MSA cannot be answered by this study design. Alternatively, fecal calprotectin levels may also be elevated as a consequence of bowel dysfunction in these disorders. We note that there are some limited data at the group level suggesting a relative stability of fecal calprotectin over the short term (several weeks) in PD.[39,40] Commercial calprotectin assays have variable performance, and currently, there is a lack of assay standardization and universally accepted cutoff values.[14] Furthermore, in our study, there was a substantial overlap in fecal calprotectin levels between patients and controls; therefore, individual patient values may have very limited utility to distinguish PD or MSA versus controls. Other fecal proteins have been assayed as noninvasive markers of intestinal inflammation, such as lactoferrin or S100A12/calgranulin C; however, currently, these are much less well established than calprotectin.[14] Colonoscopy remains the gold standard for diagnosing colonic inflammation. Since this was not done as part of our study protocol because of concerns about the procedural risk, particularly in MSA patients (due to autonomic and laryngeal dysfunction), subclinical intestinal disorders such as IBD or diverticulitis affecting calprotectin levels could not be excluded in this study.

Advantages to assaying calprotectin include its relatively low cost, excellent temperature stability and requirement for only a small amount of fecal sample for analysis, making it likely that this marker will be more widely researched in future studies. Re-

cently, fecal calprotectin was explored in PD clinical trials as a potential therapeutic biomarker for GI-targeted treatments.[39,40] One study documented a significant drop in fecal calprotectin levels after eight weeks of prebiotic treatment using resistant starch;[40] however, another study showed no significant change in fecal calprotectin levels in the face of improved constipation symptoms with probiotic treatment.[22,39]

In conclusion, PD and MSA patients had higher levels of fecal calprotectin than controls, providing further support for the presence of intestinal inflammation in these disorders. Beyond observational research, mechanistic and interventional studies are necessary to develop a more complete understanding of the role of intestinal inflammation in these neurodegenerative disorders.

Supplementary Materials

The online-only Data Supplement is available with this article at https://doi.org/10.14802/jmd.21085.

Conflicts of Interest

The authors have no financial conflicts of interest.

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Supplementary Table 1. Correlations between fecal calprotectin with sociodemographic and clinical characteristics in controls

Controls (*n* = 60)	r_s	*p*-value
Age	0.056	0.668
Sex	0.243	0.062
Smoking status	-0.006	0.964
Body mass index	-0.051	0.702
Constipation severity		
Constipation severity score (PAC-SYM) (0–36)	0.109	0.408
Less than 3 bowel movements per week	0.002	0.985

Spearman's rank correlation coefficient, denoted as r_s in the table. PAC-SYM, Patient Assessment of Constipation Symptoms.

Supplementary Table 2. Correlations between fecal calprotectin with sociodemographic, clinical features and disease severity in patients with PD

PD (*n* = 71)	r_s	*p*-value
Age	0.252	0.034*
Sex	0.065	0.592
Smoking status	0.080	0.506
Body mass index	-0.174	0.146
Constipation severity		
Constipation severity score (PAC-SYM) (0–36)	0.050	0.759
Less than 3 bowel movements per week	0.085	0.479
Disease duration	0.118	0.326
Age of onset	0.080	0.506
LEDD	-0.004	0.973
PD motor severity (MDS-UPDRS part III)	0.044	0.722
Severity of motor response complications (MDS-UPDRS part IV)	0.132	0.278
Cognitive function (MoCA)	0.097	0.508

Spearman's rank correlation coefficient, denoted as r_s in the table. *denotes correlation is significant at the 0.05 level (2-tailed). PD, Parkinson's disease; LEDD, levodopa equivalent daily dose; PAC-SYM, Patient Assessment of Constipation Symptoms; MDS-UPDRS, International Parkinson and Movement Disorder Society-Unified Parkinson's Disease Rating Scale; MoCA, Montreal Cognitive Assessment.

Supplementary Table 3. Correlations between fecal calprotectin with sociodemographic, clinical features and disease severity in patients with MSA

MSA (*n* = 38)	r_s	*p*-value
Age	0.113	0.498
Sex	0.184	0.269
Smoking status	0.192	0.256
Body mass index	-0.198	0.247
Constipation severity		
Constipation severity score (PAC-SYM) (0–36)	-0.100	0.556
Less than 3 bowel movements per week	0.104	0.532
Disease duration	0.116	0.502
Age of onset	0.178	0.299
LEDD	-0.055	0.744
MSA severity (UMSARS parts I and II)	0.113	0.506
Cognitive function (MoCA)	-0.271	0.223

Spearman's rank correlation coefficient, denoted as r_s in the table. MSA, multiple system atrophy; LEDD, levodopa equivalent daily dose; PAC-SYM, Patient Assessment of Constipation Symptoms; UMSARS, Unified Multiple System Atrophy Rating Scale; MoCA, Montreal Cognitive Assessment.

Supplementary Table 4. Correlation between fecal calprotectin level with blood immunological markers in Parkinson's disease and multiple system atrophy

Blood biochemical and immunological markers	Parkinson's disease		Multiple system atrophy	
	r_s	*p*-value	r_s	*p*-value
White cell count	0.401	0.052	0.256	0.339
Neutrophil count	0.311	0.148	0.282	0.289
Lymphocyte count	0.119	0.590	0.059	0.829
Monocyte count	0.417	0.048*	0.212	0.431
Eosinophil count	0.542	0.008*	-0.283	0.288
Erythrocyte sedimentation rate	0.020	0.929	0.029	0.914
C-reactive protein	-0.010	0.962	0.273	0.273
Caspase-1	-0.195	0.349	-0.226	0.339
Interferon-γ	-0.155	0.458	-0.140	0.556
Interleukin-1β	-0.178	0.395	-0.126	0.596
Interleukin-4	0.305	0.138	-0.226	0.339
Interleukin-6	0.125	0.550	-0.164	0.490
Interleukin-17	-0.443	0.027*	-0.182	0.443
Matrix metallopeptidase-3	0.241	0.246	-0.233	0.323
Nuclear factor-κβ	0.260	0.209	-0.233	0.323
Tumor necrosis factor-α	0.007	0.974	-0.105	0.659

Spearman's rank correlation coefficient, denoted as r_s in the table. *denotes correlation is significant at the 0.05 level (2-tailed).