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*Research Article*

# Serum Biomarkers of Olfactory Identification Deficits in Patients with Parkinson's Disease

**Fu-Jia Li, Yang-Dan-Yu Li, Xu Liu, Jie Zu, Wei Zhang, Qi-Hua Xiao, Xue-Bin Niu, Li Du, Chen-Chen Cui, Ru-Yu Zhang, Xiao-Qing He, Gui-Yun Cui, and Chuan-Ying Xu**

*Department of Neurology, The Affiliated Hospital of Xuzhou Medical University, Xuzhou, China*

Correspondence should be addressed to Gui-Yun Cui; teachercuigy@126.com and Chuan-Ying Xu; xucy2022@yeah.net

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To investigate whether glial fibrillary acidic protein (GFAP), neurofilament light chain (NFL), and 12 cytokines can serve as serum biomarkers of olfactory identification dysfunction in patients with Parkinson's disease (PD). GFAP and NFL levels were measured in 75 patients with PD and 36 healthy controls (HCs). The levels of 12 cytokines were assayed in 41 patients with PD. The 16-item Sniffin' Sticks test and the Mini-Mental State Examination (MMSE) were used to assess olfactory identification ability and cognitive function, respectively. Linear regression models were applied to control for confounding effects. Receiver operating characteristic curves were used to examine the diagnostic accuracy of serum NFL, GFAP, and interleukin-6 (IL-6) levels. The cut-off value for the SS-16 test in diagnosing dysosmia was equal to 9.5 points. Serum GFAP levels were higher in patients with PD with olfactory identification dysfunction than in those without. GFAP, NFL, and IL-6 levels were correlated with SS-16 scores. Moreover, combining these three biomarkers yielded the best-fitting model for distinguishing patients with PD with or without dysosmia. We found a prominent indirect effect of GFAP on MMSE scores through its contribution to SS-16 scores. GFAP, NFL, and IL-6 can serve as serum biomarkers for olfactory identification dysfunctions in PD. We inferred that astrogliosis might promote the occurrence of dysosmia by releasing proinflammatory factors and causing neuronal damage and may indirectly impair cognition through its effect on olfactory function.

## 1. Introduction

Olfactory dysfunction is a common nonmotor symptom in patients with Parkinson's disease (PD) that often predates motor symptoms [1–4]. Moreover, olfactory dysfunction is closely correlated with cognitive dysfunction and dementia conversion in PD [5]. There is a research gap regarding biomarkers for dysosmia, especially in PD. Many studies have shown that the deposition of $\alpha$-synuclein ($\alpha$-syn) in olfactory structures contributes to dysosmia in patients with PD [6–8]. Neurofilament light chains (NFLs) are a marker of axonal injury, and their serum levels reflect the severity of many symptoms of PD [9–11]. Glial fibrillary acidic protein (GFAP) is particularly abundant in astrocytes and is a marker of astroglial cell activation following central nervous system (CNS) injuries and neurodegeneration, also known as astrogliosis [12]. Evidence in animal models and clinical pathology shows close relationships between $\alpha$-syn deposition and regional astrogliosis [8, 13, 14], as well as NFL release in PD [14–16]. Whether serum GFAP or NFL levels are related to dysosmia in patients with PD remains unclear.

Furthermore, inflammation-related pathology was also closely related to dysosmia in PD [7]. In a pathological setting, overactivated cells such as microglia [17] and astrocytes [18] release large amounts of proinflammatory cytokines, causing substantial neural damage. Whether cytokines can serve as biomarkers for dysosmia remains to be elucidated. To address these gaps, we measured serum GFAP, NFL, and cytokine levels in this study as a preliminary step to investigate their roles in olfactory identification dysfunction in PD.

## 2. Materials and Methods

*2.1. Study Participants.* Patients with PD were recruited from the Department of Neurology of the Affiliated Hospital of

Xuzhou Medical University from March to August 2022. Those who fulfilled the Movement Disorder Society clinical diagnostic criteria for PD (2015) were included. We excluded patients who had (1) a history of nose surgery, (2) a history of smoking, (3) suffered from chronic rhinitis, or (4) recently caught a cold. Healthy controls (HCs) were recruited among caregivers of patients with PD or individuals hospitalized for medical examinations exempt from severe neurological, mental, or systemic diseases. A power analysis was performed to assess the observed power of our sample size after recruiting participants. Written informed consent was obtained from all participants before conducting this study. This research project was authorized by the Ethics Committee of the Affiliated Hospital of Xuzhou Medical University (No. XYFY2022-KL073).

*2.2. Assessment of Clinical Characteristics.* Data on clinical variables such as sex, age, years of education, and disease duration were collected. The Unified Parkinson Disease Rating Scale part III (UPDRS-III) and modified Hoehn and Yahr stage (HY stage) were used to assess the severity of motor symptoms. The Mini-Mental State Examination (MMSE) was used to evaluate cognitive function.

*2.3. Evaluation of Odor Identification Ability.* The 16-item Sniffin' Sticks test (SS-16) evaluated olfactory identification ability. During the test, 16 odor sticks were individually provided to the participants, who were then asked to name the odor they smelled. The score for each correct answer was 1 point. The total score ranged from 0 to 16 points.

*2.4. Laboratory Assessment.* Serum GFAP and NFL levels were measured in 75 patients with PD and 36 HCs using the ultrasensitive Simoa technology on the automated Simoa HD-X platform (GBIO, Hangzhou, China) and the multiplex Neurology 2-Plex B (cat. no. 103520, Quanterix, Billerica, MA, USA) assay kit according to the manufacturer's instructions. The mean limit of detection for NFL and GFAP was 0.0688 and 0.5635 pg/mL, respectively. The mean lower limit of quantitation for NFL and GFAP was 0.427 and 4.88 pg/ml, respectively. The levels of 12 cytokines (interleukin (IL)-1$\beta$, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-17; interferon (IFN)-$\alpha$, IFN-$\gamma$; and tumor necrosis factor (TNF)-$\alpha$) were quantified in 41 patients with PD using the multiplex bead-based flow fluorescent immunoassay technology on the Beckman Coulter NAVIOS flow cytometer (Xuzhou, China). A 12-cytokine assay kit (cat. no. R701001, Raisecare, Qingdao, Shandong, China) was purchased and used according to the manufacturer's instructions. The operator did not know the medical status of any of the participants.

*2.5. Statistical Analyses.* All data were analyzed using SPSS 26.0 and R version 4.2.1. Differences with a $P$ value $< 0.05$ were deemed to be significant. When calculating the cut-off for the SS-16, we utilized the SS-16 data from 140 HCs sourced from our hospital's Neurology Department. First, we assessed the normal distribution of these data. Considering that lower olfactory identification ability is regarded as a pathological state, we estimated a one-sided lower 95% confidence interval (CI) for the SS-16 score as 9.5 points. Patients scoring below this threshold were considered to exhibit impaired olfactory identification function. As the criteria for the test of normality were very strict and were hardly met for any continuous variable, if the absolute values of its skewness and kurtosis were less than 3 and 10, respectively, the variable was identified as normally distributed. Presented in terms of means and standard deviation values, these continuous variables were compared using an independent-sample $t$-test. For markedly skewed data, variables were presented as the median and interquartile range, compared using the Mann–Whitney $U$ test, and the difference between the medians of the two groups was compared using the Hodges-Lehmann estimation. Categorical variables were expressed as proportions and compared using the chi-squared test. The Spearman and Pearson correlation analyses were performed to test for relationships between variables. Multiple linear regression was used to control for confounding effects. Receiver operating characteristic curves were used to examine the diagnostic accuracy of serum NFL, GFAP, and IL-6 levels. Mediation analysis was performed to evaluate whether dysosmia, as measured by the SS-16, may mediate the association between GFAP levels and cognitive impairment, as measured by the MMSE. The statistical significance was tested for the indirect effects of dysosmia through a bootstrapping approach (replicated 100 times), and 95% CIs were identified.

## 3. Results

*3.1. Exploration of the Cut-Off for SS-16 Scores.* To confirm the cut-off value for the SS-16 scores, data (collected from 140 HCs who previously underwent SS-16 testing but did not participate in this study) from the clinical database of the Department of Neurology of the Affiliated Hospital of Xuzhou Medical University were analyzed. The cut-off value was equal to 9.5 points and estimated by the 95% CI of the SS-16 scores of the 140 HCs, with a sensitivity of 78.7% and a specificity of 71.4% in distinguishing patients with PD from controls (Figure 1(a)). Patients with scores below this cut-off value were deemed to have olfactory identification dysfunction, accounting for 78.7% of patients with PD (Figure 1(b)).

*3.2. Comparisons of Clinical Characteristics.* A total of 111 participants were included in this study: 36 HCs and 75 patients with PD. Of those with PD, there were 16 patients without dysosmia and 59 with dysosmia, according to the classification criteria mentioned above. No significant differences in age, sex, or years of education were observed between the HC and PD groups. Furthermore, age, sex, years of education, disease duration, HY stage, UPDRS-III scale, and MMSE scores did not vary across patients with PD with and without dysosmia (Table 1).

*3.3. Comparisons of Serum GFAP and NFL Levels.* In the comparison of serum NFL and GFAP concentrations, both levels were higher in the PD group than in the HC group (GFAP: PD vs. HC = 132.69 ± 76.60 vs. 84.86 ± 35.77 pg/mL,

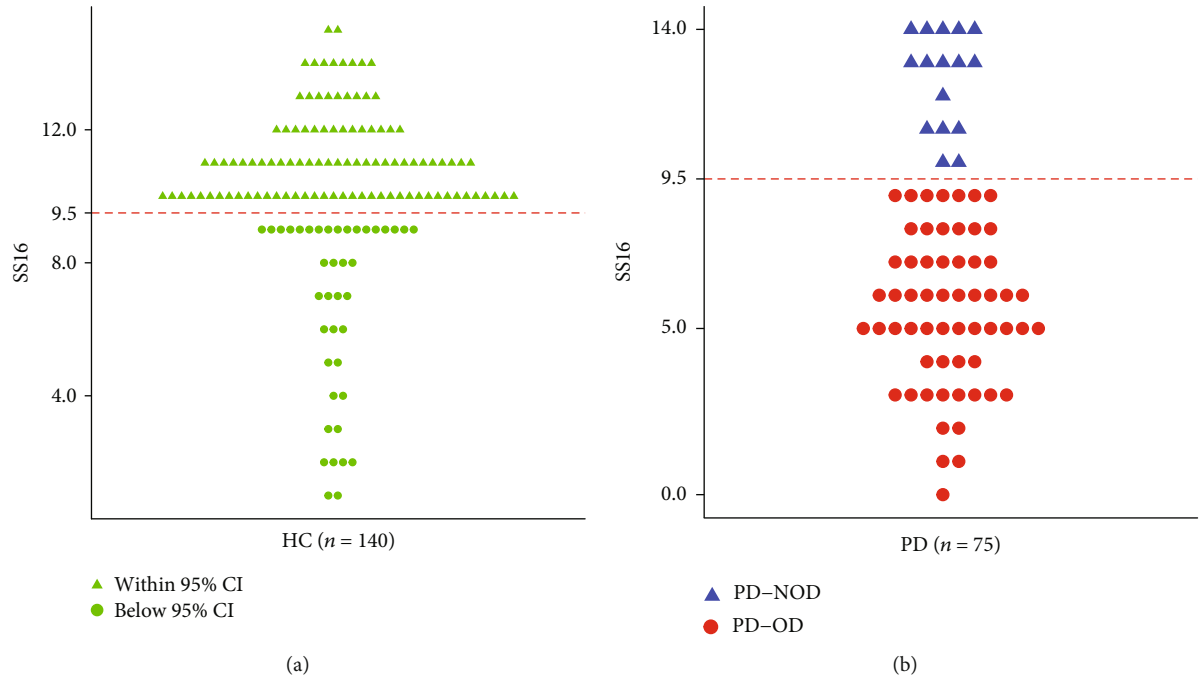



Figure 1: The SS-16 score threshold was 9.5 points. (a) This value was estimated by the 95% CIs of the SS-16 scores of a total of 140 HCs. (b) Patients with PD with scores below this cut-off value were deemed to have PD with olfactory dysfunction (PD-OD), while those with scores above this cut-off were deemed to have PD without olfactory dysfunction (PD-NOD). Abbreviations: SS-16 = 16-item Sniffin' Sticks test; CI = confidence interval; HC = healthy control; PD = Parkinson's disease.

Table 1: Comparisons of clinical characteristics, serum NFL, and GFAP levels of study participants.

| Characteristic | HC ($n = 36$) | PD ($n = 75$) | $P$ | PD without dysosmia ($n = 16$) | PD with dysosmia ($n = 59$) | $P$ |
|---|---|---|---|---|---|---|
| Age (years) | 61.11 ± 8.44 | 64.40 ± 8.12 | 0.051 | 63.56 ± 7.69 | 64.63 ± 8.28 | 0.645 |
| Sex (male) | 36 (19) | 75 (35) | 0.689 | 16 (8) | 59 (27) | 0.985 |
| Education (years) | 8.46 ± 3.54 | 7.45 ± 4.20 | 0.221 | 8.19 ± 3.08 | 7.24 ± 4.47 | 0.428 |
| Disease duration (years) | NA | 5.11 ± 3.49 | NA | 4.84 ± 2.64 | 5.19 ± 3.71 | 0.731 |
| HY stage (≥3) | NA | 27 (36) | NA | 4 (25) | 23 (39) | 0.459 |
| UPDRS-III | NA | 41.74 ± 20.72 | NA | 35.69 ± 14.20 | 43.54 ± 22.08 | 0.185 |
| MMSE | NA | 24.32 ± 5.46 | NA | 26.53 ± 3.29 | 23.70 ± 5.80 | 0.076 |
| SS-16 | NA | 7.01 ± 3.57 | NA | 12.50 ± 1.46 | 5.53 ± 2.27 | <0.001 |
| NFL (pg/mL) | 8.45 (6.89, 11.27) | 13.77 (10.54, 20.43) | <0.001 | 11.13 (10.33, 15.46) | 15.24 (11.05, 22.35) | 0.103 |
| GFAP (pg/mL) | 84.86 ± 35.77 | 132.69 ± 76.60 | 0.001 | 93.18 ± 33.34 | 143.41 ± 81.56 | 0.019 |

Abbreviations: HC = healthy controls; PD = Parkinson's disease; HY stage = Hoehn-Yahr stage; UPDRS-III = Unified Parkinson Disease Rating Scale part III; MMSE = Mini-Mental State Examination; NFL = neurofilament light chain; GFAP = glial fibrillary acidic protein.

$P = 0.001$, estimated difference (95%CI) = 47.84 (26.70 to 68.98), Figure 2(a); NFL: PD vs. HC = 13.77 (10.54, 20.43) vs. 8.45 (6.89, 11.27) pg/mL, $P < 0.001$, estimated difference (95 %CI) = 5.16 (3.36 to 7.60), Figure 2(b)). Serum GFAP levels were higher in patients with PD with dysosmia than in those without dysosmia (GFAP: with dysosmia vs. without dysosmia = 143.41 ± 81.56 vs. 93.18 ± 33.34 pg/mL, $P = 0.019$, estimated difference (95%CI) = 50.22 (23.24 to 77.21), Figure 2(a)). Furthermore, serum NFL levels were higher in PD patients without dysosmia than in controls (PD patients without dysosmia vs. HCs = 11.13 (10.33, 15.46) vs. 8.45 (6.89, 11.27) pg/mL, $P = 0.005$, estimated difference (95%CI) = 3.30 (1.16 to 5.09), Figure 2(b)). However, serum GFAP levels did not differ significantly between these two groups.

*3.4. Relationships between Serum GFAP and NFL Levels and SS-16 Scores.* Pearson's correlation analysis shows a negative association between the GFAP concentration and the SS-16 score (Figure 3(a); $r$ (Pearson's correlation coefficient), (95%CI) = −0.41 (-0.60 to -0.25), $P < 0.001$). Multiple linear

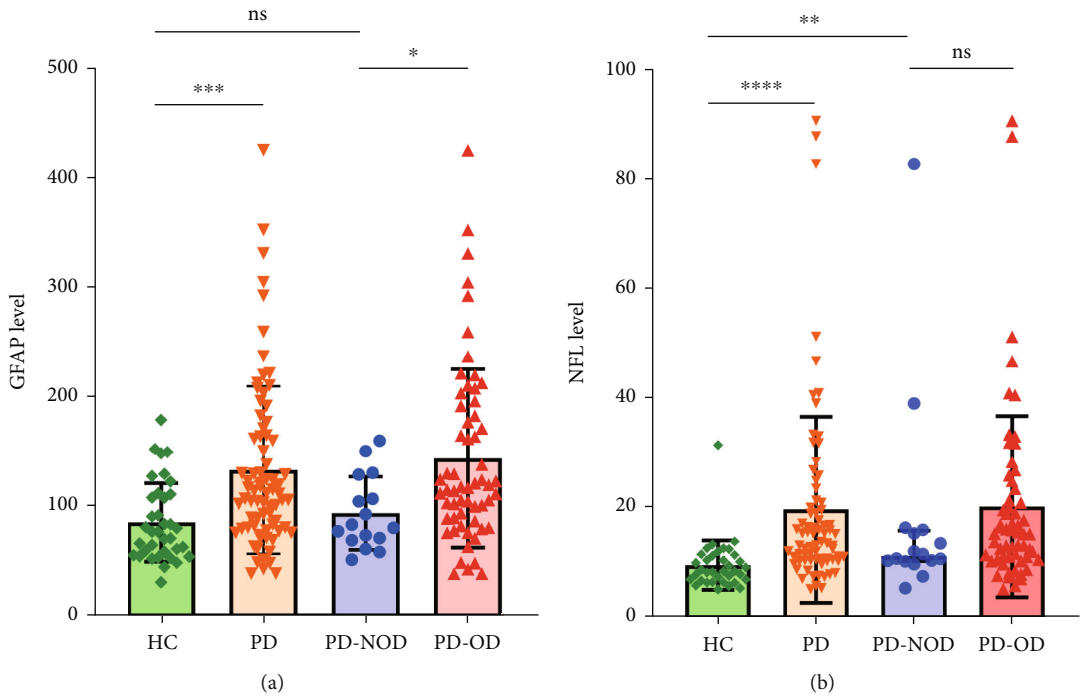



Figure 2: Comparison of serum GFAP (a) and NFL (b) levels across HCs and patients with PD, who were further stratified into two groups: PD-OD and PD-NOD. Abbreviations: PD-OD = PD with olfactory dysfunction; PD-NOD = PD without olfactory dysfunction; HC = healthy control; PD = Parkinson's disease.

regression analysis demonstrated that GFAP (coefficient (95%CI) = −0.018 (-0.031 to -0.005), $P = 0.008$) was an important independent contributor to the SS-16 score, even after controlling for confounding effects such as age, sex, years of education, disease duration, and HY stage. Similarly, Spearman's rank correlation analysis showed that serum NFL levels were negatively associated with SS-16 scores (Figure 3(b); coef (Spearman's correlation coefficient), (95%CI) = −0.3 (-0.001 to -0.48), $P = 0.03$).

*3.5. Relationships between the GFAP or NFL Level and the Identification Accuracy of Every Subitem in the SS-16.* According to the data from 75 patients with PD in our study, the odor item with the lowest identification accuracy in the SS-16 was leather (accuracy = 34%). In contrast, garlic had the highest accuracy (accuracy = 63%). The 75 patients were divided into two groups for each of the 16 subitems in the SS-16 test based on whether they correctly identified the odorant or not. Serum GFAP and NFL levels were compared between groups. We found that higher GFAP levels were associated with lower identification accuracy for peppermint, apple, pineapple, rose, anise, and fish (Figure 4(a)), while higher NFL levels were related to lower identification accuracy for coffee, rose, anise, and fish (Figure 4(b)).

*3.6. Relationships between 12 Cytokine Levels and GFAP, NFL, and SS-16 Scores.* A correlation matrix depicting relationships between GFAP, NFL, 12 cytokine levels, the total SS-16 test score, and the score of each SS-16 subitem was calculated through Spearman's correlation analysis (Figure 5). IL-6 level was associated with the SS-16 total score ($P = 0.013$), even after controlling for sex, age, disease duration, and HY stage by multiple linear regression ($P = 0.043$). Regarding the SS-16 subitem score, a higher IL-2 level was associated with lower banana identification accuracy. The IL-4 level was positively related to the scores for peppermint and anise. IL-5, IL-6, and IL-10 levels were negatively associated with the identification accuracy of cloves. The IL-8 level was positively related to the score for garlic. IL-12p70 was positively associated with the score for peppermint but negatively associated with the score for turpentine. A negative relationship between IFN-$\alpha$ and the peppermint score was also found.

Furthermore, serum GFAP level was positively associated with NFL, IL-6, and IFN-$\gamma$ while negatively related to IL-4 and IL-8 levels. Positive relationships between the NFL level and serum IL-1$\beta$, IL-6, IL-17, and IFN-$\gamma$ levels were also observed. Notably, IL-6 was positively related to GFAP and NFL levels and negatively related to the total SS-16 score (Figure 5).

*3.7. Discriminatory Value of Serum GFAP, NFL, and IL-6 Levels.* Binary logistic regression analysis of serum GFAP and NFL levels as single biomarkers revealed an area under the curve (AUC) of 0.706 and 0.802 for GFAP and NFL, respectively, in distinguishing patients with PD from controls. The combination of GFAP and NFL yielded the best-fitting model with an AUC of 0.810 (Figure 6(a)), which increased the discriminative ability compared with GFAP alone. An AUC of 0.694, 0.619, and 0.649 for GFAP, NFL, and IL-6, respectively, was revealed in distinguishing patients with PD with or without dysosmia. The

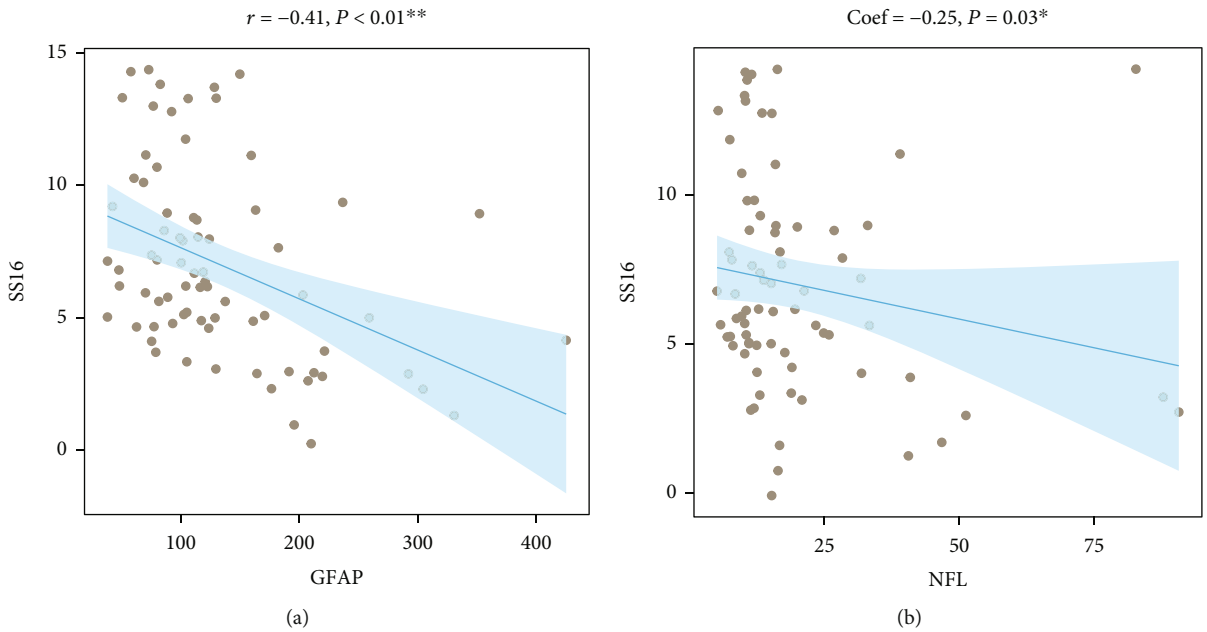



Figure 3: Correlations between serum GFAP (a) and NFL (b) levels and SS-16 scores. Abbreviations: $r$ = Pearson's correlation coefficient; coef = Spearman's correlation coefficient.

combination of GFAP, NFL, and IL-6 yielded the best-fitting model with an AUC of 0.781 (Figure 6(a)).

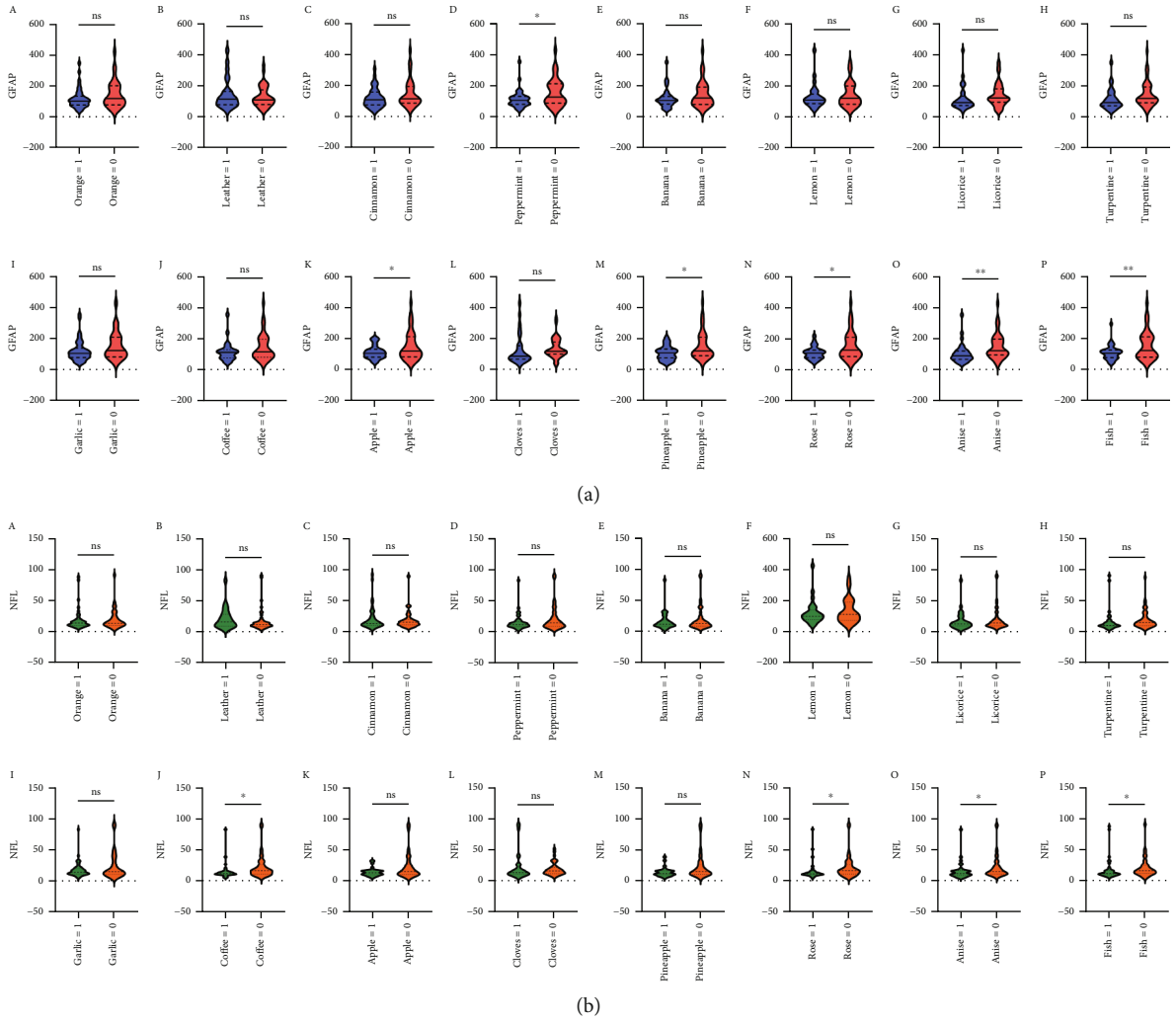
*3.8. Relationship between the GFAP Level, SS-16 Scores, and MMSE Scores.* Mediation analysis revealed a significant indirect effect of GFAP level on the MMSE score through its effects on the SS-16 score (coefficient = −0.006, 95% CI = −0.012 to 0.00, $P = 0.02$; Figure 7, the proportion of mediation = 50.6%) after adjusting for age, sex, disease duration, education years, and HY stage.

## 4. Discussion

In this study, we explored serum biomarkers of olfactory identification dysfunction in PD and found that (1) serum GFAP levels were higher in patients with PD with dysosmia than those without dysosmia; (2) GFAP, NFL, and IL-6 levels were associated with the olfactory identification dysfunction after adjusting for confounding effects; and (3) the combination of GFAP, NFL, and IL-6 yielded the best-fitting model in distinguishing patients with PD with or without dysosmia. These indexes can serve as serum biomarkers of olfactory identification dysfunction. Furthermore, we observed an indirect impact of GFAP on the MMSE score via its effect on the SS-16 score. Our findings suggest that astrogliosis may impair olfactory identification and have subsequent negative effects on cognitive function.

In our study, the threshold for SS-16 scores in discriminating patients with PD from HCs was 9.5 points and was estimated by the 95% CI of the SS-16 scores of 140 HCs, with a 78.7% sensitivity and a 71.4% specificity. This result was consistent with that of a previous study in China [19], with a cut-off value of 9.5 (87% sensitivity and 85% specificity), and similar to the results of a study in southern Brazil, with a cut-off value of 9 (88% sensitivity and 86% specificity) [20]. We found that patients with dysosmia accounted for 77.8% of the total patients with PD, contradicting the findings of Baert et al. and Casjens et al., who reported olfactory impairment in 93.3% and 97.6% of patients with PD, respectively [21, 22]. These differences may be attributed to the discrepancy in the dysosmia criteria and our study's stricter inclusion criteria. Regarding the identification ability of the individual odors in the SS-16, we found that the odor with the highest recognition accuracy was that of garlic, consistent with the results of Casjens et al.'s study [22]. This indicates that patients with PD may retain the ability to identify garlic odors. The high identification accuracy of garlic may be attributed to its volatile sulfur compound [23], which humans are highly sensitive to and thus, are more easily identified. The odor with the lowest recognition accuracy was that of leather (33%), contradictory to the results of Chen et al.'s [19] and Mahlknecht et al.'s studies [24], in which apples had the lowest identification accuracy. Discrepancies in odorant exposure, such as cultural backgrounds and personal life histories, could account for the conflicts between studies.

GFAP, an intermediate filament protein in astrocytes, is a marker of astrocytic activation [12]. NFL, a protein highly expressed in large-caliber myelinated axons, is a byproduct of neurodegeneration [10]. IL-6, a cytokine mainly secreted by astrocytes, microglia, and neurons, could trigger neuronal damage during neurodegeneration [25]. Previous studies found patients with PD have significantly higher serum NFL [9, 10] and GFAP levels than controls [26, 27], consistent with our results. For the first time, we found a higher serum GFAP level in patients with olfactory identification dysfunction, defined as an SS-16 score below 9.5 points, than in patients without.




(a)

(b)

Figure 4: Relationships between the GFAP and NFL level and the identification accuracy of every subitem in the SS-16.

Moreover, we found that a high GFAP level was an independent risk factor for olfactory identification dysfunction after adjusting for age, sex, years of education, disease duration, and HY stage. We also found a relationship between NFL levels and the severity of olfactory identification dysfunction in patients with PD. The molecular mechanisms underlying this correlation remain unclear, but axonal damage after the deposition of $\alpha$-syn in olfactory structures [7] may play a role in the elevation of NFL [15, 16]. In previous studies, IL-6 levels were inversely associated with MMSE scores and gait speed in patients with PD [28, 29]. Our study found that IL-6 was related to dysosmia after controlling for confounding effects. Compared to each serum biomarker, improved discriminatory potential was observed with the combination of GFAP, NFL, and IL-6 in differentiating patients with PD with or without olfactory identification dysfunction.

With regard to the SS-16 subitems, we found that higher GFAP levels were associated with lower identification accuracy for peppermint, apple, pineapple, rose, anise, and fish. Higher NFL levels were related to lower identification accuracy for coffee, rose, anise, and fish. A higher IL-2 level was associated with lower banana identification accuracy. The IL-4 level was positively related to the scores for peppermint and anise. IL-5, IL-6, and IL-10 levels were negatively associated with the identification accuracy of cloves. The IL-8 level was positively related to the score for garlic. IL-12p70 was positively associated with the score for peppermint but negatively associated with the score for turpentine. A negative relationship between IFN-$\alpha$ and the peppermint score was also found. The mechanisms underlying the correlation between GFAP, NFL, cytokines, and the ability to identify different odors remain unclear. Odor identification relies on variations among odorant receptors (ORs) expressed by olfactory sensory neurons (OSNs) [30]. OSNs of the same OR project axons to neighboring sites in the olfactory bulb (OB). Odor binding to ORs triggers an electrical signal that transmits along these axons to the main OB, conveying information to other brain regions and facilitating odor perception. Thus, the correlation between elevated levels of GFAP and NFL and impaired recognition of specific odors may stem from immunoinflammatory responses induced by astrogliosis and localized damage within the OB or cortex responsible for odor identification. The relationship between

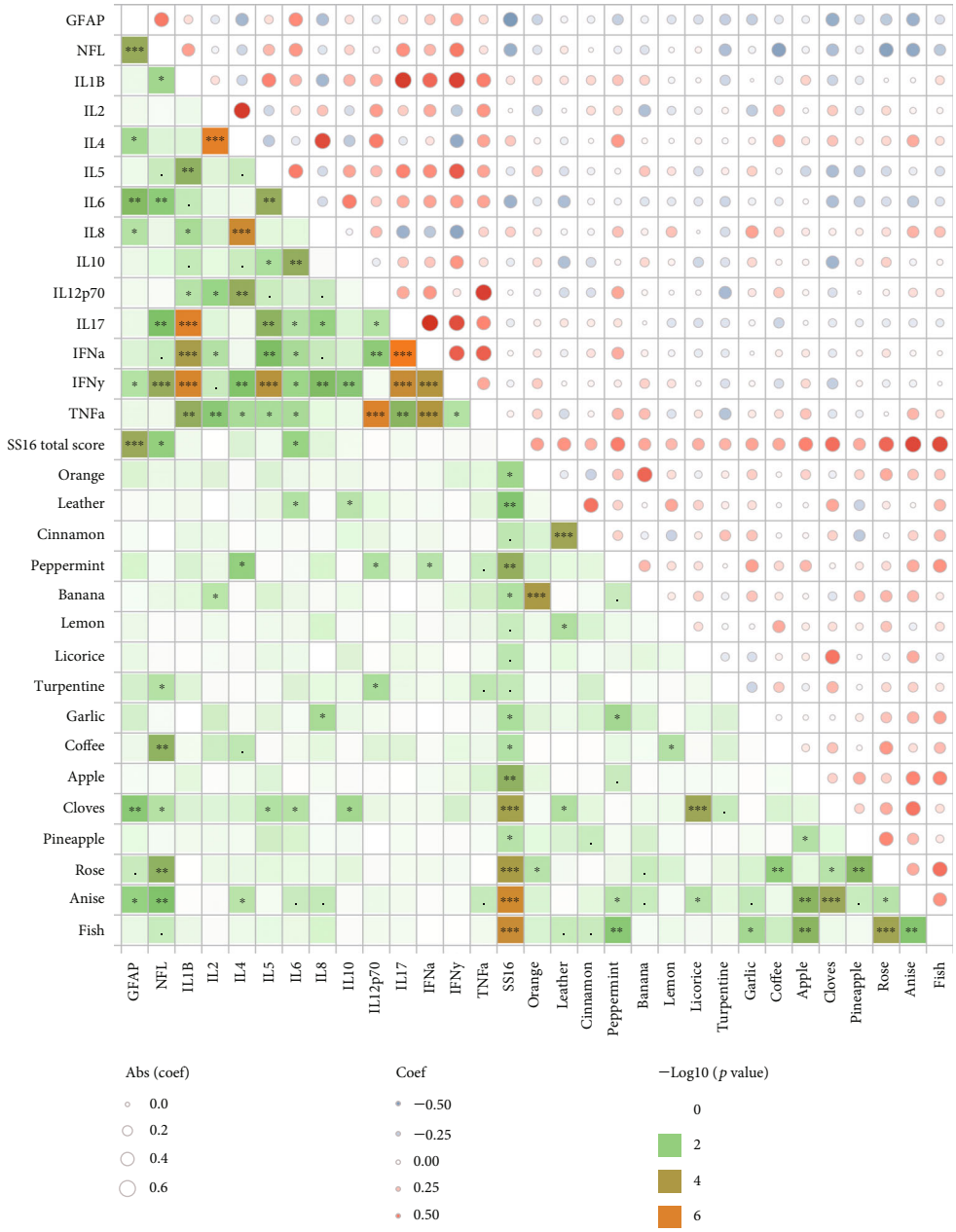



Figure 5: Relationships between 12 cytokine levels and GFAP, NFL, and SS-16 scores. Abbreviations: IL = interleukin; IFN = interferon; TNF = tumor necrosis factor. $^{\cdot}P < 0.1$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

cytokines and odor identification may be associated with their influence on inflammatory/anti-inflammatory responses, receptor protein expression, and cell functionality [31]. For example, higher IL-2 levels and a reduced ability to identify the banana odor may be attributed to IL-2 disrupting normal function in the olfactory sensory cells responsible for detecting that particular odor via immune-inflammatory responses [32]. Further research is required to elucidate the underlying mechanisms. Further studies are required to elucidate the underlying mechanisms.

GFAP is mainly expressed in the CNS [12] and released from disintegrated astrocytes into the bloodstream through the blood-brain barrier [12, 33]. Consequently, high serum GFAP levels can reflect high levels of astrogliosis in the CNS. Whether astrogliosis plays a role in olfactory identification deficits remains unclear. One of the most important contributors to dysosmia is the aggregation and propagation of $\alpha$-syn in olfactory structures [6–8]. Previous studies have shown that $\alpha$-syn from neurons can be internalized by astrocytes [34] and cause regional astrogliosis: (1) in a mouse model, severe astrogliosis was induced in the brain following the exogenous expression of human A53T $\alpha$-syn in astrocytes [35]; (2) when treated with $\alpha$-syn in vitro, astrocytes dramatically increased the expression of GFAP [8]; (3) injection of $\alpha$-syn fibrils in animal models induced astrogliosis two months after injection [14, 36]; and (4) pathological brain sections of patients with PD showed that $\alpha$-syn pathology is usually accompanied by astrogliosis. Based on these

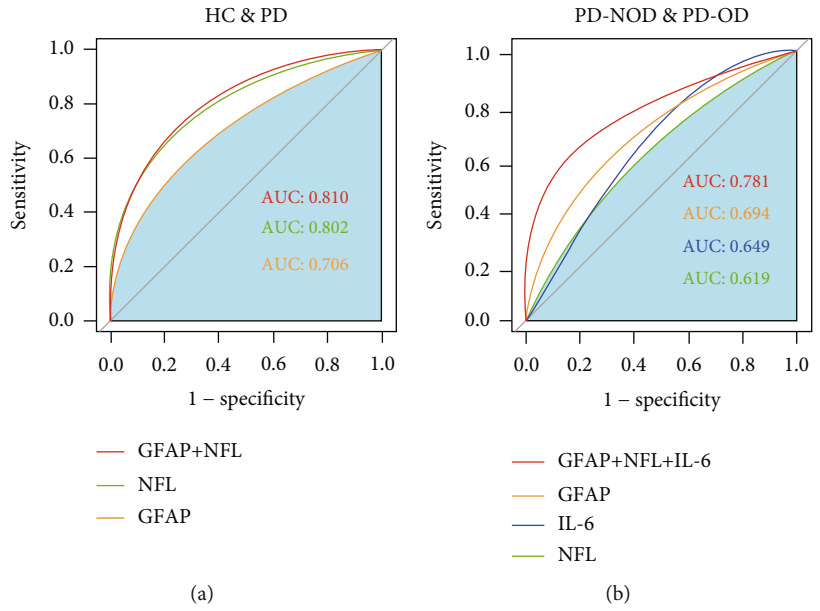



Figure 6: Receiver operating characteristic curves for GFAP (orange), NFL (green), IL-6 (blue), and the combination of these biomarkers (red) in discriminating patients with PD from HCs and patients with PD-OD from those with PD-NOD. Abbreviations: GFAP+NFL+IL-6 = the combination of GFAP, NFL, and IL-6.

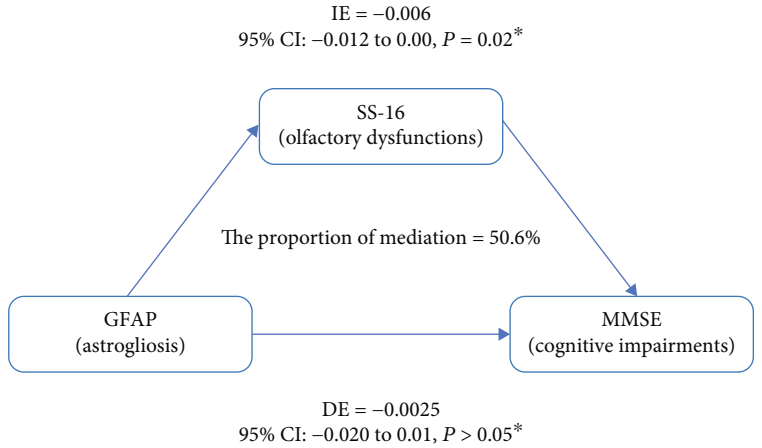



Figure 7: GFAP was found to exert an indirect effect on the MMSE scores through its effect on SS-16 scores, accounting for 50.6% of the total effects. Abbreviations: DE = the coefficient of direct effects; IE = the coefficient of indirect effects. $^*P < 0.05$.

studies, we inferred that $\alpha$-syn deposition in olfactory structures could also cause olfactory astrogliosis in the corresponding regions. Through immunohistochemical analysis, Flores-Cuadrado et al. found that $\alpha$-syn aggregates and astrogliosis (demonstrated by the increased area fraction of GFAP labeling) were significant in the OB in the PD group compared with the control group [37], which supports our inference.

Subsequently, (1) we inferred that $\alpha$-syn deposition in olfactory structures could result in regional astrogliosis, and (2) we found that astrogliosis (GFAP) levels were associated with the severity of olfactory identification deficits (SS-16 scores) in our study. Consequently, we further speculated that $\alpha$-syn deposition could cause olfactory impairment through astrogliosis. Astrocytes play an indispensable role in physiological activities, such as regulating the blood-brain barrier, nourishing neurons, and modulating neurogenesis [38]. Nevertheless, $\alpha$-syn deposits can result in astrocyte dysfunction in several ways [34, 35]. Furthermore, astrocytic function dysregulation may activate a "cascade of pathological events that contribute to and/or worsen pathology [39]." In our study, the relationship between astrogliosis (GFAP) and axonal injury (NFL) supports this assertion. IL-6 was found to be associated with astrogliosis levels (GFAP), nerve damage (NFL), and olfactory dysfunction (SS-16) in our study. Serum IL-6 levels have been shown to correlate with cerebrospinal fluid IL-6 levels in patients with PD [40]. As a proinflammatory

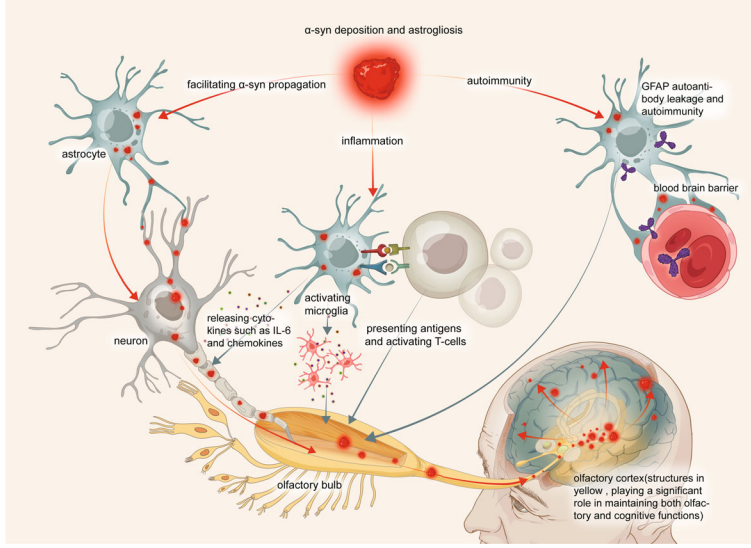



FIGURE 8: Our study suggests that astrogliosis may play a key role in olfactory and cognitive dysfunction. The olfactory structures involved in this process are shown in yellow, including the olfactory bulb and olfactory cortex, as well as other structures such as the amygdala, piriform cortex, orbitofrontal cortex, hippocampus, and entorhinal cortex. These structures are important for both olfactory processing and cognitive function. According to our inference, $\alpha$-syn first deposits in the olfactory bulbs and will be internalized by astrocytes, causing astrocyte dysfunction and regional astrogliosis in the olfactory bulbs. Then, these hyperplastic, activated, and dysfunctional astrocytes may cause olfactory structure damage in the following ways: (1) facilitating $\alpha$-syn propagation from the olfactory bulbs to other structures through olfactory tracts; (2) causing inflammatory reactions through (a) the release of a variety of neurotoxic cytokines, such as IL-6 and chemokines, (b) recruiting and activating microglia, and (c) presenting antigens and activating T-cells; or (3) providing antigens for the anti-GFAP antibodies, causing autoimmunity. Astrogliosis occurs in structures that play a crucial role in both olfactory and cognitive functions, leading to damage in both areas. Moreover, it can also facilitate the spread of $\alpha$-syn from olfactory structures to neocortical regions, further exacerbating the situation. This suggests that astrogliosis can indirectly impact cognition by affecting olfactory function.

cytokine, IL-6 is increasingly released from astrocytes when exposed to $\alpha$-syn [18, 41–43], causing structural injuries. Consequently, astrogliosis may impair olfactory function by releasing IL-6.

Based on previous studies' conclusions and our study's results, we speculated that these hyperplastic, activated, and dysfunctional astrocytes could cause olfactory structure damage in the following ways. First, astrogliosis may facilitate the propagation of $\alpha$-syn to adjacent astrocytes, microglia, and neurons through "prion-like" properties [44], endocytosis [41, 45–47], tunneling nanotubes [48], and exosomes [49–51]. Second, astrocytes can (1) release a variety of neurotoxic chemokines and cytokines, such as IL-6 [18, 41, 52, 53]; (2) recruit and activate microglia, causing inflammatory damage [54]; and (3) present antigens and activate T-cells [55], causing immune damage. Third, astrocytes can provide antigens for anti-GFAP autoimmunoreactivity from peripheral blood in the context of leakage of the blood-brain barrier because of $\alpha$-syn deposition [56, 57]. Therefore, $\alpha$-syn deposition can result in astrogliosis, astrocytic dysfunction, and eventually dysosmia. These periods are summarized in Figure 8.

Our study also found a significant indirect effect of astrogliosis on cognition through its effects on olfaction. We can interpret this result using the following two aspects of astrogliosis. First, the olfactory cortex comprises the amygdala, piriform cortex, orbitofrontal cortex, hippocampus, entorhinal cortex, and other structures. Previous studies have shown that the amygdala and piriform cortex significantly mediate the relationship between olfactory and cognitive function [58, 59]. In contrast, the orbitofrontal cortex is closely related to executive function [60]. Neurotoxic lesions in the hippocampus and entorhinal cortex can impair memory [61]. Consequently, astrogliosis injury to these structures can result in both olfactory and cognitive dysfunction. Second, according to Braak staging, $\alpha$-syn pathology may propagate through the peripheral olfactory system and infiltrate neocortical regions [62]. Therefore, for patients with dysosmia, astrogliosis may facilitate the propagation of $\alpha$-syn, accelerating the conversion process to dementia (Figure 8).

Our study has several strengths. We explored serum biomarkers of olfactory identification dysfunction in patients with PD and elucidated the role of astrogliosis in this period. Nevertheless, this study had some limitations. Dysosmia encompasses deficits in various facets of olfaction, including odor identification, detection threshold, discrimination, and memory [63]. Our study, using the Sniffin' Sticks 16-item odor identification test, was confined to exploring olfactory identification dysfunction. Second, SS-16, a relatively subjective assessment approach, is prone to the influence of differences in odorant exposure, especially when considering variations in patients' cultural backgrounds. Despite incorporating education level as a covariate in our analyses, the unfavorable impact of these factors could not be fully mitigated. Third, cognitive function was estimated using the MMSE; however, the Montreal Cognitive Assessment may be a superior method for studying cognition. Fourth, because the HCs participating in this study did not undergo

SS-16 and cytokine testing, we could not investigate the diagnostic potential of the SS-16, in combination with GFAP, NFL, and IL-6 levels, in distinguishing patients with PD from HCs. Finally, further studies are warranted to test our hypothesis through the activation and suppression of astrocytes in animal models of PD.

## 5. Conclusions

Our study suggests that serum GFAP, NFL, and IL-6 may be potential biomarkers for olfactory identification deficits in PD. We propose that astrogliosis could contribute to olfactory dysfunction and adversely impact cognitive function. These findings shed new light on the pathophysiology of PD and hint at promising therapeutic targets. Specifically, mitigating the overactivation of astrocytes and the subsequent immune-inflammatory response could potentially ameliorate olfactory deficits in PD patients. It may even exert a protective effect on their cognitive function.

## Data Availability

The data presented in this study are available on request from the corresponding authors. The data are not publicly available due to privacy and ethical restrictions.

## Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

## Authors' Contributions

FJL was responsible for the methodology, software, formal analysis, data curation, original draft preparation, visualization, and project administration. YDYL, XL, LD, and XBN performed the validation studies. FJL, RYZ, QHX, CCC, and XQH were involved in the investigation. JZ, WZ, CYX, and GYC prepared resources. FJL, CYX, and GYC were responsible for reviewing and editing the manuscript. GYC and CYX supervised and acquired funding for the project. All authors have read and approved the submitted version of the manuscript.

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