

Corneal Nerve Fiber Loss Relates to Cognitive Impairment in Patients with Parkinson's Disease

Ning-Ning Che

Henan Provincial People's Hospital, School of Clinical Medicine, Henan University

<https://orcid.org/0000-0002-5470-2003>

Qiu-Huan Jiang

Nursing department, Henan Provincial People's Hospital

Guan-Xiao Ding

Henan Provincial People's Hospital

Si-Yuan Chen

Henan Provincial People's Hospital

Zhen-Xiang Zhao

Henan Provincial People's Hospital

Xue Li

Henan Provincial People's Hospital

Rayaz A Malik

Department of Medicine, Weill Cornell Medicine-Qatar, Doha, Qatar

Jian-Jun Ma

Henan Provincial People's Hospital

Hong-Qi Yang (✉ ericynq@163.com)

Henan Provincial People's Hospital, School of Clinical Medicine, Henan University

<https://orcid.org/0000-0002-9277-0920>

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Ning-Ning Che^{1,2†}, Qiu-Huan Jiang^{1,3†}, Guan-Xiao Ding¹, Si-Yuan Chen¹,
Zhen-Xiang Zhao¹, Xue Li¹, Rayaz A Malik^{4,5}, Jian-Jun Ma^{1*}, Hong-Qi Yang^{1,6}

*Correspondence: majj1124@163.com; ericyng@163.com

†Ning-Ning Che and Qiu-Huan Jiang contributed equally to this work.

Ning-Ning Che: cnn9209@163.com

Qiu-Huan Jiang: qiuhuan1890@163.com

Guan-Xiao Ding: dingguanxiao@163.com

Si-Yuan Chen: siyuanyuexue@163.com

Zhen-Xiang Zhao: zhenxiangzhao@sina.cn

Xue Li: xueli371@163.com

Rayaz A Malik: ram2045@qatar-med.cornell.edu

Jian-Jun Ma: majj1124@163.com

Hong-Qi Yang: ericyng@163.com

¹Department of Neurology, Henan Provincial People's Hospital, School of Clinical Medicine, Henan University, Zhengzhou 450003, China

²Department of Neurology, Affiliated BenQ Hospital of Nanjing Medical University, Nanjing 210019, China

³Nursing department, Henan Provincial People's Hospital, Zhengzhou 450003, China

⁴Department of Medicine, Weill Cornell Medicine-Qatar, Doha, Qatar

⁵Division of Cardiovascular Sciences, School of Medical Sciences, University of Manchester, Manchester, UK

⁶Department of Neurology, People's Hospital of Zhengzhou University, School of Clinical Medicine, Zhengzhou University, Zhengzhou 450003, China

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Ning-Ning Che^{1,2†}, Qiu-Huan Jiang^{1,3†}, Guan-Xiao Ding¹, Si-Yuan Chen¹, Zhen-Xiang Zhao¹, Xue Li¹, Rayaz A Malik^{4,5}, Jian-Jun Ma^{1*}, Hong-Qi Yang^{1,6*}

Abstract

Background Cognitive impairment in Parkinson's disease (PD) adversely influences quality of life. There is currently no available biomarker to predict cognitive decline in PD. PD involves both the central and peripheral nervous system and especially small fiber damage occurs in PD. Corneal confocal microscopy (CCM) has been used as a non-invasive tool for quantifying small nerve fibre damage in PD. The present study investigated whether corneal nerve measures were associated with cognitive function in PD.

Methods Patients with PD were classified into those with normal cognitive function (PD-CN), mild cognitive impairment (PD-MCI), and dementia (PDD). Corneal nerve fiber density (CNFD), corneal nerve branch density (CNBD) and corneal nerve fiber length (CNFL) were quantified with CCM and compared with a control group.

Results Sixty-five PD patients (44.62% male; mean age 64.60±6.95 years; mean disease duration 4.63±2.53 years) and 30 controls (53.33% male; mean age 62.43±6.16 years) were studied. CNFD was decreased and CNBD was increased in PD patients compared to controls ($P<0.05$). CNFD decreased progressively with decline in cognitive function in PD patients. CNBD and CNBD/CNFD ratio was higher in PD-CN compared to controls but decreased with worsening cognitive function in PD-MCI and PDD patients. CNFD correlated with the Montreal cognitive assessment (MoCA) score ($r=0.683$, $P<0.0001$), unified Parkinson disease rating scale (UPDRS)-part III ($r=-0.481$, $P<0.0001$) and total UPDRS scores ($r=-0.401$, $P<0.0001$) in PD patients. CNFD, CNBD, CNFL were lower and CNBD/CNFD ratio was higher with increasing Hoehn and Yahr stage. There was no correlation between CNFD and Levodopa equivalent daily

dose (LEDD) ($r=0.176$, $P=0.161$). CNFD, CNBD and CNFL could discriminate between PD-MCI and PD-CN with an area under the curve (AUC) of 82.85%, 67.47%, and 78.74%, respectively. CNFD, CNBD and CNFL could discriminate between PDD and PD-CN with an AUC of 96.67%, 90.12% and 84.44%. A combination of all three CCM parameters further increased the AUC value.

Conclusions PD patients show evidence of corneal nerve loss compared with controls and corneal nerve parameters are associated with the severity of cognitive and motor dysfunction in PD. CCM could serve as an objective in vivo ophthalmic imaging technique to assess neurodegeneration in PD.

Keywords: Parkinson's disease, cognitive impairment, small fiber neuropathy, corneal nerve fiber, corneal confocal microscopy

Background

Parkinson's disease (PD) is the second most common neurodegenerative disorder affecting the elderly worldwide [1]. Although the primary focus is on motor symptoms in PD, cognitive impairment, hyposmia, autonomic dysfunction and sleep problems, referred to as non-motor symptoms (NMS), are increasingly recognized [2]. With disease progression, patients will experience deterioration of both motor function and NMS with worsening cognitive impairment, hallucinations, psychosis symptoms, and eventually dementia. Cognitive impairment is the most common NMS, and has an adverse effect on activity of daily life, care-giver burden and quality of life [3]. Cognitive decline in PD encompasses the full spectrum of cognitive impairment with mild subjective cognitive decline (SCD), mild cognitive impairment (PD-MCI) and Parkinson's disease dementia (PDD) [4]. In SCD, cognitive impairment may be noted by the patient, family members or clinician, but cognitive test performance is normal [5]. In PD-MCI, insidious cognitive decline may be noticed by the patient, relative or clinician, but it does not interfere significantly with daily life [5]. While in PDD, cognitive decline can severely impair daily life and functional independence [6]. PD patients have a much higher risk of dementia as compared with the general population and the majority of patients with PD will develop some degree of cognitive impairment [7]. At present, there are no reliable biomarkers for predicting patients with increased risk of cognitive decline.

PD is traditionally regarded as a central neurodegenerative disorder, but peripheral nerve degeneration has been observed [8-10]. The overall incidence of peripheral neuropathy in PD patients is 19 to 55% [11] and it may be associated with worse cognitive decline [12]. Skin biopsy has confirmed the deposition of phosphorylated α -synuclein in and loss of the cutaneous nerve fibers [10]. Additionally, three studies utilising corneal confocal microscopy (CCM) in patients with PD have shown corneal nerve loss which correlated with the severity of autonomic dysfunction and motor deficits [8,9,13]. More recently two studies have also shown a progressive loss of corneal nerve fibers in subjects with MCI and dementia using CCM [14,15].

Whether patients with PD-MCI and PDD have corneal nerve fiber loss is still unknown and is the focus of present study. We have quantified corneal nerve morphology in PD patients with different stages of cognitive decline and assessed

the association of corneal nerve fiber loss with cognitive and motor function. We have also assessed the diagnostic utility of CCM for PD-MCI and PDD.

Materials and Methods

Participants inclusion criteria

Patients with PD were recruited from the department of Neurology, Henan Provincial People's Hospital between March 2017 and October 2019. PD was diagnosed according to the 2015 MDS clinical diagnostic criteria for Parkinson's disease through a two-step process [16]. The first step is the diagnosis of parkinsonism defined as bradykinesia with either resting tremor, rigidity, or both. Then clinically established PD (absence of absolute exclusion criteria, at least two supportive criteria, and without red flags) or clinically probable PD (absence of absolute exclusion criteria, if no more than 2 red flags are present, the red flags must be counterbalanced by supportive criteria) was defined according to this criterion. All cardinal manifestations of PD were assessed by evaluating the MDS-unified Parkinson disease rating scale (UPDRS). Patients diagnosed with atypical parkinsonism (progressive supranuclear palsy, cortical basal ganglia degeneration and multiple system atrophy) and secondary parkinsonism (drug-induced, immune-mediated, inflammatory, infectious, traumatic or neoplasm, etc.) were excluded from the study. Healthy controls were included from either volunteers or spouse of PD patients who had no history of movement disorder or cognitive impairment. Patients or healthy controls younger than 40 or older than 85 years of age were excluded from the study. Participants with a history of eye surgery, eye inflammation, glaucoma, corneal disease, thyroid eye disease, diabetes, peripheral neuropathy, alcoholism, vitamin B₁₂ and folate deficiency were also excluded. All subjects agreed to participate in the study and written informed consent was obtained. The clinical profiles of each participant were carefully reviewed by experienced neurologists who were specialized in movement disorders (H.-Q. Y. and J.-J. M.). The study was approved by the ethics committee of Henan Provincial People's Hospital.

Definitions of cognitive impairment

The clinical spectrum of cognitive impairment in PD including SCD, PD-MCI and PDD. The purpose of this study is to explore the corneal nerve functions through CCM in PD, thus SCD is not included in this study because the cognitive test is in

normal range, making diagnosis difficult.

The definition of PD-MCI requires the cognitive impairment in the background of PD diagnosis as previously mentioned. This definition emphasized that PD-MCI have the same underlying disease process with PD. Also, the cognitive decline should not interfere significantly with daily life and functional independence. The relevant cognitive domains in PD-MCI include attention and working memory, executive, language, memory, and visuospatial functions. Since comprehensive testing is not always available in our team and the purpose of the present study was to measure corneal nerve function, abbreviated assessment of cognitive decline was performed. That is, the use of the MoCA alone with cutoff score of <26 was determined as a level I category for PD-MCI according to the Movement Disorder Society Diagnostic Criteria for Mild Cognitive Impairment in Parkinson's Disease [17].

The diagnosis of PDD requires a dementia syndrome (presence of deficits in at least two of the cognitive domains) developed in the context of established PD, and the cognitive decline must be severe enough to affect daily life and normal functioning. The cutoff score of <21 for MoCA was used for PDD diagnosis in this study [18].

Demographics

Age, gender, education levels, BMI, serum vitamin B12, homocysteine and cholesterol were assessed in all subjects. Disease duration was defined as the time between presentation with first motor symptoms and the present study. Levodopa equivalent daily dose (LEDD) was assessed according to the levodopa conversion formula [19]. In brief, 100mg levodopa=133mg entacapone=1mg pramipexole=5mg ropinirole=10mg selegiline=1mg rasagiline=100mg amantadine.

Motor symptoms and non-motor symptom evaluation

Part I, II and III sub-scales of the unified Parkinson's disease rating scale (UPDRS) and Hoehn and Yahr staging (H-Y) were undertaken for all patients with PD [20]. Olfactory function was assessed with an olfactory kit for Parkinson's disease (Jiangsu Parkinsense Biotech Co., Ltd, Nanjing, China). The olfactory kit consists of twelve smell cards, with four olfactory options each and the subject is required to choose the correct one. All subjects are required to complete all odor tests and the result is represented by the overall score.

Olfactory dysfunction was identified by a score <8 . Autonomic symptoms were evaluated by the scale for outcomes Parkinson's disease for autonomic symptoms (SCOPA-AUT) [21]. These include symptoms of the gastrointestinal tract, urinary tract, cardiovascular system, thermoregulation, pupil activity and sexual function with a higher score indicating more severely autonomic dysfunction. Quality of life was evaluated with the 39-item Parkinson's disease questionnaire (PDQ-39) with a higher score indicating a poorer quality of life [22]. The 14-item Hamilton anxiety rating scale (HAMA-14) and the 24-item Hamilton depression rating scale (HAMD-24) were used to assess anxiety and depression, respectively. The evaluation of motor and non-motor symptoms in PD patients were all performed in the "on" state.

Corneal confocal microscopy

Images of the corneal sub-basal plexus were prepared using a Heidelberg Retina Tomograph III with a Rostock Cornea Module (HRT III RCM; Heidelberg Engineering GmbH, Heidelberg, Germany) [23]. Lidocaine was used to anesthetize each eye and the subject was seated comfortably and instructed to fixate on an outer fixation light. The CCD camera was used to correctly position the applanating cap onto the cornea. Images from the central cornea at the level of the sub-basal plexus were captured using the "section" mode by an experienced examiner according to an established protocol [24]. We chose 4-6 best quality CCM images from the center corneal and the corneal nerves were analyzed using validated, semi-automated, purpose-written software (CCMetrics, Imaging Science and Biomedical Engineering, Manchester, UK) and the automated version (ACCMetrics) [25]. Three corneal nerve parameters were analyzed: (a) corneal nerve fiber density (CNFD): the number of all main nerve fibers per square millimeter; (b) corneal nerve branch density (CNBD): the number of branch nerves originating from the main nerve; and (c) corneal nerve fiber length (CNFL): the sum of length of all nerve fibers per square millimeter [26].

Statistical analysis

The Shapiro-Wilk test was used to check the data for normality. For variables following a normal distribution, numbers are expressed as mean \pm standard deviation (SD). Two groups were compared using independent samples t-test

and multiple comparisons were performed using analysis of variance (ANOVA) with Bonferroni as post hoc test. For variables following a non-normal distribution or non-homoscedasticity, numbers are expressed as median (interquartile range). Two groups were compared with non-parametric Mann-Whitney test and multiple comparisons were performed using non-parametric Kruskal-Wallis test. Categorical variables were compared with Chi-square tests and Fisher's exact tests. The correlation between corneal nerve measures and clinical characteristics were explored with Pearson or Spearman correlation analyses, and the standardized correlation coefficients were presented. The MoCA score was set as the dependent variable. Age, gender, BMI, VitB₁₂, disease duration, H-Y stage, UPDRS-I, UPDRS-II, UPDRS-III, LEDD, SCOPA-AUT, CNFD, CNBD, and CNFL were considered as independent variables in univariate linear regression analyses. The variables with $P < 0.05$ at the bivariate level were considered in multiple linear regression analyses. Stepwise selection was used to select variables. The receiver operating characteristic (ROC) curve was used to analyze the capability of CNFD, CNBD, and CNFL for distinguishing patients with PD- MCI from PD-CN, and PDD from PD-CN. All analyses were carried out using SPSS version 22.0 (IBM Corporation, Armonk, NY, USA). Dot plots and ROC curve were generated using GraphPad Prism version 8.0 (GraphPad Software, Inc, San Diego, CA, USA). P value of < 0.05 was considered statistically significant.

Results

Clinical and demographic profiles

A total of 75 PD patients were evaluated of whom 65 were included in the study. Among the 10 patients excluded, 7 had impaired glucose tolerance and 3 had corneal disease. The demographic and clinical profiles of PD patients (44.62% male; age 64.60 ± 6.95 years; age at onset 59.97 ± 6.84 years; disease duration 4.63 ± 2.53 years) and controls (53.33% male; age 62.43 ± 6.16 years) are summarized in **Table 1**. There were no significant differences in age, gender, education levels, BMI, plasma cholesterol, VitB₁₂, and homocysteine concentrations between control and PD patients. As shown in **Table 1**, the MoCA scores were lower in PD patients than in the control group [24.00 ($21.00, 28.00$) vs 28.50 ($27.00, 29.25$), $P < 0.0001$].

CCM measurement between PD and control group

CNFD was significantly lower in PD patients compared to controls (no./mm², 25.83±4.73 vs 34.33±3.78; mean difference, 8.49; 95% CI, 6.55 to 10.42; *P*=0.000) (**Figure 1**). CNBD was higher in PD patients compared to controls (no./mm², 31.77±14.15 vs 24.58±8.23; mean difference, -7.18; 95% CI, -12.71 to -1.66; *P*=0.011). CNFL was significantly lower in PD patients compared to controls (mm/mm², 14.43±3.31 vs 15.86±2.27; mean difference, 1.43; 95% CI, 0.10 to 2.76; *P*=0.035). The CNBD/CNFD ratio was significantly higher in PD patients compared to controls (1.20±0.44 vs 0.72±0.24; mean difference, -0.49; 95% CI, -0.66 to -0.31; *P*=0.000).

CCM measurement between PD patients with differing degrees of cognitive impairment

To assess the relationship between corneal nerve morphology and severity of cognitive impairment, PD patients were classified into 3 subgroups: PD-CN [MoCA 28.00 (27.00~29.00), n=27], PD-MCI [MoCA 23.00 (22.00~24.00), n=23], and PDD (MoCA 13.40±3.83, n=15). The representative morphology of the corneal nerve fibers in each subgroup is shown in **Figure 2** and **Figure 3**. CNFD was lower in all three PD subgroups compared to controls (*P*<0.001). CNFD in PD-MCI (no./mm², 25.04±2.67 vs 29.26±3.21; mean difference, 4.22; 95% CI, 1.51 to 6.94; *P*=0.000) and PDD (no./mm², 20.86±4.67 vs 29.26±3.21; mean difference, 8.39; 95% CI, 5.32 to 11.48; *P*=0.000) groups was significantly lower than in patients with PD-CN. CNFD in the PDD group was also significantly lower than in the PD-MCI group (no./mm², 20.86±4.67 vs 25.04±2.67; mean difference, 4.17; 95% CI, 0.99 to 7.35; *P*=0.004).

CNBD was significantly higher in patients with PD-CN compared with the control group (no./mm², 39.54±13.67 vs 24.58±8.23; mean difference, 14.96; 95% CI, 7.26 to 22.67; *P*=0.000). However, CNBD in patients with PD-MCI was lower than in the PD-CN group (*P*=0.046) and PDD patients had a significantly lower CNBD compared with PD-CN (no./mm², 18.62±10.12 vs 39.54±13.67; mean difference, 20.92; 95% CI, 11.57 to 30.28; *P*=0.000) and PD-MCI (no./mm², 18.62±10.12 vs 31.22±10.22; mean difference, 3.57; 95% CI, 2.94 to 22.22; *P*=0.004). CNFL was comparable between patients with PD-CN and the control group (mm/mm², 16.40±2.53 vs 15.86±2.27; *P*=1.000, by Kruskal-Wallis

test). CNFL in patients with PD-MCI (mm/mm^2 , 13.91 ± 1.81 vs 16.40 ± 2.53 , $P=0.007$) and PDD (mm/mm^2 , 11.69 ± 4.13 vs 16.40 ± 2.53 , $P=0.000$) was significantly lower compared to PD-CN patients.

The CNBD/CNFD ratio was significantly higher in PD-CN (1.34 ± 0.38 vs 0.72 ± 0.24 , $P=0.000$) and PD-MCI group (1.26 ± 0.44 vs 0.72 ± 0.24 , $P=0.000$) compared to controls, whilst it was lower in patients with PDD compared to patients with PD-CN ($P=0.006$).

Correlation of corneal nerve parameters with clinical and neurological outcomes

CNFD was positively associated with MoCA (*Spearman's correlation coefficient* $r=0.683$, $P<0.0001$), but negatively associated with UPDRS-III (*Pearson's correlation coefficient* $r=-0.481$, $P<0.0001$) and total UPDRS scores (*Pearson's correlation coefficient* $r=-0.401$, $P<0.0001$) in PD patients (**Figure 4**). There was no correlation between CNFD and LEDD (*Spearman's correlation coefficient* $r=0.176$, $P=0.161$). PD patients had lower CNFD, CNBD, CNFL and CNBD/CNFD ratio with higher H-Y stage (**Figure 5**).

Correlation of cognitive function with clinical and neurological outcomes

To explore the factors affecting cognitive function, univariate analysis with MoCA as the dependent variable showed a significant association with CNFD ($b=0.910$, $P<0.0001$), CNBD ($b=0.248$, $P<0.0001$), CNFL ($b=1.021$, $P<0.0001$), disease duration ($b=-1.316$, $P<0.0001$), H-Y stage ($b=-2.760$, $P<0.0001$), UPDRS-III ($b=-0.200$, $P<0.0001$), and SCOPA-AUT ($b=-0.439$, $P<0.0001$). In multiple linear regression analysis, CNFD, CNBD, CNFL, disease duration, H-Y stage, UPDRS- III, and SCOPA-AUT were independent variables and stepwise selection was used to select risk variables. After adjusting for age and sex, MoCA was associated with CNFD ($b=0.678$, $P<0.0001$), disease duration ($b=-0.713$, $P=0.001$), and SCOPA-AUT ($b=-0.165$, $P=0.036$) (adjusted $R^2=0.615$, $P<0.001$) (**Table 2**).

Diagnostic utility of CCM

ROC analysis showed that CNFD, CNBD and CNFL could discriminate between PD-MCI and PD-CN with an area under the curve (AUC) of 82.85% (95% CI, 71.82%-93.88%), 67.47% (95% CI, 52.63%-82.31%), and 78.74% (95% CI, 66.23%-91.26%). Using a CNFD cutoff of <27.98 no./ mm^2 , the

sensitivity and specificity for PD-MCI was 91.30% and 62.96%. Using a CNBD cutoff of <40.23 no./mm², the sensitivity and specificity for PD-MCI was 91.30% and 44.40%. Using a CNFL cutoff of <15.37 mm/mm², the sensitivity and specificity for PD- MCI was 78.26% and 70.37%. Moreover, a combination of all three CCM parameters resulted in an increased AUC of 85.99% (95% CI, 76.09%-95.89%), with sensitivity and specificity of 91.30% and 70.37% (**Figure 6A**).

ROC analysis showed that CNFD, CNBD and CNFL could discriminate between PDD and PD-CN with an area under the curve (AUC) of 96.67% (95%CI, 91.62%-100%), 90.12% (95%CI, 80.12%-100%), 84.44% (95%CI, 69.87%-99.02%), respectively. Using a CNFD cutoff of <25.00 no./mm², the sensitivity and specificity for PDD was 86.67% and 96.30%. Using a CNBD cutoff of <20.47 no./mm², the sensitivity and specificity for PDD was 73.33% and 96.30%. Using a CNFL cutoff of <12.29 mm/mm², the sensitivity and specificity for PDD was 66.67% and 100%. Furthermore, a combination of all three CCM parameters resulted in an increased AUC of 98.27% (95% CI, 95.36%-100%), the sensitivity and specificity was 100% and 88.89% respectively (**Figure 6B**).

Discussion

The main finding of this study is that corneal nerve loss was associated with cognitive impairment and neurological disability in patients with PD. Indeed, clinicopathological studies indicate involvement of the peripheral nervous system in PD. Two kinds of peripheral neuropathy have been proposed in PD according to the peripheral nerves involved [27,28]. One, is a medium-large fiber neuropathy (A α / β fibers involved), which is thought to be related to levodopa therapy and consequent vitamin B12 deficiency [11,29]. It usually develops in advanced PD patients [28] and can be diagnosed with nerve conduction studies. This large fiber neuropathy may affect balance and gait, and increases the chance of falls [30]. A recent study has shown that the frequency of falls almost tripled in PD patients with neuropathy as compared to PD patients without neuropathy [31]. Ceravolo et al have shown that longer time exposure to levodopa and increasing age are associated with the development of neuropathy [11]. The other type of neuropathy is characterized by a small fiber

neuropathy (A δ and C fibers involved) observed in the early stage of PD. It can be measured with a skin biopsy and/or CCM [9, 27,28]. Skin biopsy has confirmed deposition of phosphorylated α -synuclein in intraepidermal nerve fibers which correlated negatively with intraepidermal nerve fiber density (IENFD) [10]. Nolano et al have found that IENFD loss is associated with disease duration and severity [32]. There was no significant difference in IENFD in PD patients who were levodopa-naive or on levodopa treatment [33]. Our data has also revealed no association between CNFD and LEDD, which is consistent with other studies [9,13] indicating that small fiber neuropathy is not related to levodopa treatment. The present study showed a lower CNFD and higher CNBD in patients with PD which is consistent with Kass-Iliyya et al [13], indicating concomitant nerve degeneration and regeneration [34]. We further show that CCM alterations correlate with cognitive status and CNFD was independently associated with cognitive function after adjusting for age and sex. It is speculated that in the early stage of cognitive decline in PD-CN, a reduction in CNFD may activate compensatory mechanism which lead to increased CNBD and CNBD/CNFD ratio. With increasing cognitive decline nerve regeneration may become impaired and the CNBD is reduced. Indeed, the patients with PD-CN had relative mild PD with a disease duration of 3.29 years and H-Y score of 2, compared to PD-MCI and PDD and had an increased CNBD/CNFD ratio compared to the other groups. However, in the PDD group, disease duration was 7.0 years and the H-Y score was 4 indicatives of greater loss of both peripheral and central dopaminergic neurones [35]. Ponirakis et al have shown reduced CNBD in MCI and dementia patients compared to controls [14]. These subjects with MCI may have differed from PD-MCI as they were recruited from the general population and the patients with dementia included those with Alzheimer's disease, vascular dementia and mixed dementia. Our PD patients all had an underlying synucleinopathy [36]. Patients with other neurodegenerative conditions like multiple sclerosis, Wilson's disease and Friedreich's ataxia have showed a decrease in CNBD compared to controls [37-39]. Thus, different etiologies may impact differently on corneal nerve fibers [40]. Another important finding is that patients with PD-MCI and PDD have more serious autonomic symptoms compared to PD-CN patients and indeed multiple linear

regression analyses showed that MoCA was associated with CNFD and SCOPA-AUT. Autonomic nerve fibers are thinly myelinated or unmyelinated nerve fibers [41] and autonomic dysfunction may indicate small fiber neuropathy in PD. In a newly proposed clinical subtypes in PD, patients have been divided into three subgroups: the mild motor predominant, intermediate, and diffuse malignant [42,43]. The last type have not only more severe motor symptoms, but also autonomic dysfunction and cognitive impairment and show faster progression, worse prognosis and shorter survival [43]. Association between small fiber impairment (represented by autonomic dysfunction and CCM) and cognitive decline may suggest that small fiber neuropathy may be a clinical sign of diffuse malignant subtype of PD.

Our study has some limitations. The small sample size limits generalization of this result to all PD patients. The cross-sectional design cannot address whether CCM parameters at baseline might be used to predict cognitive decline and a longitudinal follow-up study with a larger sample size would be required. The PD patients are also clinically diagnosed and did not undergo a more definitive DAT PET scan, although this cannot exclude atypical PD. Cognitive function was determined by the MoCA score, instead of comprehensive cognitive testing. This was a hospital-based study and patient selection bias was unavoidable. We are also aware that dry eye, which is more frequent in PD may influence corneal nerve morphology, although this has not been found to have a major influence [44].

Conclusion

For the first time, we show that CCM demonstrates an association between corneal nerve loss and cognitive function in PD. The underlying basis for this association is not clear but it may suggest a common underlying neurodegenerative process involving both the central and peripheral nervous system. Given the relatively good diagnostic outcomes, corneal nerve fiber may be a useful marker to differentiate patients with PD and differing degrees of cognitive impairment and neurological disability.

Abbreviations

PD: Parkinson's disease; CCM: Corneal confocal microscopy; SCD: subjective cognitive decline; CN: cognitively normal; MCI: mild cognitive impairment; PDD: Parkinson's disease with dementia; CNFD: corneal nerve fiber density;

CNBD: corneal nerve branch density; CNFL: corneal nerve fiber length; UPDRS: unified Parkinson's disease rating scale; H-Y stage: Hoehn and Yahr stage; LEDD: levodopa equivalent daily dose; Hcy: Homocysteine; MoCA: Montreal cognitive assessment; SCOPA-AUT: the scale for outcomes in PD for autonomic symptoms; HAMA-14: the 14-item Hamilton anxiety rating scale; HADA-24: the 24-item Hamilton depression rating scale; PDQ-39: the 39-item Parkinson's disease Questionnaire.

Declarations

Ethics approval and consent to participate

The study was approved by the ethics committee of Henan Provincial People's Hospital. All participants provided informed written consent.

Consent for publication

Not applicable.

Availability of supporting data

The datasets used and/or analyzed in the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

N-N Che and H-Q Yang made substantial contributions to conception and design,

acquisition of data, and was involved in drafting the manuscript. Q-H Jiang was responsible for collecting patients' data and literature search. G-X Ding, S-Y Chen and Z-X Zhao participated in data collection. X Li and J-J M contributed in interpretation of the data. RA Malik helped critically in data analysis and revised the manuscript. H-Q Yang undertook general supervision of the research group, acquisition of funding, and was involved in revising the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

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Author details

¹Department of Neurology, Henan Provincial People's Hospital, School of Clinical Medicine, Henan University, Zhengzhou 450003, China

²Department of Neurology, Affiliated BenQ Hospital of Nanjing Medical University, Nanjing 210019, China

³Nursing department, Henan Provincial People's Hospital, Zhengzhou 450003, China

⁴Department of Medicine, Weill Cornell Medicine-Qatar, Doha, Qatar

⁵Division of Cardiovascular Sciences, School of Medical Sciences, University of Manchester, Manchester, UK

⁶Department of Neurology, People's Hospital of Zhengzhou University, School of Clinical Medicine, Zhengzhou University, Zhengzhou 450003, China

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Figures

Figure 1. Corneal nerve fiber parameters in controls and patients with PD. CNFD and CNFL were decreased (A, C) and CNBD and CNBD/CNFD ratio were increased (B, D) in PD as compared to the control group ($*P<0.05$, $**P<0.01$, $***P<0.001$ with control group). Abbreviations: PD, Parkinson's disease; CNFD, corneal nerve fiber density; CNBD, corneal nerve branch density; CNFL, corneal nerve fiber length.

Figure 2. Representative CCM images of corneal nerve fibers in a healthy control (A, E, and I), PD-CN (B, F, and J), PD-MCI (C, G, and K), and PDD (D, H, and L) patients. The corneal nerve plexus is beaded, linear homogeneous and highly reflective (A-D). Nerve fiber trunks are highlighted in red; branch origins are represented by the green dots (E~H) and corneal nerve fiber length is shown in green line (I~L). Images (E~H) were analyzed with the manual software (CCMetrics) and images (I~L) were marked with the automated version (ACCMetrics). PD-CN patients showed decreased CNFD and increased CNBD compared to the control group. PD-MCI and PDD patients showed a progressive reduction in CNFD, CNBD and CNFL. Abbreviations: CCM, corneal confocal microscopy; PD-CN, Parkinson's disease with cognitively normal; PD-MCI, Parkinson's disease with mild cognitive impairment; PDD, Parkinson's disease dementia; CNFD, corneal nerve fiber density; CNBD, corneal nerve branch density; CNFL, corneal nerve fiber length.

Figure 3. Corneal nerve fiber parameters in healthy controls and PD patients with varying degree of cognitive dysfunction classified into PD-CN, PD-MCI and PDD. CNFD, CNBD, CNFL and CNBD/CNFD ratio in each group was compared with the control group and dot plots were generated. CNFD decreased with cognitive decline. CNBD and CNBD/CNFD ratio was increased in PD-CN compared to controls with a trend for a decrease with cognitive impairment in PD ($*P<0.05$, $**P<0.01$, $***P<0.001$). Abbreviations: PD, Parkinson's disease; PD-CN, Parkinson's disease with cognitively normal; PD-MCI, Parkinson's disease with

mild cognitive impairment; PDD, Parkinson's disease dementia; CNFD, corneal nerve fiber density; CNBD, corneal nerve branch density; CNFL, corneal nerve fiber length.

Figure 4. Association between CNFD with MoCA, part III and total UPDRS score and LEDD using *Pearson* (A, D) or *Spearman* (B, C) correlation analyses with standardized correlation coefficients. Abbreviations: CNFD, corneal nerve fiber density; MoCA, Montreal cognitive assessment; UPDRS, unified Parkinson's disease rating scale; LEDD, levodopa equivalent daily dose.

Figure 5. Association between corneal nerve fiber parameters and disease severity according to the H-Y stage in Parkinson's disease. CNFD, CNBD, CNFL and CNBD/CNFD ratio decreased progressively with increased H-Y stage ($*P<0.05$, $**P<0.01$, $***P<0.001$). Abbreviations: H-Y, Hoehn-Yahr stage; PD-CN, Parkinson's disease with cognitively normal; PD-MCI, Parkinson's disease with mild cognitive impairment; PDD, Parkinson's disease dementia; CNFD, corneal nerve fiber density; CNBD, corneal nerve branch density; CNFL, corneal nerve fiber length.

Figure 6. The ROC for CNFD, CNBD and CNFL and a combination of all three parameters to distinguish PD-MCI and PDD from PD-CN. Abbreviations: ROC, receiver operating characteristic; CNFD, corneal nerve fiber density; CNBD, corneal nerve branch density; CNFL, corneal nerve fiber length; PD-MCI, Parkinson's disease with mild cognitive impairment; PDD, Parkinson's disease with dementia; PD-CN, Parkinson's disease with normal cognitive function; AUC, area under the curve.

Table 1. Demographic characteristics of participants

Numbers are expressed as mean \pm SD or median (interquartile range).

Abbreviations: NA, not available; PD-CN, Parkinson's disease with normal cognitive function; PD-MCI, Parkinson's disease with mild cognitive impairment;

PDD, Parkinson's disease dementia; UPDRS, unified Parkinson's disease rating scale; LEDD, levodopa equivalent daily dose; Hcy, Homocysteine; SCOPA-AUT, the scale for outcomes in PD for autonomic symptoms; HAMA-14, the 14-item Hamilton anxiety rating scale; HADA-24, the 24-item Hamilton depression rating scale; PDQ-39, the 39-item Parkinson's disease questionnaire; MoCA, Montreal cognitive assessment; CNFD, corneal nerve fiber density; CNBD, corneal nerve branch density; CNFL, corneal nerve fiber length.

(* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

a Significant difference between Controls and PD

b Significant difference between PD-CN and P-MCI

c Significant difference between PD-MCI and PDD

d Significant differences between PD-CN and PDD

Table 2. Multiple linear regression analysis for cognitive function in Parkinson's disease

In this model, MoCA was set as the dependent variable, with disease duration, H- Y, CNFD, CNBD, CNFL, UPDRS-III, and SCOPA-AUT considered as independent variables. Adjusted for age and gender, all the variables considered in the fitted model had $P < 0.05$, adjusted $R^2=0.615$, $P < 0.001$. Abbreviations: MoCA, Montreal cognitive assessment; CNFD, corneal nerve fiber density; SCOPA-AUT, the scale for outcomes in PD for autonomic symptoms; H-Y, Hoehn and Yahr Stage; CNBD, corneal nerve branch density; CNFL, corneal nerve fiber length; UPDRS, unified Parkinson's disease rating scale.

Figures

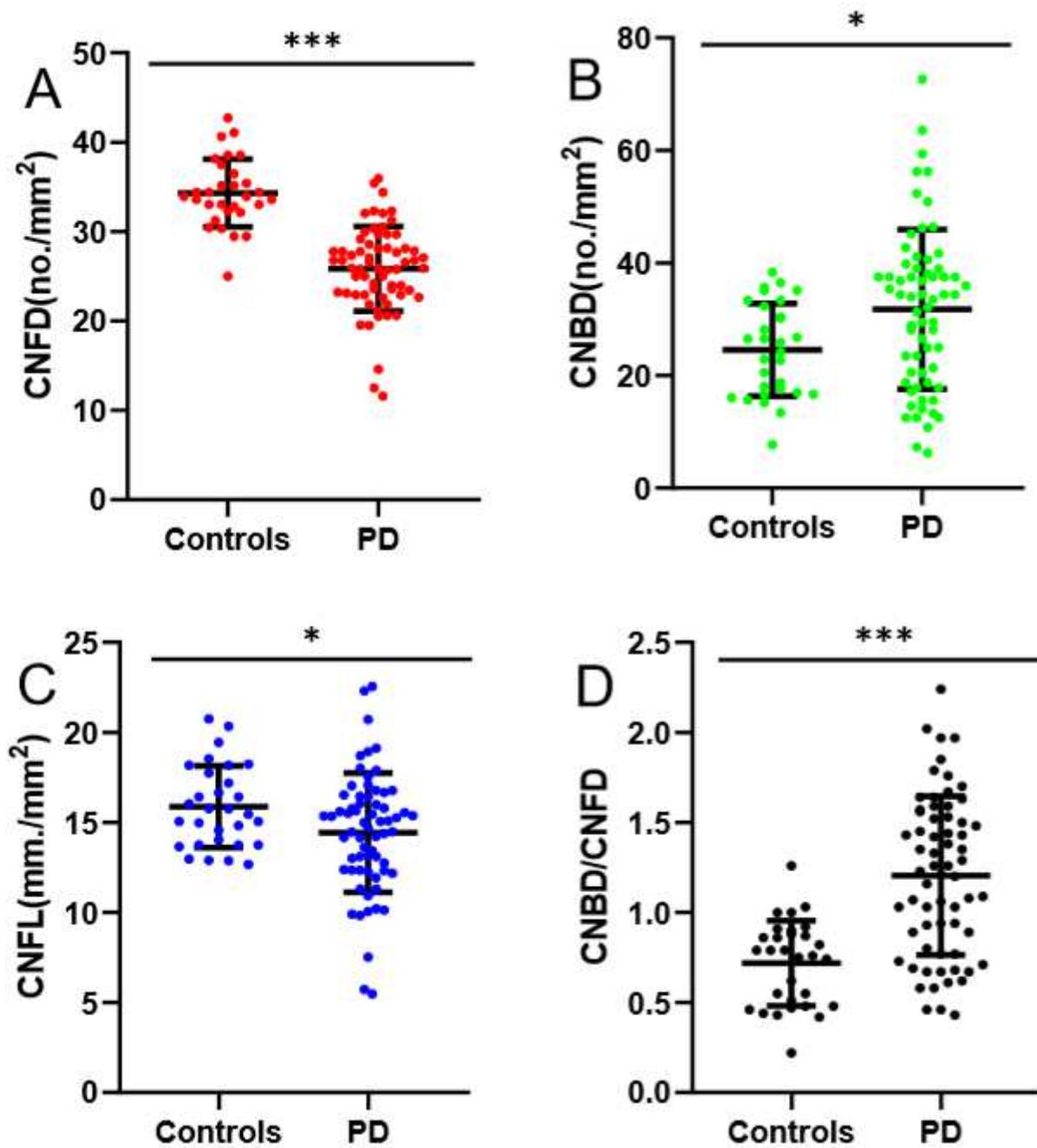


Figure 1

Corneal nerve fiber parameters in controls and patients with PD. CNFD and CNFL were decreased (A,C) and CNBD and CNBD/CNFD ratio were increased (B,D) in PD as compared to the control group (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ with control group). Abbreviations: PD, Parkinson's disease; CNFD, corneal nerve fiber density; CNBD, corneal nerve branch density; CNFL, corneal nerve fiber length.

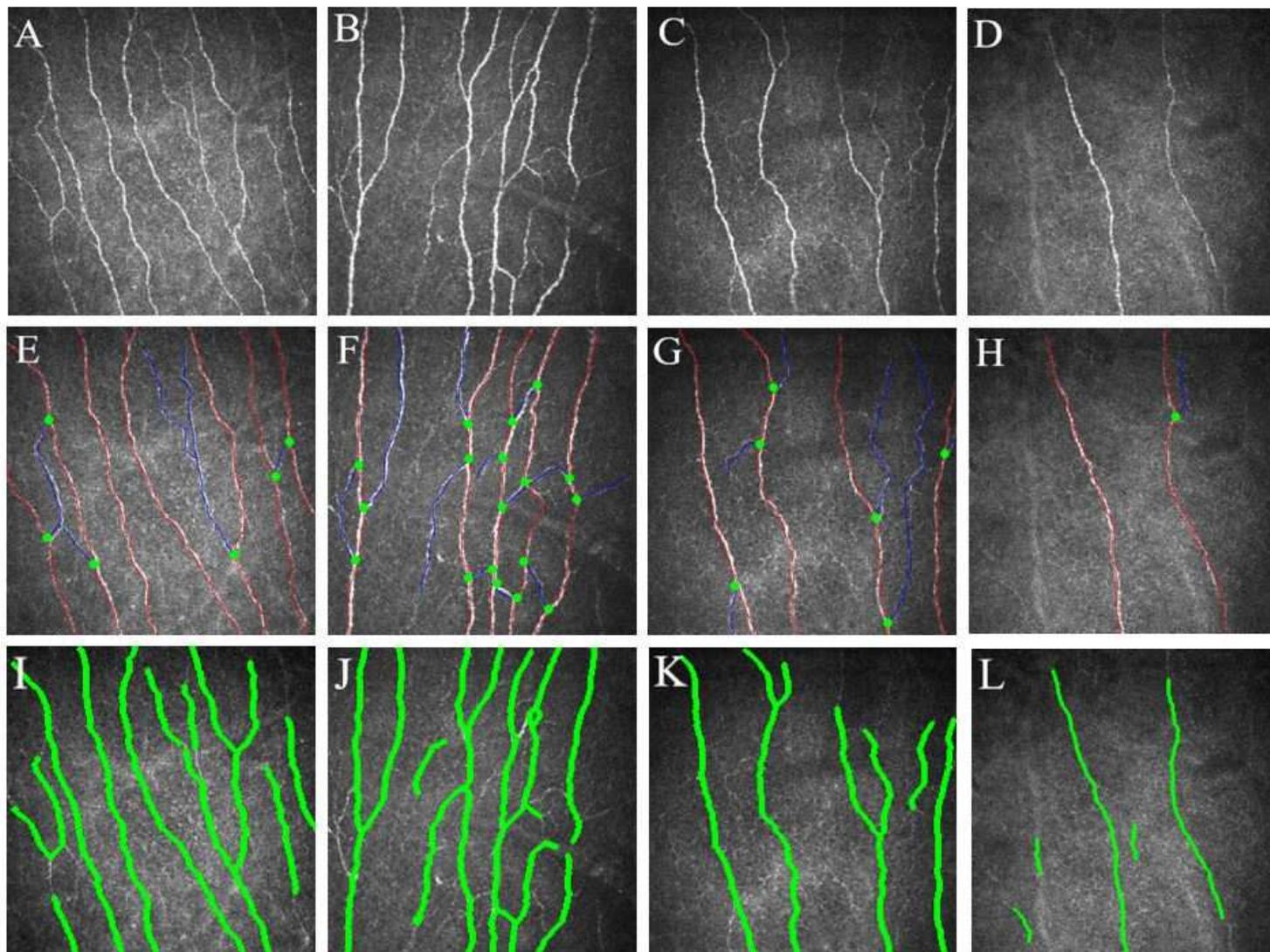


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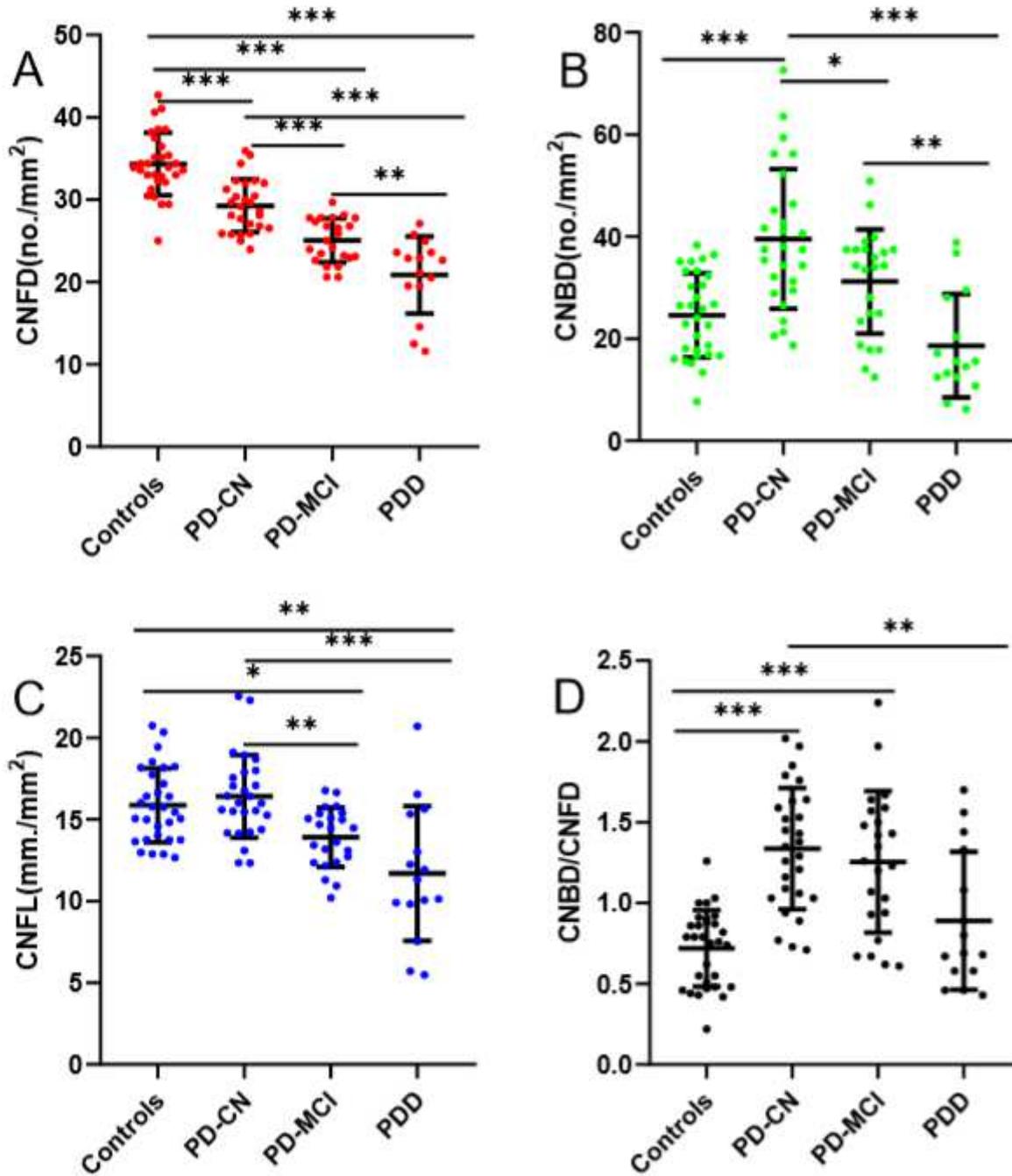


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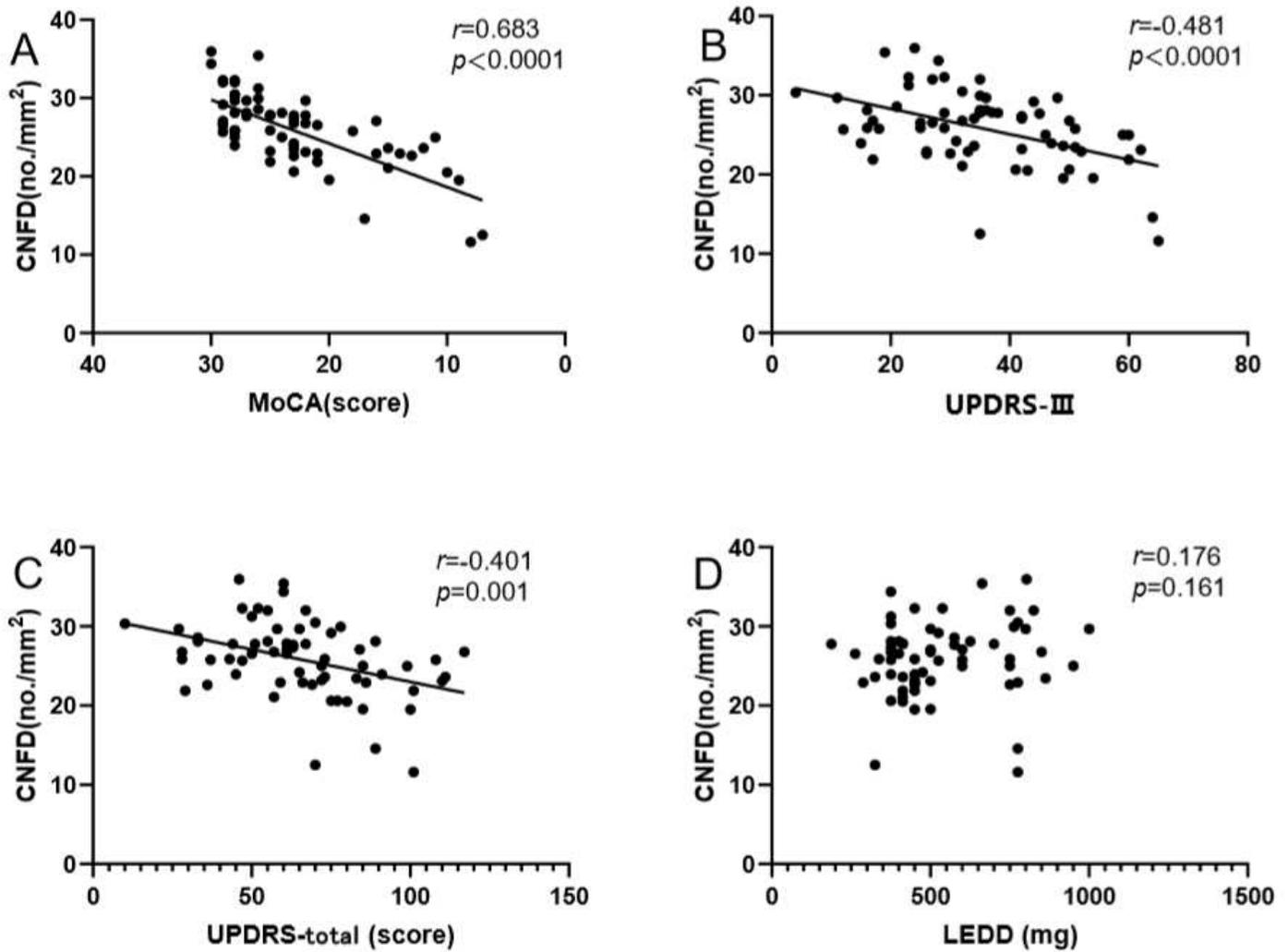


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Association between CNFD with MoCA, part III and total UPDRS score and LEDD using Pearson (A, D) or Spearman (B, C) correlation analyses with standardized correlation coefficients. Abbreviations: CNFD, corneal nerve fiber density; MOCA, Montreal cognitive assessment; UPDRS, unified Parkinson's disease rating scale; LEDD, levodopa equivalent daily dose.

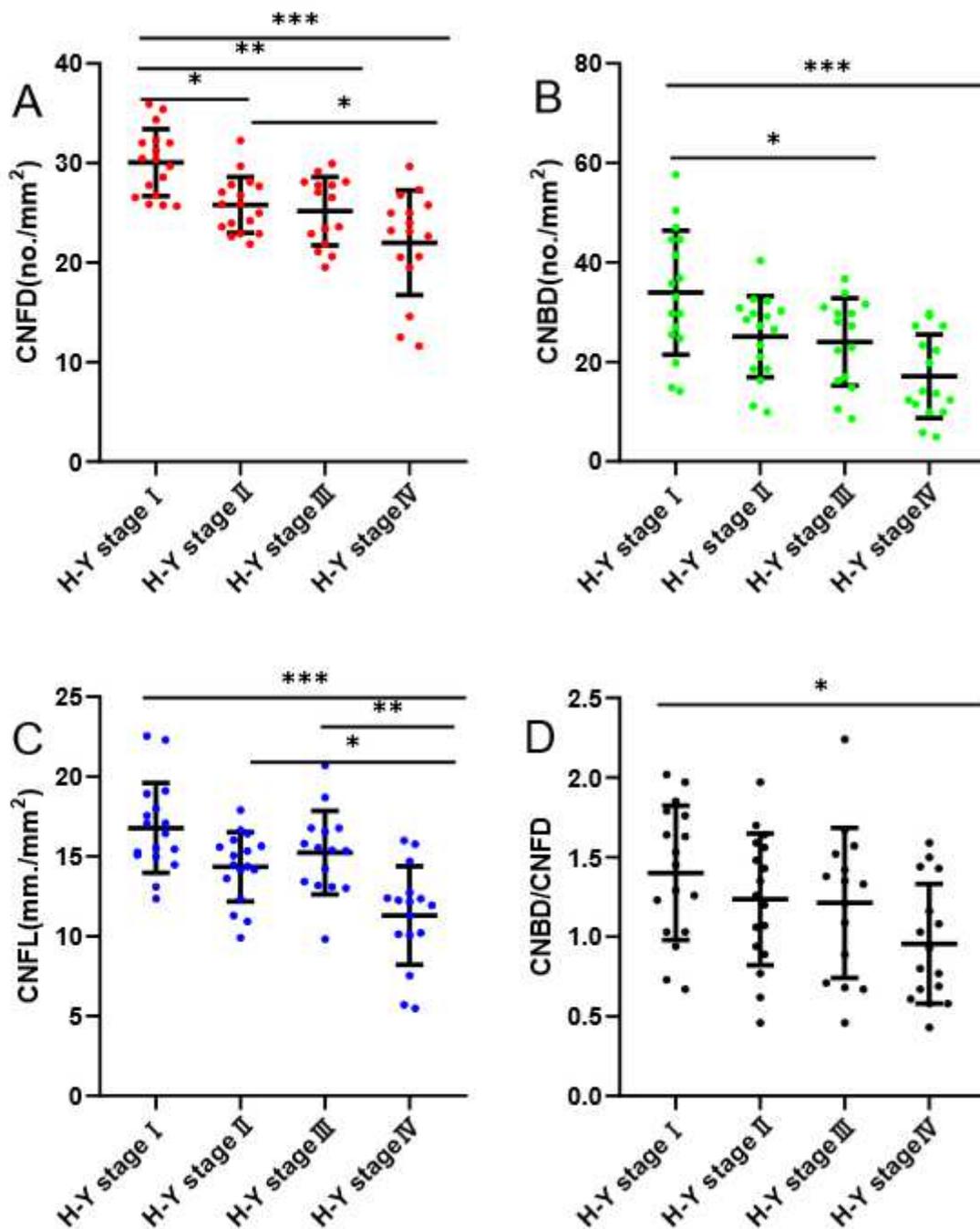


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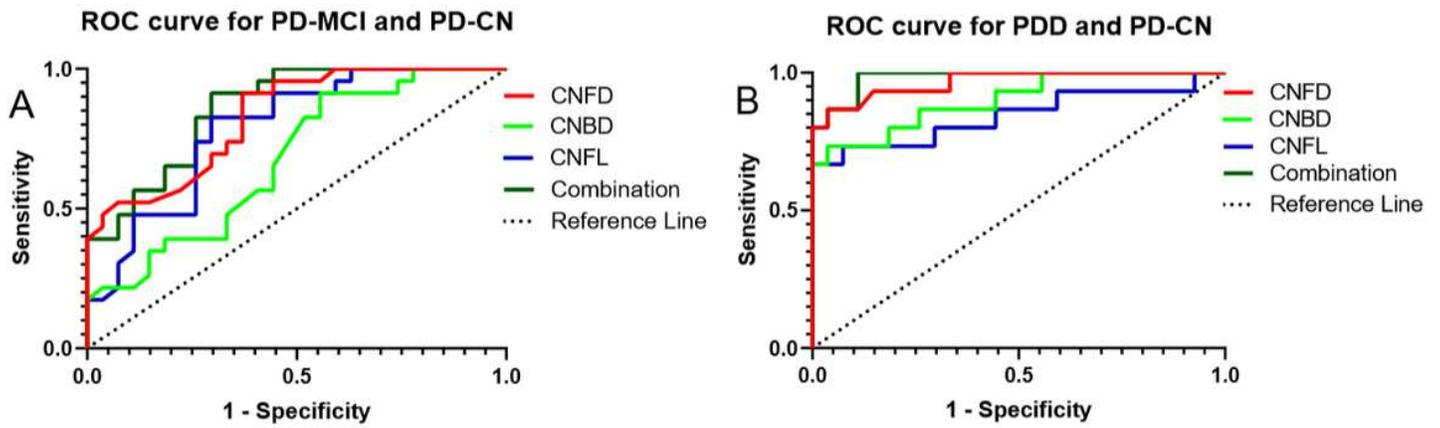


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The ROC for CNFD, CNBD and CNFL and a combination of all three parameters to distinguish PD-MCI and PDD from PD-CN. Abbreviations: ROC, receiver operating characteristic; CNFD, corneal nerve fiber density; CNBD, corneal nerve branch density; CNFL, corneal nerve fiber length; PD-MCI, Parkinson's disease with mild cognitive impairment; PDD, Parkinson's disease with dementia; PD-CN, Parkinson's disease with normal cognitive function; AUC, area under the curve.