



A nomogram based on neuron-specific enolase and substantia nigra hyperechogenicity for identifying cognitive impairment in Parkinson's disease

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Background: One in four individuals with Parkinson's disease (PD) experience cognitive impairment (CI). However, few practical models integrating clinical and neuroimaging biomarkers have been developed to address CI in PD. This study aimed to evaluate the correlation between circulating neuron-specific enolase (NSE) levels, substantia nigra hyperechogenicity (SNH), and cognitive function in PD and to develop a nomogram based on clinical and neuroimaging biomarkers for predicting CI in patients with PD.

Methods: A total of 385 patients with PD who underwent transcranial sonography (TCS) from January 2021 to December 2022 at Beijing Tiantan Hospital, Capital Medical University, were recruited as the training cohort. For validation, 165 patients with PD treated from January 2023 to December 2023 were enrolled. Data for SNH, plasma NSE, and other clinical measures were collected, and cognitive function was assessed using the Montreal Cognitive Assessment (MoCA). Logistic regression analysis was employed to select potential risk factors and establish a nomogram. The receiver operating characteristic curve and calibration curve were generated to evaluate the performance of the nomogram.

Results: Patients with PD exhibiting CI displayed advanced age, elevated Unified PD Rating Scale-III (UPDRS-III) score, an increased percentage of SNH, higher levels of plasma NSE and homocysteine (Hcy), a larger SNH area, and lower education levels compared to PD patients without CI. Gender [odds ratio (OR) =0.561, 95% confidence interval (CI): 0.330–0.954, P=0.03], age (OR =1.039; 95% CI: 1.011–1.066; P=0.005), education level (OR =0.892; 95% CI: 0.842–0.954; P<0.001), UPDRS-III scores (OR =1.026; 95% CI: 1.009–1.043; P=0.003), plasma NSE concentration (OR =1.562; 95% CI: 1.374–1.776; P<0.001), and SNH (OR =0.545; 95% CI: 0.330–0.902; P=0.02) were independent predictors of CI in patients with PD. A nomogram developed using these six factors yielded a moderate discrimination performance with an area under the curve (AUC) of 0.823 (95% CI 0.781–0.864; P<0.001). The calibration curve demonstrated acceptable agreement between predicted outcomes and actual values. Validation further confirmed the reliability of the nomogram, with an AUC of 0.864 (95% CI: 0.805–0.922; P<0.001).

Conclusions: The level of NSE in plasma and the SNH assessed by TCS are associated with CI in

patients with PD. The proposed nomogram has the potential to facilitate the detection of cognitive decline in individuals with PD.

Keywords: Parkinson's disease (PD); cognition; substantia nigra (SN); transcranial sonography (TCS); neuron-specific enolase (NSE)

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Introduction

Parkinson's disease (PD) is the second most prevalent neurodegenerative disease worldwide, and its global burden has more than doubled over the past two decades (1,2). Both motor and nonmotor symptoms (NMSs) are clinical manifestations of PD, with cognitive impairment (CI) being one of the most common and devastating NMSs, influencing approximately 25–30% of the PD population (3). One study indicated that almost half of the patients with PD and normal cognition will develop CI within 6 years, and all newly diagnosed mild CI cases will progress to dementia within 5 years (4). CI not only severely impairs functioning and reduces quality of life but also decreases life expectancy while increasing mortality rates (5), imposing a tremendous burden on families and public healthcare systems. In 2020, the Lancet Commission reported that over 40% of dementia cases worldwide could be theoretically prevented or delayed, especially in low-income and middle-income countries (6). Given the heterogeneous nature of the mechanisms underlying CI in PD, different biomarker patterns suggest unique yet interconnected alternations in the brain. Therefore, identifying and integrating several types of markers to create different models for accurately predicting individuals at higher risk of early or rapid cognitive deterioration is important for prognosis and effective patient management.

Several cerebrospinal fluid (CSF) biomarkers, such as $A\beta_{42}$, total tau, p-tau, and α -synuclein, have been studied to determine their association with CI in PD (7). Nevertheless, clinically administering a lumbar puncture to retrieve a CSF sample can be uncomfortable for certain patients. Although neuron-specific enolase (NSE), a glycolytic enzyme found in neuronal and neuroendocrine tissues, is not biologically released, its elevated level in the circulation can indicate neuronal damage. A meta-analysis revealed that CSF NSE levels are significantly higher in patients with Alzheimer disease (AD), PD with dementia (PDD), and dementia with

Lewy bodies but not in those with PD without dementia or multiple system atrophy (8).

The use of structural, functional, and molecular neuroimaging has significantly enhanced researchers' understanding of clinical features and pathological processes (9–11). Nevertheless, studies exploring the pathological link between the substantia nigra (SN) and CI in PD have yielded conflicting results (12). One study found that iron deposition in the SN assessed via quantitative susceptibility mapping (QSM) increased as early as the prodromal stage of PD but was not significantly associated with Montreal Cognitive Assessment (MoCA) scores (13). Another study discovered that the susceptibility value of the bilateral hippocampus and SN might predict CI in patients with chronic cerebral hypoperfusion (14). However, functional magnetic resonance imaging (MRI) is not a routine clinical examination; in contrast, transcranial sonography (TCS) is an economical and convenient method with a high sensitivity in detecting SN hyperechogenicity (SNH) (15). In our previous study, SN grading and certain clinical characteristics demonstrated good predictive potential for CI in PD (16). Therefore, we conducted this subsequent study to investigate the correlation between circulating NSE levels, SNH, and cognitive function in PD. We also sought to develop a nomogram based on clinical and neuroimaging biomarkers to predict CI in patients with PD. We present this article in accordance with the TRIPOD reporting checklist (available at <https://qims.amegroups.com/article/view/10.21037/qims-23-1778/rc>).

Methods

Patient selection and study design

This cross-sectional study was conducted in accordance with the Declaration of Helsinki (as revised in 2013) and was approved by the Ethics Board of Beijing Tiantan Hospital, Capital Medical University (No. KT2022-015-

04). Informed consent was obtained from all the patients. Patients who were treated in the Department of Dyskinesia and who underwent TCS examinations from January 2021 to December 2022 were selected as the training set. A separate test cohort was recruited from patients who attended the same medical center from January 2023 to December 2023 to validate the reliability of the prediction model. The allocation ratio of the training set and test set was 7:3. Eligible participants were individuals with PD aged 18 years and above, with complete clinical data and a TCS result. The exclusion criteria were the following: Parkinson syndrome caused by cerebrovascular disease, craniocerebral tumors, trauma, or medications; insufficient clinical history; ambiguous SN results from TCS examination; and patients with implanted deep brain stimulation (DBS) electrodes, stem cell therapy, or pallidotomy. The diagnosis of PD adhered to the criteria set by the International Parkinson and Movement Disorder Society (17).

Cognitive and behavioral assessments

Two neurologists, each with more than 5 years of experience in handling patients with movement disorders, conducted neurological examinations on the participants and evaluated them using the Movement Disorder Society Unified Parkinson's Disease Rating Scale (MDS-UPDRS). If a consistent diagnosis could not be reached, the final diagnosis would be made by a senior expert with over 10 years of experience in managing movement disorders. Prior to the clinical assessments, the patients refrained from antiparkinsonian medications for at least 12 hours. All patients underwent neuropsychological tests via the MoCA (18) and the Mini-Mental State Examination (MMSE) (19). Global cognitive function was evaluated using MoCA according to the critiques and recommendations set by the International Parkinson and Movement Disorders Society (18). The overall MoCA score ranges from 0 to 30, with a value below 26 indicating CI (20). The participants were then classified into two groups: PD patients with normal cognition (PD-NCI) and PD patients with CI (PD-CI).

Clinical data collection

Demographic and clinical data, including gender, age, disease duration, education level, body mass index, and smoking and drinking habits, in addition to the medical history of hypertension, diabetes mellitus (DM), and

hyperlipemia, were collected. A fasting venous blood sample of 5 ml was collected in the morning, and the serum was immediately centrifuged, preserved at -80°C , and analyzed using an immunoturbidimetric assay. Ferritin, folate, and vitamin B12 (VitB12) levels were measured using a chemiluminescent assay (Cobas E601, Roche Diagnostics, USA), and homocysteine (Hcy) levels were determined through a cyclase assay with an electrochemiluminescence immunoassay analyzer (AU 5800, Roche Diagnostics). The plasma concentration of NSE was determined using the Elecsys NSE assay (Roche Diagnostics).

TCS examination

Two sonographers with at least 5 years of expertise in neuroimaging performed the ultrasound examination. Hyperechoic areas of the SN were identified and measured by two experienced sonographers, each with over 10 years of experience in neuroimaging, who were blinded to the patient's clinical diagnosis. The ultrasound examination was conducted with an ultrasound machine (Aplio i900, Canon, Japan) equipped with an I6SX1 phased array probe (2.0–3.0 MHz) under a scanning setting of 14–16 cm of depth and a dynamic range of 45–55 dB. Image brightness and gain compensation were adjusted according to the patient's condition.

The patient was placed in the supine position, with the temporal window fully exposed. The probe was placed close to the temporal window on one side, and the different axial sections of the midbrain were explored via slight adjustments of the angle of the sound beam in the direction of the auricular frame line (the line connecting the tip of the ear and the corner of the eye). A butterfly-shaped midbrain is located near the cerebral midline, and its peripheral and internal nuclei could be observed in the presence of a good bone window. The area of SNH was calculated if there was hyperechogenicity observed on the left or right sides of the SN. The echogenicity of the SN was graded as described by Bartova *et al.* (21) (*Figure 1*). Interclass correlation coefficients (ICCs) were employed to test interobserver agreement for SN grading and the SNH area calculation.

Statistical analysis

The measurement data are reported as the mean \pm standard deviation (SD) or median with interquartile range (IQR), whereas discrete variables are expressed as percentages. Differences between groups were compared using

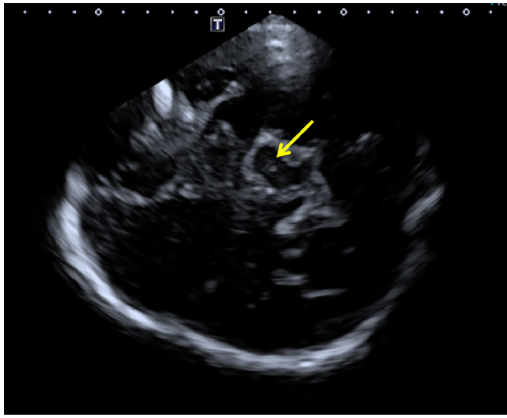


Figure 1 Transcranial ultrasonography showing butterfly-shaped midbrain and hyperecho of the left substantia nigra (yellow arrow).

unpaired independent *t*-tests for continuous variables with normal distributions, the nonparametric Mann-Whitney test for continuous variables without normal distribution, and the chi-squared test for discrete variables. Individual missing values were replaced with the average of adjacent values rather than deleted. Spearman correlation analysis and partial correlation analysis were performed to detect the correlation between NSE, area of SNH, and demographic data. Univariate logistic regression analysis was used to identify potential risk factors for CI. Variables with significance ($P < 0.1$) in the univariate analysis were incorporated into the multivariable model. The final prediction model was visualized with a nomogram for predicting CI. The model's discriminative ability was assessed using the concordance index (C-index) or area under the curve (AUC) of the receiver operating characteristic (ROC) curve, while the model's calibration was evaluated with a calibration curve. $P < 0.05$ was considered statistically significant. All statistical analyses were performed using SPSS version 25.0 (IBM Corp., USA) or RStudio (version 4.3.2).

Results

Demographics and clinical characteristics

The demographic and clinical data of the training and test cohorts are summarized in *Table 1*. The training set consisted of 385 patients with an average age of 63.33 ± 9.69 years, 246 of whom were males. The mean disease duration in this set was 6.0 ± 4.83 years. The test set comprised 165 patients with an average age of 64.68 ± 8.83 years, 99 of

whom were male. The patient selection process is presented in *Figure S1A,S1B*. The mean MoCA score was 23 ± 5.85 in the training set and 21.47 ± 5.38 in the test set. Apart from the MoCA score, status of dementia, and history of hyperlipemia, there were no statistically significant differences in the characteristics between the two sets. More details can be found in *Table S1*.

Based on the MoCA threshold of 26, 53.0% (204/385) of patients in the training set had CI, 47.5% (97/204) of whom were diagnosed with PDD. In the test set, 69.7% (115/165) exhibited CI, with 49.6% (57/115) diagnosed with PDD. In the training set, significant differences were observed in gender, age, hyperlipidemia, Hoehn and Yah (H-Y) stage, education level, PDD, and UPDRS-III score, whereas differences in other variables, such as disease duration, BMI, history of smoking and drinking, hypertension, and DM, were not statistically significant. These findings were consistent with those of the test cohort (*Table 1*).

Serum and imaging biomarkers

The ICC values for SN grading and calculation of SNH area were 0.80 (range, 0.78–0.83) and 0.73 (range, 0.71–0.77), respectively, indicating an acceptable level of agreement between raters. In both sets, the levels of NSE ($P < 0.001$), Hcy concentration ($P = 0.03$), and percentage of SNH ($P < 0.001$) were higher in the PD-CI group than in the PD-NCI group. In the training set, a larger area of SN ($P = 0.001$) was found in the PD-CI group. There were no significant differences observed in the levels of plasma folate, VitB12, or ferritin between the two groups (*Table 1*).

Factor selection for the predictive model

In the training set, Spearman correlation analysis showed that age and disease duration were associated with education level, H-Y stage, UPDRS-III score, Hcy, and NSE (*Table S2*). Additionally, gender differences in education level, Hcy, and area of SNH were significantly different (*Table S3*). These three factors were employed as covariates in subsequent analyses. Spearman partial correlation analysis indicated that H-Y stage ($r = -0.198$; $P < 0.001$), UPDRS-III ($r = -0.269$; $P < 0.001$), education status ($r = 0.401$; $P < 0.001$), Hcy ($r = -0.134$; $P < 0.001$), and NSE level ($r = 0.303$; $P < 0.001$) were all correlated to MoCA score after adjustments were made for age, disease duration, and gender (*Table 2*).

The ROC curve showed that plasma NSE alone exhibited a moderate discriminatory power for identifying

Table 1 Baseline demographic and clinical data of participants

Parameter	Training group			Test group		
	PD-CI (n=204)	PD-NCI (n=181)	P value	PD-CI (n=115)	PD-NCI (n=50)	P value
Male gender (%)	140 (68.6)	106 (58.6)	0.04 ^a	75 (65.2)	24 (48.0)	0.03 ^a
Age (years)	64.85±9.15	61.61±10.02	0.001 ^b	65.86±8.87	61.96±8.17	0.009 ^b
BMI (kg/m ²)	24.12±3.83	24.28±3.26	0.67 ^b	21.43±3.61	23.87±3.46	0.35 ^b
Duration of disease (years)	6.33±4.84	5.63±4.81	0.15 ^b	6.76±4.06	6.78±3.62	0.97 ^b
Education level (years)	9 (0, 19)	12 (0, 21)	<0.001 ^c	9 (0,22)	12 (6,17)	<0.001 ^c
Smoking, n (%)	52 (25.5)	34 (18.8)	0.11 ^a	22 (19.1)	9 (18.0)	0.86 ^a
Drinking, n (%)	47 (23.0)	32 (17.7)	0.19 ^a	22 (19.1)	8 (16.0)	0.63 ^a
Hypertension, n (%)	70 (34.3)	53 (59.3)	0.29 ^a	49 (42.6)	17 (34.0)	0.30 ^a
Diabetes mellitus, n (%)	34 (16.7)	24 (13.3)	0.35 ^a	19 (16.5)	6 (12.0)	0.45 ^a
Hyperlipidemia, n (%)	39 (19.1)	18 (9.9)	0.01 ^a	27 (23.5)	9 (18.0)	0.43 ^a
H-Y stage	2.81±1.00 ^d	2.47±0.86	<0.001 ^b	2.93±0.82	2.56±0.93 ^d	0.01 ^b
MoCA score	18.50±5.16	27.30±1.34	<0.001 ^b	19.05±4.59	27.025±1.61	<0.001 ^b
PDD, n (%)	97 (47.5)	0	<0.001 ^a	57 (49.6)	0	<0.001 ^a
UPDRS-III	41.70±16.79	33.51±16.02	<0.001 ^b	42.21±16.94 ^e	35.19±13.88	0.01 ^b
Hcy (μmol/L)	16.59±8.62	14.70±7.88	0.02 ^b	17.02±9.52	13.93±4.65	0.03 ^b
Folate (ng/mL)	7.67±5.67	8.11±4.54	0.40 ^b	8.75±6.49	7.32±4.17 ^f	0.15 ^b
Vitamin B12 (pg/mL)	470.38±103.15	512.38±306.47	0.30 ^b	490.93±376.15	487.14±331.92 ^g	0.95 ^b
Ferritin (ng/mL)	124.78±119.02	116.93±103.15	0.53 ^b	137.42±100.51	108.17±79.5 ^h	0.72 ^b
NSE (ng/mL)	16.38±3.66	13.96±2.07	<0.001 ^b	16.22±3.65	13.19±2.57	<0.001 ^b
SNH, n (%)	139 (68.1)	91 (50.3)	<0.001 ^a	83 (72.2)	27 (54.0)	0.02 ^a
Area of SNH (cm ²)	0.32 (0, 1.11)	0 (0,1.03)	0.001 ^c	0.19 (0,0.83)	0.32 (0,0.85)	0.09 ^c

Education level and area of SNH are presented as the median (interquartile range); gender, smoking, drinking, hypertension, diabetes mellitus, hyperlipidemia, PDD, and SNH are presented as number (frequency). The other parameters are presented as the mean ± standard deviation. ^a, χ^2 test; ^b, unpaired independent *t*-test; ^c, nonparametric Mann-Whitney test; ^d, missing value: H-Y stage, training group (PD-CI) 1 and test group (PD-NCI) 1; ^e, missing value: UPDRS-III, test group (PD-CI) 1; ^f, missing value: folate, test group (PD-NCI) 1; ^g, missing value: vitamin B12, test group (PD-NCI) 1; ^h, missing value: ferritin, test group (PD-NCI) 1. PD-CI, Parkinson's disease with cognitive impairment; PD-NCI, Parkinson's disease without cognitive impairment; BMI, body mass index; H-Y, Hoehn and Yahr; MoCA, Montreal Cognitive Assessment; PDD, Parkinson's disease with dementia; UPDRS-III, Unified Parkinson's Disease Rating Scale Part III; Hcy, homocysteine; NSE, neuron-specific enolase; SNH, substantia nigra hyperechogenicity.

cognitive decline in PD, with an AUC of 0.774 [95% confidence interval (CI): 0.725–0.823; $P < 0.001$]. The sensitivity and specificity of NSE in diagnosing CI in PD were 79.4% and 82.7%, respectively, with a cutoff value of 15.255 (*Figure 2A*). In the test group, the AUC for NSE in predicting PD-CI was 0.760 (95% CI: 0.684–0.837; $P < 0.001$), and the corresponding sensitivity and specificity were 79.6% and 85.7%, respectively (*Figure 2B*).

For the identification of risk factors affecting cognitive

function, univariate logistic regression showed that age, gender, hyperlipidemia, H-Y stage, education level, UPDRS-III, Hcy, NSE, SNH, and area of SNH were potential risk factors. After the influence of age, disease duration, and gender was accounted for, the final multivariable logistic model incorporated six predictors (age, gender, education level, UPDRS-III, NSE, and SNH) in both the training and test sets (*Table 3*, *Table S4*). A nomogram was generated based on these risk factors and presented in *Figure 3*.

Table 2 Spearman partial correlation analysis after adjustments for age, disease duration, and gender in the training set

Parameter	H-Y stage	UPDRS-III	MoCA	Education level	Hcy	Folate	Vitamin B12	Ferritin	NSE	Area of SNH
H-Y stage	–	–0.399	0.198	–0.132	–0.222	0.017	–0.085	–0.006	–0.087	0.060
P		<0.001	<0.001	0.01	<0.001	0.74	0.10	0.90	0.09	0.24
UPDRS-III	0.399	–	–0.269	0.177	0.109	–0.111	–0.035	–0.005	0.111	0.003
P	<0.001		<0.001	0.001	0.03	0.03	0.49	0.92	0.03	0.95
MoCA	–0.198	–0.269	–	0.401	–0.134	0.030	0.034	–0.061	–0.303	–0.082
P	<0.001	<0.001		<0.001	0.009	0.56	0.5	0.23	<0.001	0.10
Education level	–0.132	–0.177	0.401	–	–0.109	0.035	–0.015	–0.169	–0.089	0.049
P	0.01	0.001	<0.001		0.03	0.50	0.76	0.001	0.08	0.34
Hcy	–0.222	0.109	–0.134	–0.109	–	–0.264	–0.234	0.076	0.005	0.037
P	<0.001	0.03	0.009	0.03		<0.001	<0.001	0.14	0.92	0.47
Folate	0.017	–0.111	0.03	0.035	–0.264	–	0.192	–0.060	–0.024	–0.024
P	0.74	0.03	0.56	0.50	<0.001		<0.001	0.24	0.64	0.64
Vitamin B12	–0.085	–0.035	0.034	–0.015	–0.234	0.192	–	0.070	–0.042	–0.042
P	0.10	0.49	0.50	0.76	<0.001	<0.001		0.17	0.41	0.41
Ferritin	–0.006	–0.005	–0.061	–0.169	0.076	–0.063	0.070	–	–0.012	–0.023
P	0.90	0.92	0.23	0.001	0.14	0.24	0.17		0.81	0.65
NSE	–0.087	0.111	–0.303	–0.089	0.005	–0.024	–0.042	–0.012	–	0.106
P	0.09	0.03	<0.001	0.08	0.92	0.64	0.41	0.81		0.03
Area of SNH	0.06	0.003	–0.082	0.049	0.037	–0.024	–0.042	–0.023	0.106	–
P	0.24	0.95	0.10	0.34	0.47	0.64	0.41	0.65	0.03	

H-Y, Hoehn and Yahr; UPDRS-III, unified Parkinson's Disease Rating Scale Part III; MoCA, Montreal Cognitive Assessment; Hcy, homocysteine; NSE, neuron-specific enolase; SNH, substantia nigra hyperechogenicity.

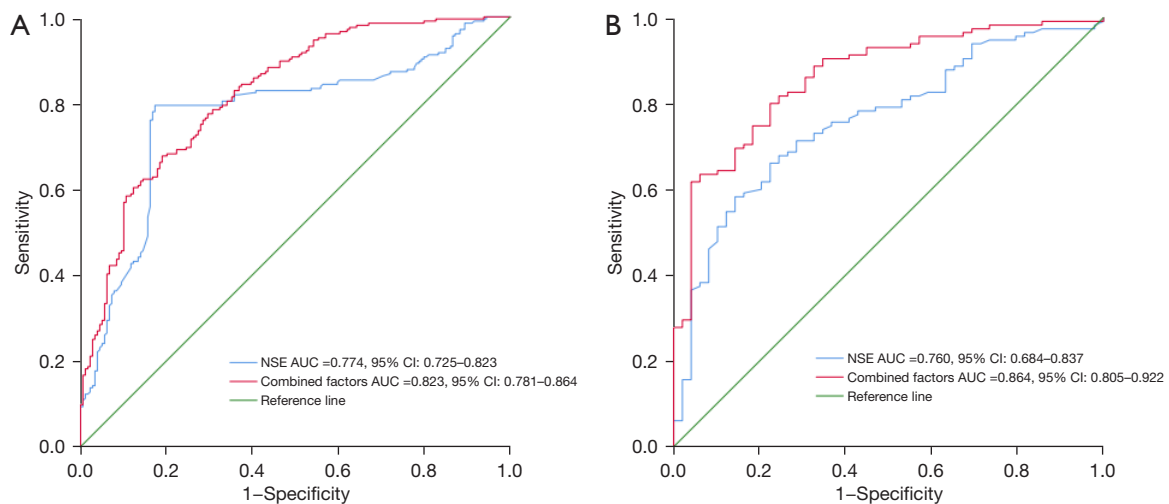


Figure 2 Receiver operating characteristic curves for evaluating the discrimination capability of plasma NSE and the final model. (A) Training set. (B) Test set. NSE, neuron-specific enolase; AUC, area under the curve; CI, confidence interval.

Table 3 Logistic regression analysis of possible risk factors and cognitive impairment in the test set

Parameter	Cognitive impairment (univariate analysis)		Cognitive impairment (multivariate analysis)	
	OR (95% CI)	P value	OR (95% CI)	P value
Gender (male)	0.492 (0.251, 0.967)	0.03	0.645 (0.220, 0.972)	0.03
Age	1.053 (1.012, 1.095)	0.01	1.081 (1.030, 1.134)	0.002
BMI	1.046 (0.951, 1.150)	0.35		
Duration of disease	0.986 (0.918, 1.058)	0.69		
Education year	0.846 (0.774, 0.925)	<0.001	0.804 (0.714, 0.905)	<0.001
Drinking history	0.805 (0.332, 1.956)	0.63		
Smoking history	0.928 (0.393, 2.189)	0.86		
Hypertension	0.694 (0.347, 1.386)	0.30		
Diabetes mellitus	0.689 (0.257, 1.845)	0.45		
Hyperlipidemia	0.715 (0.309, 1.658)	0.44		
H-Y stage	1.723 (1.128, 2.631)	0.01	1.091 (0.565, 2.108)	0.79
PDD	1.096 (0.547, 2.196)	0.79		
UPDRS-III	1.029 (1.006, 1.052)	0.01	1.023 (1.009, 1.051)	0.03
Hcy	1.070 (1.005, 1.138)	0.03	1.048 (0.974, 1.127)	0.20
Folate	1.047 (0.983, 1.115)	0.15		
Ferritin	1.004 (1.000, 1.008)	0.08	1.001 (0.935, 1.013)	0.05
Vitamin B12	1.00 (0.999, 1.001)	0.95		
NSE	1.398 (1.214, 1.609)	<0.001	1.434 (1.218, 1.688)	<0.001
SNH	0.453 (0.227, 0.902)	0.02	0.527 (0.208, 0.804)	0.03
Area of SNH	0.324 (0.093, 1.132)	0.07	0.320 (0.091, 1.127)	0.08

OR, odds ratio; CI, confidence interval; BMI, body mass index; H-Y, Hoehn and Yahr; PDD, Parkinson's disease with dementia; UPDRS-III, Unified Parkinson's Disease Rating Scale Part III; Hcy, homocysteine; NSE, neuron-specific enolase; SNH, substantial nigra hyperechogenicity.

Validation and calibration of the nomogram

To verify the accuracy of the nomogram, internal validation was conducted using the bootstrap method with 1000 resamples. The final model had a C-index of 0.823 (95% CI: 0.781–0.864; $P < 0.001$), along with a sensitivity of 67.6% and a specificity of 81.0% (Figure 2A). In the test group, the final model achieved an AUC of 0.864 (95% CI: 0.805–0.922; $P < 0.001$) for predicting PD-CI (Figure 2B), with a sensitivity and specificity of 80.0% and 77.6%, respectively. These results indicated the nomogram's discriminatory ability. Furthermore, the calibration curves in both cohorts demonstrated a high level of agreement between the predicted and ideal models (Figure S2A,S2B).

Discussion

The predictive model for clinical diagnosis and prognosis is a valuable tool and has been partially applied in clinical practice (22,23). In a previous study, a nomogram was constructed by combining dopamine transporter (DAT) imaging, CSF biomarkers (p-tau/ α -syn and p-tau), and the rs55785911 genotype to predict mild CI in patients with PD and rapid-eye-movement sleep behavior disorder (24). However, the high costs associated with both DAT imaging and genotypic sequencing, as well as the unsuitability of lumbar puncture for certain individuals, limit the broad application of this model in clinical settings. TCS is a cost-effective and reproducible technique, and its qualitative and

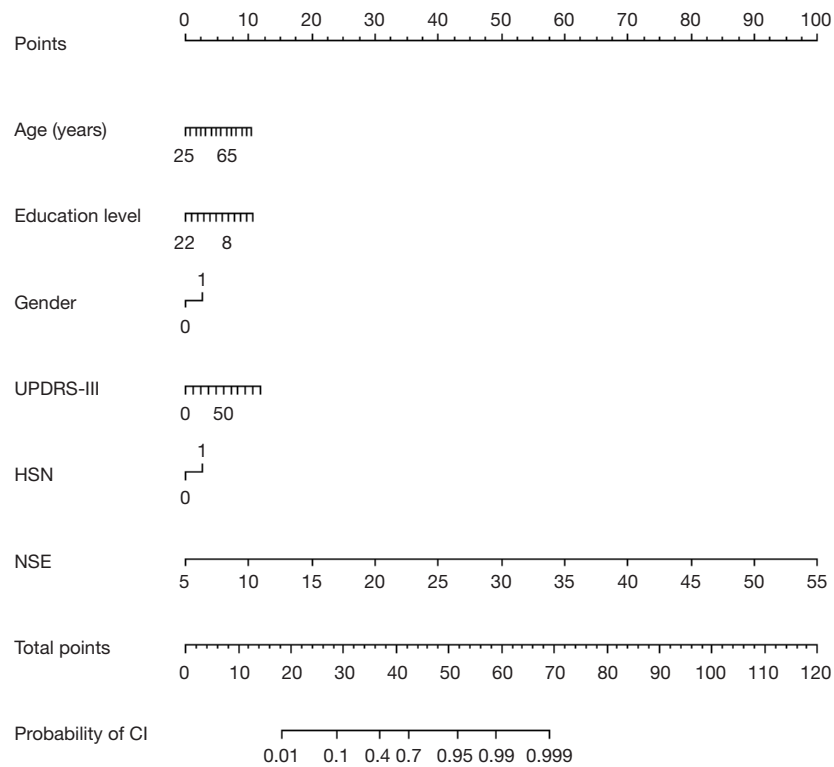


Figure 3 The nomogram constructed to visualize the individual probabilities of diagnosing cognitive impairment in Parkinson's disease. UPDRS-III, Unified Parkinson's Disease Rating Scale part III; HSN, hyperechogenic substantia nigra; NSE, neuron-specific enolase; CI, cognitive impairment.

quantitative analysis of SN echogenicity has been clinically recommended as an auxiliary diagnostic indicator of PD (25,26). Plasma markers are also readily available parameters in daily practice. Therefore, the primary purpose of this study was to develop an objective and practical nomogram based on clinical and TCS imaging biomarkers to assist clinicians in identifying CI in patients with PD. Our results identified six independent risk predictors of CI in PD, including age, gender, UPDRS-III scores, education status, plasma NSE level, and SNH. A visualizable nomogram was developed based on these factors, which yielded a satisfactory diagnostic power for CI in PD with an AUC of 0.823 (95% CI: 0.781–0.864; $P < 0.001$). The AUC of the test set also supported the reliability of the model.

The prominent neurodegeneration in the SN is the main cause of striatal dopamine dysfunction, leading to cardinal motor symptoms and cognitive deficits in PD (27). Iron deposition and microglia activation play an important role in the progression of SNH. In an animal study, the injection of a dopamine-6-hydroxylase-induced PD model resulted in poor results in cognitive-behavioral tests, and

immunofluorescence analysis also indicated massive death of ipsilateral SN tyrosine hydroxylase-positive neurons (28), providing indirect evidence of the correlation between the decline in cognitive function and the loss of SN dopaminergic neurons. Recently, several studies have explored the correlation between SN and NMSs (29–31). Increased SN echogenicity is correlated with visual hallucinations (31), and the MoCA score has been reported to have a positive correlation with the correlated phase value of SN as assessed by QSM (30). Our previous research found significant differences in the ultrasound grading of the SN between NCI and CI in patients with PD (16), but the relationship between CI and area of SNH has not been investigated to date. In this study, we quantified CI in patients with PD by employing the MoCA test since it has a better performance and a higher sensitivity in identifying mild CI than does the MMSE (32,33). Univariate logistic analysis showed that SNH and area of SNH were potential risk factors for CI in PD, but multivariable logistic analysis revealed only SNH assessed by TCS was an independent marker of CI. Furthermore, correlation analysis revealed

there to be no obvious correlation between the area of SNH and the severity of disease (H-Y stage and UPDRS-III score), which is consistent with a prior study (34). This suggests that the alternation of SN echogenicity evaluated by TCS may be able to qualitatively assess changes in cognitive function in patients with PD but cannot be used as a quantitative index. The SN is subdivided into two distinct parts: the SN pars compacta (SNc) and the SN pars reticulata (SNr). Dorsolateral sensorimotor, dorsomedial limbic, and ventral associative are three main subregions in the SNc (35). Studies reported spatiotemporal changes in nigrostriatal iron content in PD (36), which were correlated with MoCA and could be quantified with QSM (37). However, TCS exhibits limitations in the subregional segmentation of the SN. Therefore, further exploration of the relationship between the subregional distribution characteristics of SNH and cognitive function is needed. This exploration can be guided by TCS-MRI fusion imaging in conjunction with volumetric imaging, which may offer a means to gaining a deeper understanding of the pathology of SNH development.

NSE is a soluble protein found mostly in the cytoplasm of neurons within the cortex and subcortical areas, constituting a considerable portion of the brain's total soluble protein. Elevated CSF and circulating NSE reflect neuronal damage after traumatic brain injury and oxidative damage associated with neurodegenerative diseases (38). However, the literature on NSE in PD is contradictory (8,39). Regarding the correlation between NSE and CI, circulation NSE values were found to be an independent predictor of CI 6 months after patients experienced out-of-hospital cardiac arrest with a seemingly favorable outcome (40). These values also play a crucial role in predicting CI in patients undergoing hemodialysis (41). In our study, an increased plasma NSE was observed in patients with PD and CI, which aligns with the result of a meta-analysis by Katayama *et al.* (8). Univariate and multivariable regression analyses further indicated NSE level as an independent factor affecting cognition in PD. Although the pathological mechanism of CI in PD is unclear, amyloid- and tau-related pathological changes, as well as other pathologies, such as mitochondrial and neurotransmitter systems and α -synuclein in the brain (which can induce neurotoxicity and alleviate neuroinflammation and apoptotic neuronal cell death), are likely to contribute to cognitive dysfunction in PD (3). Circulating NSE level has been recommended as a biomarker for predicting the neurological outcome of adults who are comatose after cardiac arrest (42). Elevated NSE

levels can stimulate inflammatory glial cell proliferation and actin remodeling, influencing the migration of activated macrophages and microglia to the injury site and encouraging neuronal cell death (38).

Besides NSE and SNH, our results demonstrated that age, education level, and UPDRS-III were independent determinants of CI in patients with PD. It is widely recognized that cognitive ability tends to diminish with advancing age, primarily due to the correlation between age and brain atrophy, which directly affects cognitive function. Higher education levels correlate with better cognitive performance. Nguyen *et al.*'s systematic review indicated that education has the potential to act as a preventive factor against dementia (43). A study in China involving 1,286 older adult individuals supports the key role of education in maintaining cognitive health in disadvantageous settings (44). The UPDRS-III is a test used to assess motor function in patients with PD. A higher initial UPDRS-III score suggests a more severe underlying pathology. Research has shown that the UPDRS-III score is positively correlated with the improvement rate of cognitive function after DBS implantation (45). Chen and colleagues generated a predictive model for CI in PD that combines age of onset, hypertension, baseline MoCA scores, UPDRS-III scores, and APOE ϵ 4, achieving an AUC of 0.80 (46). These investigations provide additional evidence for the association between cognitive status and the progression of PD.

Notably, male individuals in our study exhibited inferior cognitive performance compared to females. This finding was consistent with other research (47) indicating a positive correlation between increased striatal dopaminergic activity in females compared to males. This difference may be attributed to the protective effects of estrogen, which decreases DAT activity and enhances dopamine availability at synapses (48). Therefore, gender differences should be taken into account when using a precision medicine approach to preventing cognition decline in PD.

Some limitations to this study need to be addressed. First, although we applied MoCA to evaluate the cognitive state of participants, we did not perform a detailed analysis of the correlation between the seven cognitive domains of MoCA and the risk factors, nor did we carry out a comprehensive analysis of the specific cognitive aspects affected by elevated NSE concentration and SNH. Second, it should be mentioned that PD CI encompasses mild cognitive impairment (MCI) and dementia. In this cross-sectional study, we categorized participants into two

groups based on the MoCA criteria: those with normal cognition and those experiencing cognitive decline. Future studies should consider further subdividing CI into MCI and dementia and conducting follow-up assessments to validate the discriminative efficacy and predictive power of the model in MCI and dementia, as well as dementia conversion. Third, we did not examine the impact of PD treatment on cognitive performance, which is an important factor that should not be overlooked. Furthermore, the role of NSE is diverse and somewhat unclear, so other explanations for the levels of serum NSE in PD-related CI cannot be ruled out.

To conclude, we found that circulating NSE concentration and SNH evaluated by TCS were associated with CI in PD. We developed a simple and practical model by combining age, education status, UPDRS-III scores, plasma NSE level, and SNH to predict cognitive condition in patients with PD. The integration of clinical and neuroimaging biomarkers demonstrated good diagnostic performance for identifying CI in patients with PD.

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Footnote

Reporting Checklist: The authors have completed the TRIPOD reporting checklist. Available at <https://qims.amegroups.com/article/view/10.21037/qims-23-1778/rc>

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <https://qims.amegroups.com/article/view/10.21037/qims-23-1778/coif>). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This cross-sectional study was approved by the Ethics Board of Beijing Tiantan Hospital, Capital Medical University (No. KT2022-015-04) and was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Informed consent was obtained from all the patients.

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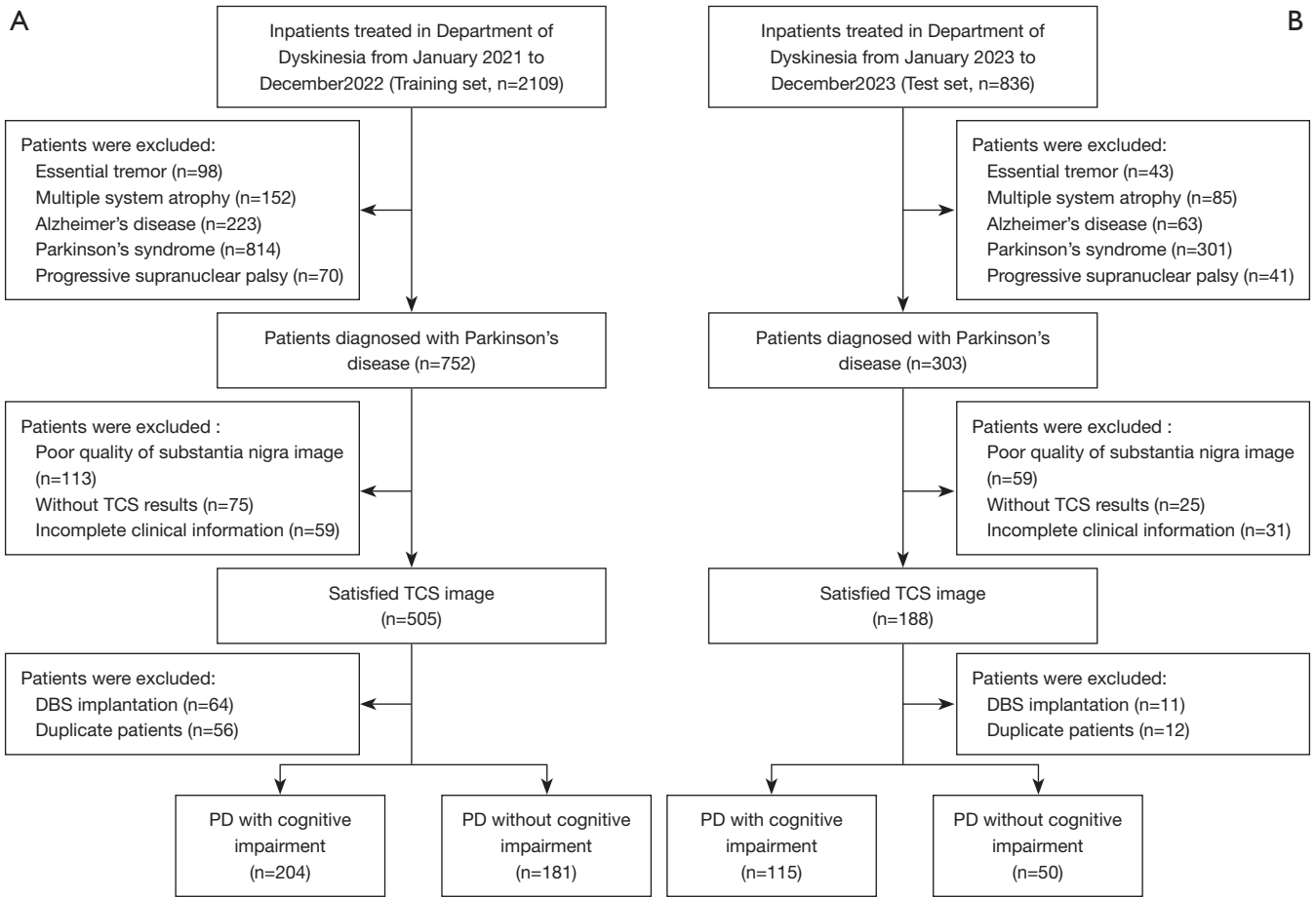


Figure S1 The flowchart of patient selection in the (A) training set and the (B) test set. TCS, transcranial sonography; PD, Parkinson's disease; DBS, deep brain stimulation.

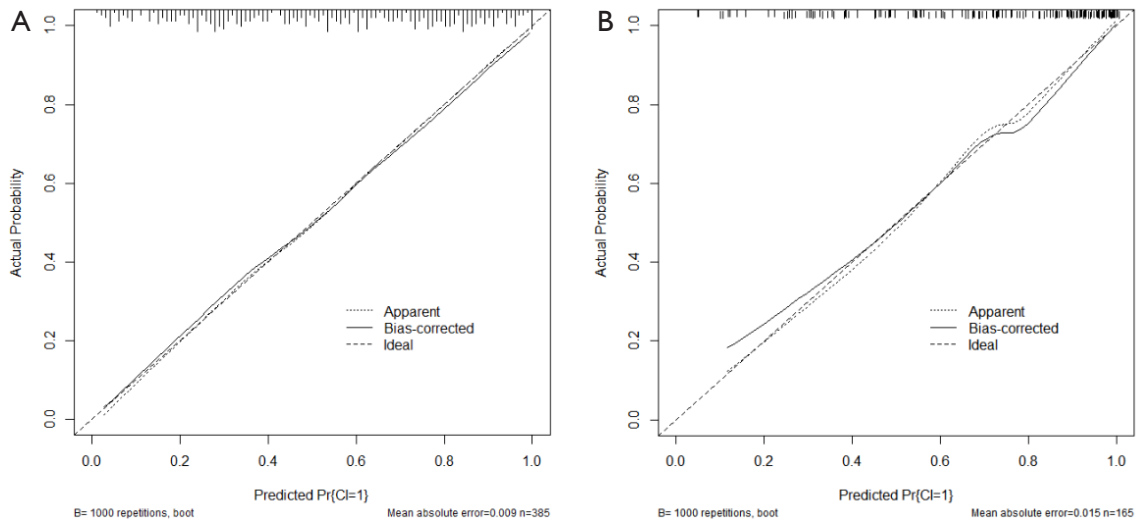


Figure S2 Calibration curves for the nomogram indicating good agreement between the predictors and observations. (A) Training set. (B) Test set. Pr, probability; CI, Class.

Table S1 Comparison of baseline demographic and clinical data of the training group and test group

Parameter	PD		P value
	Training group (n=385)	Test group (n=165)	
Male gender (%)	246 (63.9)	99 (60.0)	0.387 ^a
Age (years)	63.33±9.69	64.68±8.83	0.09 ^b
BMI (kg/m ²)	24.19±3.57	24.26±3.57	0.856 ^b
Duration of disease (years)	6.00±4.83	6.77±3.93	0.182 ^b
Education level (years)	12 (0,21)	12 (0,22)	0.766 ^c
Smoking, n (%)	86 (22.3)	31 (18.8)	0.351 ^a
Drinking, n (%)	79 (20.5)	30 (18.2)	0.529 ^a
Hypertension, n (%)	123 (31.9)	66 (40.0)	0.068 ^a
Diabetes mellitus, n (%)	58 (15.1)	25 (15.2)	0.979 ^a
Hyperlipidemia, n (%)	57 (14.8)	36 (21.8)	0.044 ^a
H-Y stage	2.7±0.95 ^d	2.82±0.87 ^d	0.058 ^b
MoCA score	23±5.85	21.47±5.38	0.029 ^b
PDD, n (%)	97 (25.2)	57 (34.5)	0.025 ^a
UPDRS-III	37.8±16.92	40.08±16.36 ^e	0.152 ^b
Hcy (μmol/L)	15.70±8.33	16.08±8.45	0.627 ^b
Folate (ng/mL)	7.88±85.17	8.32±5.92 ^f	0.377 ^b
Vitamin B12 (pg/mL)	490.11±402.44	489.79±362.49 ^g	0.993 ^b
Ferritin (ng/mL)	121.09±113.37	128.68±95.44 ^h	0.482 ^b
NSE (ng/mL)	15.24±3.25	15.30±3.63	0.849 ^b
SNH, n (%)	230 (59.7)	110 (66.7)	0.125 ^a
Area of SNH (cm ²)	0.21 (0,1.11)	0.25 (0,0.85)	0.94 ^c

Education level and area of SNH are presented as median (interquartile range); gender, smoking, drinking, hypertension, diabetes mellitus, hyperlipidemia, PDD, and SNH are presented as number (frequency); and the other parameters are presented as the mean ± standard deviation. ^a, χ^2 test; ^b, unpaired independent *t*-test; ^c, nonparametric Mann-Whitney test; ^d, missing value: H-Y stage, training group 1 and test group 1; ^e, missing value: UPDRS-III, test group 1; ^f, missing value: folate, test group 1; ^g, missing value: vitamin B12, test group 1; ^h, missing value: ferritin, test group 1. PD-CI, Parkinson's disease with cognitive impairment; PD-NCI, Parkinson's disease without cognitive impairment; BMI, body mass index; H-Y, Hoehn and Yahr; MoCA, Montreal Cognitive Assessment; PDD, Parkinson's disease with dementia; UPDRS-III, Unified Parkinson's Disease Rating Scale Part III; Hcy, homocysteine; NSE, neuron-specific enolase; SNH, substantia nigra hyperechogenicity.

Table S2 Spearman correlation analysis of age, disease duration, and other variables in the training set

Parameter	Education level	H-Y stage	UPDRS-III	MoCA	Hcy	Folate	Vitamin B12	Ferritin	NSE	Area of SNH
Age	-0.152	0.153	0.051	-0.214	0.029	0.057	-0.063	0.011	0.053	0.065
	P=0.003	P=0.003	P=0.320	P<0.001	P=0.573	P=0.265	P=0.216	P=0.829	P=0.297	P=0.205
Disease duration	-0.023	0.384	0.304	-0.188	0.177	0.126	-0.119	-0.049	0.10	0.030
	P=0.654	P<0.001	P<0.001	P=0.002	P<0.001	P=0.013	P=0.02	P=0.334	P=0.050	P=0.561

H-Y, Hoehn and Yahr; UPDRS-III, Unified Parkinson's Disease Rating Scale Part III; MoCA, Montreal Cognitive Assessment; Hcy, homocysteine; NSE, neuron-specific enolase; SNH, substantial nigra hyperechogenicity.

Table S3 Comparisons of gender difference in various parameters in the training set

Parameter	Male (n=246)	Female (n=139)	P value
Age (years)	63.27±9.91	63.43±9.33	0.874 ^a
BMI (kg/m ²)	24.29±3.73	24.03±3.27	0.490 ^a
Duration of disease (years)	6.21±5.21	5.65±4.07	0.276 ^a
Education level	12 (0, 21)	10 (0, 21)	0.018 ^c
Smoking, n (%)	82 (33.3)	4 (2.9)	<0.001 ^b
drinking, n (%)	76 (30.9)	3 (2.2)	<0.001 ^b
Hypertension, n (%)	83 (33.7)	40 (28.8)	0.316 ^b
Diabetes mellitus, n (%)	37 (15.0)	21 (15.1)	0.986 ^b
Hyperlipidemia, n (%)	43 (17.5)	14 (10.1)	0.049 ^b
H-Y stage	2.66±0.93	2.65±0.99 ^d	0.860 ^a
MoCA score	22.46±5.71	22.94±6.12	0.483 ^a
UPDRS-III	37.91±16.89	37.74±17.03	0.924 ^a
Hcy (μmol/L)	16.68±8.99	13.98±6.71	0.002 ^a
Folate (ng/mL)	7.33±5.18	8.84±5.04	0.006 ^a
Vitamin B12 (pg/mL)	467.91±429.34	529.41±347.82	0.15 ^a
Ferritin (ng/mL)	138.91±138.40	89.55±82.31	<0.001 ^a
NSE (ng/mL)	15.25±2.83	15.23±3.90	0.938 ^a
SNH, n (%)	165 (67.1)	65 (46.8)	<0.001 ^b
Area of SNH (cm ²)	0.33 (3, 1.11)	0 (0, 0.93)	<0.001 ^c
CI, n (%)	140 (56.9)	64 (46.0)	0.04 ^b

^a, unpaired independent *t*-test; ^b, χ^2 test; ^c, nonparametric Mann-Whitney test; ^d, missing value: H-Y stage, female 1. Education level and area of SNH are presented as median (interquartile range); smoking, drinking, hypertension, diabetes mellitus, hyperlipidemia, SNH, and CI are presented as number (frequency); and the other parameters are presented as the mean \pm standard deviation. BMI, body mass index; H-Y, Hoehn and Yahr; MoCA, Montreal Cognitive Assessment; UPDRS-III, Unified Parkinson's Disease Rating Scale Part III; Hcy, homocysteine; NSE, neuron-specific enolase; SNH, substantial nigra hyperechogenicity; CI, cognitive impairment.

Table S4 Logistic regression analysis of possible risk factors and cognitive impairment in the training set

Parameter	Cognitive impairment (univariate analysis)		Cognitive impairment (multivariate analysis)	
	OR (95% CI)	P value	OR (95% CI)	P value
Gender (male)	0.646 (0.425,0.982)	0.041	0.561 (0.330, 0.954)	0.033
Age	1.036 (1.014, 1.059)	0.001	1.039 (1.011, 1.066)	0.005
BMI	0.988 (0.934, 1.045)	0.671		
Duration of disease	1.032 (0.988, 1.079)	0.159		
Education year	0.889 (0.847, 0.934)	<0.001	0.892 (0.842, 0.954)	<0.001
Drinking history	0.717 (0.434, 1.185)	0.195		
Smoking history	0.676 (0.415, 1.102)	0.116		
Hypertension	0.793 (0.515, 1.220)	0.291		
Diabetes mellitus	0.764 (0.434, 1.346)	0.352		
Hyperlipidemia	0.467 (0.257, 0.850)	0.013	0.528 (0.259,1.074)	0.078
H-Y stage	1.480 (1.184, 1.850)	0.001	0.991 (0.733, 1.340)	0.953
PDD	0	0.996		
UPDRS-III	1.031 (1.018, 1.045)	<0.001	1.026 (1.009,1.043)	0.003
Hcy	1.031 (1.003, 1.059)	0.032	1.020 (0.989, 1.052)	0.216
Folate	0.984 (0.946, 1.022)	0.403		
Ferritin	1.001 (0.999, 1.002)	0.535		
Vitamin B12	1.00 (0.999,1.000)	0.314		
NSE	1.590 (1.409, 1.794)	<0.001	1.562 (1.374, 1.776)	<0.001
SNH	0.473 (0.312, 0.716)	<0.001	0.545 (0.330, 0.902)	0.018
Area of SNH	3.020 (1.397, 6.528)	0.005	1.115 (0.562, 2.213)	0.755

OR, odds ratio; CI, confidence interval; BMI, body mass index; H-Y, Hoehn and Yahr; PDD, Parkinson's disease with dementia; UPDRS-III, Unified Parkinson's Disease Rating Scale Part III; Hcy, homocysteine; NSE, neuron-specific enolase; SNH, substantial nigra hyperechogenicity.