ORIGINAL PAPER

Submitted: 29.11.2022
Accepted: 29.05.2023
Early publication date: 08.08.2023

Endokrynologia Polska
DOI: 10.5603/EP.a2023.0056
ISSN 0423–104X, e-ISSN 2299–8306
Volume/Tom 74; Number/Numer 4/2023

Ultrasound characteristics of the cervical vagus nerve in patients with type 2 diabetes and diabetic peripheral neuropathy

Fan Xiong[1]*, Qian Wang[1, 3]*, Yun Hu[1], Xiao Jiang[2], Lin Liu[1], Yumeng Han[1], Qing Jiang[1], Shiqin Yuan[1], Lan Xu[1]

[1]Department of Endocrinology, The Affiliated Wuxi People's Hospital of Nanjing Medical University, Wuxi People's Hospital, Wuxi Medical Centre, Nanjing Medical University, Wuxi, China
[2]Department of Ultrasound, The Affiliated Wuxi People's Hospital of Nanjing Medical University, Wuxi People's Hospital, Wuxi Medical Centre, Nanjing Medical University, Wuxi, China
[3]Department of Geriatrics, Nanjing Drum Tower Hospital, the Affiliated Hospital of Nanjing University Medical School, Nanjing, China
**These authors contributed equally to this article.*

Abstract

Introduction: Diabetic peripheral neuropathy (DPN) and autonomic neuropathy are commonly coexistent in patients with type 2 diabetes mellitus (T2DM). Current assessment tools for diabetic neuropathy remain complicated and limited. We aimed to investigate the sonographic changes of the cervical vagus nerve in DPN patients with T2DM.

Material and methods: Patients with T2DM were divided into a DPN group (DPN, n = 44) and non-DPN controls (NDPN, n = 43) based on electromyogram results. Another 43 healthy controls (CON) were included. High-frequency ultrasound (HFU) of the vagus nerve was performed in all participants.

Results: Compared with controls, the honeycomb structure of the vagus nerve in patients with T2DM decreased, $p < 0.001$. The DPN group had higher cross-sectional area (CSA) of the right vagus nerve than the NDPN group (1.60 ± 0.52 *vs.* 2.00 ± 0.57 mm^2, $p = 0.001$). Logistic regression showed that right vagus nerve CSA was a risk factor of DPN (odds ratio [OR] = 3.924, $p = 0.002$). Right vagus nerve CSA was positively correlated with diabetes duration ($p = 0.003$), and negatively correlated with the motor conduction velocity (MCV) of the ulnar, median, and common peroneal nerves ($p < 0.001$ for all), as well as the sensor conduction velocity (SCV) of the ulnar and median nerve (both $p < 0.005$).

Conclusion: HFU shows thickening of the cervical vagus nerve in patients with DPN, which is a potential diagnostic feature of diabetic neuropathy. **(Endokrynol Pol 2023; 74 (4): 421–429)**

Key words: *high-frequency ultrasound; vagus nerve cross-sectional area; diabetic peripheral neuropathy*

Introduction

Type 2 diabetes mellitus (T2DM) is a common clinical metabolic disease. The global diabetes prevalence in 20-79-year-olds in 2021 was estimated to be 10.5% (536.6 million people), with a predicted rise to 12.2% (783.2 million) by 2045 [1]. Diabetic neuropathy is the most common and troublesome complication of diabetes, affecting up to half of all patients with diabetes [2, 3], and it increases the risk of foot ulceration, amputation, cardiovascular dysfunction, myocardial infarction, and sudden death [2, 4]. Therefore, early recognition and appropriate management of neuropathy in patients with diabetes is important. However, the diagnosis of diabetic neuropathy at present is mainly based on clinical manifestations such as limb pain, numbness, paraesthesia, muscle weakness, and muscle atrophy, but half of the patients missed the diagnosis due to a lack of obvious symptoms [5, 6].

With the advancement of ultrasound technology and the improvement of resolution, high-frequency ultrasound (HFU) is safer and more targeted, allowing noninvasive examination of nerves in real-time. Several studies have shown that the peripheral nerve cross-sectional area (CSA) of diabetic peripheral neuropathy (DPN) patients is larger than that of the control group, which may help to detect neuropathy early and guide the diagnosis and treatment of DPN [7, 8]. Moreover, the increased CSA is positively correlated with the severity of diabetic neuropathy [9].

Lan Xu, Department of Endocrinology, The Affiliated Wuxi People's Hospital of Nanjing Medical University, Nanjing Medical University, Wuxi, No. 299, Qingyang Road, Wuxi City, Jiangsu Province, China, tel: 0510-85350835; e-mail: xulan126@126.com
Xiao Jiang, Department of Ultrasound, The Affiliated Wuxi People's Hospital of Nanjing Medical University, Nanjing Medical University, Wuxi, No. 299, Qingyang Road, Wuxi City, Jiangsu Province, China; tel: 0510-85350835; e-mail: yixingjx790908@163.com

As the longest cranial nerve and an important part of the autonomic nerve, the vagus nerve has the widest distribution in the body and runs a superficial course in the neck, and its anatomical location and characteristic appearance is easily measured using HFU [10]. However, the HFU measurement of the vagus nerve in diabetes was very limited. The study showed that the CSA of the vagus nerve was significantly smaller in patients with diabetes compared with normal controls [11]. Autonomic neuropathies are commonly coexistent with DPN [12], both of which account for 90% of diabetic neuropathy [13]. Tahniyah et al. found that all patients with electrophysiologically diagnosed DPN had cardiac autonomic neuropathy [12]. The DCCT/EDIC study demonstrated that DPN and cardiovascular autonomic neuropathy had similar risk factors, such as older age, sustained albuminuria, longer duration, higher mean pulse rate, and β-blocker use [14].

Therefore, the present study investigated the characteristics of the cervical vagus nerve on HFU in patients with T2DM and DPN.

Material and methods

The study protocol was approved by the Ethics Committees of Nanjing Medical University Affiliated Wuxi People's Hospital (ethical batch number: KY21068), and it was registered with the Chinese Clinical Trial Registry (ChiCTR2200065538). All participants provided written informed consent before study enrolment.

Participants

Healthy controls and patients with T2DM were from the physical examination centre and endocrinology department of Wuxi People's Hospital, respectively. All patients met the 1999 World Health Organization diagnostic criteria for diabetes. According to the results of the nerve conduction study (NCS), the patients with T2DM were divided into those with non-diabetic peripheral neuropathy (NDPN group) and those with DPN (DPN group) based on abnormal nerve conduction velocity (NCV) (2 or more abnormal nerves) [15]. The exclusion criteria were: (1) pregnancy and lactation; (2) type 1 diabetes or other special types of diabetes; (3) acute complications of diabetes (diabetic ketoacidosis, non-ketotic hyperosmolar coma); (4) various nondiabetic neurological diseases, including vitamin B12 deficiency, cervical and lumbar spine diseases, cerebral infarction, chronic inflammatory demyelinating neuropathy, hereditary neuropathy, etc.; (5) a history of neurotoxic drugs (such as chemotherapy drugs); (6) heart failure, respiratory failure, severely impaired liver and kidney functions, acute metabolic disorders, and infectious fever; and (7) a history of neck injury or surgery, and scars that may influence the examination.

The inclusion criteria of healthy controls were as follows: age over 18 years, body mass index (BMI) between 18 and 28 kg/m^2, fasting blood glucose (FBG) < 5.6 mmol/L, and no history of other diseases. The exclusion criteria were T2DM and the above exclusion criteria for patients with T2DM.

Collection of general data and biochemical indicators

The age, sex, weight, height, heart rate, systolic and diastolic blood pressure, smoking history, duration of diabetes, medication history, diabetes complications, and comorbidities of the participants were recorded. BMI was calculated as weight divided by the square of the height (kg/m^2).

All subjects took a venous blood test in the morning after fasting for 8 hours. Glycosylated haemoglobin (HbA_{1c}) was measured using a high-performance liquid chromatograph, triglyceride and total cholesterol were measured using the enzyme endpoint method, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol were measured using the direct method, and FBG was measured using the hexokinase endpoint method. The detection instrument for the above indicators is the American Beckman Coulter AU5800 automatic biochemical analyser, and the kits were purchased from the American Beckman Coulter company. The detection method of fasting C-peptide (FCP) was electrochemiluminescence, and the detection instrument was a Roche cobas®8000 automatic biochemical immunoassay analyser; the kit was purchased from Roche.

The FBG and FCP were used to calculate homeostasis model assessment 2 estimates of insulin resistance (HOMA2-IR) and homeostasis model assessment 2 estimates of β-cell function (HOMA2-B) with the HOMA2 calculator (Website: http://www.dtu.ox.ac.uk/homa). Eight patients (DPN = 4, NDPN = 4) with values beyond the defined range of HOMA2 calculators were removed. Islet β-cell function was evaluated using the C-peptide index, which was calculated by the formula: FCP (ng/mL) × 100 / [FBG (mmol/L) × 18] [16].

Electrodiagnostic examinations

NCS was performed for all patients with T2DM using an electromyography/evoked potential instrument (Alpine bioMed ApS, Danish, represented by Shanghai Bendi Medical Equipment Co. Ltd.) on both lower and upper extremities. This test was done on the skin surface with a temperature of 32–34°C and in a room at 20–25 . Each sensory NCS was performed for the median nerve, ulnar nerve, and superficial peroneal nerve. Motor NCS was performed for the median nerve, ulnar nerve, and common peroneal nerve. NCS was evaluated using 5 parameters: latency, amplitude, time limit, conduction distance, and conduction velocity in the patients.

Measurement instruments

All subjects completed the Survey of Autonomic Symptoms (SAS) and the 31-item Composite Autonomic Symptom Score (COMPASS 31) independently in a quiet environment to assess autonomic symptoms, and those who were unable to complete them independently due to the their educational level were questioned by the same physician.

SAS is an important scale for the assessment of early diabetic autonomic neuropathy, involving the following autonomic symptom domains: gastrointestinal, urinary, orthostatic, sudomotor, vasomotor, and sexual dysfunction [17]. This survey consists of 11 questions for women and 12 for men, and each item is rated by an impact score ranging from 1 (least severe) to 5 (most severe). Thus, the total number of symptoms reported was 0 to 11 for women and 0 to 12 for men, and the total score ranged from 0 to 55 points for women and 0 to 60 points for men.

COMPASS 31 is a simplified and validated version of the original COMPASS scale (164 items) that quantifies symptom severity in 6 domains of autonomic nerves, with a total of 31 questions [18]. It comprises 6 different domains including orthostatic intolerance (4 items), vasomotor dysfunction (3 items), secretomotor dysfunction (4 items), gastrointestinal dysfunction (12 items), bladder dysfunction (3 items), and pupillomotor dysfunction (5 items). The scores in each domain are weighted and summed to obtain an autonomic symptom score ranging from 0 to 100. The higher the scores, the more severe the autonomic symptoms.

Ultrasonographic studies

HFU tests were performed via nerve tracing with a high-resolution linear transducer (4–15 MHz, L12-5, Philips IU Elite, Netherlands). Participants were placed in a supine position with the head

slightly extended and then rotated to the opposite side of the scan. In most cases, the vagus nerve was located in the carotid sheath, antero-laterally to the common carotid artery, and dorsally to the internal jugular vein [19]. The probe was placed at the thyroid gland level where the vagus nerve could clearly be differentiated [20]. Each nerve was studied in a position where it had a nearly elliptic cross-sectional shape, by the same sonographer, holding the transducer in an axial position. Then the long diameter a and the short diameter b (perpendicular to a) were measured separately, and the ellipse area formula was used to calculate the CSA of vagus nerve = $a \times b \times \varpi/4$. The vagus nerve consists of several hypoechoic fascicles surrounded by hyperechoic epineurial tissue, giving the nerve a honeycomb appearance. The study finally observed and recorded whether the characteristic structure was visible in HFU. The image and data acquisition process were completed by the same experienced professional sonographer, who was blinded to the grouping of the subjects.

Statistical analysis

Sample size calculation: according to the preliminary test, the average CSA of the vagus nerve in 20 patients with T2DM was 1.45 mm^2, and the average area of 20 healthy controls was 1.81 mm^2, with a standard deviation of 0.5 mm^2. In the PASS 15.0 sample size calculation software, the 2 independent samples t-test calculation module in the mean was selected, the test level α was taken as 0.05, and the test power was taken as 90% to calculate the sample size, and at least 42 subjects were required for each of the 2 groups. SPSS 25.0 statistical software was used for data analysis. The Shapiro-Wilk test was used to test the normality of the measurement data, and those with normal distribution were expressed as the mean ± standard deviation; the independent samples t-test was used for comparison between the 2 groups. Those without normal distribution were expressed as the median (quartile), and the independent-sample Mann-Whitney U-test was used for comparison between the 2 groups. Enumeration data were expressed by frequency (proportion) [n (%)], and the χ^2 test was used. Binary logistic regression analysis identified the influencing factors of patients with DPN, and the receiver operating characteristic (ROC) curve was drawn to calculate the area under the curve (AUC) to analyse the possibility of evaluating the vagus nerve ultrasound measurement parameters as predictors of DPN. Pearson test was used for normally distributed data, and Spearman test was used for non-normally distributed data, to analyse the correlation between ultrasound measurement parameters and general clinical indicators, electromyography conduction velocity, SAS, and COMPASS 31 scales. $p < 0.05$ was regarded as a statistically significant difference.

Results

Comparison between the T2DM group and the healthy control group

A total of 87 patients with T2DM and 43 healthy controls were finally included in this study according to the calculation results of the aforementioned sample size. It is worth mentioning that after 43 NDPN patients were enrolled, 44 DPN patients were included as the intergroup control according to age and gender matching. The comparison of clinical indicators between the 2 groups are presented in Table 1. In patients with T2DM, age, weight, BMI, FBG, and triglyceride levels were higher than in controls. The comparison of vagus nerve ultrasound measurement indexes between the 2 groups is shown in Table 2. The honeycomb structure in patients with T2DM decreased (51.2% *vs*. 18.4%, $p < 0.001$, after adjustment for age, $p < 0.001$). Typical ultrasound images of the right vagus nerve in healthy controls and T2DM groups are shown in Figure 1. Healthy people (Fig. 1A) have a typical honeycomb structure, but no honeycomb structure was observed in patients with T2DM (Fig. 1B).

Comparison of the DPN group and the NDPN group

According to the NCS examination results, among the 87 patients with T2DM, 43 were not combined with DPN (NDPN group), and 44 were combined with DPN (DPN group). The comparison of clinical indicators between the 2 groups is shown in Table 3. The duration of diabetes in the DPN group was longer than that in the NDPN

Table 1. *Comparison of clinical indicators between the type 2 diabetes mellitus (T2DM) group and the healthy control group*

	Control (n = 43)	T2DM (n = 87)	p-value
Age [years]	45 (34,48)	50 (40,57)	0.001
Gender [men/women]	17/26	48/39	0.093
Height [cm]	164.9 ± 7.9	165.5 ± 8.0	0.716
Weight [kg]	62.9 ± 10.5	70.7 ± 12.3	< 0.001
BMI [kg/m^2]	23.06 ± 2.76	25.73 ± 3.45	< 0.001
Smoking history	19 (44.2)	31 (35.6)	0.346
FBG [mmol/L]	4.82 (4.55,5.17)	7.72 (6.32,9.87)	< 0.001
Triglycerides [mmol/L]	1.02 (0.71,1.41)	1.78 (1.13,2.56)	< 0.001
Total cholesterol [mmol/L]	4.55 (4.38,4.92)	4.70 (3.86,5.84)	0.902
High-density lipoprotein [mmol/L]	1.38 ± 0.30	0.96 ± 0.21	< 0.001
Low-density lipoprotein [mmol/L]	2.77 (2.35,3.09)	2.67 (2.02,3.47)	0.670

Date shown as mean ± standard deviation, median (IQR), or n; BMI — body mass index; FBG — fasting blood glucose

Table 2. *Comparison of the vagus nerve ultrasound measurement indexes between the type 2 diabetes mellitus (T2DM) group and the healthy control group*

	Control (n = 43)	T2DM (n = 87)	p-value	p-value[a]
Left long diameter [mm]	1.60 ± 0.23	1.59 ± 0.32	0.919	0.646
Right long diameter [mm]	1.68 ± 0.30	1.72 ± 0.33	0.510	0.549
Left short diameter [mm]	1.20 ± 0.20	1.20 ± 0.24	0.997	0.940
right short diameter [mm]	1.29 ± 0.21	1.31 ± 0.24	0.723	0.960
Left CSA [mm^2]	1.50 ± 0.32	1.53 ± 0.51	0.747	0.993
Right CSA [mm^2]	1.73 ± 0.51	1.80 ± 0.58	0.515	0.730
Honeycomb structure(n/%)	22 / 51.2	16 / 18.4	< 0.001	< 0.001

Date shown as mean ± standard deviation (SD), n, or %; aafter correcting for age; CSA — cross-sectional area

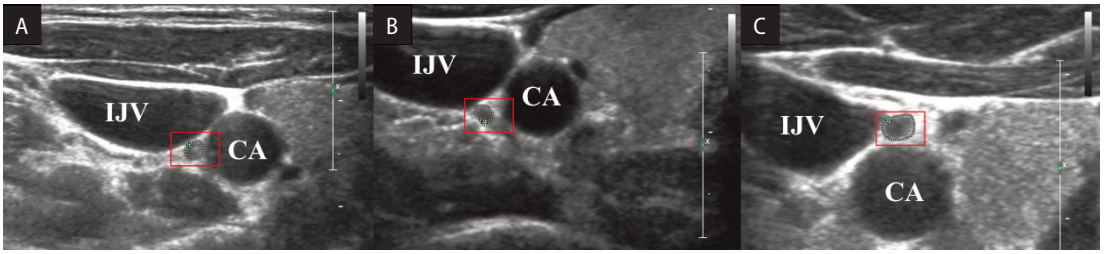

Figure 1. *Typical ultrasound images of the right vagus nerve in the control and type 2 diabetes mellitus (T2DM) groups (non-diabetic peripheral neuropathy [NDPN] and diabetic peripheral neuropathy [DPN] group). The vagus nerve is an oval structure in the red box between the carotid artery (CA) and the internal jugular vein (IJV). Healthy people (***A***) have a typical honeycomb structure, but no honeycomb structure was observed in patients with T2DM (***B, C***). The vagus nerve cross-sectional area was smaller in NDPN patients (***B***) than in DPN patients (***C***), and no honeycomb structure was observed*

Table 3. *Comparison of clinical indicators between the diabetic peripheral neuropathy (DPN) group and the non-diabetic peripheral neuropathy (NDPN) group*

	NDPN (n = 43)	DPN (n = 44)	p-value
Age [years]	47 (39,54)	52 (43,57)	0.090
Gender [men/women]	23/20	25/19	0.755
Height [cm]	164.2 ± 8.0	166.7 ± 7.9	0.149
Weight [kg]	71.2 ± 12.3	70.3 ± 12.5	0.719
BMI [kg/m^2]	26.34 ± 3.72	25.13 ± 3.08	0.103
DM duration [year]	1.0 (0.0,4.8)	6.7 (1.0,15.3)	0.002
Smoking history [n/%]	16 (37.2)	15 (34.1)	0.761
Hypertension [n/%]	15 (34.9)	22 (50.0)	0.154
Diabetic retinopathy [n/%]	15 (34.9)	23 (52.3)	0.102
Diabetic nephropathy [n/%]	17 (39.5)	19 (43.2)	0.730
Atherosclerosis [n/%]	23 (53.5)	24 (54.5)	0.922
Insulin therapy [n/%]	17 (39.5)	25 (56.8)	0.107
Metformin treatment [n/%]	27 (62.8)	30 (68.2)	0.597
Glycated haemoglobin (%)	9.88 ± 2.10	9.87 ± 1.93	0.986
FBG [mmol/L]	7.72 (6.67,9.04)	7.70 (5.94,11.05)	0.410
FCP [ng/mL]	1.97 ± 1.11	1.81 ± 0.91	0.467
HOMA2-IR	1.83 ± 0.91	1.78 ± 0.80	0.776
HOMA2-b	55.60 (38.60,70.00)	45.95 (27.28,66.53)	0.171

→

Table 3. *Comparison of clinical indicators between the diabetic peripheral neuropathy (DPN) group and the non-diabetic peripheral neuropathy (NDPN) group*

	NDPN (n = 43)	DPN (n = 44)	p-value
C-peptide index	1.49 (0.78,1.80)	1.11 (0.74,1.55)	0.112
Triglycerides [mmol/L]	1.65 (1.11,2.32)	1.92 (1.34,2.67)	0.188
Total cholesterol [mmol/L]	4.80 ± 1.20	4.94 ± 1.44	0.635
High-density lipoprotein [mmol/L]	0.97 (0.85,1.06)	0.91 (0.77,1.12)	0.393
Low-density lipoprotein [mmol/L]	2.76 ± 0.91	2.79 ± 1.11	0.903

Date shown as mean ± standard deviation (SD), median (IQR), n, or %; BMI — body mass index; FBG — fasting blood glucose; FCP — fasting C-peptide; HOMA2-IR — insulin resistance index; HOMA2-β — pancreatic islet beta cell function index

Table 4. *Comparison of the vagus nerve ultrasound measurement indexes between the non-diabetic peripheral neuropathy (NDPN) group and the diabetic peripheral neuropathy (DPN) group*

	NDPN (n = 43)	DPN (n = 44)	p-value	p-value[a]
Left long diameter [mm]	1.53 ± 0.34	1.65 ± 0.29	0.076	0.140
Right long diameter [mm]	1.63 ± 0.28	1.81 ± 0.34	0.008	0.043
Left short diameter [mm]	1.15 ± 0.23	1.24 ± 0.24	0.094	0.195
Right short diameter[mm]	1.22 ± 0.23	1.39 ± 0.22	0.001	0.008
Left CSA [mm^2]	1.42 ± 0.53	1.62 ± 0.48	0.070	0.163
Right CSA [mm^2]	1.60 ± 0.52	2.00 ± 0.57	0.001	0.009
Honeycomb structure [n/%]	7 / 16.3	9 / 20.5	0.615	0.794

Date shown as mean ± standard deviation (SD), n, or %; CSA — cross-sectional area; [a]after correction for course of disease

group (p = 0.002). The comparison of vagus nerve ultrasound measurement indexes between the NDPN group and the DPN group is shown in Table 4. The right long diameter (1.63 ± 0.28 *vs*. 1.81 ± 0.34, p = 0.008), short diameter (1.22 ± 0.23 *vs*. 1.39 ± 0.22, p = 0.001), and CSA (1.60 ± 0.52 *vs*. 2.00 ± 0.57, p = 0.001) of the DPN group were larger than those of the NDPN group. Typical ultrasound images of the right vagus nerve in patients with NDPN and DPN are shown in Figure 1. The vagus nerve cross-sectional area was smaller in NDPN patients (Fig. 1B) than in DPN patients (Fig. 1C), and no honeycomb structure was observed.

Regression analysis

Taking DPN or not as the dependent variable and the duration of diabetes, the long diameter, short diameter, and CSA of the right vagus nerve as independent variables, a stepwise binary logistic regression analysis was performed. The results showed that among the parameters measured by HFU, the CSA of the right vagus nerve (OR = 3.924, p = 0.002) was an independent risk factor for DPN. The possibility of right vagus nerve CSA as a predictor of DPN was assessed by ROC curve analysis (Fig. 2A). The results showed that AUC = 0.712 (95% CI: 0.602–0.822, p = 0.001), the best cut-off value was 1.71 mm^2, the sensitivity was 72.7%, and the specificity was 69.8%.

Correlation analysis of vagus nerve ultrasound measurement parameters and other variables

The correlation analysis between the CSA of the right vagus nerve and clinical indicators in patients with T2DM showed that the right CSA was positively correlated with diabetes duration (r = 0.317, p = 0.003), and negatively correlated with C-peptide index (r = –0.236, p = 0.029). There was no correlation in the CSA with other clinical indicators including age, gender, height, weight, BMI, heart rate, systolic blood pressure, diastolic blood pressure, HbA_{1c}, FBG, FCP, HOMA2-IR, HOMA2-β, and lipid levels.

Electromyography conduction velocity reflects the function of peripheral nerves. The correlation analysis between the CSA of the right vagus nerve in patients with T2DM and the conduction velocity of the right related nerve in electromyography is shown in Figure 2B-F. The CSA of the right vagus nerve was negatively correlated with the conduction velocities of the ulnar, median, and common peroneal nerves. Among them, the correlation with median nerve motor conduction velocity was the largest (r = –0.425, p < 0.001).

The correlations between vagus nerve ultrasound measurement parameters with SAS and COM-

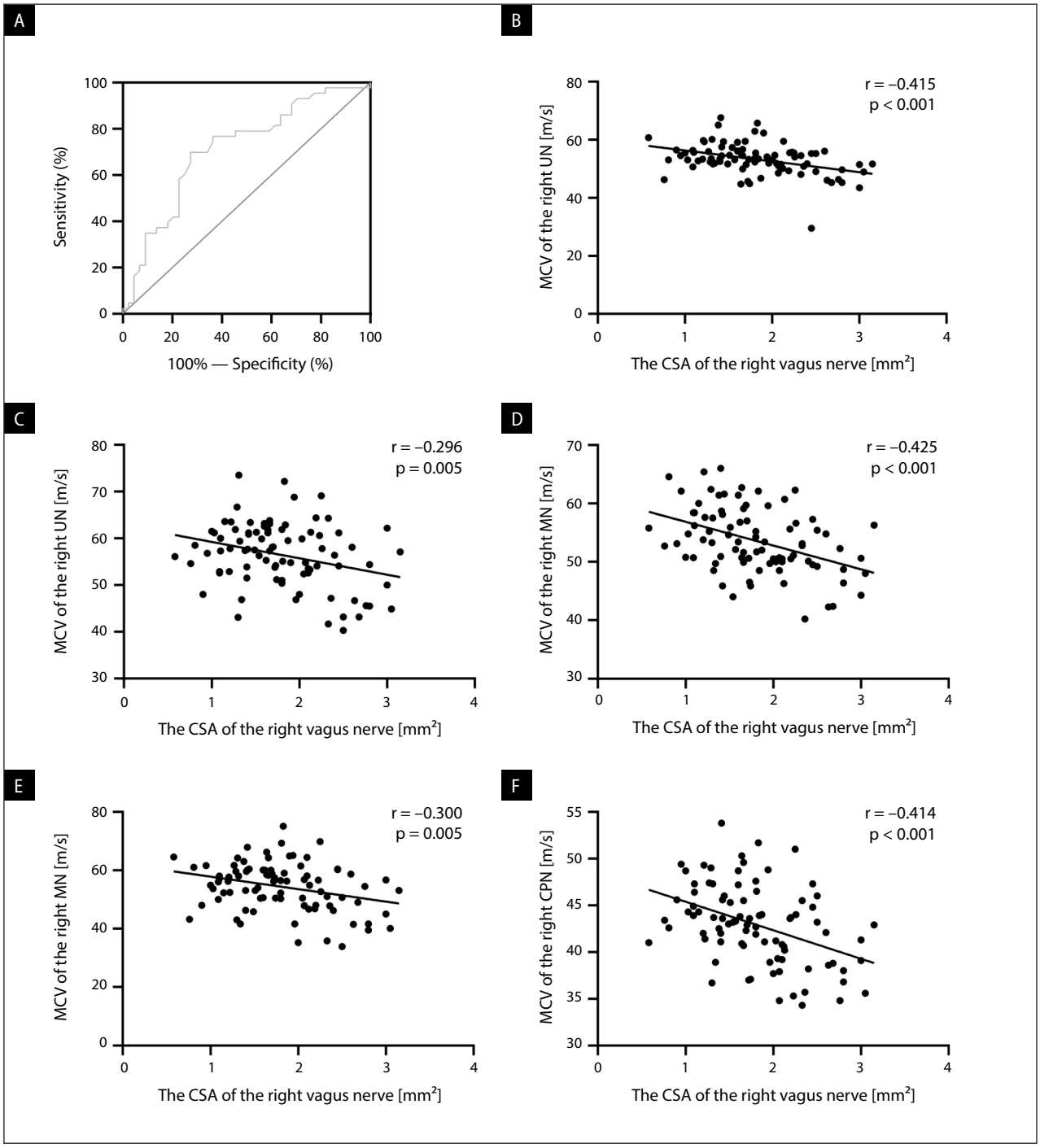

Figure 2. A. *Receiver operating characteristic (ROC) curve analysis of the cross-sectional area of the right vagus nerve in patients with diabetic peripheral neuropathy (DPN). The results showed that the area under the curve (AUC) = 0.712 (95% confidence interval (CI): 0.602–0.822, p = 0.001), the best cut-off value was 1.71 mm², the sensitivity was 72.7%, and the specificity was 69.8%;* **B–F.** *Correlation analysis of the right vagus nerve cross-sectional area and electromyography conduction velocity in patients with type 2 diabetes mellitus (T2DM). The cross-sectional area (CSA) of the right vagus nerve was negatively correlated with the conduction velocities of the ulnar, median, and common peroneal nerves. Among them, the correlation with median nerve motor conduction velocity was the largest (***D***, r = –0.425, p < 0.001). MCV — motor conduction velocity; SCV — sensor conduction velocity; UN — ulnar nerve; MN — median nerve; CPN — common peroneal nerve*

PASS 31 scales are shown in Table 5. There was no correlation between the long diameter, short diameter, and CSA of the vagus nerve with the number of symptoms, the total score of SAS, and COMPASS 31.

Table 5. *Correlation analysis of the ultrasound measurement parameters with Survey of Autonomic Symptoms (SAS) and the 31-item Composite Autonomic Symptom Score (COMPASS 31) scales*

	SAS				COMPASS 31	
	Total score		Number of symptoms		Total score	
	r	p-value	r	p-value	r	p-value
Left long diameter [mm]	-0.050	0.576	0.035	0.694	0.025	0.782
Right long diameter [mm]	0.062	0.490	0.087	0.329	0.059	0.511
Left short diameter [mm]	0.000	0.999	0.026	0.768	0.029	0.744
right short diameter [mm]	0.074	0.407	0.08	0.372	0.000	0.999

CSA — cross-sectional area

Discussion

The present study found impairment of the honeycomb structure of the cervical vagus nerve in patients with T2DM using HFU assessment. Moreover, we think that our study investigated the characteristics of cervical vagus nerve with HFU in patients with DPN for the first time. The results showed that the right vagus nerve CSA was negatively correlated with the conduction velocity of the peripheral nerves and may become a predictive factor of DPN.

In previous ultrasound studies of the vagus nerve, the parameters commonly used are the CSA and the 2 vertical diameters on the cross-section [19]. Our study firstly observed the absence of honeycomb structure in the vagus nerve of patients with T2DM, which was in accordance with the changes of peripheral nerves in these patients [21]. The honeycomb structure usually reflects the inner membrane of the nerve fibre bundle. The destruction of the honeycomb structure in patients with T2DM in our study may be due to the hyperglycaemia in these patients. The hyperglycaemic state affects membrane structures, resulting in reduced blood supply to the nerves, lack of oxygen, and swelling, disintegration, and demyelination of nerve cells [22].

Tawfik et al. found that the CSA of the vagus nerve in patients with diabetes was smaller than that of controls [11], which was not observed in the present study. One reason may be the different measurement positions of the vagus nerve: Tawfik et al. captured the nerve at the level of thyroid cartilage while we chose the thyroid gland level because of the least statistical heterogeneity at the thyroid gland level [20]. Therefore, the CSA of normal controls was lower than that in the study of Tawfik et al., which has been reported in previous studies [20, 23]. On the other hand, the study of Tawfik et al. recruited 7 patients with type 1 diabetes, which may also affect the results.

Although the change of vagus nerve CSA in patients with DPN has never been reported (as we are aware), the changes in vagus nerve CSA in patients with DPN in our study were consistent with previous studies on peripheral nerves: the CSAs of the ulnar nerve, median nerve, common peroneal nerve, tibial nerve, and sural nerves were significantly larger in the DPN group and significantly correlated with electrophysiological findings [7, 8, 24–26]. Therefore, our study demonstrated that in patients with DPN, the structural damage of peripherals and vagus nerves is synchronized. The possible explanation is that the conversion of glucose to sorbitol mediated by aldose reductase increases, which leads to an increase in nerve water content [27], which is manifested as a thickening of nerves under ultrasound. However, the aetiology and pathogenesis of diabetic neuropathy have not been fully elucidated.

In the present study, the right vagus nerve CSA mainly correlated with the duration of diabetes and C-peptide index. Long duration of diabetes has been demonstrated as a risk factor of DPN and autonomic neuropathy [14]. The C-peptide index reflected the β-cell function [16]. DPN is more popular in patients with insulin deficiency than in patients with insulin resistance [28] due to the increased glycaemic variability [29] and the high risk of hypoglycaemia [30]. Previous studies have also demonstrated that the vagus nerve plays an important role in the insulin secretion of β-cells [31, 32], and impairment of the vagus nerve may decrease β-cell function in patients with diabetic neuropathy.

The present study showed that the right vagus nerve CSA was positively correlated with the diagnosis of DPN, while the left side was not meaningful. This may be related to the asymmetry of the vagus nerve innervating the abdominal organs. The right vagus nerve mainly sends branches to the small intestine and the colon, while the left side dominantly innervates the stomach, the liver, and the upper part of the duodenum [33]. Several studies have also compared bilateral vagus nerves and found that the right CSA is larger than the left CSA of vagus [11, 34, 35]. As the results of the ROC analysis, the sensitivity and specificity of the cut-off value of 1.71 mm^2 for

the right vagus nerve CSA were 72.7% and 69.8%, respectively, which was similar to the studies using several peripheral nerves [7], but testing just one vagus nerve is more convenient and easier than testing multiple peripheral nerves. On the other hand, whether the combination of vagus and peripheral nerve assessment using HFU will further increase the specificity and sensitivity of the diagnosis of DPN deserves further investigation.

Consistent with previous studies, there were no significant differences in vagus nerve CSA between patients with and without autonomic symptoms [11, 36]. In addition, CSA was not correlated with the number of autonomic symptoms or the SAS impact score. The result was similar to that of a study of Parkinson's patients using the SCOPA-AUT questionnaire to assess autonomic symptoms [37]. Changes in vagus nerve ultrasound characteristics may occur earlier than the onset of autonomic symptoms. The relationship between vagus nerve CSA and the assessment of early stages of autonomic neuropathy, such as heart rate variability, should be further investigated. A limitation of our study was the availability of objective measures of autonomic function. The assessment of autonomic symptoms using the SAS and COMPASS 31 may not reflect early autonomic neuropathy, and the relationship between the vagus nerve CSA and autonomic neuropathy in the present study was incomplete. The present study also lacked data on the vagus nerve tested at other levels, such as the carotid sinus and thyroid cartilage. The effectiveness of vagus nerve assessment at different levels in predicting DPN needs to be further evaluated.

Conclusions

HFU represents a convenient and noninvasive examination. This new technique leads to the expansion of neuroimaging in patients with diabetes, which can help us further understand the damage of nerves caused by diabetes in vivo. HFU shows a loss of honeycomb structure of the vagus nerve in T2DM patients and an increase of vagus nerve CSA in DPN patients. Although the damage to peripherals and vagus nerves seems similar in patients with DPN, symptoms of autonomic neuropathy appear later than those of peripheral neuropathy. Therefore, cervical vagus nerve HFU may be a useful tool to assist in the diagnosis of DPN and early screening of autonomic neuropathy.

Acknowledgements
We thank the staff from the Electromyogram Room of the Department of Neurology, the Affiliated Wuxi People's Hospital of Nanjing Medical University, for their help.

Authorship confirmation/Contribution statement
L.X.: Conceptualization (equal); Funding acquisition (lead); Methodology (equal); Supervision (lead). X.J.: Conceptualization (equal); Investigation (equal). F.X.: Data curation (equal); Formal analysis (equal); Investigation (equal); Methodology (equal); Visualization (equal); Writing — original draft (equal). Q.W.: Data curation (equal); Investigation (equal); Visualization (equal); Writing — original draft (equal). Y.H.: Formal analysis(equal); Project administration (lead); Writing — review & editing (lead). L.L., Q.J., Y.H. and S.Y.: Writing — review & editing (supporting).

Conflict of interests
There are no conflicts of interest to declare.

Funding statement
This work was funded by the General project of Wuxi science and Technology Bureau, China (N20202006) and the scientific research project of Wuxi Municipal Health Commission (M202011).

Ethics statement
This study protocol was reviewed and approved by the Ethics Committee of Wuxi People's Hospital (ethical batch number: KY21068). The patients/participants provided written informed consent to participate in this study. Written informed consent was obtained from the individual to publish any potentially identifiable images or data contained in this article.

Data sharing
Data supporting the findings of this study are available from the corresponding author (LX) upon reasonable request.

References

1. Sun H, Saeedi P, Karuranga S, et al. IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. Diabetes Res Clin Pract. 2022; 183: 109119, doi: 10.1016/j.diabres.2021.109119, indexed in Pubmed: 34879977.
2. Didangelos T, Veves A. Treatment of Diabetic Cardiovascular Autonomic, Peripheral and Painful Neuropathy. Focus on the Treatment of Cardiovascular Autonomic Neuropathy with ACE Inhibitors. Curr Vasc Pharmacol. 2020; 18(2): 158–171, doi: 10.2174/1570161117666190521101342, indexed in Pubmed: 31109280.
3. Vinik AI, Nevoret ML, Casellini C, et al. Diabetic neuropathy. Endocrinol Metab Clin North Am. 2013; 42(4): 747–787, doi: 10.1016/j.ecl.2013.06.001, indexed in Pubmed: 24286949.
4. Vinik AI, Casellini C, Névoret ML. Alternative Quantitative Tools in the Assessment of Diabetic Peripheral and Autonomic Neuropathy. Int Rev Neurobiol. 2016; 127: 235–285, doi: 10.1016/bs.irn.2016.03.010, indexed in Pubmed: 27133153.
5. Zhao Z, Ji L, Zheng L, et al. Effectiveness of clinical alternatives to nerve conduction studies for screening for diabetic distal symmetrical polyneuropathy: A multi-center study. Diabetes Res Clin Pract. 2016; 115: 150–156, doi: 10.1016/j.diabres.2016.01.002, indexed in Pubmed: 27116903.
6. Pop-Busui R, Boulton AJM, Feldman EL, et al. Diabetic Neuropathy: A Position Statement by the American Diabetes Association. Diabetes Care. 2017; 40(1): 136–154, doi: 10.2337/dc16-2042, indexed in Pubmed: 27999003.
7. Narayan S, Goel A, Singh AK, et al. High resolution ultrasonography of peripheral nerves in diabetic patients to evaluate nerve cross sectional area with clinical profile. Br J Radiol. 2021; 94(1121): 20200173, doi: 10.1259/bjr.20200173, indexed in Pubmed: 33733810.
8. Ma X, Li T, Du L, et al. Applicability of High-Frequency Ultrasound to the Early Diagnosis of Diabetic Peripheral Neuropathy. Biomed Res Int. 2021; 2021: 5529063, doi: 10.1155/2021/5529063, indexed in Pubmed: 33829060.
9. Ishibashi F, Taniguchi M, Kojima R, et al. Morphological changes of the peripheral nerves evaluated by high-resolution ultrasonography are associated with the severity of diabetic neuropathy, but not corneal nerve fiber pathology in patients with type 2 diabetes. J Diabetes Investig. 2015; 6(3): 334–342, doi: 10.1111/jdi.12299, indexed in Pubmed: 25969719.
10. Chen HH, Chen TC, Yang TL, et al. Transcutaneous Sonography for Detection of the Cervical Vagus Nerve. Ear Nose Throat J. 2021; 100(3): 155–159, doi: 10.1177/0145561319875432, indexed in Pubmed: 31547704.

11. Tawfik EA, Walker FO, Cartwright MS, et al. Diagnostic Ultrasound of the Vagus Nerve in Patients with Diabetes. J Neuroimaging. 2017; 27(6): 589–593, doi: 10.1111/jon.12452, indexed in Pubmed: 28524416.
12. Haq T, Ahmed T, Latif ZA, et al. Cardiac autonomic neuropathy in patients with type 2 diabetes mellitus having peripheral neuropathy: A cross-sectional study. Diabetes Metab Syndr. 2019; 13(2): 1523–1528, doi: 10.1016/j.dsx.2019.03.015, indexed in Pubmed: 31336516.
13. Dyck PJ, Albers JW, Andersen H, et al. Toronto Expert Panel on Diabetic Neuropathy. Diabetic polyneuropathies: update on research definition, diagnostic criteria and estimation of severity. Diabetes Metab Res Rev. 2011; 27(7): 620–628, doi: 10.1002/dmrr.1226, indexed in Pubmed: 21695763.
14. Braffett BH, Gubitosi-Klug RA, Albers JW, et al. DCCT/EDIC Research Group. Risk Factors for Diabetic Peripheral Neuropathy and Cardiovascular Autonomic Neuropathy in the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Study. Diabetes. 2020; 69(5): 1000–1010, doi: 10.2337/db19-1046, indexed in Pubmed: 32051148.
15. Shabeeb D, Najafi M, Hasanzadeh G, et al. Electrophysiological measurements of diabetic peripheral neuropathy: A systematic review. Diabetes Metab Syndr. 2018; 12(4): 591–600, doi: 10.1016/j.dsx.2018.03.026, indexed in Pubmed: 29610062.
16. Funakoshi S, Fujimoto S, Hamasaki A, et al. Utility of indices using C-peptide levels for indication of insulin therapy to achieve good glycemic control in Japanese patients with type 2 diabetes. J Diabetes Investig. 2011; 2(4): 297–303, doi: 10.1111/j.2040-1124.2010.00096.x, indexed in Pubmed: 24843502.
17. Zilliox L, Peltier AC, Wren PA, et al. Assessing autonomic dysfunction in early diabetic neuropathy: the Survey of Autonomic Symptoms. Neurology. 2011; 76(12): 1099–1105, doi: 10.1212/WNL.0b013e3182120147, indexed in Pubmed: 21422460.
18. Sletten DM, Suarez GA, Low PA, et al. COMPASS 31: a refined and abbreviated Composite Autonomic Symptom Score. Mayo Clin Proc. 2012; 87(12): 1196–1201, doi: 10.1016/j.mayocp.2012.10.013, indexed in Pubmed: 23218087.
19. Curcean AD, Rusu GM, Dudea SM. Ultrasound appearance of peripheral nerves in the neck: vagus, hypoglossal and greater auricular. Med Pharm Rep. 2020; 93(1): 39–46, doi: 10.15386/mpr-1273, indexed in Pubmed: 32133445.
20. Abdelnaby R, Elsayed M, Mohamed KA, et al. Sonographic Reference Values of Vagus Nerve: A Systematic Review and Meta-analysis. J Clin Neurophysiol. 2022; 39(1): 59–71, doi: 10.1097/WNP.0000000000000856, indexed in Pubmed: 34144573.
21. Huang H, Wu S. Application of High-Resolution Ultrasound on Diagnosing Diabetic Peripheral Neuropathy. Diabetes Metab Syndr Obes. 2021; 14: 139–152, doi: 10.2147/DMSO.S292991, indexed in Pubmed: 33469331.
22. Jack M, Wright D. Role of advanced glycation endproducts and glyoxalase I in diabetic peripheral sensory neuropathy. Transl Res. 2012; 159(5): 355–365, doi: 10.1016/j.trsl.2011.12.004, indexed in Pubmed: 22500508.
23. Pelz JO, Belau E, Henn P, et al. Sonographic evaluation of the vagus nerves: Protocol, reference values, and side-to-side differences. Muscle Nerve. 2018; 57(5): 766–771, doi: 10.1002/mus.25993, indexed in Pubmed: 29053902.
24. Singh KP, Gupta K, Kataria N, et al. High-resolution ultrasonography of the sural nerve in diabetic peripheral neuropathy. J Ultrason. 2020; 20(81): e83–e89, doi: 10.15557/JoU.2020.0013, indexed in Pubmed: 32609965.
25. Singh K, Gupta K, Kaur S. High resolution ultrasonography of the tibial nerve in diabetic peripheral neuropathy. J Ultrason. 2017; 17(71): 246–252, doi: 10.15557/JoU.2017.0036, indexed in Pubmed: 29375899.
26. Kang S, Kim SeH, Yang SN, et al. Sonographic features of peripheral nerves at multiple sites in patients with diabetic polyneuropathy. J Diabetes Complications. 2016; 30(3): 518–523, doi: 10.1016/j.jdiacomp.2015.12.008, indexed in Pubmed: 26782023.
27. Lee D, Dauphinée DM. Morphological and functional changes in the diabetic peripheral nerve: using diagnostic ultrasound and neurosensory testing to select candidates for nerve decompression. J Am Podiatr Med Assoc. 2005; 95(5): 433–437, doi: 10.7547/0950433, indexed in Pubmed: 16166459.
28. Zaharia OP, Strassburger K, Strom A, et al. German Diabetes Study Group. Risk of diabetes-associated diseases in subgroups of patients with recent-onset diabetes: a 5-year follow-up study. Lancet Diabetes Endocrinol. 2019; 7(9): 684–694, doi: 10.1016/S2213-8587(19)30187-1, indexed in Pubmed: 31345776.
29. Akaza M, Akaza I, Kanouchi T, et al. Nerve conduction study of the association between glycemic variability and diabetes neuropathy. Diabetol Metab Syndr. 2018; 10: 69, doi: 10.1186/s13098-018-0371-0, indexed in Pubmed: 30214502.
30. Mohseni S. Hypoglycemic neuropathy. Acta Neuropathol. 2001; 102(5): 413–421, doi: 10.1007/s004010100459, indexed in Pubmed: 11699552.
31. Makhmutova M, Weitz J, Tamayo A, et al. Pancreatic β-Cells Communicate With Vagal Sensory Neurons. Gastroenterology. 2021; 160(3): 875–888.e11, doi: 10.1053/j.gastro.2020.10.034, indexed in Pubmed: 33121946.
32. Charpentier J, Waget A, Klopp P, et al. Lixisenatide requires a functional gut-vagus nerve-brain axis to trigger insulin secretion in controls and type 2 diabetic mice. Am J Physiol Gastrointest Liver Physiol. 2018; 315(5): G671–G684, doi: 10.1152/ajpgi.00348.2017, indexed in Pubmed: 30070580.
33. Al-Kureischi K. Verlauf und Nervenfaserarten des Truncus vagalis des Menschen . Acta Anat (Basel). 1979; 103(3): 252–258, indexed in Pubmed: 433561.
34. Holzapfel K, Naumann M. Ultrasound Detection of Vagus Nerve Atrophy in Bulbar Amyotrophic Lateral Sclerosis. J Neuroimaging. 2020; 30(6): 762–765, doi: 10.1111/jon.12761, indexed in Pubmed: 33167079.
35. Pelz JO, Belau E, Menze I, et al. Correlation between sonographic morphology and function of the cervical vagus nerves. Auton Neurosci. 2019; 220: 102552, doi: 10.1016/j.autneu.2019.05.004, indexed in Pubmed: 31126827.
36. Sartucci F, Bocci T, Santin M, et al. High-resolution ultrasound changes of the vagus nerve in idiopathic Parkinson's disease (IPD): a possible additional index of disease. Neurol Sci. 2021; 42(12): 5205–5211, doi: 10.1007/s10072-021-05183-5, indexed in Pubmed: 33821361.
37. Sijben LCJ, Mess WH, Walter U, et al. The cross-sectional area of the vagus nerve is not reduced in Parkinson's disease patients. eNeurologicalSci. 2022; 27: 100400, doi: 10.1016/j.ensci.2022.100400, indexed in Pubmed: 35592106.