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Association of platelet count and plateletcrit with nerve conduction function and peripheral neuropathy in patients with type 2 diabetes mellitus

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ABSTRACT

Aims/Introduction: Diabetes has been considered as a 'pro-thrombotic state' with enhanced platelet reactivity. Abnormality in platelet aggregation has been found in patients with its most common chronic complication – diabetic peripheral neuropathy (DPN). The purpose of this study was to investigate the potential association of platelet indices with nerve conduction function and the presence of DPN in Chinese patients with type 2 diabetes mellitus.

Materials and Methods: This study involved a total of 211 inpatients with type 2 diabetes mellitus and 55 healthy individuals for whom nerve conduction studies were carried out. DPN was diagnosed according to the American Diabetes Association recommendation. Clinical data were retrospectively collected.

Results: Patients with diabetes in whom neuropathy developed had lower levels of platelet count (PLT) and plateletcrit (PCT) than healthy controls ($P < 0.05$). Statistically significant associations of low PLT and PCT levels with the reduction of summed amplitude/velocity Z-score, and the prolongation of F-wave minimum latency in nerve conduction studies were found. Furthermore, after multivariate adjustment, logistic regression analysis showed that low levels of PLT (odds ratio 2.268, 95% confidence interval 1.072–4.797; $P < 0.05$; PLT <226 vs PLT ≥226) and PCT (odds ratio 2.050, 95% confidence interval 1.001–4.201; $P < 0.05$; PCT <0.222 vs PCT ≥0.222) in type 2 diabetes mellitus patients were risk factors for the presence of DPN.

Conclusions: Lower PLT and PCT levels are closely associated with poorer peripheral nerve conduction functions and the presence of neuropathy in patients with type 2 diabetes mellitus, which suggests that PLT and PCT might be potential biomarkers for showing DPN.

INTRODUCTION

Diabetic peripheral neuropathy (DPN) is one of the most common chronic complications of type 2 diabetes mellitus, and it occurs in more than half of type 2 diabetes mellitus patients[1]. Symptoms of DPN include pain, paresthesia, loss of sensation, weakness, ataxia and so on[2,3], of which distressing neuropathic pain, and impaired balance and gait are often unresponsive to therapy[4]. Apart from the considerable morbidity, mortality and diminished quality of life of patients, DPN is also considered as the strongest initiating risk factor for diabetic foot ulceration and non-traumatic lower limb amputation[5–7]. Furthermore, DPN has put a tremendous financial burden on healthcare systems and the entire society. For example, it was estimated that up to 27% of the direct medical cost of diabetes might be attributed to DPN and its complications[8].

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Patients can benefit from early diagnosis of DPN by receiving good multidisciplinary care in the early stages of the disease, which substantially reduces the risk of complications and hospitalization. Currently, nerve conduction studies (NCSs) are recognized as the gold standard for diagnosing DPN[4]. However, NCSs are impractical to implement in routine clinical care, because they are expensive, labor intensive and time-consuming. Therefore, simple and effective biomarkers are required to detect the population with type 2 diabetes mellitus who are at high risk of DPN.

Typical DPN is defined as a 'symmetrical, length-dependent sensorimotor polyneuropathy attributable to metabolic and microvessel alterations as a result of chronic hyperglycemia exposure and cardiovascular risk covariates'[9]. A combination of the metabolic abnormalities (such as hyperglycemia, insulin resistance, oxidative stress, dyslipidemia and neuroinflammation) and vascular dysfunctions (including endothelial injury and impaired endoneurial blood flow) results in progressive nerve fiber loss and endoneurial microangiopathic change in patients with DPN[3,7,10–12].

Due to activation of procoagulant mechanisms and dysfunction of anti-aggregants, platelets in type 2 diabetes mellitus patients adhere to endothelium and aggregate more readily compared with normal people. In addition, abnormal platelet aggregation has been found in DPN patients[13,14]. Hyperactivated platelets will not only trigger thrombus formation, but also release oxidative and vasoconstrictive substances, which would in turn induce local vascular lesions development[11,12]. Platelet indices, such as platelet count (PLT) and plateletcrit (PCT), can reflect the function of platelets and are easily obtained in clinical practice. Notably, low PLTs have been reported in patients with diabetes, and it is likely to be associated with an increased risk of microvascular disease[15,16].

However, the relationship between platelet parameters and peripheral nerve conduction function in type 2 diabetes mellitus patients is rarely reported. Also, their predictive effect over DPN remains controversial. Therefore, the present study was carried out to explore the potential associations of platelet indices (PLT and PCT) with nerve conduction function, as well as the presence of DPN in patients with type 2 diabetes mellitus.

MATERIALS AND METHODS

Participants and inclusion criteria

A total of 211 patients with type 2 diabetes mellitus were enrolled from inpatients at the First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China, between February 2018 and October 2019. We divided them into the diabetes mellitus with no peripheral neuropathy group (diabetes group, 100 patients) and DPN group (111 cases). In addition, 55 healthy people were selected as the healthy control group (HC) during the same time period. Diagnostic criteria for type 2 diabetes mellitus complies with the recommendation of the American Diabetes Association[17,18], which suggests that both fasting and 2-h plasma glucose values of the oral glucose tolerance test and hemoglobin (HbA1c) can be used as criteria for diagnosing diabetes and prediabetes. DPN was diagnosed according to the recommendations of the American Diabetes Association and the Toronto Consensus on Diabetic Neuropathies considering both the presence of clinical symptoms or signs and abnormality in NCSs. Peripheral nerve defects of non-diabetic origin were strictly excluded[19–21].

The study protocol was approved by the ethics committee of the First Affiliated Hospital of Wenzhou Medical University. The participants provided their written informed consent to participate in this study.

Exclusion criteria

Patients with type 1 diabetes, gestational diabetes mellitus and any other type of diabetes;

Patients with neuropathy due to other causes, such as cervical and lumbar spondylopathy, Guillain–Barré syndrome, epilepsy and severe arteriovenous vascular disease;

Patients with other serious diseases, such as progressive malignancy, acute infection, severe renal insufficiency and heart failure;

Patients with a history of medications affecting platelets (e.g., aspirin) or a history of coronary heart disease, cerebral infarction and blood system disease that would possibly affect platelet-related indices.

Clinical neurological examination

All type 2 diabetes mellitus patients enrolled underwent a complete neurological examination. Experienced neurologists carried out the vibration perception threshold test, and assessed the symptoms, signs and neurological deficits of DPN using the neuropathy disability score (NDS) and neuropathy symptom score. The NDS assesses ankle reflexes (using a tendon hammer), vibration (using a 128-Hz tuning fork), pinprick sensation (using Neurotip) and temperature sensation (using warm and cool rods) at the great toe of each side, and the score ranges from 0 to 10[22,23]. Neuropathy symptom score is used for the assessment of quality (pain, burning, numbness, paresthesia, muscular fatigue, muscular spasms), localization (feet, calves or elsewhere), time of exacerbation (daytime, night or both) and maneuver to achieve relief of symptoms (lying down, standing or walking) with a maximum score of 9[24]. The minimum acceptable criteria for a clinical diagnosis of peripheral neuropathy is an NDS score of $\geq$6, or an NDS score of 3–5 with an neuropathy symptom score of $\geq$5[25].

Neuroelectrophysiological examination

Nerve conduction studies were carried out by electrophysiological experts using an electromyography machine (Kipoint-4 type, Vidi; NDI-200P + type; Poseidon). During the test, participants remained calm and relaxed, and local skin temperature was kept constant (32–33.8°C). Briefly, the motor action potential amplitude, distal latency and conduction velocity (CV) of the bilateral ulnar, median, tibial and common peroneal nerves,

and sensory amplitude, distal latency and CV of the bilateral ulnar, median and superficial peroneal nerves were determined. The F-wave latency of the tibial nerve on both sides was also assessed and recorded. The reduction of action potential amplitude and CV, and the prolongation of F-wave minimum latency in NCSs are objective signs of DPN[26–28]. When abnormality (normal deviates ≤2.5th/≥97.5th percentile) of one or more attributes in two or more nerves was found, NCSs were considered abnormal[29,30]. The diagnosis of abnormal NCSs was judged by an electrophysiologist and the NCSs parameters of the severe side were included into the clinical data.

Clinical data collection

The participants' clinical data were collected by viewing the electronic medical record. Clinical data includes demographic information, diabetic duration, body mass index, smoking history, hypertension history, hyperlipidemia history and whether they are affected by diabetic complications. Diabetic complications, including diabetic foot, diabetic retinopathy, diabetic nephropathy and peripheral vascular disease in type 2 diabetes mellitus, were diagnosed by physicians according to the criteria defined in the relevant guidelines[18,31–35]. Laboratory data include HbA1c, fasting plasma glucose, triglyceride, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, PLT, PCT, mean platelet volume (MPV), platelet distribution width standard deviation value (PDW-SD) and platelet larger cell ratio. The inspection results were compared with the normal reference range of the laboratory.

Calculation of mean value of NCSs parameters and summed Z-score

We calculated the mean motor nerve CV (MNCV) using the following formula: MNCV = (ulnar nerve motor CV + median nerve motor CV + tibial nerve motor CV + common peroneal nerve motor CV) / 4. A similar method was used to calculate the mean motor nerve amplitude, mean sensory nerve amplitude and mean sensory nerve conduction velocity[36]. Furthermore, action potential amplitude and CV of each peripheral nerve were normalized and summarized as the total Z-score of each patient, following the calculation formula[26]:

Table 1 | Baseline characteristics of 266 participants among the healthy control, diabetes mellitus with no peripheral neuropathy and diabetic peripheral neuropathy groups

	HC	DM	DPN	*P*-value
Samples (*n*)	55	100	111	
Age (years)	52 (40–61)	56 (46–63)	61 (54–68)*	<0.001
Male sex (%)	61.8	56.0	55.0	0.689
Smoking (%)	25.5	25.0	26.1	0.982
Hypertension (%)	10.9	33*	54.1*	<0.001
Hyperlipidemia (%)	5.5	35.4*	33.3*	<0.001
Diabetic duration (years)	–	6 (1–10)	10 (5–18)	<0.001
BMI (kg/m^2)	23.30 (21.37–25.70)	23.84 (22.14–26.28)	23.62 (22.20–26.80)	0.199
HbA1c (%)	5.60 (5.50–5.70)	9.10 (7.30–10.88)*	9.20 (7.60–10.90)*	<0.001
FPG (mmol/L)	5.10 (4.50–6.00)	7.15 (5.90–9.30)*	7.60 (5.80–10.00)*	<0.001
TC (mmol/L)	4.71 (4.09–5.52)	4.90 (4.03–5.67)	4.74 (4.17–5.54)	0.793
TG (mmol/L)	1.51 (1.11–2.05)	1.70 (1.08–2.20)	1.53 (1.05–2.33)	0.450
HDL-C (mmol/L)	1.08 (0.91–1.27)	0.97 (0.82–1.15)*	1.01 (0.87–1.12)	0.028
LDL-C (mmol/L)	2.56 (2.14–3.08)	2.56 (2.06–3.37)	2.43 (2.00–3.08)	0.510
PLT (10^9/L)	223 (196–257)	211 (185–256)	201 (171–233)*	0.017
MPV (fL)	10.91 ± 0.13	10.97 ± 0.10	11.18 ± 0.09	0.166
PCT (%)	0.24 (0.22–0.27)	0.23 (0.20–0.27)	0.23 (0.20–0.25)*	0.040
PDW-Sd (fL)	12.70 (11.90–13.70)	13.20 (12.20–14.70)	13.50 (12.30–15.50)*	0.026
PLCR (%)	31.72 ± 0.94	32.77 ± 0.77	34.47 ± 0.76	0.070
Complication (%)†	–	23.0%	64.0%	<0.001
DR (%)	–	19.0%	58.6%	<0.001
DN (%)	–	5.0%	10.8%	0.121
DF (%)	–	1.0%	7.2%	0.037
PVD (%)	–	2.0%	12.6%	0.004

Data are the mean ± standard error of the mean, median (25th–75th percentiles) or n (%). BMI, body mass index; DM, diabetes mellitus with no peripheral neuropathy group; DPN, diabetic peripheral neuropathy group; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MPV, mean platelet volume, PCT, plateletcrit; PDW-Sd, platelet distribution width standard deviation value; PLCR, platelet larger cell ratio; TC, total cholesterol; TG, triglyceride. *Indicates to $P < 0.05$ when compared with the healthy control (HC) group. †Diabetic complication including diabetic retinopathy (DR), diabetic nephropathy (DN), diabetic foot (DF), peripheral vascular disease in type 2 diabetes mellitus (PVD).

$$\text{Amplitude}: Z_{k_i} = \frac{X_{k_i} - \overline{X}_k}{S_k}, \text{ conduction velocity}: Z_{k_i} = \frac{X_{k_i} - \overline{X}_k}{S_k}$$

$$\text{Summed amplitude } Z \text{ score}: Z_i = \sum Z_{k_i}, \text{ Summed velocity } Z \text{ score}: Z_i = \sum Z_{k_i}$$

k = (ulnar nerve motor, ulnar nerve sensory. . .each nerve) and i = 1, 2, 3. . .n (all participants: n = 266; type 2 diabetes mellitus patients: n = 211).

Statistical analysis

IBM SPSS 25.0 software (IBM Corp., Armonk, NY, USA) was used for statistical analysis. Continuous variables are presented as the mean ± standard error of mean or medians (25th–75th percentiles), whereas categorical data were expressed as a percentage (%). The differences between groups were evaluated using Student's *t*-test, one-way ANOVA test, Kruskal–Wallis test or χ^2-test accordingly. Correlation analysis, such as Pearson's and Spearman's test and multiple linear regression analysis, were carried out to explore the relationship between platelet indices and NCSs parameters. Binary logistic regression models were used to evaluate the odds ratios (OR) for associations between risk factors and the presence of DPN. All tests were two-sided, and $P < 0.05$ was considered statistically significant.

RESULTS

Baseline characteristics

Table 1 shows the detailed baseline characteristics of 266 participants among the HC, diabetes and DPN groups. The diabetes group and DPN group showed higher levels of HbA1c, fasting plasma glucose, and higher proportions of hypertension and hyperlipidemia population when compared with the HC group ($P < 0.001$). Patients in whom neuropathy developed were on average 5 years older, had diabetes for 4 years

Table 2 | Presence of diabetic peripheral neuropathy and nerve conduction studies parameters of quartiles (quartile1–quartile4) of platelet count level

	PLT (10^9/L)				
	Q1 ≤178	Q2 179–210	Q3 211–251	Q4 >251	*P* for trend
Samples (*n*)	67	66	67	66	–
PLT	158 (144–169)	197 (191–204)	229 (218–240)	284 (261–321)	<0.001
PCT	0.180 (0.168–0.198)	0.222 (0.204–0.233)	0.243 (0.232–0.258)	0.302 (0.277–0.339)	<0.001
DPN (%)	53.70%	39.40%	40.30%	33.30%	0.079
Motor amplitude (mv)					
Ulnar	12.02 ± 0.30	12.73 ± 0.27	13.49 ± 0.28	13.17 ± 0.28	0.002
Median	12.32 ± 0.35	13.42 ± 0.41	13.91 ± 0.43	12.40 ± 0.36	0.018
Tibial	13.46 ± 0.71	15.32 ± 0.76	15.27 ± 0.66	15.47 ± 0.69	0.377
Common peroneal	6.45 ± 0.40	7.28 ± 0.43	7.91 ± 0.50	7.42 ± 0.38	0.109
Motor CV (m/s)					
Ulnar	51.61 ± 0.67	54.08 ± 0.63	54.27 ± 0.59	53.77 ± 0.64	0.010
Median	52.13 ± 0.67	54.03 ± 0.55	53.96 ± 0.58	53.46 ± 0.67	0.158
Tibial	44.31 ± 0.65	45.80 ± 0.50	45.73 ± 0.48	46.34 ± 0.60	0.214
Common peroneal	43.87 ± 0.60	44.99 ± 0.59	45.97 ± 0.55	45.73 ± 0.56	0.127
Sensory amplitude (mv)					
Ulnar	34.73 ± 2.29	41.29 ± 2.31	36.93 ± 2.08	40.99 ± 2.14	0.101
Median	36.89 ± 2.12	43.31 ± 1.89	40.40 ± 2.10	39.32 ± 2.12	0.174
Superficial peroneal	9.62 ± 0.64	12.24 ± 0.62	11.60 ± 0.59	12.34 ± 0.76	0.015
Sensory CV (m/s)					
Ulnar	51.22 ± 0.62	53.28 ± 0.66	52.81 ± 0.65	53.52 ± 0.72	0.026
Median	51.25 ± 0.87	53.71 ± 0.73	52.95 ± 0.95	51.56 ± 0.81	0.054
Superficial peroneal	43.94 ± 0.62	45.77 ± 0.57	45.09 ± 0.49	45.20 ± 0.63	0.265
F-wave minimum latency (ms)	44.25 ± 0.54	42.72 ± 0.42	41.67 ± 0.45	41.89 ± 0.48	0.002
MNAmp	11.06 ± 0.33	12.23 ± 0.33	12.60 ± 0.37	12.11 ± 0.31	0.009
MNCV	48.03 ± 0.56	49.73 ± 0.43	50.05 ± 0.40	49.89 ± 0.53	0.051
SNAmp	27.08 ± 1.51	32.26 ± 1.38	29.54 ± 1.34	31.06 ± 1.53	0.030
SNCV	48.81 ± 0.55	50.92 ± 0.50	50.28 ± 0.54	50.02 ± 0.60	0.052
Summed amplitude Z-score	−1.77 ± 0.59	0.66 ± 0.53	0.77 ± 0.59	0.36 ± 0.57	0.005
Summed velocity Z-score	−1.78 ± 0.68	0.74 ± 0.53	0.61 ± 0.52	0.45 ± 0.68	0.046

Data are the mean ± standard error of the mean, median (25th–75th percentiles) or *n* (%). CV, conduction velocity; DPN, diabetic peripheral neuropathy; MNAmp, mean motor nerve amplitude; MNCV, mean motor nerve conduction velocity; PCT, plateletcrit; PLT, platelet count; Q1, quartile 1; Q2, quartile 2; Q3, quartile 3; Q4, quartile 4; SNAmp, mean sensory nerve amplitude; SNCV, mean sensory nerve conduction velocity.

longer, and had higher proportions of hypertension and diabetes complications ($P < 0.05$) than those type 2 diabetes mellitus patients in whom neuropathy did not develop. Furthermore, compared with the HC group, the DPN group had a lower level of PLT and PCT, and a higher level of PDW-SD ($P < 0.05$), whereas there was no significant difference in platelet indices between the HC and diabetes groups ($P > 0.05$).

NCSs parameters and presence of DPN in different PLT/PCT quartiles

We divided these 266 participants into quartiles according to PLT and PCT levels, respectively. The NCSs data, including nerve conduction amplitude, velocity and the F-wave minimum latency in each quartile, were presented in Tables 2 and S1. With increasing levels of PLT and PCT, the motor or sensory amplitude and CV of certain peripheral nerves, as well as the summed amplitude and velocity Z-score, increased ($P < 0.05$; Figure 1), whereas the F-wave minimum latency was shortened significantly ($P < 0.01$). Additionally, the highest quartile level of PCT (group Q4) showed the lowest proportion of DPN patients. (Q1: 44.80%; Q2: 52.90%; Q3: 37.90%; Q4: 30.80%; $P < 0.05$; Table S1).

Correlation between PLT/PCT level and NCSs parameters

Correlation analysis and multiple linear regression analysis were carried out to investigate the relationship of PLT and PCT levels with NCSs parameters. As is shown in Table 3, PLT and PCT levels were positively correlated with mean motor nerve amplitude ($r = 0.154$, $P < 0.05$; $r = 0.127$, $P < 0.05$), MNCV ($r = 0.204$, $P < 0.01$; $r = 0.203$, $P < 0.01$), summed amplitude Z-score ($r = 0.172$, $P < 0.01$; $r = 0.145$, $P < 0.05$) and summed velocity Z-score ($r = 0.143$, $P < 0.05$; $r = 0.136$, $P < 0.05$), whereas negatively correlated with the F-wave minimum latency ($r = -0.242$, $P < 0.001$; $r = -0.220$, $P < 0.001$).

For single motor nerve conduction, PLT and PCT levels were positively correlated with the action potential amplitude, and the CV of the ulnar nerve and tibial nerve, as well as the CV of the common peroneal nerve ($P < 0.05$). Similarly, as for single sensory nerve conduction, PLT and PCT levels were positively correlated with superficial peroneal nerve amplitude and ulnar nerve CV ($P < 0.05$; Table 3).

In multiple linear regression models, after adjusting for age, sex, diabetic duration, HbA1c, body mass index, smoking, hypertension, hyperlipidemia, diabetic retinopathy and diabetic nephropathy[26,37–39], we found that PLT and PCT levels were correlated with conduction amplitude of a certain nerve (Table S2). Furthermore, PCT level was positively correlated with MNCV ($\beta = 10.263$, $P < 0.05$), and negatively correlated with the F-wave minimum latency ($\beta = -9.713$, $P < 0.05$).

Risk factors for DPN

After adjusting for age, sex and other previously reported neuropathy-related factors, including diabetic duration, HbA1c,

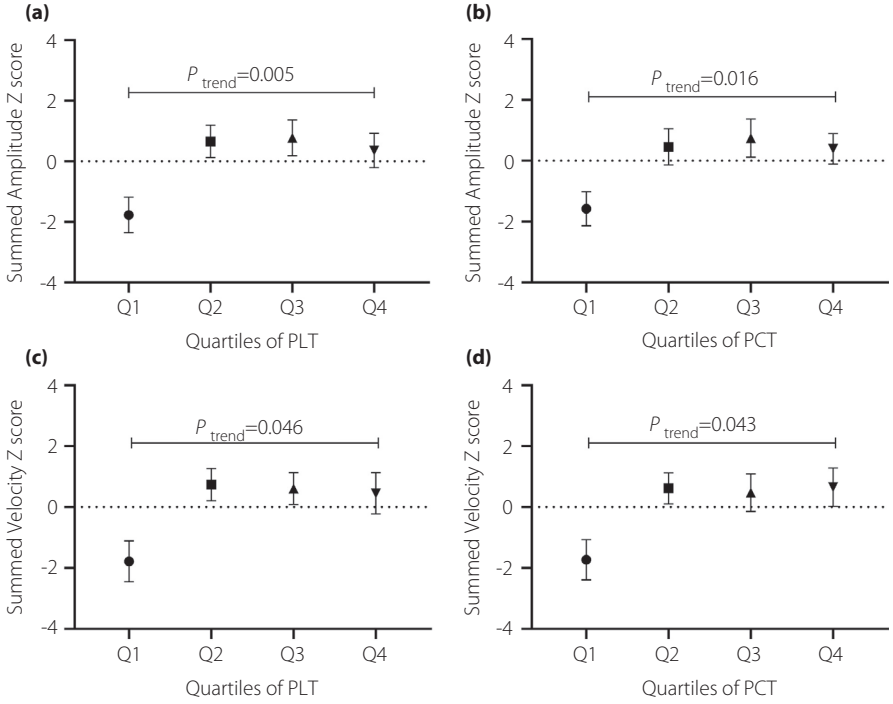

Figure 1 | The summed amplitude and velocity Z-score in quartiles of platelet count (PLT)/plateletcrit (PCT) levels, respectively. (a) Summed amplitude Z-score across PLT quartiles. (b) Summed amplitude *Z* score across PCT quartiles. (c) Summed velocity Z-score across PLT quartiles. (d) Summed velocity Z-score across PCT quartiles. The values presented are the mean ± standard error of the mean.

Table 3 | Correlation analysis of platelet count/plateletcrit with nerve conduction studies parameters

	PLT (10^9/L)		PCT (%)	
	r	*P*	*r*	*P*
Motor amplitude (mv)				
Ulnar	0.175	0.004	0.136	0.027
Median	0.031	0.616	0.011	0.854
Tibial	0.149	0.015	0.136	0.027
Common peroneal	0.123	0.045	0.096	0.117
Motor CV (m/s)				
Ulnar	0.172	0.005	0.188	0.002
Median	0.084	0.172	0.085	0.166
Tibial	0.138	0.024	0.134	0.029
Common peroneal	0.184	0.003	0.180	0.003
Sensory amplitude (mv)				
Ulnar	0.107	0.082	0.106	0.085
Median	0.051	0.409	0.038	0.542
Superficial peroneal	0.176	0.004	0.165	0.007
Sensory CV (m/s)				
Ulnar	0.146	0.017	0.149	0.015
Median	0.034	0.583	0.027	0.664
Superficial peroneal	0.059	0.334	0.051	0.408
F-wave minimum latency (ms)	−0.242	<0.001	−0.220	<0.001
MNAmp	0.154	0.012	0.127	0.039
MNCV	0.204	0.001	0.203	0.001
SNAmp	0.119	0.052	0.109	0.075
SNCV	0.105	0.086	0.101	0.101
Summed amplitude Z-score	0.172	0.005	0.145	0.018
Summed velocity Z-score	0.143	0.020	0.136	0.027

CV, conduction velocity; MNAmp, mean motor nerve amplitude; MNCV, mean motor nerve conduction velocity; PCT, plateletcrit; PLT, platelet count; SNAmp, mean sensory nerve amplitude; SNCV, mean sensory nerve conduction velocity.

body mass index, smoking, hypertension, diabetes complications and blood lipid profile (total cholesterol, triglyceride, low-density lipoprotein cholesterol)[26,37], patients with diabetes who had a level of PLT <226 showed a higher risk of diabetic peripheral neuropathy compared with those with PLT ≥ 226 (OR 2.268, 95% confidence interval 1.072–4.797; $P < 0.05$), whereas patients with PCT <0.222 showed a higher presence of DPN compared with those with PCT ≥0.222 (OR 2.050, 95% confidence interval 1.001–4.201; $P < 0.05$). The results of binary logistic regression analysis show that lower levels of PLT and PCT are risk factors for DPN (Tables 4 and 5). Figures 2 and 3 show ORs for associations between different PLT/PCT subgroups and the presence of DPN using logistic regression models.

DISCUSSION

Diabetes is considered as a 'prothrombotic state' resulting from increased intravascular thrombin generation, enhanced platelet aggregability reactivity and reduced fibrinolytic potential[40,41]. The morbidity and mortality of diabetes mainly depend on its vascular complications[42]. Increased platelet activation and aggregation antedates the occurrence of microvascular disease in patients with diabetes[43], and plays a critical role in the pathophysiology of diabetic vascular complications[44]. As for DPN, studies have shown abnormal platelet aggregation in affected individuals[13,14]. Furthermore, it has been reported that after treatment, symptomatic relief of DPN can be accompanied by improved platelet abnormalities[15].

During the development of DPN, small fibers (i.e., unmyelinated C fibers) are affected at the early stages, and contribute to the early hyperalgesia and dysesthesia in patients. Later on, large fibers, such as Aβ and Aδ fibers, progressively demyelinate and degenerate, resulting in reduced nerve conduction velocity or altered vibrating perception threshold[3,45]. NCSs are the most sensitive, objective, and reliable method of detecting and quantitating DPN, especially in the pre-symptomatic stage of DPN onset[46,47]. Therefore, to investigate the potential relationship between platelet dysfunction and DPN, we analyzed the NCSs data of 266 participants. The present study showed that lower PLT and PCT levels might be risk factors for the presence of DPN in a Chinese population with type 2 diabetes mellitus.

Table 4 | Odds ratios for associations between risk factors (including platelet count) and the presence of diabetic peripheral neuropathy with the use of binary logistic regression models

Predictor variables	Model 1†		Model 2‡	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
HbA1c (%)	1.198 (1.023–1.403)	0.025	1.254 (1.055–1.491)	0.010
Diabetic duration (year)	1.096 (1.039–1.157)	0.001	1.099 (1.038–1.163)	0.001
Complication (%)	4.850 (2.463–9.550)	<0.001	5.025 (2.471–10.219)	<0.001
PLT (10^9/L)				
≥226	1, Reference		1, Reference	
<226	2.184 (1.100–4.336)	0.026	2.268 (1.072–4.797)	0.032

CI, confidence interval; OR, odds ratio; PLT, platelet count. †Model 1: adjusted for age, diabetic duration, glycated hemoglobin (HbA1c) and complication (diabetic retinopathy/diabetic nephropathy/diabetic foot/peripheral vascular disease in type 2 diabetes mellitus). ‡Model 2: adjusted for variables in model 1. and for sex, body mass index, smoking, hypertension, total cholesterol, triglyceride and low-density lipoprotein cholesterol.

Table 5 | Odds ratios for associations between risk factors (including plateletcrit) and the presence of diabetic peripheral neuropathy with the use of binary logistic regression models

Predictor variables	Model 1†		Model 2‡	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
HbA1c (%)	1.232 (1.052–1.442)	0.010	1.286 (1.082–1.529)	0.004
Diabetic duration (year)	1.100 (1.042–1.161)	0.001	1.105 (1.042–1.171)	0.001
Complication (%)	4.911 (2.498–9.652)	<0.001	4.936 (2.449–9.945)	<0.001
PCT (%)				
≥0.222	1, Reference		1, Reference	
<0.222	2.108 (1.083–4.103)	0.028	2.050 (1.001–4.201)	0.049

CI, confidence interval; OR, odds ratio; PCT, plateletcrit. †Model 1: adjusted for age, diabetic duration, glycated hemoglobin (HbA1c) and complication (diabetic retinopathy/diabetic nephropathy/diabetic foot/peripheral vascular disease in type 2 diabetes mellitus). ‡Model 2: adjusted for variables in model 1, and for gender, body mass index, smoking, hypertension, total cholesterol, triglyceride and low-density lipoprotein cholesterol.

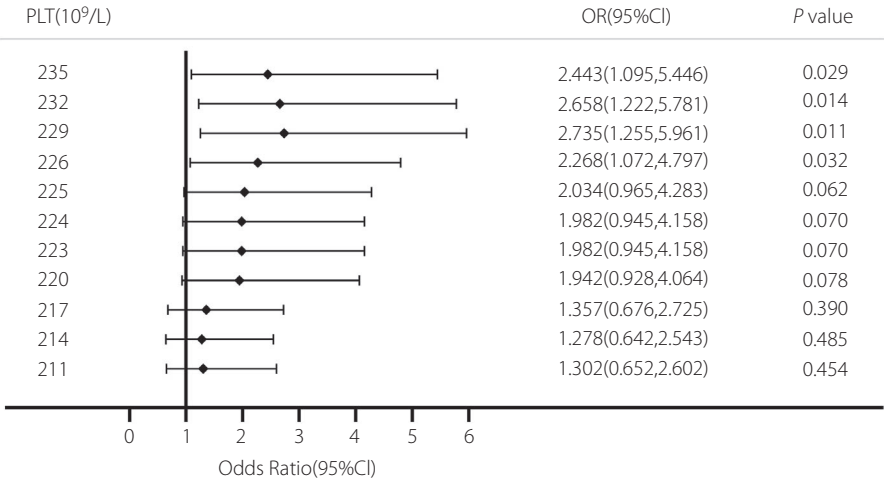

Figure 2 | Odds ratios (OR) for associations between different platelet count (PLT) subgroups and the presence of diabetic peripheral neuropathy (DPN) with the use of binary logistic regression models. Compared with patients with diabetes who had a level of PLT ≥211, those with PLT <211 did not show a significant difference in the presence of DPN ($P = 0.454$). Nor did PLT <225 compared with PLT ≥225 ($P = 0.062$). However, patients with a level of PLT <226 had a higher risk of DPN (OR 2.268, 95% confidence interval [CI] 1.072–4.797; $P = 0.032$) compared with those with PLT ≥226 (OR 1, reference); as did PLT <229 compared with PLT ≥229, PLT <232 compared with PLT ≥232 and PLT <235 compared with PLT ≥235 (all $P < 0.05$). Logistic regression models were adjusted for age, sex, diabetic duration, glycated hemoglobin, body mass index, smoking, hypertension, complication (diabetic retinopathy/diabetic nephropathy/diabetic foot/peripheral vascular disease in type 2 diabetes mellitus), total cholesterol, triglyceride and low-density lipoprotein cholesterol.

In previous studies, Buch *et al.*[15] and Hekimsoy *et al.*[48] found that PLT levels of type 2 diabetes mellitus patients were lower than in healthy people. However, few studies investigated the alteration of platelet indices in patients with DPN. In the present study, we compared the differences in platelet indices between different groups (HC group, diabetes group and DPN group). We found that patients in the DPN group had lower PLT and PCT levels, and higher PDW-SD levels compared with the HC group. A similar conclusion has been found in another type of diabetic microangiopathy; namely, diabetic retinopathy. In a meta-analysis involving 4,653 participants, Ji *et al.*[16] found an obvious decrease of the PLT level in diabetic retinopathy patients. Peripheral PLT depends on several different variables; that is, platelet production rate, mean platelet survival and the size of the exchangeable splenic platelet pool. Lower PLTs in the present DPN patients might be attributed to impaired platelet productions, shorter survival time of platelets, increased turnover of the platelet population and massive consumption during coagulation in diabetes[48].

PCT, the ratio of platelets per unit volume of blood, is a measure of total platelet mass[49]. It is determined by the combination of PLT and MPV (PLT × MPV), thus providing more

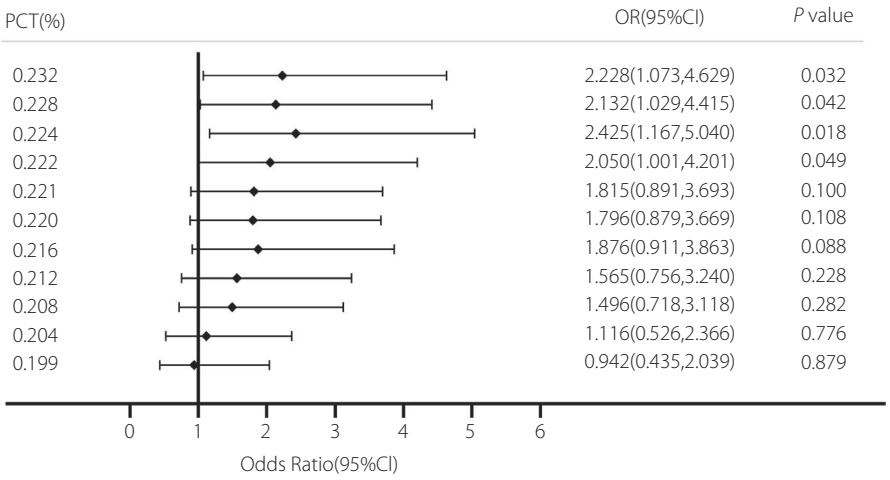

Figure 3 | Odds ratios (OR) for associations between different plateletcrit (PCT) subgroups and the presence of diabetic peripheral neuropathy (DPN) with the use of binary logistic regression models. Compared with patients with diabetes who had a level of PCT ≥0.199, those with PCT <0.199 did not show a significant difference in the presence of DPN ($P = 0.879$); nor did PCT <0.221 compared with PCT ≥0.221 ($P = 0.100$). However, patients with a level of PCT <0.222 had a higher risk of DPN (OR 2.050, 95% confidence interval [CI] 1.001–4.201; $P = 0.049$) compared with those with PCT ≥0.222 [OR 1, reference]; as did PCT <0.224 compared with PCT ≥0.224, PCT <0.228 compared with PCT ≥0.228 and PCT <0.232 compared with PCT ≥0.232 (all $P < 0.05$). Logistic regression models were adjusted for age, sex, diabetic duration, glycated hemoglobin, body mass index, smoking, hypertension, complication (diabetic retinopathy/diabetic nephropathy/diabetic foot/peripheral vascular disease in type 2 diabetes mellitus), total cholesterol, triglyceride and low-density lipoprotein cholesterol.

accurate information than PLT or MPV alone[50]. However, the correlation between PCT and the action potential conduction of peripheral nerves has been rarely reported. To our knowledge, it is the first time that a lower level of PCT has been reported as a risk factor for DPN. We found that patients with the lowest PCT quartile (PCT ≤0.199) had slower nerve conduction velocity, lower amplitude of action potential and longer F-wave minimum latency, with a higher proportion of DPN. As is shown in Table 1, there was no significant difference in MPV levels between groups, which is consistent with the study of Akinsegun *et al.*[51]. Additionally, almost no solid correlations of MPV with peripheral nerve conduction function and the presence of DPN were found (Tables S3–S5). Thus, more studies are required to examine the underlying mechanism, and confirm the relationship of PCT and DPN. PCT is likely to be a largely underestimated parameter in DPN.

Platelets from patients with diabetes show a stronger response to classical agonists, and a more notable membrane expression of adhesive molecules, such as thrombospondin, P-selectin, GPIIb-IIIa, GPVI and CD40L[52]. However, the mechanisms of platelets hyperactivity in diabetes remain controversial. Insulin is a natural antagonist of platelet hyperactivity; however, insulin resistance in type 2 diabetes mellitus patients not only decreases the sensitivity of platelets to anti-aggregants, such as PGI2 and NO, but also reduce the secretion of prostacyclin and nitric oxide by the endothelium[12]. Activated platelets might be involved in the pathophysiology of diabetes and its complications by the following mechanisms: (i) capillary microembolism; (ii) local progression of pre-existing vascular lesions by secretion of oxidative, constrictive and mitogenic substances; and (iii) trigger of an arterial thrombotic event, which will lead to a poor prognosis[40]. More scientific research must be carried out to comprehensively show the critical role of platelet dysfunction in the initiation and development of DPN.

In conclusion, the present study showed the relationship of PLT and PCT levels with peripheral nerve conduction functions, and lower levels of PLT and PCT might be useful indicators for predicting DPN in patients with type 2 diabetes mellitus. Platelet indices are easy to obtain in clinical practice, and might be of great significance for DPN early diagnosis and monitoring.

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DISCLOSURE

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 | Presence of diabetic peripheral neuropathy and nerve conduction studies parameters of quartiles (Q1–Q4) of plateletcrit level.

Table S2 | Multiple linear regression analysis of the correlation between platelet count/plateletcrit and nerve conduction studies parameters.

Table S3 | Correlation analysis of mean platelet volume with nerve conduction studies parameters.

Table S4 | Odds ratios (95% confidence interval) associated with risk factors for neuropathy in binary logistic regression models.

Table S5 | Multiple linear regression analysis of the correlation between mean platelet volume and nerve conduction studies parameters.