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Original Article

Impact of MPV and PDW on bone mineral density and their relationship with osteoporosis in Chinese patients with type 2 diabetes

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Abstract: Platelet functions are related to bone resorption and formation. This present study aimed to study the impact of mean platelet volume (MPV) and platelet distribution width (PDW) on bone mineral density (BMD) values and their relationship with the prevalence of osteoporosis in Chinese patients with type 2 diabetes mellitus (T2DM). A total of 882 elderly hospitalized T2DM patients (242 patients with osteoporosis, 323 patients with osteopenia, and 317 control subjects) undergoing BMD measurement were enrolled. Different biochemical and hematological parameters including MPV and PDW were determined, and the relationship between MPV and PDW, BMD, and osteoporosis was analyzed. There was a progressive increase in MPV and PDW levels from normal BMD subjects (11.20±1.16 fl and 14.68±2.63%, respectively) to osteopenia (11.46±1.23 fl and 15.30±2.91%, respectively) and osteoporosis (11.50±1.29 fl and 15.42±2.65%, respectively) patients (all $P<0.01$). Partial correlation analysis demonstrates that MPV and PDW concentrations are all negatively associated with BMD values at the lumbar spine, femoral neck, and total hip in all participants after adjustment for potential confounders (all $P<0.05$). Furthermore, T2DM patients in the highest quartile of MPV and PDW had significantly lower BMD values at the femoral neck and total hip, and higher prevalence of osteoporosis compared with those in the lowest MPV and PDW quartile ($P<0.01$ or $P<0.05$). Higher MPV and PDW levels were related to lower BMD values at the femoral neck and total hip and a higher prevalence of osteoporosis in T2DM patients.

Keywords: Mean platelet volume, platelet distribution width, osteoporosis, bone mineral density, type 2 diabetes mellitus

Introduction

Osteoporosis (OP) is a complex skeletal disorder characterized by a significant reduction in bone mass and deterioration of the bone microstructure, resulting in increased susceptibility to fractures [1]. Osteoporosis is one of the most common diseases encountered in T2DM patients and is a strong predictor of T2DM, now becoming a major public health problem globally with an economic burden on the society. Despite continuous advances in the treatment of osteoporosis it remains a difficult challenge. Therefore, early identification and intervention of a surrogate marker in clinical settings are urgently needed for the prevention and treatment of OP in T2DM patients.

Bone remodeling is a result of the mutual effect between osteoblasts and osteoclasts; an imbalance between bone resorption and bone formation can lead to high bone turnover and osteoporosis [2]. Osteoblasts are bone forming cells derived from multipotent mesenchymal stem cells (MSC) [1]. Osteoclasts are large multinucleated cells that are of hematopoietic origin, differentiating from the monocyte-macrophage lineage, which are responsible for bone resorption [3-4]. Therefore, it has been postulated that osteoblasts and osteoclasts may be concerned with bone hematopoiesis and that megakaryocytes (MKs) have a significant role in skeletal homeostasis [4]. Platelets are small anuclear cells of 1-2 μm in length with an average life span of 7-8 days, generated by bone

marrow-derived megakaryocytes after cytoplasmic fragmentation [5]. MKs changes are also related to platelet number and size [4]. Moreover, platelets have adenosine diphosphate (ADP) receptors and vitamin D receptors, which play an important role in bone remodeling [6-7]. Therefore, platelet function, size, and volume changes may relate to osteoporosis.

Mean platelet volume (MPV) and platelet distribution width (PDW), describing platelet size and the degree of difference in platelet sizes, respectively, are sample and useful platelet indices.

Currently, MPV and PDW are widely used to assess platelet function and activation [8, 9]. An elevated MPV and PDW, as indicators of larger and more reactive platelets resulting from an increased platelet turnover, may represent a risk factor for osteoporosis. Several previous studies have investigated the relationship between MPV and PDW, BMD values, and osteoporosis. They found that MPV and PDW in the osteoporosis patients group were higher compared with control groups, and that MPV levels were significantly and negatively correlated with femoral neck T-score, and BMD values at the lumbar spine and femoral neck [10, 11]. In contrast, Akbal et al. [4] demonstrated that MPV and PDW in osteoporosis group were lower than those in the normal BMD group. Moreover, PDW levels were positively correlated with femur and lumbar T-scores, whereas MPV levels were not associated with femur and lumbar T-scores.

Despite a vast amount of literature in the context of different disorders, relatively little information is available and the results were not consistent regarding the association between serum MPV and PDW, BMD values, and osteoporosis. Additionally, to the best of our knowledge, no report has attempted to identify differences in MPV and PDW levels of T2DM patients with osteoporosis, osteopenia and normal BMD, or to explore their potential association with BMD values at the lumbar spine and hip, as well as osteoporosis. Therefore, the aim of this study was to investigate the levels of MPV and PDW in Chinese T2DM patients and to explore the relation between these markers, BMD values at various sites, and osteoporosis which may provide evidence in support of using low cost readily available clinical hematological parameters to guide future prevention and treatment efforts.

Materials and methods

Study population

This cross-sectional analysis initially consisted of 3,514 consecutive patients with T2DM who were admitted to the Inpatient Ward of our Endocrinology Department of the Affiliated Hospital of Southwest Medical University for screening of osteoporosis between August 2012 and September 2015. The diagnosis of T2DM was based on oral glucose tolerance tests and the 1999 World Health Organization (WHO) criteria. Inclusion criteria were: 1) postmenopausal women aged 45 years or older who had not menstruated for at least 1 year and men aged 50 years or older; 2) estimated glomerular filtration rate (eGFR) ≥30 ml/min/1.73 m^2; and 3) long-term residence (≥5 years) in China's Sichuan Province. Exclusion criteria were: 1) subjects who exhibited premature menopause (<40 years) and those who had other known metabolic bone diseases such as osteomalacia, aluminium related bone disease, Paget's disease, and so forth. Also, subjects that had taken any drugs that might influence bone metabolism for more than 6 months or within the previous 12 months, such as thiazolidinediones (rosiglitazone and pioglitazone), calcitonin, bisphosphonates, loop diuretics, high-dose thiazide diuretics, systemic glucocorticoids, immunosuppressant, and estrogens; 2) subjects having diseases known to effect bone metabolism and/or oxidant-antioxidant status like malignancy, thyroid diseases, hyperparathyroidism, hypogonadism, Cushing syndrome, infective and inflammatory diseases, chronic liver disease , chronic kidney disease (CKD) stage 3, 4 and 5 (eGFR<60 mL/min/1.73 m^2), cardiovascular disease (CVD), cerebrovascular disease, and chronic obstructive pulmonary disease. Also, subjects using medications that may affect the oxidant/antioxidant system, including vitamins (A, C, and E) and minerals (zinc and selenium) during the previous 6 months; 3) subjects who were diagnosed with acute complications of diabetes including diabetic hyperosmolar coma, ketoacidosis, and hypoglycemic coma; 4) subjects having congenital or acquired platelet disease, hematological disorders, or taking platelet inhibitors (e.g., aspirin and clopidogrel) within

one week before blood sampling; 5) patients with lumbar vertebrae hyperosteogeny and fractures; 6) patients who smoked and drank; and 7) subjects with incomplete baseline data and missing available information. All of the patients met the inclusion and exclusion criteria. Consequently, the remaining 882 T2DM participants (322 men and 560 women) were included in the analyses.

The study protocol conformed to all ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Human Research Ethics Committee of the Affiliated Hospital of Southwest Medical University in Sichuan Province. Since the study is an observation of existing datasets the need for informed consent was waived.

Subject's classification

All of the subjects were subdivided into three groups according to according to their BMD measurements, using the T-score [12]: normal BMD (T-score ≥ -1.0 SD, n=242), osteopenia (-2.5 SD < T-score < -1.0 SD, n=323), and osteoporosis (T-score ≤ -2.5 SD, n=317).

Clinical and biochemical measurements

We used a structured interview questionnaire and reviewed medical records of each patient to collect information regarding the demographic characteristics (gender, age, age at menopause), diabetes duration, use of medications, and history of comorbidities. Body weight and height, body mass index (BMI), and blood pressure were measured with the use of standard methods, as described previously [13]. Blood samples were collected following overnight fasting to measure alanine aminotransferase (ALT), aspartate aminotransferase (AST), total serum alkaline phosphatase (ALP), total bilirubin (TBIL), direct bilirubin (DBIL), indirect bilirubin (IBIL), total cholesterol (TC), triglycerides (TG), albumin, serum creatinine (Cr), serum calcium, fasting blood glucose (FBG), glycated hemoglobin A1C (HbA1c) , platelet counting (PLT), plateletcrit (PCT), mean platelet volume (MPV), platelet distribution width (PDW), hemoglobin (Hb), neutrophil and lymphocyte count. Neutrophil to lymphocyte ratio (NLR) was quantified as total neutrophil counts divided by lymphocyte counts. eGFR was calculated using CKD-EPI formula [14]. Corrected calcium concentration (mg/dL)=serum calcium concentration (mg/dL)+0.8×[4.0 (g/dL)-serum albumin concentration (g/dl)].

Ankle-brachial index (ABI) measurements were obtained per standard protocol [15]. Peripheral arterial disease (PAD) was considered as present if the ABI value on either leg was <0.9.

Vibrating perception threshold (VPT) values were measured by a neurothesiometer (Bio-Thesiometer; Bio-Medical Instrument Co., Newbury, OH, USA) as XIAO et al. [16] described. In our present study, diabetic peripheral neuropathy (DPN) was considered as present if the VPT value on either limb was >25 V (volt). The areal BMD at the lumbar spine and hip (femoral neck and total hip) in all of the participants was measured by dual energy x-ray absorptiometry (DEXA) and was expressed as g/cm^2. All measurements were taken by the same well-trained and qualified operator on the same machine using standardized protocols for participant positioning to ensure machine accuracy of greater than 98%. The BMD coefficients of variation were 0.84%, 1.96%, and 1.72% for the lumbar spine, femoral neck, and total hip, respectively.

Statistical analysis

All data were first analyzed for normality of distribution using the Kolmogoro-Smirnov test of normality. Data were shown as mean ± SD for continuous variables or percentages (%) for categorical variables, respectively. Otherwise, specified comparisons of clinical and biochemical parameters among groups were performed by Chi-square (χ^2) tests for categorical variables, one-way analysis of variance (ANOVA) for normally distributed continuous variables, with post-hoc analysis in two-group comparisons performed by LSD tests, and Kruskal-Wallis test followed by multiple pairwise comparisons with Bonferroni's post hoc adjustment for non-parametric distributed covariates. Thereafter, Pearson correlation for normally distributed variables and Spearman's rank correlation for abnormally distributed parameters were employed to assess the relationship between MPV and PDW levels and clinical and biochemical parameters. Subsequently, the association between MPV and PDW levels and BMD values at various sites were tested by partial correlation analyses.

Table 1. Baseline characteristics of T2DM patients with normal BMD, osteopenia and osteoporosis ($\bar{x}$ ±s)

	Normal (n=242)	Osteopenia (n=323)	Osteoporosis (n=317)	*P* for trend
Male/Female	141/101	135/188**	46/271**,##	0.000
Diabetes duration (years)	7.46±5.81	7.86±6.59	8.77±6.82	0.057
Age (years)	59.21±9.35	62.00±8.38**	66.17±8.10**,##	0.000
Height (cm)	162.62±7.59	158.96±7.16**	154.59±6.63**,##	0.000
Weight (kg)	66.23±10.76	61.31±9.87**	55.71±9.78**,##	0.000
BMI (kg/m^2)	24.99±3.38	24.27±3.86*	23.31±3.81**,##	0.000
SBP (mmHg)	132.86±20.81	133.38±21.09	134.90±21.87	0.541
DBP (mmHg)	71.90±12.33	71.75±12.51	70.21±12.30	0.181
TC (mmol/L)	4.86±1.15	4.90±1.16	4.84±1.18	0.838
TG (mmol/L)	2.24±2.12	2.25±1.97	1.92±1.45#	0.010
FBG (mmol/L)	10.59±4.94	10.84±5.69	9.87±4.90	0.035
HbA1c (%)	9.24±2.31	9.44±2.58	8.96±2.60#	0.014
ALT (U/L)	20.81±8.88	18.39±8.44**	17.12±8.09**	0.000
AST (U/L)	19.26±5.58	18.83±6.22	18.79±6.09	0.205
TBIL (μmol/L)	12.91±5.70	12.25±5.34	11.60±4.29*	0.042
DBIL (μmol/L)	4.43±1.91	4.24±1.88	4.09±1.56	0.139
IBIL (μmol/L)	8.48±4.21	8.01±3.86	7.51±3.17*	0.034
ALP (U/L)	80.07±25.71	81.25±28.68	86.08±32.83	0.153
Corrected calcium (mg/dL)	9.16±0.50	9.26±0.58	9.02±0.59*,##	0.000
Cr (μmol/L)	70.00±26.98	66.72±24.02	64.49±24.66**	0.011
eGFR (mL/min/1.73 m^2)	93.71±21.27	91.81±20.34	87.21±20.54**,##	0.000
Neutrophil count (*10^9/L)	4.33±1.82	4.54±2.14	4.52±2.26	0.738
Lymphocyte count (*10^9/L)	1.66±0.66	1.76±0.65	1.55±0.56##	0.000
NLR	3.08±2.36	2.97±2.10	3.42±2.66#	0.028
Hb (g/L)	132.23±20.86	130.57±16.18	122.26±17.39**,##	0.000
Albumin (g/l)	42.17±4.41	41.19±4.33*	40.61±4.75**	0.000
PLT (*10^9/L)	204.61±61.53	205.37±64.87	200.67±68.54	0.452
PCT	0.23±0.06	0.23±0.06	0.23±0.07	0.396
MPV(fl)	11.20±1.16	11.46±1.23	11.50±1.29**	0.005
PDW (%)	14.68±2.63	15.30±2.91	15.42±2.65*	0.008
ABI	1.05±0.13	1.04±0.11	1.00±0.16**,##	0.000
VPT	15.15±9.17	16.23±9.09	17.41±10.09**	0.006
Incidence of DPN (n, %)	25 (10.33)	36 (11.15)	45 (14.20)	0.316
Incidence of PAD (n, %)	16 (6.61)	22 (6.81)	32 (10.09)	0.206
BMD values (g/cm^2)				
Lumbar spine (L1-L4)	1.132±0.182	0.982±0.094**	0.810±0.105**,##	0.000
Femoral neck	0.939±0.111	0.807±0.088**	0.691±0.096**,##	0.000
Total hip	0.840±0.108	0.715±0.084**	0.588±0.086**,##	0.000

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglyceride; FBG, fasting blood glucose; HbA1c, glycated hemoglobin A1c; ALT, alanine amino transferase; AST, aspartate amino transferase; TBIL, total bilirubin; DBIL, direct bilirubin; IBIL, indirect bilirubin; ALP, alkaline phosphatase; Cr, creatinine; eGFR, estimated glomerular filtration rate; NLR, neutrophil to lymphocyte ratio; Hb, hemoglobin; PLT, platelet counting; PCT, plateletcrit; MPV, Mean platelet volume; PDW, platelet distribution width; ABI, ankle-brachial index; VPT, vibrating perception threshold; Corrected calcium concentration (mg/dL)=serum calcium concentration (mg/dL)+0.8×[4.0 (g/dL)-serum albumin concentration (g/dl)]. Values were given as means ± SD. *P<0.05, **P<0.01 compared with normal BMD group; #P<0.05, ##P<0.01 compared with osteopenia group.

Finally, we divided the study population into quartiles of MPV with cut-off points of 10.5, 11.4, and 12.2. We also divided the study population into quartiles of PDW with cut-off points

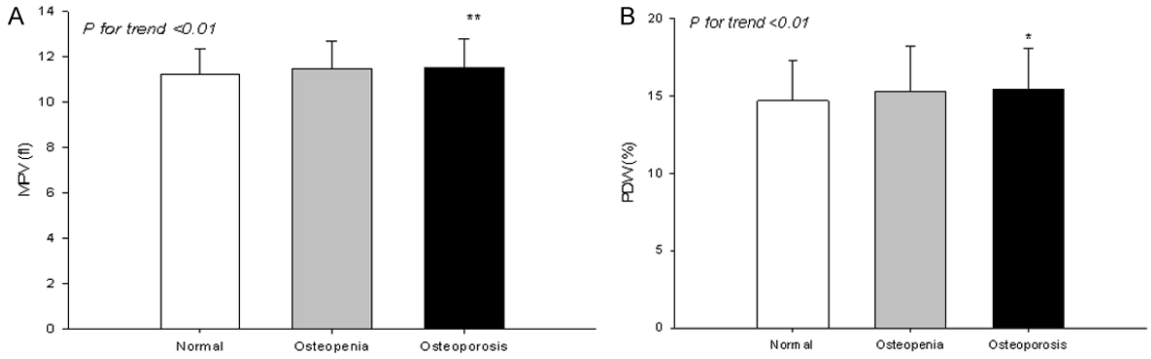

Figure 1. Comparison of MPV (A) and PDW (B) levels in T2DM patients with normal BMD and osteopenia, and osteoporosis. vs. Normal, *P<0.05, **P<0.01.

of 13.1, 15.3, and 16.6. We then compared differences in BMD values at the lumbar spine and hip using ANOVA for normally distributed continuous variables. We used a Kruskal-Wallis test for nonparametric distributed covariates and the prevalence of osteoporosis using χ^2 tests among four groups. All statistical analyses were performed using the Statistical Program for Social Sciences (SPSS) statistical software (version 20.0, Chicago, IL, USA). All reported P values were two-sided. A P value of <0.05 was considered statistically significant.

Results

Baseline characteristics of the study population

A total of 882 T2DM patients (mean age, 62.73±9.00 years; male/female, 322/560; and mean diabetes duration, 8.08±6.49 years) were enrolled in this study. The baseline characteristics of the study subjects are shown and listed in **Table 1**. Compared with patients with T2DM and with normal BMD and osteopenia, those with osteoporosis are significantly older. They are more likely to be female and have significantly lower levels of eGFR, ABI, Hb, corrected calcium concentration, body weight and height, and BMI (P<0.01 or P<0.05). Levels of serum Cr, albumin, TBIL, IBIL, ALT, and BMD values at all skeletal sites decreased, and TG, HbA1c, and VPT elevated in osteoporosis patients compared to normal BMD patients. There was a significant increase in NLR and a significant decrease in lymphocyte count in osteoporosis patients in comparison to osteopenia patients (P<0.01 or P<0.05). However, there were no differences among three groups in terms of DBP, SBP, TC, AST, ALP, neutrophil count, PLT, PCT, and incidence of PAD and DPN (all P>0.05).

Serum MPV and PDW levels among T2DM patients with normal BMD, osteopenia, and osteoporosis

Data on MPV and PDW levels in T2DM patients with normal BMD, osteopenia, and osteoporosis groups are depicted in **Figure 1**. We observed that there was a progressive increase in MPV and PDW levels from normal BMD subjects (11.20±1.16 fl and 14.68±2.63%, respectively) to osteopenia (11.46±1.23 fl and 15.30±2.91%, respectively) and osteoporosis (11.50±1.29 fl and 15.42±2.65%, respectively) patients (all P<0.01). MPV and PDW levels were significantly higher in subjects with osteoporosis than in normal BMD subjects (P<0.01 or P<0.05).

Correlation analysis among MPV and PDW levels, clinical characteristics, and BMD values in all T2DM patients combined

Correlation analysis results between MPV and PDW levels, clinical characteristics, and BMD values are summarized in **Table 2**. MPV and PDW concentrations were all positively associated with gender and VPT and negatively associated with body weight, BMI, TG, PLT, PCT, ALT, AST, TBIL, DBIL, ALP, Hb, albumin, and BMD values at the lumbar spine, femoral neck, and total hip (P<0.01 or P<0.05). Moreover, PDW concentrations were also positively correlated with age and are negatively associated with body height, IBIL, DBP (P<0.01 or P<0.05), while the association between MPV concentrations, DBP, and IBIL was marginally significant (P=0.064 and P=0.056, respectively). No sig-

Table 2. Correlation analysis among MPV and PDW levels, clinical, and biochemical parameters in all T2DM patients combined

Index	MPV		PDW	
	r	*P*	*r*	*P*
Gender	0.067	0.048	0.089	0.008
Diabetes duration	0.028	0.405	0.043	0.199
Age	0.049	0.143	0.085	0.011
Body height	-0.031	0.366	-0.075	0.029
Body weight	-0.135	0.000	-0.174	0.000
BMI	-0.110	0.001	-0.156	0.000
SBP	0.013	0.706	0.031	0.365
DBP	-0.062	0.064	-0.069	0.040
TC	0.017	0.618	-0.037	0.275
TG	-0.067	0.049	-0.104	0.002
FBG	0.008	0.819	-0.014	0.680
HbA1c	0.039	0.249	-0.045	0.186
ALT	-0.172	0.000	-0.191	0.000
AST	-0.117	0.000	-0.125	0.000
TBIL	-0.087	0.010	-0.107	0.001
DBIL	-0.127	0.000	-0.132	0.000
IBIL	-0.064	0.056	-0.088	0.009
ALP	-0.084	0.012	-0.077	0.023
Corrected calcium	-0.052	0.136	-0.029	0.403
Albumin	-0.077	0.026	-0.080	0.021
Cr	-0.006	0.862	-0.027	0.421
eGFR	-0.044	0.190	-0.048	0.157
PLT	-0.500	0.000	-0.442	0.000
PCT	-0.172	0.000	-0.212	0.000
MPV	-	-	0.768	0.000
PDW	0.768	0.000	-	-
Neutrophil count	0.005	0.889	-0.021	0.545
Lymphocyte count	-0.006	0.856	0.008	0.817
NLR	0.010	0.778	-0.015	0.662
Hb	-0.159	0.000	-0.166	0.000
ABI	-0.017	0.641	-0.006	0.870
VPT	0.095	0.008	0.101	0.005
BMD values				
Lumbar spine	-0.093	0.006	-0.124	0.000
Femoral neck	-0.126	0.000	-0.145	0.000
Total hip	-0.132	0.000	-0.166	0.000

nificant relationship was observed between MPV and PDW concentrations and other clinical and biochemical parameters (all $P>0.05$).

Adjusted associations of MPV and PDW concentrations with BMD values at the lumbar spine and hip

Partial correlation analysis demonstrates that MPV and PDW concentrations are all negatively associated with BMD values at the femoral neck and total hip in all participants after adjustment for gender, age, BMI, corrected calcium, eGFR, ALP, SBP, DBP, TC, TG, FBG, and HbA1c (all $P<0.05$) (**Table 3**).

BMD values and the prevalence of osteoporosis across quartiles of MPV and PDW levels in all T2DM patients

As we indicate in **Table 4**, the BMD values at the lumbar spine, femoral neck, and total hip decrease significantly with a progressive increase in MPV quartiles ($P<0.01$ or $P<0.05$). Similarly, the BMD values at all sites of lumbar spine, femoral neck, and total hip decrease significantly along with an increase in PDW quartiles (all $P<0.01$). T2DM patients in the highest quartile of MPV have significantly lower BMD values at the femoral neck and total hip compared with those in the lowest MPV quartile (all $P<0.01$). Similar increase was noted for BMD values at the lumbar spine, femoral neck, and total hip in the highest quartile of PDW when compared with those in the lowest PDW quartile (all $P<0.01$). Consistently, the prevalence of osteoporosis increased along with an increase in MPV and PDW quartiles and the prevalence of osteoporosis in the highest quartile of MPV and PDW was higher than that in the lowest MPV and PDW quartile (all $P<0.05$) (**Figure 2**).

Discussion

To the best of our knowledge, this is the first report comparing MPV and PDW levels in T2DM patients with osteoporosis, osteopenia and normal BMD group, and showing that MPV and

Table 3. Partial correlation analysis between MPV and PDW concentrations, and BMD values at the lumbar spine and hip in all T2DM patients

Model	BMD	MPV		PDW	
Model 1	Lumbar spine	-0.013	0.716	-0.021	0.543
	Femoral neck	-0.074	0.031	-0.062	0.073
	Total hip	-0.078	0.023	-0.075	0.029
Model 2	Lumbar spine	-0.015	0.667	-0.035	0.331
	Femoral neck	-0.077	0.030	-0.082	0.020
	Total hip	-0.080	0.024	-0.093	0.009
Model 3	Lumbar spine	-0.017	0.636	-0.038	0.287
	Femoral neck	-0.080	0.027	-0.084	0.019
	Total hip	-0.082	0.023	-0.093	0.010

Model 1: adjusted for gender, age and BMI; Model 2: adjusted for gender, age, BMI, ALP, serum calcium and eGFR; Model 3: adjusted for gender, age, BMI, ALP, corrected calcium, eGFR, SBP, DBP, TC, TG, FBG and HbA1c.

PDW levels in osteoporosis are significantly higher than those in normal BMD subjects ($P<0.01$ or $P<0.05$). Moreover, a negative association is observed between MPV and PDW, BMD values at the femoral neck and total hip in all participants even after adjustment for possible confounders including gender, age, BMI, corrected calcium, eGFR, ALP, SBP, DBP, TC, TG, FBG, and HbA1c (all $P<0.05$). Aligned with this observation , we also noticed that T2DM patients in the highest quartile of MPV and PDW had significantly lower BMD values at the femoral neck and total hip, and a higher prevalence of osteoporosis compared with those in the lowest MPV and PDW quartile ($P<0.01$ or $P<0.05$). Collectively, these data demonstrate that MPV and PDW levels may play a crucial role in the pathogenesis of osteoporosis in T2DM patients.

Circulating platelets vary in both size and functional activity within an individual. It is recognized that platelet morphological and functional properties are determined during MKs development. MKs with high ploidy are the source of large hyper-reactive platelets. The increase in platelet volume, greater platelet size, expressed as MPV which are more active metabolically, biochemically, and functionally than smaller ones [17, 18]. In addition, changes in platelet shape, and consequently in volume and PDW, may also occur at the sites of activation [15]. These findings provide further evidence that elevated MPV and PDW are important surrogate markers of platelet activation and function. Recently, authors have argued that platelet functions are related to bone mineralization, suggesting that MPV and PDW may play a pivotal role in the development of osteoporosis [4]. Several studies have shown that MPV and PDW played an important role in development of osteoporosis and fractures. Resorlu and his colleagues [11] found that MPV was higher in the osteopenia group than normal BMD group, and MPV was significantly and negatively correlated with femoral neck T-score in 50 patients diagnosed with ankylosing spondylitis. Similarly, Li et al. [10] also found that osteoporosis patients had higher MPV levels and MPV levels were negatively correlated with BMD at the lumbar spine and femoral neck. Additionally, the same author has shown that increased MPV was a significant factor with decreased BMD in multivariate linear regression analysis. Recently, Cure and his workers [19] have demonstrated that a high MPV was associated with fractures, even in old age. Increased MPV may be associated with osteoporosis and an impaired blood supply, which consequently may increase the risk of fracture. These findings were in line with our study which suggests that MPV and PDW levels in osteoporosis are significantly higher than those in normal BMD subjects, and MPV and PDW are negatively correlated with BMD values at the femoral neck and total hip even after adjustment for possible confounders. This suggests that MPV and PDW are related to bone mineralization and higher levels of MPV and PDW may play an important role in the development of osteoporosis. Contrary to our findings, Akbal et al. [4] demonstrated that MPV and PDW in the osteoporosis group were significantly lower than those in normal BMD group. Moreover,

Table 4. BMD values and the prevalence of osteoporosis across quartiles of MPV and PDW levels in all T2DM patients

BMD value	MPV quintiles					PDW quintiles				
	Q1 (n=198)	Q2 (n=264)	Q3 (n=204)	Q4 (n=216)	*P*	Q1 (n=215)	Q2 (n=235)	Q3 (n=215)	Q4 (n=217)	*P*
Lumbar spine	0.983±0.155	0.958±0.156	0.959±0.220	0.943±0.172	0.047	0.997±0.210	0.963±0.160	0.948±0.169	0.936±0.177**	0.003
Femoral neck	0.833±0.135	0.797±0.132	0.788±0.137	0.790±0.144**	0.002	0.831±0.145	0.805±0.132*	0.794±0.128	0.776±0.142**	0.000
Total hip	0.736±0.131	0.702±0.131	0.689±0.135	0.688±0.141**	0.000	0.736±0.138	0.709±0.133*	0.694±0.127	0.676±0.140**	0.000

Vs. Q1, *P<0.05, **P<0.01

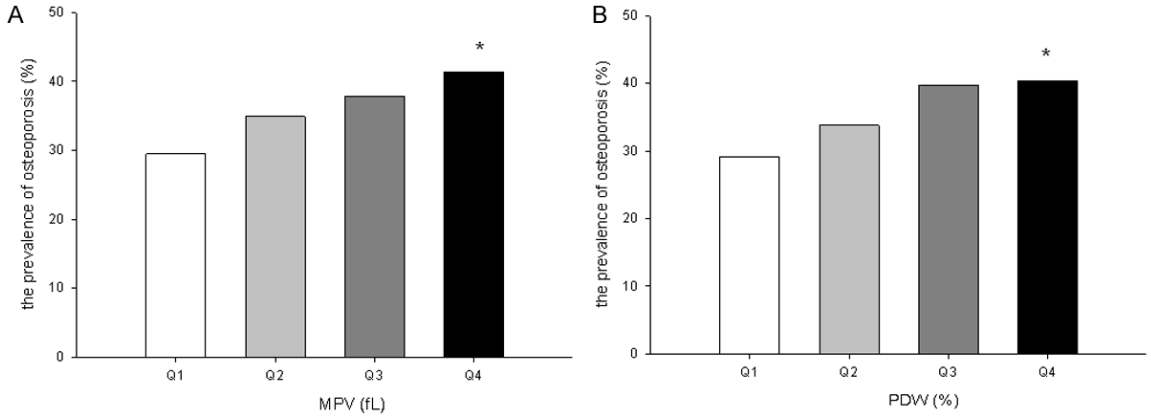

Figure 2. The distribution of the prevalence of osteoporosis across quartiles of MPV (Figure 2A) and PDW (Figure 2B) levels in all T2DM patients. vs. Q1, *P<0.05.

PDW was positively correlated with BMD measurements and independently related to femur and lumbar T-scores. These conflicting results may be due to differences in study population, variation in design, limitations in sample size, suboptimal statistical analysis, confounders adjustment, limited age, gender and race range, and factors that affect MPV levels including MPV measurement method, anticoagulant, temperature, and time interval between blood sampling and examination.

The mechanism by which MPV and PDW influence BMD values and osteoporosis are not fully understood. There are, potentially, several explanations for the mechanism. First, oxidative stress could represent a possible mechanism linking MPV and PDW with the development of osteoporosis. Accumulating evidence has shown that oxidative stress and low antioxidant levels play a role in the initiation and progression of osteoporosis [20, 21]. Experimental and clinical studies have shown that reactive oxidant species (ROS) triggers platelet activation via multiple pathways including enhanced formation of isoprostanes (a maker of oxidative stress) [22], regulations of proliferation, differentiation and maturation of megakaryocytes, and MPV directly shows the response of the thrombocytes to the stress [23]. It has been reported that oxidative stress leads to increased MPV levels [24, 25] while regular flavonoid antioxidant intake decreases MPV levels [22, 26]. Collectively, these findings demonstrated that MPV may be associated with oxidative stress, and MPV may be used as a marker of oxidative stress. Our study indicates that patients with osteoporosis have significantly lower levels of TBIL, IBIL, and albumin. Moreover, MPV levels are negatively associated with TBIL, DBIL, and albumin, while PDW levels are negatively correlated with IBIL. Bilirubin (TBIL, DBIL, and IBIL), the end product of heme metabolism, plays an important physiologic role as a strong antioxidant through efficient scavenging of peroxyl radicals and suppression of oxidation [27]. Albumin is also an important contributor to total antioxidant status, like bilirubin [28]. In agreement with our findings, Cure et al. [29] found that MPV level was lower in the Gilbert's syndrome (a congenital form of hyperbilirubinemia) patients than healthy individuals, indicating that antioxidant bilirubin can decrease oxidative stress, which in turn decreases MPV levels. Based on these results, we speculate that higher levels of MPV and PDW levels may be a result of low circulating levels of antioxidants and consequently increased oxidative stress, which increases megakaryopoiesis in bone marrow and raises the release of large-sized thrombocyes to blood [29] in osteoporosis patients. However, the exact mechanisms await further experimental verification.

Second, a possible explanation for the increase of MPV and PDW in patients with osteoporosis might be atherosclerosis and diabetic vascular complications. T2DM is an important disorder which is accompanied by early and progressive atherosclerosis. It easily develops macrovascular and microvascular complications, ultimately resulting in the initiation and progression of osteoporosis. It has been demonstrated that

elevated MPV levels may result from increased oxidative stress [24, 25], while increased oxidative stress play an important role in the pathogenesis of diabetic vascular complications, indirectly demonstrating that increased MPV may be involved in the initiation and progression of diabetic vascular complications. ABI, an indicator of PAD that is a common diabetic macrovascular complication, can reflect the severity of systemic atherosclerosis. VPT, an indicator of DPN that is a common diabetic microvascular complication, can provide important clinically meaningful information about large nerve fiber dysfunction in diabetes [30, 31]. More importantly, abnormal VPT values have been shown to predict foot ulceration and lower limb amputation [32, 33]. In our present study, we found that osteoporosis patients have significantly lower levels of ABI and higher VPT values compared with normal BMD patients, supporting the concept that prevalence of osteoporosis is associated with macrovascular and microvascular complications in T2DM patients. Moreover, MPV and PDW levels are positively associated with VPT, suggesting that elevated MPV and PDW levels are related to the development of DPN, and that DPN may represent a possible mechanism linking elevated MPV and PDW levels with the development of osteoporosis. Our results are consistent with previous studies. XIAO et al. [15] found that T2DM subjects in the highest tertile of PDW had significantly higher VPT values as compared with those in the lowest tertile of PDW and that both MPV and PDW were associated with VPT values in T2DM patients, independent of traditional cardiovascular risk factors. Papanas et al. [34] and Dindar et al. [35] also have shown that MPV levels were significantly higher in patients with neuropathy than those without neuropathy. Further studies are undoubtedly warranted to establish the association between these biomarkers and vascular complications, especially microvascular complications in diabetic patients.

Third, another explanation for the relationship between serum MPV and PDW and osteoporosis in T2DM patients might be metabolic syndrome (MetS). MetS represents a cluster of cardiometabolic risk factors including central obesity, elevated blood pressure, impaired glucose metabolism, and atherogenic dyslipidemia [36], which have been reported to be associated with osteoporosis. In our study, we find that compared with patients with T2DM patients with normal BMD and osteopenia, osteoporosis patients have significantly lower levels of body weight, BMI, TG, FBG, and HbA1c, suggesting an important role of clustered components of MetS in the pathogenesis of osteoporosis in T2DM patients. Moreover, MPV and PDW concentrations are negatively associated with body weight, BMI, and TG. Additionally, serum PDW concentrations are negatively correlated with DBP. These findings suggest that MPV and PDW levels in osteoporosis are negatively correlated with part components of MetS, which is consistent with two recent studies. Aypak et al. [37] found that Turkish children with MetS had lower MPV levels and MPV levels were inversely correlated with FBG, HOMA-IR, LDL-C, LDL-C/HDL-C ratio, and MetS in girls. Additionally, the risk analyses of MetS in terms of MPV quartiles demonstrated that the adjusted OR for the lowest vs. the highest quartile was 7.71 in girls. Likewise, a study carried out by Park et al. [36] found that MPV was significantly lower in adult female subjects with MetS, and MPV was inversely associated with MetS in only women, independently of confounding factors. Based on these results, we speculate that there is a potential and complex relationship between MPV and PDW, MS and osteoporosis, which needs more study to confirm.

There are some limitations to our study. First, the study was a cross-sectional study with single-center data. We could not determine if a causal relationship exists between MPV and PDW and osteoporosis. Second, we could not ascertain whether these findings may be generalizable to the general population which likely would eliminate the variability necessary to capture some important associations in our study, as participants were elderly T2DM patients and admitted to the Inpatient Ward of our Endocrinology Department undergoing strict screening. Third, even if we have adjusted for multiple potential confounders, there may have been uncontrolled or unmeasured confounders not included in the analyses such as intake of calcium and vitamin D, indictors of oxidative stress, and other antioxidants such as carotenoids, malonyldialdehyde, and superoxide dismutase. However, the results from the unadjusted and fully adjusted models are similar and the potential for presence of further

residual confounders capable of drastically changing the results is low. Finally, laboratory evaluation including MPV and PDW were performed only once, so the accuracy of the data can decrease due to measurement error. Despite these limitations, our study has several strengths including a relatively large sample size in a single center and BMD measurements done with DEXA, considered the current "gold standard". Most importantly, our study is, to our knowledge, the first to report the association between MPV and PDW levels, BMD values and osteoporosis in Chinese T2DM patients. Therefore, this study could be an important beginning and provide background information for future prospective studies.

In conclusion, our results show that MPV and PDW levels are positively associated with BMD values and are negatively correlated with the prevalence of osteoporosis in T2DM patients. MPV and PDW could be potentially used as readily available diagnostic and therapeutic markers for osteoporosis in clinical settings. Larger and prospective studies are needed to validate our findings and to establish the precise mechanism of action.

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Disclosure of conflict of interest

None.

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