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The Peripheral Blood Mononuclear Cell Count Is Associated With Bone Health in Elderly Men

A Cross-Sectional Population-Based Study

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Abstract: The peripheral blood mononuclear cell (PBMC) count is a routinely used and meaningful index for infection and blood diseases. PBMCs may be closely related to osteoclasts and include osteoclast precursors; we examined the association between the PBMC count and bone health. This research included 2806 community men aged ≥ 50 years who underwent full health examinations from October 2007 through December 2011 in four medical centers. The PBMC count was significantly high among subjects with “at least osteopenia”

compared with controls. In analysis of covariance adjusted for potential confounders, the bone mineral density (BMD) value and T-score had a significant decreasing trend across the quartiles of PBMC count. In univariate analysis, the PBMC count had a strong association with “at least osteopenia” (odds ratio [OR] = 2.520, 95% confidence interval [CI]: 1.397–4.547). After adjustment for confounding factors (multivariate analysis) from Model 1 to 4, PBMC count remained as an independent risk factor for “at least osteopenia” (OR = 2.481, 95% CI: 1.176–5.236). Moreover, after adjusting for all confounding variables, participants had a significantly high OR in the body mass index (BMI) < 25 group (OR = 2.798, CI: 1.122–6.973; $P = 0.027$) and systolic blood pressure (SBP) < 140 group (OR = 2.519, CI: 1.059–5.993; $P = 0.037$). In conclusion, the PBMC count is significantly associated with bone loss in elderly men and the exact mechanism requires further clarification.

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Abbreviations: ALT = alanine aminotransferase, AST = aspartate transaminase, BMD = bone mineral density, BMI = body mass index, CI = confidence interval, DXA = dual-energy x-ray absorptiometry, eGFR = estimated glomerular filtration rate, OCP = osteoclast precursor, OR = odds ratio, PBMC = peripheral blood mononuclear cell, RBC = red blood cell, SBP = systolic blood pressure.

INTRODUCTION

Osteoporosis is a systemic skeletal disease characterized by reduced bone mineral density (BMD) and deteriorated microarchitecture.¹ Osteopenia refers to a state of bone loss less severe than that in osteoporosis.² Many studies have been undertaken to reveal the exact mechanisms of bone loss. It is generally accepted that an imbalance between osteoblastic bone formation and osteoclastic bone resorption is the major pathogenesis. However, the definite etiology and pathogenesis are yet to be determined.^{3,4}

Osteoclasts, derived from the monocyte/macrophage hematopoietic lineage, are the only cells capable of bone resorption and thus play an important role in the pathological process of bone loss.⁵ Before bone loss occurs, osteoclast precursor (OCP) cells are mobilized from bone marrow into the circulation and then enter bone tissue to initiate bone resorption.⁶ Thus, OCP cells exist in peripheral blood and constitute the proliferative monocyte subpopulation of the peripheral blood mononuclear cell (PBMC) population.⁷ Therefore, PBMCs may play a role in osteoclastic bone loss.

In clinical conditions, the PBMC count is a routinely conducted and meaningful indicator for health monitoring and disease diagnosis.^{8,9} In the physiological condition, the number of circulating monocytes can temporarily change with strenuous exercise and excessive stress.¹⁰ In most cases, a change in circulating monocyte number indicates severe infection such as sepsis.¹¹ In

recent years, increasing number of studies have revealed that the PBMC count is associated with cancer prognosis, cerebral vascular accident, and cardiovascular disease.^{12–15} For instance, preoperative high level of PBMC count may provide worse prognostic information for patients with hepatocellular carcinoma (HCC) after hepatic resection.¹² In contrast, low PBMC count is associated with aortic valve stenosis, and there is a progressive decrease in PBMC count with increasing aortic valve stenosis severity.¹⁶

However, the relationship between the PBMC count and bone mass remains unknown. To our knowledge, this study is the first to investigate whether the PBMC count was associated with bone loss. We selected a well-characterized elderly men population for the reason that bone health has been recognized as important age-related public health problem in men.¹⁷ Moreover, evidence show that the prevalence of osteopenia has an 80% increase in men aged ≥ 60 years compared with those aged ≥ 50 years.¹⁸ The aim of this study is to find a key relevant indicator of bone health for preliminary diagnosis, thus help to provide clues for further dual-energy x-ray absorptiometry (DXA) screening or treatment.

MATERIALS AND METHODSD

Study Participants

We conducted a retrospective and consecutive cohort study in four medical centers including the Taizhou Medical Center, Shaoxing People's Hospital, First Affiliated Hospital of Wen Zhou Medical University, and Sir Run Run Shaw Hospital Affiliated to Zhejiang University. A population of Chinese men aged ≥ 50 years who had undergone full health examinations from October 2007 through December 2011 was included in this study. The examinations also included extensive screening tests for the early detection of some age-related diseases such as malignancy and osteoporosis. All subjects with available PBMC count and DXA measurement data were included in this study. Patients were excluded with a history of alcohol consumption (>20 g alcohol per day currently or previously); smoking (>10 cigarettes per day currently or previously); diabetes; rheumatoid arthritis; hyperthyroidism; renal diseases; recent infection (anomalies on routine blood test); and specific blood diseases including Hodgkin lymphoma, acute monocytic cell lymphoma, and acute myelomonocytic leukemia. Patients with chronic diseases that are known to alter bone metabolism were also excluded, including those with liver, renal, or thyroid disorders. Participants with a history of medications (>3 months) that could influence bone metabolism were also excluded. Finally, a cohort of 2806 subjects was included in our study. The research protocol was approved by the Ethics Committees of the four hospitals and informed consent was waived owing to the retrospective nature of the study.

Baseline Measurements

Participants' baseline characteristics were collected. Age, smoking history, alcohol consumption, and past medical history were all recorded using a standardized questionnaire. Blood pressure was measured at rest with a standard mercury sphygmomanometer. Height and weight were measured by trained nurses with patients wearing a light gown, and body mass index (BMI) was calculated for all participants.

Laboratory Assays and Measurements

Blood samples were obtained from these healthy participants after 12 h of fasting and analyzed in laboratories according

to the same standard test and operating procedures. We extracted the following parameters: glucose; calcium; phosphorus; alkaline phosphatase; aspartate transaminase (AST); alanine aminotransferase (ALT); total protein; and complete blood count including PBMC count, neutrophil count, eosinophil count, basophil count, lymphocyte count, red blood cell (RBC) count, and platelet count. The PBMC count had a reference range of 0.1 to $0.6 \times 10^9/L$ and was divided into four quartiles as follows: Q1: $<0.34 \times 10^9$, Q2: 0.34×10^9 to 0.41×10^9 , Q3: 0.41×10^9 to 0.53×10^9 , and Q4: $\geq 0.53 \times 10^9$. Estimated glomerular filtration rate (eGFR) was calculated using the Cockcroft–Gault formula.

BMD Measurements

The BMD was measured using DXA with a Lunar system (Prodigy; Lunar, Madison, WI). BMD measurements provided absolute values (g/m^2) and T-scores at the lumbar spine (L1 to L4). All measurements were taken by experienced operators on the same parameter settings following the standardized procedures. A standard quality control program including daily calibrations with machine-specific phantoms was performed in these medical centers. The coefficients of variation for quality-control BMD measurements in the four medical centers were 0.95%, 0.89%, 0.82%, and 0.91%. The World Health Organization recommends evaluating bone status based on the T-scores of DXA by comparing the scores with those of healthy young Chinese adults of the same sex and ethnicity. Osteoporosis is defined when the T-score is <-2.5 , and osteopenia when the T-score is between -1 and -2.5 .⁴ In this research, the subjects with osteoporosis or osteopenia were defined as "at least osteopenia" and the control group included subjects with a T-score of >-1 .

Statistical Analysis

Statistical analysis was performed using SPSS 16.0 software (SPSS Inc., Chicago, IL). Continuous variables were compared using the Wilcoxon signed-rank test or Mann–Whitney U test as appropriate; categorical variables were compared using the χ^2 test or Fisher's exact test. The statistical results were presented as the mean \pm standard deviation. Analysis of covariance and multiple linear regression were used to examine the relationship between the PBMC count, BMD value, and T-score. We adjusted for the potential confounding factors from Model 1 to 4, Model 1 included PBMC count, neutrophil count, eosinophil count, basophil count, lymphocyte count, RBC count, platelet count, age, height, weight, SBP, and glucose; Model 2 included PBMC count, neutrophil count, eosinophil count, basophil count, lymphocyte count, RBC count, platelet count, age, height, weight, SBP, glucose, serum calcium, serum phosphorus, and alkaline phosphatase; Model 3 included PBMC count, neutrophil count, eosinophil count, basophil count, lymphocyte count, RBC count, platelet count, age, height, weight, SBP, glucose, serum calcium, serum phosphorus, alkaline phosphatase, AST, and AST; Model 4 included PBMC count, neutrophil count, eosinophil count, basophil count, lymphocyte count, RBC count, platelet count, age, height, weight, SBP, glucose, serum calcium, serum phosphorus, alkaline phosphatase, AST, AST, eGFR, and total protein. To further inspect the underlying association between the PBMC count and bone health, we performed univariate analysis and calculated the ORs (odds ratios) and 95% confidence intervals (CIs) for the population. Multivariate analysis was performed to further confirm the association between the

PBMC count and bone health after considering the above confounding factors. Because BMD is also affected by obesity and hypertension, we created further subgroups based on BMI (BMI <25 and BMI ≥25) and SBP (SBP <140 and SBP ≥140). A two-sided *P*-value <0.05 was considered statistically significant.

RESULTS

Baseline Characteristics

A total of 2806 subjects were enrolled. According to exclusion criteria, 481 elderly men were eliminated; 2325 participants were included in the analysis (Figure 1), 569 in the at least osteopenia group and the remainder in the control group. The clinical and biochemical characteristics of the participants are shown in Table 1. Ages ranged from 50 to 87 years, with a mean (±SD) age of 59.94 ± 7.48 years in the at least osteopenia group and 57.98 ± 7.03 in the control group. The PBMC count was 0.47 ± 0.18 (×10⁹/L) in the at least osteopenia group and a relatively low value of 0.45 ± 0.15 (×10⁹/L) in the control group (*P* = 0.002). The mean BMD value and T-score were significantly lower in elderly men with at least osteopenia than controls (0.92 ± 0.08 vs. 1.20 ± 0.14 and -1.74 ± 0.59 vs. 0.72 ± 1.19, respectively).

Relationship between PBMC Count and BMD

The participants were divided into four quartiles, Q1 (< 0.34 × 10⁹/L), Q2 (0.34 × 10⁹–0.41 × 10⁹/L), Q3 (0.41 × 10⁹–0.53 × 10⁹/L), and Q4 (≥ 0.53 × 10⁹/L), according to the PBMC count. A covariance analysis was performed before adjusting for potential confounders; a wave-type downward trend was found across the quartiles in elderly men (*P* = 0.262; Figure 2A). After adjusting for age, height, weight, systolic blood pressure (SBP), glucose, calcium, phosphorus, alkaline phosphatase, AST, ALT, total protein, eGFR, neutrophil count, eosinophil count, basophil count, lymphocyte count,

RBC count, and platelet count, BMD had a linear decreasing trend from Q1 to Q4 (*P* = 0.034; Figure 2B). Multiple linear regression analysis was also applied in the study. In this analysis, the PBMC count was found to be significantly correlated with BMD after adjusting for the confounding factors from Model 1 to 4 (β = -0.049 to -0.037, *P* < 0.000; Supplemental Table 1, <http://links.lww.com/MD/A889>).

Relationship between PBMC Count and T-score

As a BMD-related variable, the T-score is the key relevant indicator for evaluating bone health. Before adjustment, the T-score was found to fluctuate across the quartiles (*P* = 0.188; Figure 3A). After adjusting for potential confounders, the T-score was found to decrease across the quartiles significantly (*P* = 0.020; Figure 3B). Besides, in multiple linear regression analysis, the PBMC count was still found to be negatively correlated to T-scores after adjusting for variables from Model 1 to 4 (β = -0.500 to -0.439, *P* < 0.000; Supplemental Table 1, <http://links.lww.com/MD/A889>).

Prevalence of at Least Osteopenia According to PBMC Count

Univariate and multivariate analyses were performed to evaluate whether the PBMC count is a risk factor for at least osteopenia. As shown in Supplemental Table 2, <http://links.lww.com/MD/A889> the univariate analysis revealed that the PBMC count was a risk factor for elderly men with at least osteopenia (OR = 2.520, CI: 1.397–4.547; *P* = 0.002). It was further confirmed by multivariate analysis after adjusting for all potential confounding factors (OR = 2.481, CI: 1.176–5.236; *P* = 0.017; Supplemental Table 2, <http://links.lww.com/MD/A889> and Figure 4). Moreover, a subgroup analysis was performed for obesity and hypertension. Participants had a significantly high OR in the BMI <25 group after adjusting for all confounding variables (OR = 2.798, CI: 1.122–6.973; *P* = 0.027; Supplemental Figure 1, <http://links.lww.com/MD/A889>). Consistently, subjects had a significantly high OR in the SBP <140 group after adjusting for all confounding variables (OR = 2.519, CI: 1.059–5.993; *P* = 0.037; Supplemental Figure 1, <http://links.lww.com/MD/A889>).

DISCUSSION

To our knowledge, our study is the first to investigate the relationship between the PBMC count and bone health. PBMC count may be an independent risk predictor for osteopenia or osteoporosis in a cross-sectional elderly men population.

The PBMC count is routinely available marker of inflammatory diseases and identified as a prognostic parameter for hematolysis, such as monocytic leukemia and multiple myeloma.^{19,20} However, increasing number of clinical studies have demonstrated the prognostic value of PBMC count mainly in cancer prognosis and vascular diseases. A higher pretreatment PBMC count was found to link with worse outcome for disease-free survival or overall survival in patients with esophageal squamous cell carcinoma,²¹ HCC,¹² breast cancer,²² and cervical cancer.²³ The common mechanisms may be related to the activation of innate immunity²⁴ and the change of tumor microenvironment with the release of a great diversity of cytokines.¹² Increased PBMC count is also associated with poor outcome in patients with heart failure,²⁵ intracerebral hemorrhage,²⁶ atherosclerosis,²⁷ thrombosis,²⁸ for the mechanism that PBMC are involved in tissue damage with the

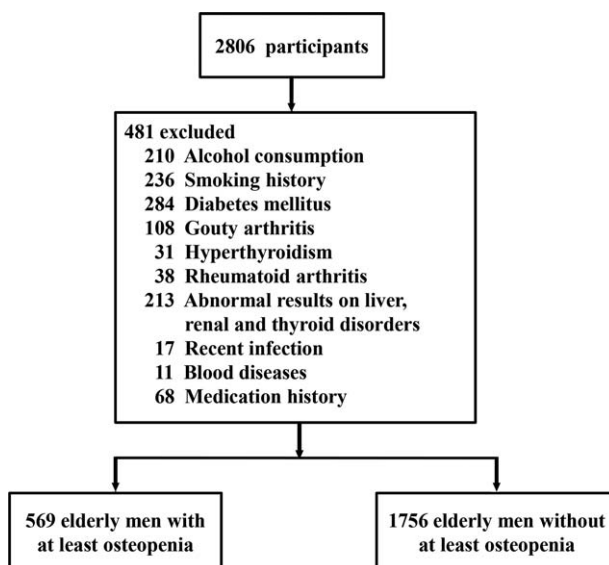


FIGURE 1. Study participants' flow chart. A total of 2806 participants were enrolled. According to the exclusion criteria, 481 participants were excluded. Finally, 2325 participants were included.

TABLE 1. Clinical and Biochemical Characteristics of the Study Cohort Grouped as Subjects With and Without at Least Osteopenia (n = 2325)

	At Least Osteopenia (n = 569)	Control (n = 1756)	P Value
PBMC count ($\times 10^9/L$)	0.47 \pm 0.18	0.45 \pm 0.15	0.002
Neutrophil count ($\times 10^9/L$)	3.75 \pm 1.41	3.62 \pm 1.24	0.038
Eosinophil count ($\times 10^9/L$)	0.17 \pm 0.14	0.17 \pm 0.16	0.514
Basophil count ($\times 10^9/L$)	0.02 \pm 0.03	0.01 \pm 0.02	0.504
Lymphocyte count ($\times 10^9/L$)	2.03 \pm 0.89	1.97 \pm 0.83	0.162
RBC count ($\times 10^{12}/L$)	4.82 \pm 0.37	4.89 \pm 0.37	0.000
Platelet count ($\times 10^9/L$)	204.69 \pm 62.44	196.88 \pm 65.22	0.012
BMD	0.92 \pm 0.08	1.20 \pm 0.14	0.000
T-scores	-1.74 \pm 0.59	0.72 \pm 1.19	0.000
Age (years)	59.94 \pm 7.48	57.99 \pm 7.03	0.000
Height (cm)	165.33 \pm 6.60	167.01 \pm 6.34	0.000
Weight (kg)	64.46 \pm 8.96	70.21 \pm 9.17	0.000
SBP (mm Hg)	126.89 \pm 14.55	129.10 \pm 14.44	0.002
Glucose (mmol/L)	5.71 \pm 1.06	5.78 \pm 1.06	0.145
Serum calcium (mmol/L)	2.36 \pm 0.11	2.36 \pm 0.10	0.220
Serum phosphorus (mmol/L)	1.06 \pm 0.16	1.06 \pm 0.15	0.645
Alkaline phosphatase (U/L)	81.04 \pm 19.43	75.15 \pm 17.74	0.000
AST (U/L)	25.43 \pm 9.64	25.50 \pm 9.67	0.879
ALT (U/L)	27.19 \pm 16.93	29.18 \pm 17.92	0.020
eGFR (mL/min/1.73 m ²)	89.32 \pm 14.56	91.72 \pm 14.09	0.000
Total protein (g/L)	75.10 \pm 4.48	75.16 \pm 4.28	0.769

ALT = alanine aminotransferase, AST = aspartate transaminase, BMD = bone mineral density, eGFR = estimated glomerular filtration rate, PBMC = peripheral blood mononuclear cell, RBC = red blood cell, SBP = systolic blood pressure.

expression of cell adhesion molecules,²⁹ as well as monocyte-mediated pathways of inflammation and apoptosis.^{26,28}

Our study is the first to explore the diagnostic value of PBMC count in orthopedic disease. DXA screening has been accepted as the golden standard for the diagnosis of osteoporosis. However, it hasn't been widely used for the screening of osteoporosis in most developing countries including China. Moreover, most organizations suggest DXA screening in men aged 70 years, or an earlier screening if there is a fragility fracture or other clinical risk factors.³⁰ Therefore, it's easy to be omitted in the diagnosis and treatment of osteoporosis or

osteopenia for those aged <70. PBMC count may represent as an inexpensive, easy-to-measure risk marker, which can serve as an important indicator for further DXA measurement.

In the present study, we retrospectively collected 2806 participants aged ≥ 50 , and found that PBMC count was significant higher in "at least osteopenia" group ($P = 0.002$). In covariance analysis, BMD and T-score both present a significant decline with the increasing of PBMC count ($P = 0.034$ and 0.020). These trends don't appear before the consideration of the potential factors, such as age and some laboratory parameters; it may indicated that these uncontrollable factors

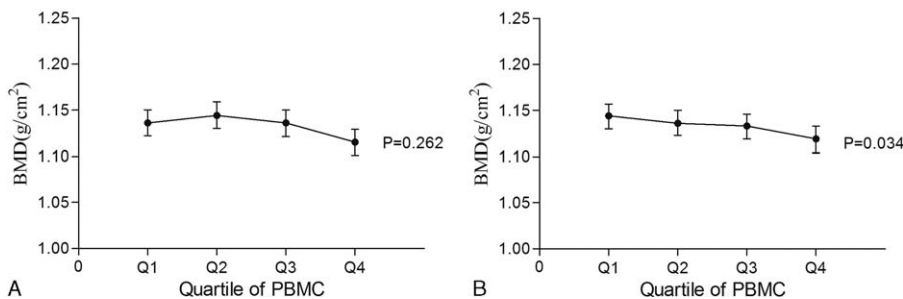


FIGURE 2. Bone mineral density (BMD) value in each quartile of the peripheral blood mononuclear cell (PBMC) count (Q1, Q2, Q3, and Q4). (A) Unadjusted BMD value. (B) BMD value adjusted for neutrophil count, eosinophil count, basophil count, lymphocyte count, red blood cell (RBC) count, platelet count, age, height, weight, systolic blood pressure (SBP), glucose, calcium, phosphorus, alkaline phosphatase, aspartate transaminase (AST), alanine aminotransferase (ALT), total protein, and estimated glomerular filtration rate (eGFR). Results are presented as the survey-weighted least-square means and 95% confidence intervals from regression analysis. P-values are from the test for a linear trend across the PBMC quartiles. ALT = alanine aminotransferase, AST = aspartate transaminase, BMD = bone mineral density, eGFR = estimated glomerular filtration rate, PBMC = peripheral blood mononuclear cell, RBC = red blood cell (RBC), SBP = systolic blood pressure (SBP).

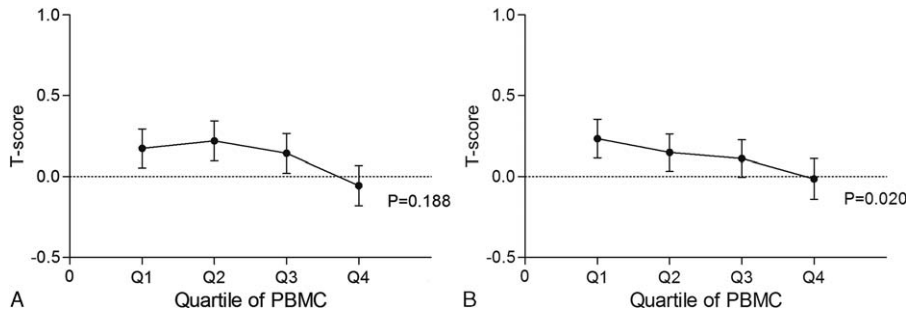


FIGURE 3. T-score in each quartile of the peripheral blood mononuclear cell (PBMC) count (Q1, Q2, Q3, and Q4). (A) Unadjusted T-score. (B) T-score adjusted for neutrophil count, eosinophil count, basophil count, lymphocyte count, red blood cell (RBC) count, platelet count, age, height, weight, systolic blood pressure (SBP), glucose, calcium, phosphorus, alkaline phosphatase, aspartate transaminase (AST), alanine aminotransferase (ALT), total protein, and glomerular filtration rate (eGFR). Results are presented as the survey-weighted least-square means and 95% confidence intervals from regression analysis. *P*-values are from the test for a linear trend across the PBMC quartiles. ALT = alanine aminotransferase, AST = aspartate transaminase, eGFR = estimated glomerular filtration rate, PBMC = peripheral blood mononuclear cell, RBC = red blood cell (RBC), SBP = systolic blood pressure (SBP).

have confounding effects on BMD, as well as T score. Thus, we further performed the multivariate logistic regression from Model 1 to 4 and concluded that higher PBMC count may be an independent predictor for “at least osteopenia” (OR = 2.48, *P* = 0.017). In subgroup analysis, we chose BMI and blood pressure as grouping variables, PBMC is still a strong risk factor, but in BMI ≥25 and HP ≥140 subgroups, the result was not statistically significant. Available data is difficult to explain this difference, more researches are needed to verify.

The mechanism of the independent association between PBMCs and “at least osteopenia” haven’t been fully understood, several reasons may account for this relationship. Firstly, increased PBMC count may indicate an increasing number of OCP cells, whereas it facilitates osteoclast formation and bone resorption. Evidence has showed that OCP cells circulate in peripheral blood and take up a certain part of the PBMC population,³¹ and it is positively related to destructive bone diseases, such as psoriatic arthritis.^{32,33} Using of risedronate, an

anti-osteoporosis drug, has been found to decrease the number of circulating OCP cells in postmenopausal women.³⁴ Secondly, PBMC may have the potential of spontaneous osteoclast formation in pathological conditions.³⁵ It has been demonstrated that cytokines, including TNF-α and IL-1, are able to induce osteoclasts formation from PBMCs.³⁶ And an increase production of TNF-α in PBMCs from women after oophorectomy has been found.³⁷ Thirdly, PBMC may secrete cytokines to change the microenvironment around osteoclast and then enhance its resorption ability.

However, our study also has certain limitations. Although the significant negative correlation between the PBMC count and bone health in elderly men has been established, it still needs to be confirmed in general population in future researches. Additionally, this was a cross-sectional study, it’s difficult to conclude the causal relationship between the PBMC count and bone loss. Follow-up would yield important and meaningful information.

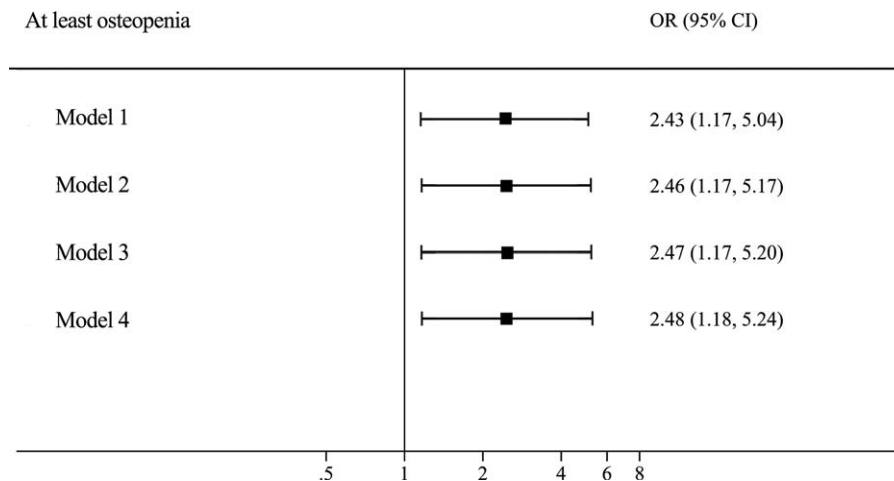


FIGURE 4. Odds ratios (ORs) and 95% confidence intervals (CIs) for at least osteopenia (T-score < -1.0) according to the peripheral blood mononuclear cell (PBMC) count after adjusting for confounders from Model 1 to 4 in all of the participants. Model 1: PBMC count, neutrophil count, eosinophil count, basophil count, lymphocyte count, red blood cell (RBC) count, platelet count, age, height, weight, systolic blood pressure (SBP), and glucose. Model 2: Model 1 plus calcium, phosphorus, and alkaline phosphatase. Model 3: Model 2 plus aspartate transaminase (AST) and alanine aminotransferase (ALT). Model 4: Model 3 plus total protein and estimated glomerular filtration rate (eGFR). ALT = alanine aminotransferase, AST = aspartate transaminase, eGFR = estimated glomerular filtration rate, ORs = odds ratios, PBMC = peripheral blood mononuclear cell, RBC = red blood cell (RBC), SBP = systolic blood pressure (SBP).

In conclusion, the PBMC count is significantly associated with bone loss after adjustment for confounding variables in elderly men population. It may serve as an important indicator for DXA measurement for those aged ≥ 50 years, but further studies are needed to elucidate the exact mechanisms.

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