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Divergent associations of inflammatory markers with bone turnover markers in elderly patients with osteoporotic fractures

Jian Xu[1,5], Yue-qin Guo[2,5], Shao-han Guo[3], Min-zhe Xu[3], Chong Li[3], Ya-qin Gong[4] & Ke Lu[3]✉

The association between inflammatory markers (IMs) and bone turnover markers (BTMs) in osteoporotic fracture patients has not been comprehensively studied. Therefore, this study examined the correlation between the platelet-to-lymphocyte ratio (PLR), neutrophil-to-lymphocyte ratio (NLR), or Monocyte-to-lymphocyte ratio (MLR) and BTMs in osteoporosis (OP) fracture patients. This retrospective cross-sectional study analyzed 740 OP fracture patients admitted to the hospital from January 2017 to July 2022. MLR, NLR, and PLR were calculated based on each patient's complete blood count. The relationship between IMs and BTMs was assessed using three models by adjusting variables. Furthermore, the potential curve relationship between IMs and BTMs was also determined via the threshold effect analysis and curve fittings. In addition, stratified analysis was performed on each adjusted variable to confirm the stability of the results. After adjusting the variables, the results showed that NLR was negatively correlated with procollagen type 1 N-terminal propeptide (P1NP) (β = -1.1788, 95% CI: -1.7230 to -0.6345, *P*-value < 0.0001) and β-C-terminal telopeptide of type I collagen (β-CTX) (β = -0.0104, 95% CI: -0.0145 to -0.0062, *P*-value < 0.0001), Furthermore, MLR was negatively correlated with P1NP (β = -17.4523, 95% CI: -27.7335 to -7.1710, *P*-value = 0.0009) and β-CTX (β = -0.1327, 95% CI: -0.2211 to -0.0443, *P*-value = 0.0034). However, PLR indicated a positive correlation with P1NP (β = 0.0326, 95% CI: 0.0007 to 0.0645, *P*-value = 0.0458) and β-CTX (β = 0.0003, 95% CI: 0.0001 to 0.0006, *P*-value = 0.0204). The threshold effect analysis and curve fittings revealed the presence of a turning point between NLR, MLR, and P1NP, β-CTX. In addition, the stratified analysis validated the result's stability. In conclusion, this study indicates a negative correlation between NLR and MLR with P1NP, while PLR shows a positive correlation with P1NP. Additionally, NLR and MLR exhibit a negative correlation with β-CTX, whereas PLR demonstrates a positive correlation with β-CTX. Further research is required to assess the intricate mechanisms linking IM with bone metabolism.

Keywords P1NP, β-CTX, Osteoporotic fractures, Inflammatory markers, Bone turnover markers

Osteoporosis (OP) is characterized by a gradual decline in bone mass, reduced bone strength, and a breakdown of bone microarchitecture, which significantly increases the risk of fractures[1]. Clinically, OP is defined by a bone mineral density (BMD) of < 2.5 standard deviations (SD) of the average peak bone mass of healthy adults[2]. Hip and vertebral fractures are the most prevalent and debilitating outcomes of OP, and these are associated with substantial healthcare costs and an increased mortality rate of 20% within the first year of post-fracture[3]. The International Osteoporosis Foundation has reported that, for adults over 50 years old, 1 in every 5 men and 1 in every 3 women are at risk of osteoporotic fracture during their lifetime[4,5]. In the United States, the prevalence

[1]Department of Orthopedics, The First People's Hospital of Kunshan, Gusu School, Nanjing Medical University, Suzhou, Jiangsu, China. [2]Endocrine Department, The Fifth People's Hospital of Kunshan, Suzhou, Jiangsu, China. [3]Department of Orthopedics, Affiliated Kunshan Hospital of Jiangsu University, Suzhou, Jiangsu, China. [4]Information Department, Affiliated Kunshan Hospital of Jiangsu University, Suzhou, Jiangsu, China. [5]Jian Xu and Yue-qin Guo contributed equally to this work. ✉email: sgu8434@sina.com

of OP in 50-year-old adults has been observed to increase from 9.4% in 2007–2008 to 12.6% in 2017–2018, with women being more susceptible[6]. Furthermore, in 2019, an estimated 32 million individuals (6.5 million men and 25.5 million women) were diagnosed with OP across 29 European countries[6,7]. Moreover, the global expenditures for osteoporotic fractures, which was $10 billion in 2010, have been expected to reach $17 billion by 2030[8]. Therefore, OP represents a significant threat to human health and well-being.

The recommended diagnostic tool for OP is BMD[9]. However, bone turnover markers (BTMs), released during the bone remodeling process, are also employed for OP diagnosis[10]. These BTMs are mainly the products of bone proteins, such as type I collagen, that are significantly modified at the post-translational level during bone synthesis[11]. Some of these biomarkers include N-terminal propeptide of type I procollagen (PINP), cross-linked C-terminal telopeptide of type I collagen (fragments beta-CTX, alpha-CTX), cross-linked C-terminal of type I collagen (ICTP), C-terminal propeptide of type I procollagen (PICP), osteocalcin (OC), and alkaline phosphatase (AP)[12]. Of these, β-CTX and P1NP are the only recommended biomarkers for investigating bone metabolism in adults[13].

Several studies have indicated a strong association between systemic immunity and inflammation with OP[8,14,15]. For instance, lymphocyte dysfunction can initiate an inflammatory cascade that accumulates macrophages and neutrophils, thereby disrupting the dynamic balance in bone formation[8,16]. Moreover, circulating immune cells, as well as increased levels of pro-inflammatory cytokines, can directly or indirectly impact osteoclastogenesis, thus altering bone resorption[17].

Recently, it was proposed that the inflammatory markers (IMs), such as the platelet-lymphocyte ratio (PLR), neutrophil-lymphocyte ratio (NLR), and Monocyte-lymphocyte ratio (MLR), which are determined during a full blood cell count (FBC), can offer insight into a body's immune response and inflammatory condition[18,19]. Furthermore, these markers are simple, economical, and reliable indicators of inflammation because of the simplicity and low cost of blood tests.

The literature has indicated that these IMs are associated with different conditions, such as depression[20], infertility[21], spontaneous preterm birth[22], and type II diabetes in patients with chronic hepatitis C-related cirrhosis[23]. However, despite the significant evidence supporting the association of inflammation with OP development, the correlation of NLR, MLR, and PLR with OP has not been comprehensively studied. Currently, no conclusive evidence supports a direct relationship between these biomarkers and OP. Therefore, this study aimed to elucidate the correlation between IMs and BTMs.

Materials and methods

Research participants and design

This study was carried out at the Affiliated Kunshan Hospital of Jiangsu University (AKHJU) in Jiangsu Province, China. This university was subsequently known as a tertiary-level A Medical Hospital in the Kunshan area with more than 3,000,000 admitted patients. This study analyzed more than 50 years old OP fracture (proximal humerus, vertebra, hip, fracture of the wrist) patients who were hospitalized between January 1, 2017, and July 27, 2022. Their electronic records were retrieved. Of these, 3,558 patients were included in this study. The exclusion criteria excluded patients who did not have BTMs, neutrophil, monocyte, lymphocyte, and platelet data, were <50 years old, and had a history of malignant tumors[8]. Based on the inclusion criteria, 740 patients were selected for the current analysis (Fig. 1). This study followed the Helsinki Declaration and acquired ethical approval from the AKHJU (2021-06-015-K01). All the patients provided written informed consent before the study, and the investigators were blinded to the patient's data during the analysis.

BTMs examination

The primary outcome variables of this study included β-CTX (reference ranges for males aged 50–70 years: 0.104–0.504 ng/mL, for males over 70 years: 0.164–0.624 ng/mL, for postmenopausal females: 0.330–0.782 ng/mL) and P1NP (reference ranges: 20–80 ng/mL) were measured by electrochemiluminescence using the Roche Cobas s8000 instrument. For laboratory analysis of β-CTX and P1NP, morning fasting venous blood samples were collected from all the participants.

Serum analysis

For routine laboratory tests, all participants fasted for at least 8 h and remained at rest without engaging in vigorous physical activity before blood collection. Then, neutrophils (reference range: $2.0–7.5\times10^9$/L), lymphocytes (reference range: $1.0–3.0\times10^9$/L), and monocytes (reference range: $0.2–0.8\times10^9$/L) were quantified using the Sysmex XN-10 (B4) hematology analyzer after flow cytometric staining. Moreover, platelet (reference range: $100–300\times10^9$/L) levels were measured *via* impedance counting. Based on the results, PLR was calculated as the ratio of platelet count to lymphocyte count, while NLR and MLR were calculated as the ratios of neutrophil count to lymphocyte count and monocyte count to lymphocyte count, respectively.

According to the National Health Commission's Clinical Laboratory Internal Quality Assessment Criteria, for routine tests, it is imperative to ensure that the intra- and inter-batch coefficient of variation (CV) for quantitative items were $\leq 1/3$ of the Total Error Allowance (TEa), respectively. The intra- and inter-batch CV for white blood cell and platelet counts should both be $\leq 6.0\%$ and $\leq 8.0\%$, respectively. For biochemical tests, internal quality control standards primarily reference the performance standards; in the absence of these, they refer to 1/3 of the TEa. For quantitative items, the intra- and inter-batch CV should be $<1/4$ and $<1/3$ of the TEa, respectively.

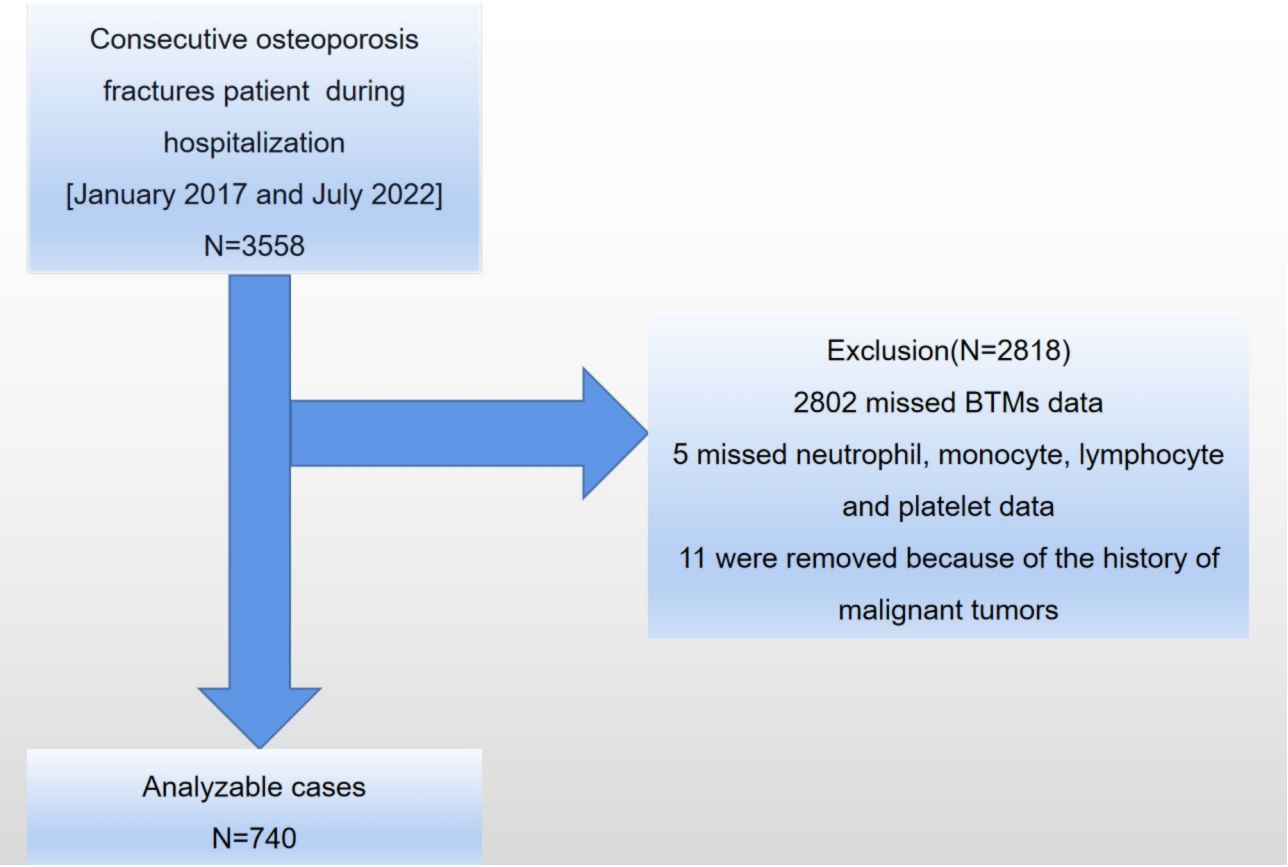

Fig. 1. A diagrammatic representation of the study's design. BTMs: bone turnover markers.

Covariates

The covariates included age, sex, smoking, drinking, calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), phosphorus (P), uric acid (UA), urea nitrogen (UN), creatinine (CR), homocysteine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), high-density lipoprotein (HDL), low-density lipoprotein (LDL), apolipoprotein A (Apo A), fibrinogen, neutrophil, monocyte, the score of American society of anesthesiologists (ASA) and body mass index (BMI), with the latter determined by dividing the weight (in kg) by the height (in meters) squared. Current or former smokers within the past 12 months were identified as ever-smokers, whereas participants who consumed alcohol at least once a week within the past 12 months were considered current drinkers. All patients fasted for 8 h before blood sampling, and all laboratory results were obtained within this period.

Statistical analyses

In this study, categorical data were analyzed by univariate analyses using Fisher's exact tests or Pearson's chi-square tests and represented as frequencies (percentages). Whereas continuous data, such as demographic, laboratory, and clinical information, were displayed as medians (including the Min and Max) or means with standard deviations (SD). The normally or not normally distributed continuous data were analyzed via the Mann-Whitney U tests and independent sample t-tests, respectively.

To evaluate the independent association between IMs and BTMs in OP fracture patients, covariates were adjusted using the generalized estimating equation. Furthermore, 3 models were developed to assess the association between IMs and BTMs in OP patients, including an unadjusted Model 1, a minimally-adjusted Model 2, and a fully-adjusted Model 3. To identify collinearity among the covariates, a variance inflation factor (VIF) analysis was performed. The covariates were then adjusted and included or excluded from the baseline or full model based on the (1) Inclusion criteria: the standardized regression coefficient (β) or matched odds ratio (OR) changed by > 10% and (2) Exclusion criteria: a covariate with a *P*-value of ≤ 0.1 in model 1 or in the univariate analysis. In Model 2, adjustments were mainly focused on rudimentary demographic data, whereas the fully adjusted Model 3 was established based on the two criteria mentioned above.

Potential non-linear correlations were also identified using a generalized additive model (GAM). In cases where an apparent correlation existed, the threshold effect of the resulting smoothed curve was determined using a two-segment linear regression model. Then, a recursive method was used to independently compute the inflection point, applying a maximum likelihood model in situations where these curves exhibited a distinct ratio. In addition, subgroup analyses were conducted on clinically relevant participant subsets to enhance the

findings' reliability, with a *P*-value of ≤ *0.05* in two-tailed tests indicating the results' significance. Empower Stats (http://www.empowerstats.com, X&Y Solutions, Inc, MA, USA) and R packages (http://www.R-project.org, The R Foundation) were employed for all the statistical analyses. The significance threshold was set at a two-sided *P*-value of ≤ *0.05*.

Results

Participants' baseline characteristics

The OP fracture patients were comprehensively analyzed, and based on the inclusion criteria, 740 patients were included in the study. The mean age of the patients was 68.65 years old, with 50 and 103 years indicating the youngest and oldest age, respectively. There were 33.51% males and 66.49% females, which indicates a higher prevalence of OP fractures among female patients. The mean (SD) and median (Min-Max) of MLR, NLR, PLR, P1NP, and β-CTX were 0.41 (0.26) and 0.33 (0.00–2.50), 5.64 (4.79) and 4.16 (0.77–47.19), 161.30 (83.97) and 140.29 (32.86–717.59), 58.15 (35.78) and 51.00 (11.00–565.00) and 0.53 (0.28) and 0.48 (0.07–2.13), respectively (Table 1).

Association between IMs and BTMs

To explore the potential relationship between IMs and BTMs, three models were constructed: an unadjusted Model 1, a Model 2 with adjustments of demographic features (including drinking, smoking, BMI, age, and sex), and a fully-adjusted Model 3.

The data in Model 3 (Table 2) indicated that NLR was negatively correlated with BTMs. For every 1-unit increase in NLR, the corresponding change in β-CTX and P1NP was −0.0104 (95% CI: -0.0145 to -0.0062; *P*-value < 0.0001) and −1.1788 (95% CI: -1.7230 to -0.6345; *P*-value < 0.0001), respectively. Furthermore, MLR was negatively correlated with BTMs as for every 1-unit increase in MLR, the corresponding change in β-CTX and P1NP were −0.1327 (95% CI: -0.2211 to -0.0443; *P*-value = 0.0034) and −17.4523 (95% CI: -27.7335 to -7.1710; *P*-value = 0.0009), respectively. However, there was no negative correlation between PLR and BTMs as in Model 3; for every 1-unit increase in PLR, there was a significant increase of 0.0003 (95% CI: 0.0001 to 0.0006; *P*-value = 0.0204) and 0.0326 (95% CI: 0.0007 to 0.0645; *P*-value = 0.0458) in β-CTX and P1NP levels, respectively.

Spline smoothing plot and threshold analyses

This study also performed curve fitting and threshold effect analyses to explore the potential relationship between IMs and BTMs. The results revealed that MLR and NLR had a curvilinear relationship with BTMs, with the presence of a turning point. In contrast, PLR showed a linear relationship with BTMs (Table 3).

The threshold effect analysis for NLR and BTMs revealed that the *P*-value for LRT was < 0.05, suggesting a turning point at k = 6 for P1NP and k = 7.1625 for β-CTX. The left side of the curve indicated a negative correlation between NLR and P1NP or β-CTX, with effect sizes, 95% CI and a *P*-value = -5.6203 (95% CI: -7.3378 to -3.9028; *P*-value < 0.0001) and −0.0336 (95% CI: -0.0442 to -0.0230; *P*-value < 0.0001), respectively. However, on the right side of the curve, the effect sizes, 95% CI, and *P*-value were 0.1640 (95% CI: -0.5633 to 0.8912; *P*-value = 0.6587) and 0.0001 (95% CI: -0.0059 to 0.0062; *P*-value = 0.9640), respectively (Fig. 2).

The threshold effect analysis for MLR and BTMs revealed a turning point at k = 0.1364 and 0.8226 for PINP and β-CTX, respectively. In this case, the left side of the curve indicated that the effect sizes, 95% CI, and *P*-value between MLR and P1NP or β-CTX were 202.4161 (95% CI: 20.1683 to 384.6639; *P*-value = 0.0299) and −0.2185 (95% CI: -0.3415 to -0.0954; *P*-value = 0.0005), respectively. In contrast, on the right side of the curve, a negative correlation was observed between MLR and P1NP, whereas a positive correlation was noted between MLR and β-CTX, with effect sizes, 95% CI, and *P*-value of -20.5459 (95% CI: -31.1007 to -9.9910; *P*-value = 0.0002) and 0.0472 (95% CI: -0.1533 to 0.2477; *P*-value = 0.6446), respectively (Fig. 3).

The threshold effect analysis for PLR and BTMs showed that the *P*-value for LRT was > 0.05, thus indicating a lack of non-linear relationship between the two variables (Fig. 4).

Subgroup analysis

Subgroup analysis was conducted using the fully adjusted Model 3 to confirm the robustness of the results. All analysis variables were adjusted except for those used for subgroup classification, and the results were consistent in all subgroups (Tables S1, S2, and S3).

Discussion

This study analyzed the data of 740 osteoporotic fracture patients admitted at Kunshan First People's Hospital to investigate the association between IMs and BTMs. The adjustment of confounding factors revealed a negative correlation between BTMs and MLR or NLR. Furthermore, a positive correlation was found between PLR and BTMs. Moreover, the threshold effect analyses and curve-fitting graphs revealed the presence of a turning point between MLR, NLR, and BTMs. To confirm the stability of the data, a stratified analysis was performed, which revealed that the results were consistent across the different strata.

In recent years, several studies have investigated the association between IMs and BMD. Öztürk et al.[24] found a significant negative correlation between lumbar spine (L2-L4) and femoral neck scores with NLR in the elderly population. Furthermore, Tang et al.[15] observed a negative correlation between NLR and BMD in postmenopausal women, which was positively associated with the risk of OP. Moreover, according to Yolaçan et al.[25], the NLR, PLR, MLR, and systemic immune-inflammation index (SII) values were negatively correlated with BMD changes in postmenopausal Turkish women. Lee et al.[26] identified a negative correlation between NLR quartiles and the average lumbar spine BMD in postmenopausal patients in South Korea. In addition,

Variables	Mean (SD) / *N* (%)	Median (Min-Max)[a]
Age, years	68.65 (11.32)	68.00 (50.00-103.00)
BMI, kg/m^2	23.02 (3.37)	23.14 (13.52–33.20)
Na, mmol/L	141.25 (2.75)	141.50 (116.40-153.70)
Mg, mmol/L	0.91 (0.09)	0.91 (0.62–1.43)
K, mmol/L	3.87 (0.43)	3.86 (2.36–6.76)
P, mmol/L	1.08 (0.19)	1.08 (0.31–1.87)
Ca, mmol/L	2.22 (0.12)	2.22 (1.76–2.62)
Fibrinogen, g/L	3.05 (0.81)	2.96 (0.83–6.10)
HDL, mmol/L	1.33 (0.29)	1.30 (0.55–2.44)
LDL, mmol/L	2.66 (0.74)	2.60 (0.79–5.89)
Apo A, g/L	1.23 (0.22)	1.21 (0.61–1.95)
Homocysteine, μmol/L	13.28 (6.98)	11.47 (0.00-68.87)
ALT, U/L	22.81 (16.35)	19.00 (5.00-226.00)
AST, U/L	25.81 (16.23)	22.00 (8.00-266.00)
UA, umol/L	280.72 (91.17)	266.00 (83.00-780.00)
Cr, μmol/L	63.22 (23.91)	59.00 (10.00-395.00)
UN, mmol/L	6.30 (5.38)	5.80 (2.40-139.81)
Neutrophil, × 10^9/L	5.64 (2.65)	4.97 (1.10–17.20)
Monocyte, × 10^9/L	0.45 (0.21)	0.40 (0.00-1.85)
Lymphocyte, × 10^9/L	1.26 (0.52)	1.20 (0.30–3.90)
Platelet, × 10^9/L	177.01 (61.40)	169.00 (42.40–540.00)
β-CTX, ng/mL	0.53 (0.28)	0.48 (0.07–2.13)
P1NP, ng/mL	58.15 (35.78)	51.00 (11.00-565.00)
MLR	0.41 (0.26)	0.33 (0.00-2.50)
NLR	5.64 (4.79)	4.16 (0.77–47.19)
PLR	161.30 (83.97)	140.29 (32.86-717.59)
N (%)		
Sex, N (%)		
Female	492 (66.49%)	
Male	248 (33.51%)	
Smoking, N (%)		
No	688 (92.97%)	
Yes	52 (7.03%)	
Drinking, N (%)		
No	709 (95.81%)	
Yes	31 (4.19%)	
ASA, N (%)		
1	64 (8.65%)	
2	513 (69.32%)	
≥3	163 (22.03%)	

Table 1. The baseline characteristics of the participants. SD, standard deviation; BMI, body mass index; NA, sodium; Mg, magnesium; K, potassium; P, phosphorus; Ca, calcium; HDL, high density lipoprotein; LDL, low density lipoprotein; Apo A, apolipoprotein A; ALT, alanine aminotransferase; AST, aspartate aminotransferase; UA, uric acid; CR, creatinine; UN, urea nitrogen; β-CTX, β-C-terminal telopeptide of type I collagen; P1NP, procollagen type 1 N-terminal propeptide; MLR, monocyte-to-lymphocyte ratio; NLR, neutrophil-to-lymphocyte ratio; PLR, plateletto-lymphocyte ratio; ASA, the score of american society of anesthesiologists. [a]For continuous variables.

Song et al.[27] revealed that postmenopausal women with rheumatoid arthritis have high baseline NLR, PLR, and MLR, which were independently associated with a higher risk of incidental vertebral fractures. Overall, these studies highlighted the detrimental impact of elevated inflammation levels on patients' bone health, which is consistent with the conclusions of the present study that high NLR and MLR levels are detrimental to bone health. However, Ma et al.[28] research revealed a positive correlation between circulating platelet concentration and BMD of the lumbar spine, left hip joint, and right hip joint. Platelets are cytoplasmic fragments derived from megakaryocytes and play a crucial role in bone homeostasis, formation, and resorption[29,30]. It has been observed that platelets have a supportive role in bone formation, particularly in platelet-derived growth factors (PDGFs), which promote bone formation by influencing cell proliferation, chemotaxis, differentiation, and

	P1NP		β-CTX	
NLR				
Model 1 β (95% CI) *P*-value	-1.0293 (-1.5627, -0.4960)	0.0002	-0.0112 (-0.0153, -0.0070)	< 0.0001
Model 2 β (95% CI) *P*-value	-1.0419 (-1.5776, -0.5062)	0.0001	-0.0114 (-0.0155, -0.0072)	< 0.0001
Model 3[a] β (95% CI) *P*-value	**-1.1788 (-1.7230, -0.6345)**	**< 0.0001**	**-0.0104 (-0.0145, -0.0062)**	**< 0.0001**
MLR				
Model 1 β (95% CI) *P*-value	-15.1813 (-25.1430, -5.2197)	0.0029	-0.1431 (-0.2210, -0.0653)	0.0003
Model 2 β (95% CI) *P*-value	-15.1736 (-25.1922, -5.1551)	0.0031	-0.1407 (-0.2189, -0.0626)	0.0004
Model 3[b] β (95% CI) *P*-value	**-17.4523 (-27.7335, -7.1710)**	**0.0009**	**-0.1327 (-0.2211, -0.0443)**	**0.0034**
PLR				
Model 1 β (95% CI) *P*-value	-0.0151 (-0.0458, 0.0156)	0.3363	0.0000 (-0.0002, 0.0002)	0.9811
Model 2 β (95% CI) *P*-value	-0.0159 (-0.0467, 0.0149)	0.3127	-0.0000 (-0.0002, 0.0002)	0.9673
Model 3[c] β (95% CI) *P*-value	**0.0326 (0.0007, 0.0645)**	**0.0458**	**0.0003 (0.0001, 0.0006)**	**0.0204**

Table 2. Associations between inflammatory markers and BTMs in different models. Model 1: no covariates were adjusted. Model 2 was adjusted for age, sex, BMI, smoking and drinking. Model 3[a] was adjusted for age, sex, BMI, smoking, drinking, Ca, UA, CR, AST and Monocyte. Model 3[b] was adjusted for age, sex, BMI, smoking, drinking, Ca, UA, UN, CR, HDL and LDL. Model 3[c] was adjusted for age, sex, BMI, smoking, drinking, K, Mg, Na, P, Fibrinogen, Neutrophil, Apo A, HDL, Homocysteine, ALT and ASA. P1NP, procollagen type 1 N-terminal propeptide; β-CTX, β-C-terminal telopeptide of type I collagen; NLR, neutrophil-to-lymphocyte ratio; CI, confidence interval; MLR, monocyte-to-lymphocyte ratio; PLR, plateletto-lymphocyte ratio; BMI, body mass index; Ca, calcium; UA, uric acid; CR, creatinine; AST, aspartate aminotransferase; UN, urea nitrogen; HDL, high density lipoprotein; LDL, low density lipoprotein; K, potassium; Mg, magnesium; Na, sodium; P, phosphorus; Apo A, apolipoprotein A; ALT, alanine aminotransferase; ASA, the score of american society of anesthesiologists.

extracellular matrix synthesis[31,32]. Increased platelets and decreased lymphocytes increase the PLR and also platelet's supportive effect on bone formation. Therefore, there is a positive correlation between PLR and BTMs.

Osteoclasts and osteoblasts predominantly modulate the bone remodeling process; however, dendritic cells, macrophages, monocytes, B cells, and T cells are also observed to be involved in inflammation and bone loss[33,34]. Oxidative and inflammatory stimuli, such as NO, IL-1β, IL-6, IL-8, IL-18, IL-15, IL-17, IL-32, and TNF-α, can activate osteoclasts-mediated bone resorption, and many of these stimuli induce osteoclastogenesis by enhancing RANKL release[35]. The literature has indicated that during the initial stages of bone healing, neutrophils exert a protective effect on bone formation[36]. However, excessive activation of neutrophils, as a result of infections or injuries, can produce chemokines that inhibit B lymphocytes or recruit pro-inflammatory cells such as Th17 cells, thereby causing inflammation-associated bone loss[37]. Moreover, activated neutrophils can secrete RANKL, which promotes osteoclast formation and maturation and results in bone loss[38]. Th17 cells are a subtype of T cells that promote osteoclastogenesis, and their numbers increase in the bone marrow, and IL-17 levels increase in the peripheral blood[39]. It has been reported that blocking the IL-17 pathway could exert a protective effect against bone loss in OVX mice[39].

The pathological pathways of inflammation are similar to those related to primary OP causes, such as aging and estrogen deficiency, as they all contribute to progressive bone density loss[40,41]. At low levels, estrogen strongly stimulates immune cells to produce inflammatory mediators[34,41]. Furthermore, low estrogen levels can activate T cells and macrophages, which, in turn, release inflammatory cytokines such as TNF-α, IL-6, and IL-1β, thereby inhibiting osteoblasts and stimulating osteoclasts to collectively promote bone resorption[34,35]. As estrogen levels fall with age, IL-1, IL-6, and TNF-α levels rise, contributing to the "inflammaging" observed in older adults[42]. In postmenopausal women, employing TNF-α inhibitors to block TNF-α has demonstrated a reduction in the serum levels of carboxy-terminal cross-linking telopeptide of type I collagen (CTX), a bone resorption marker, indicating the pivotal role of TNF-α in bone resorption[34]. In addition to increasing the inflammatory mediators, estrogen deficiency also enhances oxidative stress markers, further accelerating bone aging effects[43].

The experimental model data have indicated that aging can significantly impact the response of CD8 + and CD4 + T cells[44]. The lower diversity of CD8 + T cells markedly limits the initiation of an effective immune response, leading to chronic inflammation[38]. Inflammatory cells such as T lymphocytes produce a large number of cytokines that stimulate the RANKL/RANK/OPG pathway, thereby promoting osteoclast differentiation and bone homeostasis disruption[45]. Moreover, B lymphocytes can affect bone metabolism by regulating the balance between RANKL and OPG[46]. In a normal state, B lymphocytes inhibit osteoclast activity and slow bone resorption. However, under inflammatory conditions, B lymphocytes may also express RANKL and promote

	NLR, Model 3[a]				MLR, Model 3[b]				PLR, Model 3[c]			
	PINP β (95% CI)	*P*-value	β-CTX β (95% CI)	*P*-value	PINP β (95% CI)	*P*-value	β-CTX β (95% CI)	*P*-value	PINP β (95% CI)	*P*-value	β-CTX β (95% CI)	*P*-value
Model A[d]												
One line slope	-1.1788 (-1.7230, -0.6345)	< 0.0001	-0.0104 (-0.0145, -0.0062)	< 0.0001	-17.4523 (-27.7335, -7.1710)	0.0009	-0.1327 (-0.2211, -0.0443)	0.0034	0.0326 (0.0007, 0.0645)	0.0458	0.0003 (0.0001, 0.0006)	0.0204
Model B[e]												
Turning point (K)	6		7.1625		0.1364		0.8226		244.2857		311.6667	
< K	-5.6203 (-7.3378, -3.9028)	< 0.0001	-0.0336 (-0.0442, -0.0230)	< 0.0001	202.4161 (20.1683, 384.6639)	0.0299	-0.2185 (-0.3415, -0.0954)	0.0005	0.0470 (-0.0059, 0.1000)	0.0823	0.0002 (-0.0001, 0.0006)	0.2229
> K	0.1640 (-0.5633, 0.8912)	0.6587	0.0001 (-0.0059, 0.0062)	0.9640	-20.5459 (-31.1007, -9.9910)	0.0002	0.0472 (-0.1533, 0.2477)	0.6446	0.0141 (-0.0488, 0.0769)	0.6615	0.0005 (-0.0002, 0.0013)	0.1478
Slope 2-Slope 1	5.7843 (3.6585, 7.9101)	< 0.0001	0.0338 (0.0195, 0.0480)	< 0.0001	-222.9620 (-407.4822, -38.4418)	0.0182	0.2657 (-0.0002, 0.5315)	0.0506	-0.0330 (-0.1294, 0.0634)	0.5029	0.0003 (-0.0007, 0.0013)	0.5282
LRT[f]	**< 0.001**		**< 0.001**		**0.017**		**0.048**		0.495		0.521	

Table 3. Threshold effect analysis examining the relationship between inflammatory markers and BTMs. NLR, neutrophil-to-lymphocyte ratio; MLR, monocyte-to-lymphocyte ratio; PLR, plateletto-lymphocyte ratio; P1NP, procollagen type 1 N-terminal propeptide; β-CTX, β-C-terminal telopeptide of type I collagen; CI, confidence interval; BMI, body mass index; Ca, calcium; UA, uric acid; CR, creatinine; AST, aspartate aminotransferase; UN, urea nitrogen; HDL, high density lipoprotein; LDL, low density lipoprotein; K, potassium; Mg, magnesium; Na, sodium; P, phosphorus; Apo A, apolipoprotein A; ALT, alanine aminotransferase; ASA, the score of american society of anesthesiologists. [a]Adjusted for age, sex, BMI, smoking, drinking, Ca, UA, CR, AST and Monocyte. [b]Adjusted for age, sex, BMI, smoking, drinking, Ca, UA, UN, CR, HDL and LDL. [c]Adjusted for age, sex, BMI, smoking, drinking, K, Mg, Na, P, Fibrinogen, Neutrophil, Apo A, HDL, Homocysteine, ALT and ASA. [d]Linear analysis, *P*-value < 0.05 indicates a linear relationship. [e]Nonlinear analysis. [f]*P*-value < 0.05 means Model B is significantly different from Model A, which indicates a nonlinear relationship.

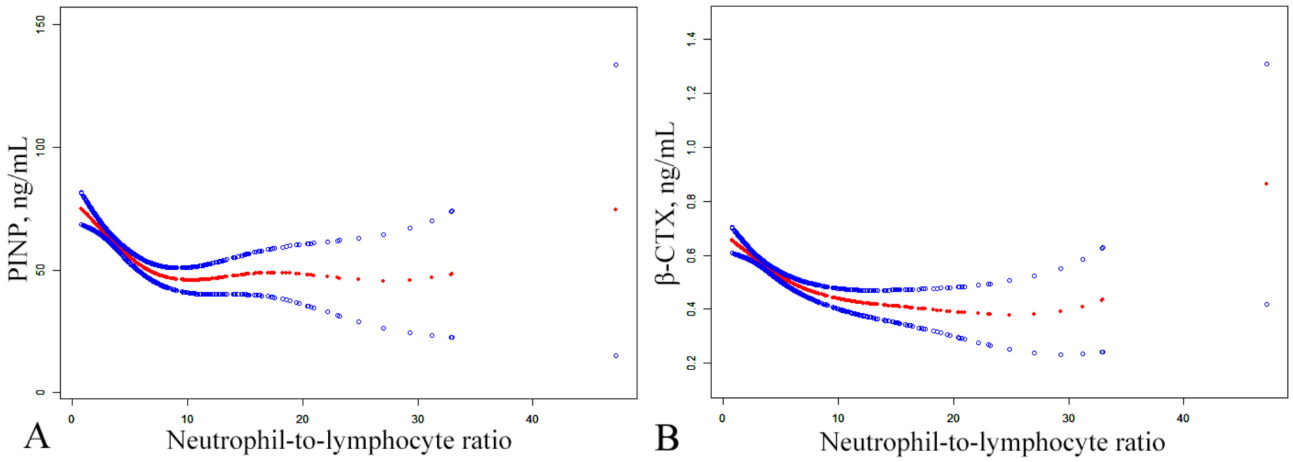

Fig. 2. The smoothed adjusted curves for NLR, β-CTX and P1NP. The red line denotes the non-linear connection between NLR and both β-CTX and P1NP, whereas the blue line indicates the CI at 95%. Models were adjusted for age, sex, BMI, smoking, drinking, Ca, UA, CR, AST and monocyte; NLR, neutrophil-to-lymphocyte ratio; P1NP, procollagen type 1 N-terminal propeptide; β-CTX, β-C-terminal telopeptide of type I collagen; CI, confidence interval; BMI, body mass index; Ca, calcium; UA, uric acid; CR, creatinine; AST, aspartate aminotransferase.

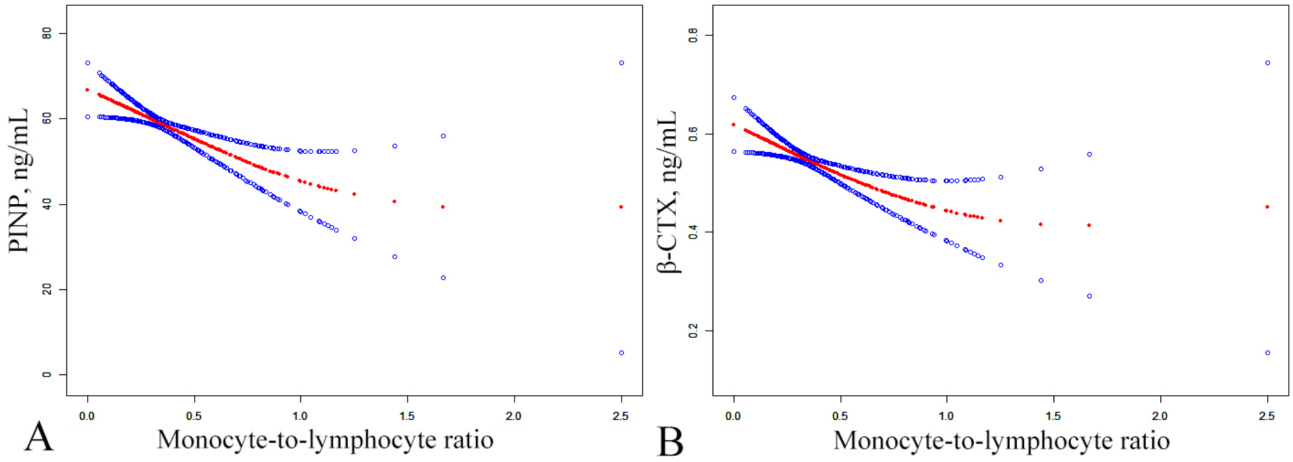

Fig. 3. The smoothed adjusted curves for MLR, β-CTX and P1NP. The red line denotes the non-linear connection between the MLR and both β-CTX and P1NP, whereas the blue line indicates the CI at 95%. Models were adjusted for age, sex, BMI, smoking, drinking, Ca, UA, UN, CR, HDL and LDL; MLR, monocyte-to-lymphocyte ratio; P1NP, procollagen type 1 N-terminal propeptide; β-CTX, β-C-terminal telopeptide of type I collagen; CI, confidence interval; BMI, body mass index; Ca, calcium; UA, uric acid; CR, creatinine; UN, urea nitrogen; HDL, high density lipoprotein; LDL, low density lipoprotein.

osteoclastogenesis[27,47,48]. Osteoclastogenesis can be regulated with RANKL from both B cells and B cell-derived plasma cells[49]. In addition, T cell subgroups may also influence the role of B cells. For instance, when activated by Th1 cytokines, the B cells can inhibit osteoclastogenesis. Furthermore, Th2 cytokines can also stimulate B cells to promote osteoclastogenesis[50,51]. Although most studies focus on B cell's association with osteoclastogenesis, a recent study found that B cells can inhibit osteoblast differentiation by activating extracellular signal-regulated kinase (ERK) and nuclear factor κ B (NF-kB) signaling pathways, thus inhibiting bone formation[52].

These results may have significant clinical implications. Firstly, the negative correlation between MLR, NLR, and BTMs suggests that inflammation impairs bone turnover, thereby increasing patient's susceptibility to OP. However, given the supportive effect of platelets on bone formation, PLR showed a positive correlation with BTMs. Furthermore, the threshold effect analysis indicated the presence of inflection points (k = 0.1364, k = 0.8226) between MLR, P1NP, and β-CTX. On the left side of the inflection point, MLR exhibited a significantly positive correlation with P1NP, while on the right side, MLR was significantly negatively correlated with P1NP. This implies that controlling the patient's MLR within 0.1364 may enhance bone formation and, to some extent, improve the patient's bone health. Moreover, MLR was significantly negatively correlated with β-CTX on the

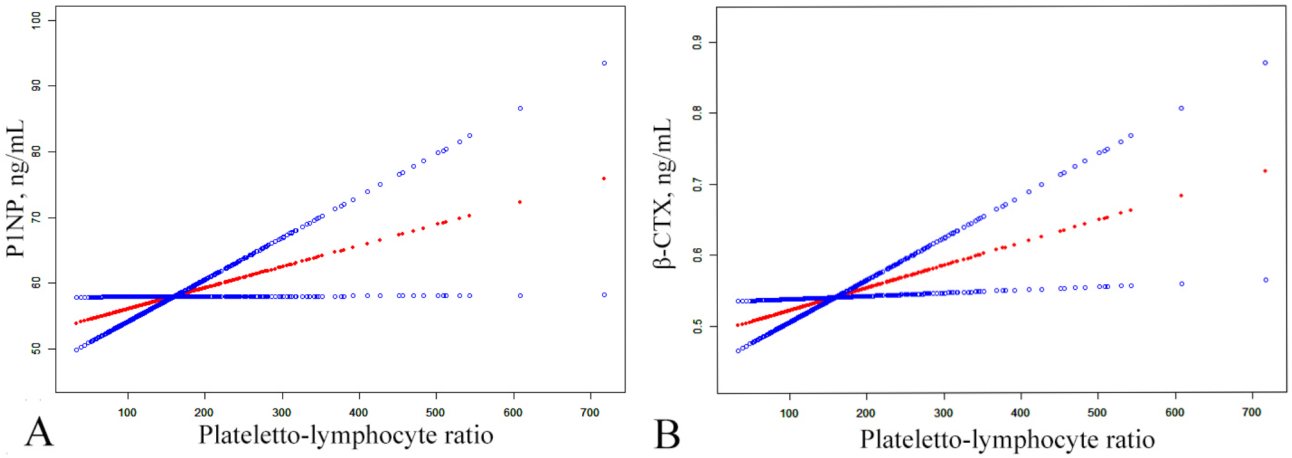

Fig. 4. The smoothed adjusted curves for PLR, β-CTX and P1NP. The red line denotes the non-linear connection between the PLR and both β-CTX and P1NP, whereas the blue line indicates the CI at 95%. Models were adjusted for age, sex, BMI, smoking, drinking, K, Mg, Na, P, fibrinogen, neutrophil, Apo A, HDL, homocysteine, ALT and ASA; PLR, plateletto-lymphocyte ratio; P1NP, procollagen type 1 N-terminal propeptide; β-CTX, β-C-terminal telopeptide of type I collagen; CI, confidence interval; BMI, body mass index; K, potassium; Mg, magnesium; Na, sodium; P, phosphorus; Apo A, apolipoprotein A; HDL, high density lipoprotein; ALT, alanine aminotransferase; ASA, the score of american society of anesthesiologists.

left side of the inflection point and positively correlated on the right side. In addition, an inflection point (k = 6, k = 7.1625) was also observed between NLR and P1NP or β-CTX, which was significantly negatively correlation on the left side, indicating that NLR could decrease P1NP and β-CTX to induce bone turnover damage. Whereas on the right side of the inflection point, the correlation was not significant (*P*-value > 0.05). The negative correlations observed between NLR, MLR, and BTMs may suggest the detrimental impact of inflammation on bone metabolism. This association provides a novel perspective for clinical strategies, suggesting that monitoring inflammation levels could be crucial when assessing the risk of osteoporotic fractures in elderly patients. Furthermore, the positive correlation between PLR and BTMs provides potential therapeutic avenues, indicating that platelet levels or function modulation might ameliorate the risk of OP in elderly individuals. These findings might offer new avenues for tailored assessment and preventive strategies for osteoporotic fractures. By integrating the relationship between IMs and BTMs, clinicians can more accurately evaluate the bone health status of elderly patients and implement appropriate interventions to reduce fracture risk, enhance their quality of life, and advance the development of preventive and therapeutic strategies for osteoporotic fractures.

This study has several strengths. For instance, three separate models were established to investigate the relationship between IMs and BTMs, and the effects of potential confounding variables were also considered. The results showed a negative correlation between MLR, NLR, and BTMs in patients with OP fractures, suggesting that high levels of MLR and NLR may impair BTMs in older populations. In addition, PLR was positively correlated with BTMs, possibly due to the supportive effect of platelets on bone formation. Therefore, monitoring the level of IMs in patients may be beneficial in reducing the high incidence and mortality rates associated with OP and fractures. In addition, these laboratory markers are simpler, more convenient, and more cost-effective compared with other tests that assess bone density. However, there are certain limitations in this study as well. Firstly, given its cross-sectional design, the study inherently harbors limitations, notably potential biases such as recall and selection biases. The researchers' inability to manipulate exposure timing posed a challenge in definitively establishing causal relationships. Data collection, susceptible to constraints from measurement tools or data sources, may introduce distortions in information. Moreover, definitively establishing the temporal relationship between IMs and the onset of OP remains a complex endeavor. Secondly, there is potential involvement of genetic and non-genetic factors in the development of OP, but the data in this study were only adjusted for certain demographic, lifestyle, and laboratory variables during analysis. Moreover, the sample size was small and only comprised 740 identifiable samples. In addition, this study did not compare the PLR, NLR, and MLR with standard inflammation markers such as proinflammatory cytokines. Therefore, their capacity to accurately reflect the inflammatory status might be restricted. Lastly, these results may not be applicable to individuals of other races, as the study was conducted at a single institute on a relatively small patient population. To ensure the consistency and validity of these findings, additional and more extensive studies should be conducted using a multi-center randomized design, a wider range of biochemical markers, and a more diverse ethnic population.

Conclusion

In conclusion, this study, the relationship between IM and BTMs in osteoporotic fracture patients was investigated. The results indicate that both the NLR and MLR have adverse effects on bone formation and metabolism, thereby increasing the risk of osteoporotic fractures. In contrast, due to the supportive role of platelets in bone formation, the PLR shows a positive correlation with P1NP and β-CTX, indicating a beneficial

effect on bone formation and metabolism to some extent. However, further prospective cohort studies and mechanistic research are necessary to validate these findings.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Author contributions
Study design: KL. Study conduct: CL and KL. Data collection: JX, SHG, MZX, KL, YQG and YQG, Data analysis: JX and SHG. Data interpretation: JX and KL. Drafting manuscript: JX. Revising manuscript content: SHG, MZX and KL. Approving the final version of the manuscript: CL, YQG and KL. JX and KL take responsibility for the integrity of the data analysis. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

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Declarations

Competing interests
The authors declare no competing interests.

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Correspondence and requests for materials should be addressed to K.L.