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Original Article

Combined detection of vitamin D, CRP and TNF-α has high predictive value for osteoporosis in elderly men

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Abstract: Objective: To observe the predictive value of 25-hydroxyvitamin D3 (25(OH)D3), C-reactive protein (CRP), and tumor necrosis factor-α (TNF-α) levels for osteoporosis in elderly men. Methods: A retrospective analysis was conducted in 122 elderly male patients that were tested in The Affiliated Hospital of Xinyang Vocational and Technical College between January 2020 and May 2022. The patients were divided into an osteoporosis group (OG, n = 77) and a control group (CG, n = 45) according to the results of bone mineral density. The formula N = Z^2*(P*(1-P))/E^2 was used to calculate the required sample size (N) for a given confidence interval (Z), total error (E), and proportion (P) of the target population. The proportion (P) is often assumed to be 0.5 and not randomly distributed across the population. The levels of cross-linked C-terminal telopeptide of type I collagen (CTX-I), procollagen type I N-terminal propeptide (PINP), intact parathyroid hormone (iPTH), osteocalcin (OC), and serum levels of 25(OH)D3, CRP, and TNF-α were measured and compared between the two groups. Pearson correlation was used to analyze the relationship between the parameters. The predictive value of 25(OH)D3, CRP and TNF-α for osteoporosis was also analyzed using receiver operating characteristic (ROC) curves. Logistic multivariate analysis was performed to analyze the risk factors for osteoporosis in elderly men. Results: Compared with the CG, the OG exhibited evidently lower serum 25(OH)D3, but significantly higher CRP and TNF-α ($P < 0.05$). Pearson correlation demonstrated that the bone mineral density was negatively correlated with CTX-I, PINP, serum CRP and TNF-α, whereas it was positively correlated with OC and 25(OH)D3 in elderly men. The areas under the ROC curve (AUCs) of serum 25(OH)D3, CRP and TNF-α were identified as 0.931, 0.878 and 0.846, respectively, and the AUC of the combined detection of the three was 0.991. Furthermore, age, CTX-I, PINP, OC, 25(OH)D3, as well as serum CRP and TNF-α were identified as risk factors for osteoporosis among elderly men. Conclusion: Serum 25(OH)D3, CRP, and TNF-α are associated with osteoporosis in elderly men, and can serve as predictors for osteoporosis.

Keywords: 25-hydroxyvitamin D3, CRP, TNF-α, osteoporosis, prediction

Introduction

Osteoporosis, a systemic skeletal disease with high incidence, is categorized into primary osteoporosis and secondary osteoporosis. Patients with osteoporosis usually have decreased bone strength and increased bone fragility due to osteopenia and deterioration of bone microstructure [1, 2]. Statistically, millions of fractures occur each year, and this incidence is expected to increase due to the aging of population, making it a global healthcare problem [3]. Without typical clinical symptoms in the early stage, most patients are diagnosed with osteoporosis after osteoporotic fractures. To reduce such risk, early identification of osteoporosis and timely interventions are substantial [4].

At present, the diagnosis of osteoporosis is based on the bone mineral density (BMD) measured by dual-energy X-ray absorptiometry. This measurement method is only available in a few hospitals, which limits the early diagnosis and treatment in general population. A simpler and easier prediction scheme is therefore needed [5, 6]. While attention to osteoporosis has long been focused on elderly women, it is necessary to shift more focus towards its impact on men [7]. Vitamin D deficiency is an important risk factor for the development and progression of osteoporosis, affecting BMD and bone microar-

chitecture by changing local bone metabolism, thereby aggravating osteoporosis. 25-hydroxyvitamin D3 (25(OH)D3) is an optimal indicator for evaluating the vitamin D level [8]. C-reactive protein (CRP) is an inflammatory mediator, which was suggested to have a negative correlation with BMD [9]. Tumor necrosis factor-α (TNF-α), secreted by T lymphocytes, is the earliest and most important multifunctional factor during inflammatory response and can effectively enhance RANKL-induced osteoclast formation, which leads to osteopenia [10].

Vitamin D, CRP, and TNF-α levels are all expected to be potential markers for predicting osteoporosis. Therefore, this study detected serum 25(OH)D3, CRP, and TNF-α levels and analyzed the predictive value of the three in elderly men with osteoporosis, so as to provide a reference for the diagnosis of osteoporosis.

Materials and methods

Clinical data

Data of 122 elderly male patients that were tested in The Affiliated Hospital of Xinyang Vocational and Technical College between January 2020 and May 2022 were retrospectively analyzed. The subjects were divided into an osteoporosis group (OG, n = 77) and a control group (CG, n = 45) according to the results of BMD. Inclusion criteria: (1) patients aged 60 years or above; (2) patients with osteoporosis confirmed by BMD testing; (3) patients with complete clinical data. Exclusion criteria: (1) patients with liver, kidney or other major organ dysfunction; (2) patients with severe infectious diseases or immune dysfunction; (3) patients with malignant tumors; (4) patients who refused to participate in the experiment. This study conforms to the Helsinki Declaration and has been approved by The ethics committee of Affiliated Hospital of Xinyang Vocational and Technical College.

Detection methods

Fasting venous blood (2 mL) was collected from subjects in both groups in the morning on the next day of admission. The blood samples were centrifuged at 4000 r/min for 5 min, and the supernatant was separated and stored for examination. A BK400 automatic biochemical analyzer and supporting reagents were used to detect 25(OH)D3 levels strictly following the instructions on the kit. The levels of inflammatory cytokines CRP (ThermoFisher, KHA0031) and TNF-α (ThermoFisher, A42898) were measured by ELISA. In addition to the primary outcome measures, levels of cross-linked carboxy-terminal telopeptide of type I collagen (CTX-I), procollagen type I N-terminal propeptide (PINP), intact parathyroid hormone (iPTH), and osteocalcin (OC) were measured using an electrochemiluminescence immunoassay analyzer.

Bone density examination

All subjects underwent BMD examination via ultrasound, and the BMD was evaluated according to criteria from World Health Organization [11]. Those with BMD below one standard deviation (SD) of the mean peak bone mass in the corresponding gender population but less than 2.5 SDs were classified as having osteopenia. Those with BMD more than 2.5 SDs below the mean peak bone mass are diagnosed with osteoporosis. Conversely, those with BMD more than 1 SD above the mean peak bone mass in the same gender population are categorized as having normal bone mass. BMD is usually expressed as t-score (T-value), that is, T-value $\geq$ -1.0 indicates normal bone mass, -2.5 < T-value < -1.0 indicates osteopenia, and T-value $\leq$ -2.5 indicates osteoporosis.

Statistical methods

The collected data were analyzed and visualized using SPSS 20.0 software and GraphPad Prism 8 software, respectively. For measurement data ($\bar{X}\pm S$), independent sample t-test was used for inter-group comparison, paired t-test was for comparison within groups (expressed as t). Chi-square test was used to process enumeration data (expressed as percentages). Receiver operating characteristic (ROC) curve was used to analyze the predictive value of 25(OH)D3, CRP and TNF-α in osteoporosis. Pearson correlation analysis was performed to analyze the correlation between indicators, and logistic multivariate regression analysis was used to analyze the independent risk factors for osteoporosis. Statistical significance level was set at $P < 0.05$.

Results

General information

There were no significant differences in general data, such as age and body mass index, between the two groups ($P > 0.05$, **Table 1**).

Table 1. Comparison of general data of patients

Variables	Osteoporosis group n = 77	Control Group n = 45	t/X^2	P
Age (years)			0.061	0.805
≥ 65	41 (53.25)	25 (55.56)		
< 65	36 (46.75)	20 (44.44)		
BMI (kg/m^2)			0.043	0.835
≥ 23	43 (55.84)	26 (57.78)		
< 23	34 (44.16)	19 (42.22)		
Smoking history			0.002	0.961
Yes	51 (66.23)	30 (66.67)		
No	26 (33.77)	15 (33.33)		
Alcohol history			0.022	0.883
Yes	40 (51.95)	24 (53.33)		
No	37 (48.05)	21 (46.67)		
Education level			0.013	0.911
Primary school or below	47 (54.02)	27 (47.37)		
Above primary school	30 (45.98)	18 (52.63)		
Combined hypertension			0.931	0.335
Yes	41 (53.25)	28 (62.22)		
No	36 (46.75)	17 (37.78)		

Note: BMI: Body Mass Index.

Table 2. Comparison of 25(OH)D3, CRP and TNF-α levels between the two groups

Variables	Osteoporosis group n = 77	Control Group n = 45	t/X^2	P
25(OH)D3 (ng/mL)	16.85±2.03	21.26±2.2	11.22	< 0.001
CRP (mg/L)	6.87±1.99	4.03±1.5	8.290	< 0.001
TNF-α (pg/mL)	7.65±2.37	4.79±1.5	7.281	< 0.001

Note: 25(OH)D3: 25-hydroxyvitamin D3, CRP: C-reactive protein, TNF-α: tumor necrosis factor-α.

Comparison of 25(OH)D3, CRP and TNF-α levels between the two groups

Compared with the CG, serum 25(OH)D3 level was lower, but the CRP and TNF-α levels were significantly higher in the OG ($P < 0.05$, **Table 2**).

Predictive value of serum 25(OH)D3, CRP and TNF-α in osteoporosis among elderly men

To determine the predictive value of serum 25(OH)D3, CRP, and TNF-α for osteoporosis in elderly men, we plotted ROC curves (**Figure 1**) and found that the areas under the curves (AUCs) of the above three were 0.931, 0.878, and 0.846 respectively, all of which are of certain diagnostic value. It is notable that 25(OH)D3, with AUC of 0.93, presented a considerably high predictive value for osteoporosis in elderly men. The AUC of combined detection of serum 25(OH)D3, CRP, and TNF-α was detected as 0.991, which was greater than that of each indicator alone (**Table 3**).

Comparison of bone metabolism related indicators

Compared with CG, the OG exhibited evidently higher CTX-I and PINP while significantly lower OC (all $P < 0.001$), but there was no significant difference in iPTH between the two groups ($P > 0.05$, **Table 4**).

Correlation analysis between BMD and various parameters

BMD was negatively correlated with CTX-I, PINP, serum CRP and TNF-α ($P < 0.001$), and positively correlated with OC and 25(OH)D3 ($P < 0.001$) in elderly men. See **Figure 2**.

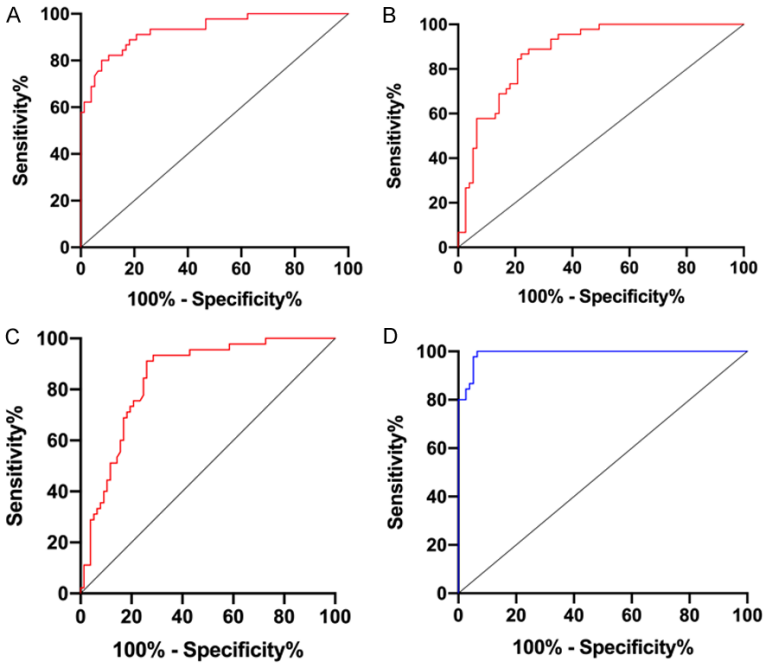

Figure 1. ROC curve of serum 25(OH)D3, CRP, and TNF-α for predicting osteoporosis in elderly men. A-C: ROC curve of 25(OH)D3, CRP, TNF-α alone in predicting osteoporosis in elderly men; D: ROC curve of combined detection of 25(OH)D3, CRP and TNF-α for osteoporosis in elderly men. 25(OH)D3: 25-hydroxyvitamin D3, CRP: C-reactive protein, TNF-α: tumor necrosis factor-α.

Multivariate logistic regression analysis

Multivariate logistic analysis was performed with the presence or absence of osteoporosis as the dependent variable, and age, CTX-I, PINP, OC, 25(OH)D3, serum CRP, and TNF-α as independent variables. The results revealed that age, CTX-I, PINP, OC, 25(OH)D3, serum CRP and TNF-α were all influencing factors of osteoporosis in elderly men ($P < 0.05$), as shown in **Table 5**.

Discussion

Osteoporosis can be attributed to various factors, and the primary mechanism involves an increase in bone resorption, leading to a reduction of bone tissue and manifesting as bone pain and susceptibility to fractures [12]. It is generally believed that women are predisposed to osteoporosis after menopause, but men also bear a high risk of this disease [13]. Unlike women, men usually have relatively slow bone loss and less pronounced symptoms, making clinical diagnosis of osteoporosis more difficult [14]. Therefore, finding predictive markers for osteoporosis can give early warning to potential male patients, thereby reducing bone loss and fracture risk.

Among the numerous bone turnover markers, PINP is a metabolite of type I collagen and is of high sensitivity and specificity for osteoporosis. It is not easily affected by hormones and can reflect osteoblast synthesis ability. An increased PINP can suggest aggravated bone metabolism diseases [15]. In addition, CTX-I is an effective marker capable of reflecting osteoclast activity, and elevated CTX-I levels have been found in various metabolic diseases [16]. OC is an essential substance for mineralization of bone matrix, and elevated OC levels are common seen in highly transformed osteoporosis [17]. Our results showed evidently higher CTX-I, higher PINP and lower OC in the OG as compared with those in the CG. We also found that BMD was negatively correlated with CTX-I and PINP and positively correlated with OC in elderly men. Previous studies [18] found that the levels of CTX-I and PINP were significantly decreased in patients with osteoporosis, which is consistent with our observations. However, despite the innovations introduced by these markers in the field of diagnosing osteoporosis, there is still a lack of standardized reference population databases and insufficient standardization of quality control. Therefore, the research on novel markers that can be used alone or in combination with existing indicators is of great significance for osteoporosis in clinical diagnosis and translational studies.

Inflammatory factors are a class of highly active and multifunctional small molecule proteins primarily produced by immune cells and related cells. Studies have shown their association with the pathogenesis of osteoporosis [19]. The levels of inflammatory factors were detected to be increased in osteoporosis patients, with CRP and TNF-α showing the most elevation, and the increase of TNF-α can lead to imbal-

Table 3. Diagnostic value of serum 25(OH)D3, CRP, and TNF-α in osteoporosis

Indicators	AUC	95 CI%	Specificity	Sensitivity	Cut-off
25(OH)D3	0.931	0.885-0.977	74.03%	93.33%	18.46
CRP	0.878	0.818-0.937	75.32%	88.89%	5.77
TNF-α	0.846	0.776-0.915	84.03%	84.44%	6.04
Combination of the three	0.991	0.980-0.999	94.81%	97.78%	0.404

Note: 25(OH)D3: 25-hydroxyvitamin D3, CRP: C-reactive protein, TNF-α: tumor necrosis factor-α.

Table 4. Comparison of bone metabolism related indicators

Variables	Osteoporosis group n = 77	Control Group n = 45	t	P
CTX-I (pg/mL)	555.08±28.04	386.66±17.56	36.31	< 0.001
PINP (ng/mL)	67.52±5.94	34.84±3.37	33.83	< 0.001
OC (ug/L)	2.85±0.39	14.87±2.85	36.53	< 0.001
iPTH (mIU/L)	41±2.13	41.27±2.28	0.658	0.512

Note: CTX-I: cross-linked carboxy-terminal telopeptide of type I collagen, PINP: amino-terminal extended telopeptide of type I procollagen, iPTH: intact parathyroid hormone, OC: osteocalcin.

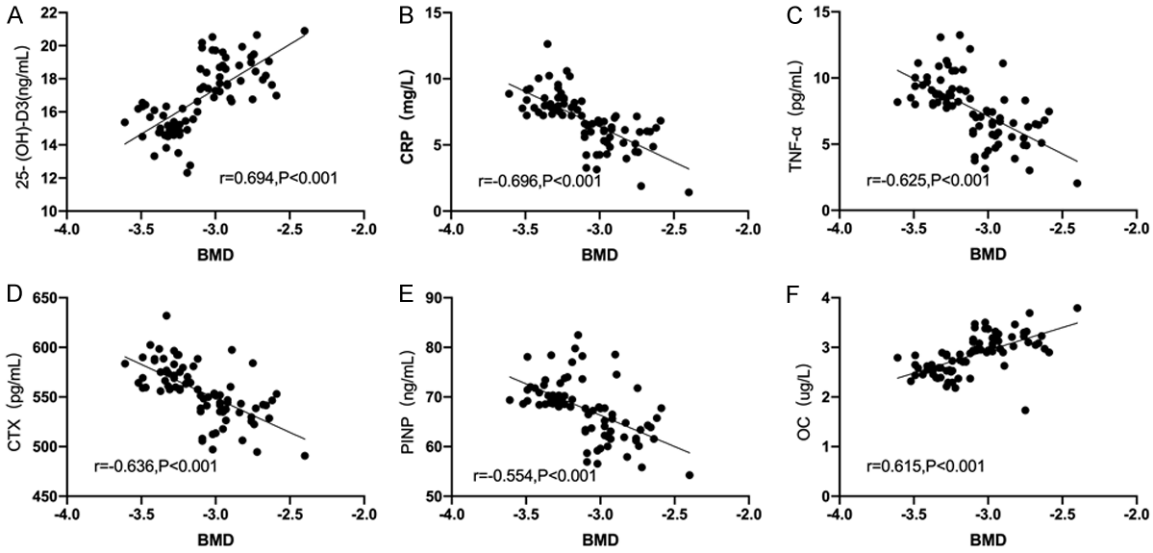

Figure 2. Correlation analysis between bone mineral density (BMD) and various parameters. A: BMD is positively correlated with 25(OH)D3; B: BMD is negatively correlated with CRP; C: BMD is negatively correlated with TNF-α; D: BMD is negatively correlated with CTX-I; E: BMD is negatively correlated with PINP; F: BMD is positively correlated with OC. 25(OH)D3: 25-hydroxyvitamin D3, CRP: C-reactive protein, TNF-α: tumor necrosis factor-α, CTX-I: C-terminal telopeptide of type I collagen, PINP: procollagen type I N-terminal propeptide, iPTH: intact parathyroid hormone, OC: osteocalcin.

ance of bone metabolism and bone loss [20]. Our results also showed that the expression of CRP and TNF-α was evidently increased in patients with osteoporosis, negatively correlating with BMD. Recent studies have pointed out that 25(OH)D3 plays a key role in regulating bone metabolism and promoting bone calcium resorption and bone formation [21]. Our results suggested that serum 25(OH)D3 was lowly expressed in osteoporosis patients. The determination of 25(OH)D3 level should be conducive to clinical screening of osteoporosis. Vitamin D is an indispensable vitamin and nutrient component of the human body, which can not only regulate the level of calcium and phosphorus metabolism of the body, but also promote the regulation of bone calcification, bone formation and growth [22]. 25(OH)D3, a metabolite of vitamin D, is processed by the liver and kidneys, regulating bone metabolism

Table 5. Multivariate logistic regression

Variables	β	SE	Wald x^2	P	OR (95% CI)
Age	0.89	0.40	4.681	0.033	2.411 (1.076-5.423)
CTX-I	0.81	0.31	7.523	0.005	2.316 (1.276-4.271)
PINP	0.95	0.42	4.867	0.004	2.671 (1.102-6.271)
OC	-1.32	-0.52	4.572	0.026	0.274 (0.092-0.904)
25(OH)D3	-1.22	-0.44	5.932	0.031	0.321 (0.127-0.801)
CRP	1.17	0.43	7.716	0.011	0.311 (0.132-0.682)
TNF-α	1.23	0.47	7.422	0.005	0.283 (0.113-0.864)

Note: 25(OH)D3: 25-hydroxyvitamin D3, CRP: C-reactive protein, TNF-α: tumor necrosis factor-α, CTX-I: C-terminal telopeptide of type I collagen, PINP: procollagen type I N-terminal propeptide, OC: osteocalcin.

and maintaining calcium and phosphorus homeostasis in the body. As an activated form of vitamin D, 25(OH)D3 is a sensitive indicator of vitamin D levels. A decreased 25(OH)D3 level can affect the absorption of calcium ions in the body, hinder bone mineralization and bone matrix formation, and cause osteoporosis. Besides, 25(OH)D3 deficiency can also cause elevated PTH levels, resulting in increased bone turnover and accelerated bone loss, thereby increasing the risk of osteoporosis [23]. Previous studies [24] have suggested that 25(OH)D3 is one of the diagnostic markers for osteoporosis, which is consistent with our findings. We found that serum 25(OH)D3 was more sensitive than CRP and TNF-α when it comes to the prediction of osteoporosis, while the predictive value of combined detection was still higher than that of any single index. This suggests that the combined detection of 25(OH)D3, CRP and TNF-α helps to improve the accuracy of osteoporosis prediction. No studies have yet analyzed the combined value of these three indicators in predicting osteoporosis, but previous studies have suggested that 25(OH)D3 is an effective marker for predicting osteoporosis, which is also consistent with our study [25]. There was a study which investigated the clinical value of serum total PINP, β-CTX, and 25(OH)D3 tests for assessing the risk of hip fragility fractures in elderly patients with osteoporosis, and they found that, compared to single test, combined test was of higher predictive value [26]. Moreover, we analyzed the risk factors for osteoporosis and found that age, CTX-I, PINP, OC, 25(OH)D3, serum CRP and TNF-α were all risk factors for osteoporosis in elderly men. Age has a substantial impact on osteoporosis. Previous studies [27] have demonstrated that atherosclerosis has adverse effects on BMD and bone metabolism. While in atherosclerosis, the levels of inflammatory factors such as CRP are increased, and these inflammatory factors can promote bone resorption, leading to the development of osteoporosis. In addition, previous studies [28] confirmed that 25(OH)D3 deficiency was a risk factor for osteoporosis, and oral vitamin D could be an important means to prevent osteoporosis.

In conclusion, serum CRP and TNF-α were significantly increased and 25(OH)D3 was significantly decreased in patients with osteoporosis. For elderly men, combined detection of 25(OH)D3, CRP, and TNF-α is helpful for early osteoporosis prediction, which enables clinicians to promptly develop coping strategies to prevent potential fractures.

Disclosure of conflict of interest

None.

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