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Diagnostic Value of a New Triple Combination in CKD-MBD: A Cross-sectional Study

Running title: Diagnostic Value in CKD-MBD

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SUMMARY

Prompt screening of CKD-MBD plays a very important role in the improvement of long-term prognosis and quality of life in such patients. To seek a triple combination of biomarkers for early diagnosis of CKD-MBD (Chronic kidney disease-mineral and bone metabolic disorder) and to explore the diagnostic efficacy of β2-microglobulin (β2-MG),

parathyroid hormone (PTH), and blood urea nitrogen (BUN) in CKD-MBD. It can play the crucial role of a sensitive indicator for the early diagnosis of CKD-MBD and in preventing or delaying the progress of CKD-MBD.

ABSTRACT

Introduction: To seek a triple combination of biomarkers for early diagnosis of Chronic kidney disease-mineral and bone metabolic disorder and to explore the diagnostic efficacy of β2-microglobulin, parathyroid hormone, and blood urea nitrogen in Chronic kidney disease-mineral and bone metabolic disorder.

Methods: A total of 864 patients with Chronic kidney disease were collected and divided into two groups according to the renal bone disease presentation in all the patients. There were 148 and 716 subjects in the Chronic kidney disease-mineral and bone metabolic disorder and the control groups, respectively. The aggregated data included basic information and various clinical laboratory indicators, such as blood lipid profile, antibody, and electrolyte levels, along with renal function-related indicators.

Results: It was observed that most renal osteopathy occurs in the later stages of Chronic kidney disease. In a comparison of clinical laboratory indicators between the two groups, 16 factors were selected for curve analysis, and the was compared. We discovered that factors with high diagnostic values were β2-microglobulin, parathyroid hormone and blood urea nitrogen.

Conclusion: The triple combination of β2-microglobulin +parathyroid hormone+blood urea nitrogen indicators can play the crucial role of a sensitive indicator for the early diagnosis of Chronic kidney disease-mineral and bone metabolic disorder and in preventing or delaying the progress of Chronic kidney disease-mineral and bone metabolic disorder.

Keywords: Blood urea nitrogen; β2-microglobulin; CKD-MBD; Parathyroid hormone; Urea nitrogen

INTRODUCTION

Chronic kidney disease-mineral and bone metabolic disorder (CKD-MBD) is one of the common serious complications in patients with chronic kidney diseases (CKD) [1]. The incidence rate of MBD increases with the progression of CKD; more than 80% of MBD patients suffer from end-stage renal disease (ESRD) [2]. Recent epidemiologic studies have indicated that abnormalities in calcium and phosphorus serum levels and secondary hyperparathyroidism lead to the risk of death in maintenance dialysis patients [3]. Early detection of CKD-MBD can provide timely treatment as well as greatly improve their quality of life [4]. Therefore, prompt screening of CKD-MBD plays a very important role in the improvement of long-term prognosis and quality of life in such patients.

At present, bone biopsy remains the gold standard for diagnosing CKD-MBD. Since bone biopsy is invasive and causes trauma, it is not used as a routine examination method in clinical practice. Henceforth, medical staff must determine the need for conducting a bone biopsy for CKD stage 3–5 patients according to their health conditions [2]. Therefore, it is an urgent need to find the diagnostic and treatment markers of CKD-MBD for improved patient outcomes.

β2-microglobulin (β2-MG) is a small molecular weight protein that is clinically significant in the examination of kidney diseases [5]. If the blood plasma β2-MG level is increased, it indicates a reduction in the glomerular filtration function [6]. It is highly sensitive to serum creatinine, which can detect the decrease of glomerular filtration rate in the early stage. However, the diagnostic value of β2-MG is limited.

It is observed that parathyroid hormone (PTH) is closely related to the occurrence and

development of CKD-MBD [7]. The main function of PTH is to regulate the metabolism of calcium and phosphorus in the body, relying on the actions of the main target organs: bone and kidney [8]. It mobilizes bone calcium into the blood, promotes the reabsorption of calcium ions by renal tubules and the excretion of phosphate, increases the blood calcium concentration, and decreases the blood phosphorus concentration. If PTH is detected early and found to be abnormal, timely correction may be able to control calcium and phosphorus metabolism disorders and alleviate and delay the development of renal bone diseases [9].

Blood urea nitrogen (BUN) is the end product of protein catabolism, and more than 90% of urea is eliminated via the kidneys [10]. When various pathological changes occur in the kidney, destroying the normal excretion function, the BUN concentration increases. Hence, the concentration of BUN in the blood is an important index of optimal renal function.

However, the evaluation accuracy of a single indicator is not as high as that of a multi-index joint evaluation. This study aimed to explore a better triple combination of diagnostic markers for the early diagnosis of CKD-MBD.

MATERIALS AND METHODS

Study Design and Participants

As this was a cross-sectional study involving CKD patients, data were collected from patient records between September 1, 2016, and June 30, 2021. The study was based on medical record data obtained from the case information system containing demographic and laboratory data in an electronic medical record (EMR) database. Because data were de-identified, institutional review board review was not required.

For CKD patients, the inclusion criteria were: 1) patients diagnosed with CKD> 3 months; 2) patients diagnosed with CKD-MBD. Exclusion criteria included: 1) patients having other diseases which might influence related indexes of renal function, for example, diabetes, cirrhosis, etc.; 2) patients having incomplete clinical data; 3) patients<18 years old. After enrollment, we reviewed the medical records for demographic and clinical data as well as laboratory data, following which the patients were divided into two groups for CKD-MBD.

Collection of Clinical Parameters

The patients' medical records were retrospectively reviewed. Patient demographic information, medical history, and laboratory data were obtained. The indicators consisted of blood lipids, antibody and electrolyte levels, renal function indexes, and other related indicators. Blood lipid profile includes total cholesterol, triglycerides, HDL cholesterol, and LDL cholesterol. The recommended reference values of the blood lipids were: total cholesterol, 3.5–5.2 mmol/L; triglycerides, 0.4–1.6 mmol/L; HDL-cholesterol, 0.8–1.8 mmol/L; and LDL-cholesterol, 2.3–3.4 mmol/L. Antibody testing included detection of serum IgA, IgG, IgM, C3, and C4 levels. The recommended reference values of the serum antibodies were: IgA, 0.7–4 g/L; IgG, 7–16 g/L; IgM, 0.4–2.3 g/L; C3, 0.9–1.8 g/L; and C4, 0.1–0.4 g/L. Serum electrolyte testing included K^+, Na^+, Cl^-, Ca^{2+}, Mg^{2+}, and P levels. The recommended reference values of the serum electrolyte levels were: K^+, 3.5–5.5 mmol/L; Na^+, 130–150 mmol/L; Cl^-, 96–110 mmol/L; Ca^{2+}, 1.8–2.6 mmol/L; Mg^{2+}, 0.6–1.2 mmol/L; P, 0.7–1.5 mmol/L. Renal function indexes included β2-microglobulin, uric acid, urea nitrogen, cystatin-C, serum creatinine, and eGFR levels. The eGFR level was estimated according to the CKD-EPI equation. In the present study, the CKD staging was done according to eGFR levels. If eGFR was >90 mL/min/1.73 m², it denoted CKD stage

1; If eGFR was 60~89 mL/min/1.73 m², CKD stage was 2; If eGFR was 30~59 mL/min/1.73 m², CKD stage was 3; If eGFR was 15~29 mL/min/1.73 m², CKD stage was 4; If eGFR was<15 mL/min/1.73 m², CKD stage was 5. The recommended reference values of the other renal function indexes were as follows: blood β2-microglobulin, 0.8–2.4 mg/L; uric acid, 150–410 μmol/L; BUN, 2.3–7 mmol/L; serum cystatin-C, 0.65–1.09 mg/L; serum creatinine, 50–132 μmol/L. Another important CKD-MBD-related indicator is blood parathyroid hormone (PTH). Its recommended reference value was 12–88 pg/mL.

Statistical Analysis

Continuous data were expressed as mean ±standard deviation (SD) or median and interquartile range (IQR), according to variable distribution, while categorical variables were expressed as frequencies and percentages. Student's t-test, Mann-Whitney, and Chi-square tests were used to compare differences between the groups. Receiver operating characteristic curves (ROC) were developed to evaluate the biomarkers predictive of CKD-MBD. An area under the curve (AUC) was considered good if > 0.7 and excellent if > 0.8. Data analysis was carried out with SPSS version 25 (IBM SPSS Statistics, Chicago, IL, USA). p-values< 0.05 were considered statistically significant.

RESULTS

Patient Selection

During the study period, a total of 3124 patients with CKD were initially selected, of which 1766 had diabetes, 302 had cardiac diseases, 26 had cirrhosis, 151 had incomplete data, and 15< 18 years old. After criteria fulfillment, 864 patients having complete data were included in this analysis. Patients were divided into two groups according to the

diagnosis of CKD-MBD. There were 716 (82.9%) and 148 (17.1%) patients in the CKD and CKD-MBD groups, respectively (Figure 1).

Demographic Information

The demographic characteristics of the patients are shown in Table 1. A total of 864 patients, including 512 men and 352 women, participated in this analysis. The mean age of all patients was 56.37 ±15.50 (range, 19~93) years, while the age in the CKD and CKD-MBD groups were 56.00 ±15.54 years and 58.14 ±15.26 years, respectively. As shown in Table 1, there were no significant differences in age, gender, current smoking, and alcohol drinking between the two groups (all $p>0.05$). BMI was significantly higher in the CKD group ($p<0.05$). In the CKD group, stage 3, 4, and 5 patients were 26%, 32.8%, and 35.2%, respectively. But in the CKD-MBD group, stage 5 accounted for 80.4% of all patients. This result shows that CKD-MBD mostly occurs in stage 5 of CKD (Table 1).

Patients' Clinical Characteristics

Table 2 shows the comparison of clinical and laboratory data between the two groups. Our results showed that there was a significant difference in triglyceride levels between the two groups; the triglyceride level in the CKD group [1.56(1.16~2.24)] was slightly higher than the triglyceride level in the CKD-MBD group [1.47(1.03~2.01)]. But from the perspective of classification indicators, no difference has been found in the distribution between the two groups. On the contrary, the HDL distribution was statistically significant between the two groups; in the CKD-MBD and CKD groups, the proportion was 10.1% and 3.1%, respectively. The antibody indicator analysis revealed that the IgA and C4 levels were lower in the CKD-MBD group, and the differences

between the two groups were statistically significant, but in terms of category distribution, no difference was found between the groups. The distribution of C3 levels between the two groups was statistically significant both in terms of continuous variables and category distribution. The level of C3 in the CKD-MBD group was relatively low [0.82(0.76~0.97)] and accounted for a 62.8% distribution. In terms of electrolyte levels, except for K^+ ions, there were significant differences observed in Na^+, Cl^-, Ca^{2+}, Mg^{2+}, and P ion levels between the two groups. Compared with the CKD group, the levels of Na^+ [138.06(135.53~140.21)], Cl^- [104.42(100.38~108.08)] and Ca^{2+} [2.09(1.94~2.19)] ions in CKD-MBD group were lower, while the levels of Mg^{2+} [1.03(0.90~1.16)] and P [1.68(1.45~2.05)] ions were higher in the CKD-MBD group. From the aspect of antibody indicators, the results show that the β2-microglobulin and Cystatin-C levels were higher in the CKD-MBD group, and the difference between the two groups was statistically significant, but in terms of category distribution, no difference was found between the two groups. Compared with the CKD group, the uric acid level of the CKD-MBD group was lower while the BUN level was higher, which was statistically significant. The serum creatinine level in the CKD-MBD group increased, and the eGFR level was reduced, but there was no significant change in urinary protein between the two groups when compared with the CKD group. It was revealed that PTH increased significantly in the CKD-MBD group; its levels in the "high" category of both the CKD-MBD and CKD groups accounted for 85.8% and 55.7%, respectively. Through the comparison of different clinical indicators between the two groups, we explored the efficacy of a few sensitive indicators for the early detection of CKD-MBD.

Comparison of the Diagnostic Efficacy of relevant indicators in the Diagnosis of CKD-MBD

Table 3 results showed that the diagnostic value of indicators related to renal function is relatively high. Among these indicators, the five indicators with the largest ROC: eGFR, β2-MG, PTH, Scr, and BUN, were selected. However, the AUC of these indicators is about 0.7. Therefore, considering the combined diagnosis of three indicators, the diagnostic value may be higher.

Table 4 displays that the AUC of the combined detection of β2-MG +PTH+BUN is significantly higher than those of their single detection and other combinations (AUC 0.804, 95% CI 0.767 to 0.841, $p< 0.05$).

DISCUSSION

Chronic kidney disease-mineral and bone metabolic disorder (CKD-MBD), caused by disturbed calcium and phosphate homeostasis, is a major complication of CKD [11]. Although the current medical scenario has greatly improved, the early detection rate of CKD-MBD is still very low. In general, a timely and accurate diagnosis helps in improving patient prognosis. Findings of CKD-MBD in CKD are very common in clinical practice and are mostly attributed to a disorder of calcium and phosphorus metabolism caused by renal failure [12]. The late onset of CKD-MBD symptoms and the absence of noninvasive diagnostic indicators may lead to delayed diagnosis and obstruct prompt therapeutic interventions. Therefore, there is an urgent need for precise clinical approaches that can timely and accurately diagnose CKD-MBD [13]. This study aimed to explore the efficacy of a triple combination of β2-MG, PTH, and BUN as early biomarkers for CKD-MBD.

In order to observe the differences in these related indexes between the two groups, we used single-factor analysis to compare these indexes. The results showed significant differences between the two groups in terms of clinical laboratory indicators, which might

provide clues for finding sensitive indicators of early diagnosis of CKD-MBD. We then used ROC curve analysis to examine the role of these indexes in the clinical prediction of CKD-MBD. There were five indexes with an AUC of 0.710. The AUC of β2-MG, BNU, Scr, eGFR, and PTH was 0.763, 0.722, 0.747, 0.767, and 0.749, respectively. Therefore, these five indexes might become potential biomarkers for the early diagnosis of CKD-MBD. Furthermore, joint index diagnoses were used to improve the diagnostic value. We constructed 10 combinations and selected the triple combination with the highest AUC: β2-MG+PTH+BUN combination.

Studies have shown that calcium and phosphorus metabolism disorders and hyperparathyroidism can promote the occurrence of cardiovascular diseases and increase the mortality of patients [14]. PTH plays an important role in the regulation of calcium and phosphorus levels. For example, high blood phosphorus and low blood calcium can promote parathyroid hyperplasia and increase parathyroid hormone synthesis and secretion [15]. Moreover, PTH also plays a crucial part in regulating bone cell remodeling. A sequential increase in PTH will stimulate osteoblast receptors, increase the number and activity of osteoclasts, increase bone resorption, initiate the formation of bone cavities and new bones, and finally lead to secondary bone diseases [16]. Therefore, PTH plays an important role in the functional mechanism of CKD-MBD. If PTH is monitored early, necessary measures might be taken on time to delay the occurrence of renal bone diseases.

β2-MG is a light chain protein of type I histocompatibility antigen on the membrane of all nucleated cells in the body [17]. It is mainly produced by lymphocytes, and its molecular weight is about 11.8 kD. Due to the small molecular weight and inability to bind with plasma protein, normal β2-MG can be freely filtered through the glomerulus.

About 99.9% of β2-MG is reabsorbed in the renal proximal convoluted tubule, decomposed, and destroyed in the renal tubular epithelial cells, while only 0.1% is excreted from the body by urination [18]. The production rate of β2-MG was constant in vivo, and the β2-MG level in plasma was not affected by age, sex, the number of muscle tissues, or other factors. Therefore, measuring the level of β2-MG in plasma is more sensitive than measuring the level of serum creatinine to evaluate renal function. Hence, β2-MG can be used as an early indicator to reflect renal damage as increased β2-MG levels in plasma can reflect the damage to glomerular filtration function or the increase of filtration load [19]. The determination of β2-MG level in plasma can also provide valuable data for the differential diagnosis, disease progression, and prognosis of renal diseases.

Urea nitrogen refers to "blood urea nitrogen" [20]. It is a nitrogen-containing compound in plasma other than proteins. It is the main end product of human protein metabolism and is filtered out of the body from the glomerulus [20]. Urea can be reabsorbed in all segments of tubules after glomerular filtration, but the faster the flow rate of urine in the tubules, the lesser the reabsorption; that is, the maximum clearance rate is reached easily [21]. BUN can be in the normal range in the early stages of renal function damage. When the eGFR drops below 50% of the normal value, the concentration of BUN increases rapidly; as the renal insufficiency is decompensated, BUN sequentially increases. Therefore, it is regarded as an index to judge glomerular filtration function in clinical practice [22]. When BUN is used alone for predicting CKD-MBD, the accuracy and predictive value becomes low and require the help of other indicators to make a joint judgment.

We analyzed the predictive value of β2-MG, PTH, and BUN in the prognosis of CKD-

MBD. The results show that the sensitivity, specificity, and accuracy of the β2-MG+PTH+BUN combination are higher than the individual diagnostic capacity of these markers. Our results indicate that the combined detection of β2-MG, PTH, and BUN is of higher value in predicting the prognosis of CKD-MBD. No other study has attempted to analyze the diagnosis value of β2-MG, PTH, and BUN combination in the diagnosis of CKD-MBD. Our study is the first one to suggest that the combined detection of β2-MG+PTH+BUN has a high diagnosis value for CKD-MBD, which will be helpful in the near future for accurate clinical diagnosis of pediatric CKD-MBD.

Ethics approval and consent to participate

This is an retrospective study. The study complies with all regulations and confirmation that informed consent was obtained. Before we conducted the study, the ethics committee gave its consent, confirming that the experiments were conducted in accordance with established ethical guidelines and that informed consent was obtained from the participants. The full name of the ethics committee: The Shanxi Provincial People's Hospital Medical Research Ethics Committee. The ethics approval number: ((2023) Provincial Medical Science and Technology Lun Audit No. 150) .

Consent for publication

All authors have read and approved the content, and agree to submit it for consideration for publication in your journal.

Availability of data and materials

The data presented in this study are available on request from the corresponding author.

Competing interests

All authors state that they have no conflicts of interest.

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Author Contributions

Z.Z., Y.G., B.H.: Conceptualization, Methodology, Software; Z.Z., Y.G., L.F., Z.W., D.Y., Z.T.Z., W.S.: Data curation, Writing- Original draft preparation. Z.Z., Y.G., Z.T.Z., Y.Z., R.L.: Visualization, Investigation; Y.Z., R.L., B.H.: Supervision; Y.G., B.H.: Software, Validation; B.H.: Writing-Reviewing and Editing.

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Table 1. Comparison of baseline information between the two groups (n = 864)

Variable	Total (n = 864)	CKD group (n = 716)	CKD-MBD group (n = 148)	*p*
Age, years	56.37 ±15.50	56.00 ±15.54	58.14 ±15.26	0.128
Male, n (%)	512(59.3)	434(60.6)	78(52.7)	0.075
Female, n (%)	352(40.7)	282(39.4)	70(47.3)	
Current smoking, n (%)				
Yes	247(28.6)	214(29.9)	33(22.3)	0.063
No	617(71.4)	502(70.1)	115(77.7)	
Alcohol drinking, n (%)				
Yes	169(19.6)	148(20.7)	21(14.2)	0.070
No	695(80.4)	568(79.3)	127(85.8)	
Body mass index, kg/m^2	24.49 ±3.90	24.69 ±3.94	23.52 ±3.54	**0.001**
CKD stage				
1	10(1.3)	8(1.1)	2(1.4)	
2	39(4.5)	35(4.9)	4(2.7)	
3	189(21.9)	186(26.0)	3(2.0)	**<0.001**
4	255(29.5)	235(32.8)	20(13.5)	
5	371(42.8)	252(35.2)	119(80.4)	

Table 2. Comparison of laboratory data between the two groups (n = 864)

Variable	Total (n = 864)	CKD group (n = 716)	CKD-MBD group (n = 148)	*p*
Cholesterol, mmol/L	4.68 ±1.63	4.72 ±1.63	4.52 ±1.64	0.175
target	412(47.7)	339(47.3)	73(49.3)	
low	198(22.9)	157(22.0)	41(27.7)	0.111
high	254(29.4)	220(30.7)	34(23.0)	
Triglycerides, mmol/L	4.50(3.62~5.42)	1.56(1.16~2.24)	1.47(1.03~2.01)	**0.047**
target	454(52.5)	370(51.7)	84(56.8)	0.260
high	410(47.5)	346(48.3)	64(43.2)	
HDL cholesterol, mmol/L	1.12 ±0.39	1.10 ±0.33	1.19 ±0.58	0.073
target	695(80.4)	580(81.0)	115(77.7)	
low	132(15.3)	114(15.9)	18(12.2)	**<0.001**
high	37(4.3)	22(3.1)	15(10.1)	
LDL cholesterol, mmol/L	2.98 ±1.21	2.99 ±1.14	2.93 ±1.47	0.658
target	374(43.3)	314(43.9)	60(40.5)	
low	250(28.9)	199(27.8)	51(34.5)	0.260
high	240(27.8)	203(28.4)	37(25.0)	
IgA, g/L	2.37(1.77~3.11)	2.39(1.81~3.16)	2.24(1.55~2.66)	**0.046**
target	777(89.9)	642(89.7)	135(91.2)	
low	12(1.4)	11(1.5)	1(0.7)	0.824
high	75(8.7)	63(8.8)	12(8.1)	
IgG, g/L	10.50(8.04~12.70)	10.50(8.16~12.75)	10.80(7.95~12.60)	0.463
target	672(77.8)	555(77.5)	117(79.1)	
low	136(15.7)	109(15.2)	27(18.2)	0.096
high	56(6.5)	52(7.3)	4(2.7)	
IgM, g/L	0.74(0.49~1.08)	0.74(0.50~1.06)	0.75(0.46~1.13)	0.973
target	754(87.3)	624(87.2)	130(87.8)	0.819
low	110(12.7)	92(12.8)	18(12.2)	
C3, g/L	0.93(0.80~1.05)	0.94(0.81~1.07)	0.82(0.76~0.97)	**<0.001**
target	484(56.0)	429(59.9)	55(37.2)	**<0.001**
low	380(44.0)	287(40.1)	93(62.8)	
C4, g/L	0.27(0.23~0.32)	0.27(0.23~0.33)	0.26(0.23~0.28)	**0.003**
target	783(90.6)	643(89.8)	140(94.6)	0.069
high	81(9.4)	73(10.2)	8(5.4)	

Potassium(K, mmol/L)	4.35(3.94~4.81)	4.33(3.94~4.78)	4.48(3.94~4.97)	0.066
target	722(83.6)	603(84.2)	119(80.4)	
low	80(9.3)	67(9.4)	13(8.8)	0.170
high	62(7.1)	46(6.4)	16(10.8)	
Sodium(Na, mmol/L)	139.33(137.38~140.95)	139.46(137.71~141.00)	138.06(135.53~140.21)	**<0.001**
target	838(97.0)	697(97.3)	141(95.3)	0.178
low	26(3.0)	19(2.7)	7(4.7)	
Chlorine(Cl, mmol/L)	107.22(105.05~110.70)	107.76(104.76~110.98)	104.42(100.38~108.08)	**<0.001**
target	581(67.2)	464(64.8)	117(79.1)	
low	30(3.5)	22(3.1)	8(5.4)	**<0.001**
high	253(29.3)	230(32.1)	23(15.5)	
Calcium(Ca, mmol/L)	2.12(2.00~2.26)	2.13(2.01~2.26)	2.09(1.94~2.19)	**0.001**
target	793(91.8)	666(93.0)	127(85.8)	
low	59(6.8)	42(5.9)	17(11.5)	**0.014**
high	12(1.4)	8(1.1)	4(2.7)	
Magnesium(Mg, mmol/L)	0.95(0.86~1.06)	0.94(0.86~1.04)	1.03(0.90~1.16)	**<0.001**
target	777(89.9)	660(92.2)	117(79.1)	**<0.001**
high	87(10.1)	56(7.8)	31(20.9)	
Phosphate(P, mmol/L)	1.40(1.18~1.69)	1.36(1.16~1.63)	1.68(1.45~2.05)	**<0.001**
target	501(58.0)	458(64.0)	43(29.1)	**<0.001**
high	363(42.0)	258(36.0)	105(70.9)	
β2-microglobulin, mg/mL	9.43(5.64~13.61)	8.49(5.22~12.20)	13.99(10.57~20.38)	**<0.001**
target	27(3.1)	24(3.4)	3(2.0)	0.603
high	837(96.9)	692(96.6)	145(98.0)	
Uric Acid, μmol/L	416.29(338.19~500.35)	420.63(346.60~508.76)	385.80(300.88~463.09)	**0.001**
target	412(47.7)	331(46.2)	81(54.7)	
low	9(1.0)	7(1.0)	2(1.4)	0.113
high	443(51.3)	378(52.8)	65(43.9)	
Blood urea nitrogen, mmol/L	16.10(10.78~22.60)	14.76(10.15~21.24)	21.60(16.89~31.03)	**<0.001**
target	75(8.7)	72(10.1)	3(2.0)	**0.002**
high	789(91.3)	644(89.9)	145(98.0)	
Cystatin C, mg/L	3.58(2.48~4.60)	3.34(2.34~4.33)	4.51(3.59~5.32)	**<0.001**
target	10(1.2)	10(1.4)	0(0)	0.226
high	854(98.8)	706(98.6)	148(100)	
Serum creatinine, μmol/L	331.06(179.29~510.70)	291.70(164.24~470.78)	495.52(369.62~734.34)	**<0.001**

Urinary protein, g/24 h	3.17 ±3.17	3.16 ±3.19	3.25 ±3.10	0.751
eGFR, mL/min/1.73 m^2	14.94(8.72~32.29)	18(10.02~35.39)	8.11(5.90~11.61)	**<0.001**
Parathyroid hormone, pg/mL	120.95(61.60~234.65)	102.70(55.85~203.30)	230.25(110.15~367.20)	**<0.001**
target	322(37.3)	306(42.7)	16(10.8)	
low	16(1.9)	11(1.5)	5(3.4)	**<0.001**
high	526(60.9)	399(55.7)	127(85.8)	

Table 3. Comparison of the diagnostic efficacy of single related indexes in the diagnosis of CKD-MBD

Variable	AUC	95%CI
TG*	0.552	0.500~0.603
IgA*	0.552	0.501~0.603
C3*	0.642	0.596~0.688
C4*	0.578	0.534~0.623
Na *	0.618	0.566~0.671
Cl *	0.671	0.622~0.720
Ca*	0.587	0.535~0.639
Mg	0.622	0.571~0.674
P	0.706	0.661~0.752
β2-MG	**0.763**	0.720~0.806
Uc*	0.586	0.534~0.638
BUN	**0.722**	0.678~0.766
Cys-C	0.702	0.657~0.747
Scr	**0.747**	0.705~0.788
eGFR*	**0.767**	0.728~0.807
PTH	**0.749**	0.703~0.801

BMI, body mass index; TG, triglycerides; Na, sodium; Cl, chlorine; Ca, calcium; Mg, magnesium; P, phosphate; β2-MG, β2-microglobulin; Uc, uric acid; BUN, blood urea nitrogen; Cys-C, cystatin c; Scr, serum creatinine; PTH, parathyroid hormone.

Table 4. Comparison of the diagnostic efficacy of the triple combination of related indexes in the diagnosis of CKD-MBD

Variable	AUC	95%CI
eGFR+β2-MG+PTH	0.796	0.758~0.834
eGFR+β2-MG+Scr	0.793	0.755~0.832
eGFR+β2-MG+BUN	0.800	0.762~0.837
eGFR+PTH+Scr	0.774	0.735~0.814
eGFR+PTH+BUN	0.777	0.738~0.816
eGFR+Scr+BUN	0.763	0.724~0.803
β2-MG+PTH+Scr	0.803	0.766~0.839
β2-MG+PTH+BUN	**0.804**	**0.767~0.841**
β2-MG+Scr+BUN	0.800	0.763~0.837
PTH+Scr+BUN	0.766	0.726~0.807

BUN, blood urea nitrogen; Cys-C, cystatin c; Scr, serum creatinine; PTH, parathyroid hormone; β2-MG, β2-microglobulin; eGFR, estimated glomerular filtration rate.

FIGURE LEGEND

Figure 1. Flow chart of patient selection and inclusion.

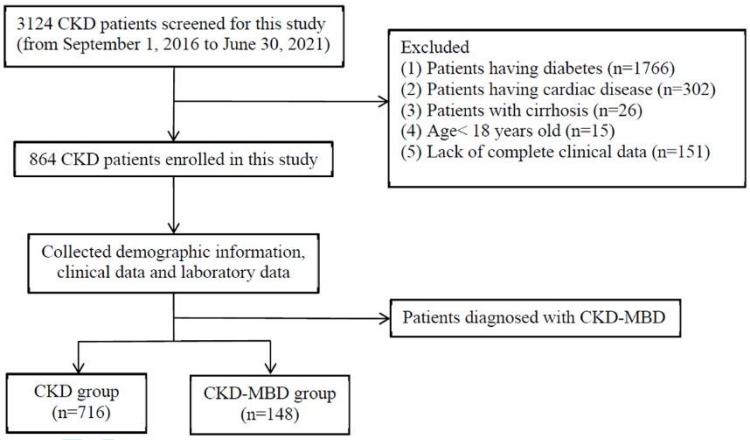

Figure 1. Flow chart of patient selection and inclusion.

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A new triple therapy for the diagnosis of CKD-MBD: A Cross-sectional Study

Running title: Diagnostic Value in CKD-MBD

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Abstract

Objectives: To seek a triple combination of biomarkers for early diagnosis of Chronic kidney disease-mineral and bone metabolic disorder and to explore the diagnostic efficacy of β2-microglobulin, parathyroid hormone, and blood urea nitrogen in Chronic kidney disease-mineral and bone metabolic disorder.

Participants: We collected medical records of 864 patients with chronic kidney disease (without direct contact with patients) and divided them into two groups based on the renal bone disease manifestations of all patients.

Primary and secondary outcome measures: There were 148 and 716 subjects in the Chronic kidney disease-mineral and bone metabolic disorder and the control groups, respectively. The aggregated data included basic information and various clinical laboratory indicators, such as blood lipid profile, antibody, and electrolyte levels, along with renal function-related indicators.

Results: It was observed that most renal osteopathy occurs in the later stages of Chronic kidney disease. In a comparison of clinical laboratory indicators between the two groups, 16 factors were selected for curve analysis, and the was compared. We discovered that factors with high diagnostic values were β2-microglobulin, parathyroid hormone and blood urea nitrogen.

Conclusions: The triple combination of β2-microglobulin +parathyroid hormone+blood urea nitrogen indicators can play the crucial role of a sensitive indicator for the early diagnosis of Chronic kidney disease-mineral and bone metabolic disorder and in preventing or delaying the progress of Chronic kidney disease-mineral and bone

metabolic disorder.

Keywords: Blood urea nitrogen; β2-microglobulin; CKD-MBD; Parathyroid hormone; Urea nitrogen

Strengths and limitations of this study:

This is a cross-sectional study aimed at identifying detection methods for CKD-MBD.

This study screened out other different detection criteria based on routine analysis data.

This study is limited and can only be analyzed based on existing medical record information. More medical record information needs to be added in the future to increase follow-up.

Introduction

Chronic kidney disease-mineral and bone metabolic disorder (CKD-MBD) is one of the common serious complications in patients with chronic kidney diseases (CKD) [1]. The incidence rate of MBD increases with the progression of CKD; more than 80% of MBD patients suffer from end-stage renal disease (ESRD) [2]. Recent epidemiologic studies have indicated that abnormalities in calcium and phosphorus serum levels and secondary hyperparathyroidism lead to the risk of death in maintenance dialysis patients [3]. Early detection of CKD-MBD can provide timely treatment as well as greatly improve their quality of life [4]. Therefore, prompt screening of CKD-MBD plays a very important role in the improvement of long-term prognosis and quality of life in such patients.

At present, bone biopsy remains the gold standard for diagnosing CKD-MBD. Since bone biopsy is invasive and causes trauma, it is not used as a routine examination method in clinical practice. Henceforth, medical staff must determine the need for conducting a bone biopsy for CKD stage 3–5 patients according to their health conditions [2]. Therefore, it is an urgent need to find the diagnostic and treatment markers of CKD-MBD for improved patient outcomes.

β2-microglobulin (β2-MG) is a small molecular weight protein that is clinically significant in the examination of kidney diseases [5]. If the blood plasma β2-MG level is increased, it indicates a reduction in the glomerular filtration function [6]. It is highly sensitive to serum creatinine, which can detect the decrease of glomerular filtration rate in the early stage. However, the diagnostic value of β2-MG is limited.

It is observed that parathyroid hormone (PTH) is closely related to the occurrence and development of CKD-MBD [7]. The main function of PTH is to regulate the metabolism of calcium and phosphorus in the body, relying on the actions of the main target organs: bone and kidney [8]. It mobilizes bone calcium into the blood, promotes the reabsorption of calcium ions by renal tubules and the excretion of phosphate, increases the blood calcium concentration, and decreases the blood phosphorus concentration. If PTH is detected early and found to be abnormal, timely correction may be able to control calcium and phosphorus metabolism disorders and alleviate and delay the development of renal bone diseases [9].

Blood urea nitrogen (BUN) is the end product of protein catabolism, and more than 90% of urea is eliminated via the kidneys [10]. When various pathological changes occur in the kidney, destroying the normal excretion function, the BUN concentration increases. Hence, the concentration of BUN in the blood is an important index of optimal renal function.

However, the evaluation accuracy of a single indicator is not as high as that of a multi-index joint evaluation. This study aimed to explore a better triple combination of diagnostic markers for the early diagnosis of CKD-MBD.

Materials and Methods

Study Design and Participants

As this was a cross-sectional study involving CKD patients, data were collected from patient records between September 1, 2016, and June 30, 2021. The study was based on medical record data obtained from the case information system containing demographic and laboratory data in an electronic medical record (EMR) database. Because data were de-identified, institutional review board review was not required.

For CKD patients, the inclusion criteria were: 1) patients diagnosed with CKD> 3 months; 2) patients diagnosed with CKD-MBD. Exclusion criteria included: 1) patients having other diseases which might influence related indexes of renal function, for example, diabetes, cirrhosis, etc.; 2) patients having incomplete clinical data; 3) patients<18 years old. After enrollment, we reviewed the medical records for demographic and clinical data as well as laboratory data, following which the patients were divided into two groups for CKD-MBD.

Collection of Clinical Parameters

The patients' medical records were retrospectively reviewed. Patient demographic information, medical history, and laboratory data were obtained. The indicators consisted of blood lipids, antibody and electrolyte levels, renal function indexes, and other related indicators. Blood lipid profile includes total cholesterol, triglycerides, HDL cholesterol, and LDL cholesterol. The recommended reference values of the blood lipids were: total cholesterol, 3.5–5.2 mmol/L; triglycerides, 0.4–1.6 mmol/L; HDL-cholesterol, 0.8–1.8 mmol/L; and LDL-cholesterol, 2.3–3.4 mmol/L. Antibody testing

included detection of serum IgA, IgG, IgM, C3, and C4 levels. The recommended reference values of the serum antibodies were: IgA, 0.7–4 g/L; IgG, 7–16 g/L; IgM, 0.4–2.3 g/L; C3, 0.9–1.8 g/L; and C4, 0.1–0.4 g/L. Serum electrolyte testing included K^+, Na^+, Cl^-, Ca^{2+}, Mg^{2+}, and P levels. The recommended reference values of the serum electrolyte levels were: K^+, 3.5–5.5 mmol/L; Na^+, 130–150 mmol/L; Cl^-, 96–110 mmol/L; Ca^{2+}, 1.8–2.6 mmol/L; Mg^{2+}, 0.6–1.2 mmol/L; P, 0.7–1.5 mmol/L. Renal function indexes included β2-microglobulin, uric acid, urea nitrogen, cystatin-C, serum creatinine, and eGFR levels. The eGFR level was estimated according to the CKD-EPI equation. In the present study, the CKD staging was done according to eGFR levels. If eGFR was >90 mL/min/1.73 m², it denoted CKD stage 1; If eGFR was 60~89 mL/min/1.73 m², CKD stage was 2; If eGFR was 30~59 mL/min/1.73 m², CKD stage was 3; If eGFR was 15~29 mL/min/1.73 m², CKD stage was 4; If eGFR was<15 mL/min/1.73 m², CKD stage was 5. The recommended reference values of the other renal function indexes were as follows: blood β2-microglobulin, 0.8–2.4 mg/L; uric acid, 150–410 μmol/L; BUN, 2.3–7 mmol/L; serum cystatin-C, 0.65–1.09 mg/L; serum creatinine, 50–132 μmol/L. Another important CKD-MBD-related indicator is blood parathyroid hormone (PTH). Its recommended reference value was 12–88 pg/mL.

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Statistical Analysis

Continuous data were expressed as mean ±standard deviation (SD) or median and

interquartile range (IQR), according to variable distribution, while categorical variables were expressed as frequencies and percentages. Student's t-test, Mann-Whitney, and Chi-square tests were used to compare differences between the groups. Receiver operating characteristic curves (ROC) were developed to evaluate the biomarkers predictive of CKD-MBD. An area under the curve (AUC) was considered good if > 0.7 and excellent if > 0.8. Data analysis was carried out with SPSS version 25 (IBM SPSS Statistics, Chicago, IL, USA). p-values< 0.05 were considered statistically significant.

Results

Patient Selection

During the study period, a total of 3124 patients with CKD were initially selected, of which 1766 had diabetes, 302 had cardiac diseases, 26 had cirrhosis, 151 had incomplete data, and 15< 18 years old. After criteria fulfillment, 864 patients having complete data were included in this analysis. Patients were divided into two groups according to the diagnosis of CKD-MBD. There were 716 (82.9%) and 148 (17.1%) patients in the CKD and CKD-MBD groups, respectively (Figure 1).

Demographic Information

The demographic characteristics of the patients are shown in Table 1. A total of 864 patients, including 512 men and 352 women, participated in this analysis. The mean age of all patients was 56.37 ±15.50 (range, 19~93) years, while the age in the CKD and CKD-MBD groups were 56.00 ±15.54 years and 58.14 ±15.26 years, respectively. As shown in Table 1, there were no significant differences in age, gender, current smoking, and alcohol drinking between the two groups (all p>0.05). BMI was significantly higher in the CKD group (p<0.05). In the CKD group, stage 3, 4, and 5 patients were 26%, 32.8%, and 35.2%, respectively. But in the CKD-MBD group, stage 5 accounted for 80.4% of all patients. This result shows that CKD-MBD mostly occurs in stage 5 of CKD (Table 1).

Patients' Clinical Characteristics

S-Table 1 shows the comparison of clinical and laboratory data between the two groups. Our results showed that there was a significant difference in triglyceride levels between

the two groups; the triglyceride level in the CKD group [1.56(1.16~2.24)] was slightly higher than the triglyceride level in the CKD-MBD group [1.47(1.03~2.01)]. But from the perspective of classification indicators, no difference has been found in the distribution between the two groups. On the contrary, the HDL distribution was statistically significant between the two groups; in the CKD-MBD and CKD groups, the proportion was 10.1% and 3.1%, respectively. The antibody indicator analysis revealed that the IgA and C4 levels were lower in the CKD-MBD group, and the differences between the two groups were statistically significant, but in terms of category distribution, no difference was found between the groups. The distribution of C3 levels between the two groups was statistically significant both in terms of continuous variables and category distribution. The level of C3 in the CKD-MBD group was relatively low [0.82(0.76~0.97)] and accounted for a 62.8% distribution. In terms of electrolyte levels, except for K^+ ions, there were significant differences observed in Na^+, Cl^-, Ca^{2+}, Mg^{2+}, and P ion levels between the two groups. Compared with the CKD group, the levels of Na^+ [138.06(135.53~140.21)], Cl^- [104.42(100.38~108.08)] and Ca^{2+} [2.09(1.94~2.19)] ions in CKD-MBD group were lower, while the levels of Mg^{2+} [1.03(0.90~1.16)] and P [1.68(1.45~2.05)] ions were higher in the CKD-MBD group. From the aspect of antibody indicators, the results show that the β2-microglobulin and Cystatin-C levels were higher in the CKD-MBD group, and the difference between the two groups was statistically significant, but in terms of category distribution, no difference was found between the two groups. Compared with the CKD group, the uric acid level of the CKD-MBD group was lower while the BUN level was higher, which

was statistically significant. The serum creatinine level in the CKD-MBD group increased, and the eGFR level was reduced, but there was no significant change in urinary protein between the two groups when compared with the CKD group. It was revealed that PTH increased significantly in the CKD-MBD group; its levels in the "high" category of both the CKD-MBD and CKD groups accounted for 85.8% and 55.7%, respectively. Through the comparison of different clinical indicators between the two groups, we explored the efficacy of a few sensitive indicators for the early detection of CKD-MBD.

Comparison of the Diagnostic Efficacy of relevant indicators in the Diagnosis of CKD-MBD

Table 2 results showed that the diagnostic value of indicators related to renal function is relatively high. Among these indicators, the five indicators with the largest ROC: eGFR, β2-MG, PTH, Scr, and BUN, were selected. However, the AUC of these indicators is about 0.7. Therefore, considering the combined diagnosis of three indicators, the diagnostic value may be higher.

S-Table 2 displays that the AUC of the combined detection of β2-MG +PTH+BUN is significantly higher than those of their single detection and other combinations (AUC 0.804, 95% CI 0.767 to 0.841, $p< 0.05$).

Discussion

Chronic kidney disease-mineral and bone metabolic disorder (CKD-MBD), caused by disturbed calcium and phosphate homeostasis, is a major complication of CKD [11]. Although the current medical scenario has greatly improved, the early detection rate of CKD-MBD is still very low. In general, a timely and accurate diagnosis helps in improving patient prognosis. Findings of CKD-MBD in CKD are very common in clinical practice and are mostly attributed to a disorder of calcium and phosphorus metabolism caused by renal failure [12]. The late onset of CKD-MBD symptoms and the absence of noninvasive diagnostic indicators may lead to delayed diagnosis and obstruct prompt therapeutic interventions. Therefore, there is an urgent need for precise clinical approaches that can timely and accurately diagnose CKD-MBD [13]. This study aimed to explore the efficacy of a triple combination of β2-MG, PTH, and BUN as early biomarkers for CKD-MBD.

In order to observe the differences in these related indexes between the two groups, we used single-factor analysis to compare these indexes. The results showed significant differences between the two groups in terms of clinical laboratory indicators, which might provide clues for finding sensitive indicators of early diagnosis of CKD-MBD. We then used ROC curve analysis to examine the role of these indexes in the clinical prediction of CKD-MBD. There were five indexes with an AUC of 0.710. The AUC of β2-MG, BNU, Scr, eGFR, and PTH was 0.763, 0.722, 0.747, 0.767, and 0.749, respectively. Therefore, these five indexes might become potential biomarkers for the early diagnosis of CKD-MBD. Furthermore, joint index diagnoses were used to

improve the diagnostic value. We constructed 10 combinations and selected the triple combination with the highest AUC: β2-MG+PTH+BUN combination.

Studies have shown that calcium and phosphorus metabolism disorders and hyperparathyroidism can promote the occurrence of cardiovascular diseases and increase the mortality of patients [14]. PTH plays an important role in the regulation of calcium and phosphorus levels. For example, high blood phosphorus and low blood calcium can promote parathyroid hyperplasia and increase parathyroid hormone synthesis and secretion [15]. Moreover, PTH also plays a crucial part in regulating bone cell remodeling. A sequential increase in PTH will stimulate osteoblast receptors, increase the number and activity of osteoclasts, increase bone resorption, initiate the formation of bone cavities and new bones, and finally lead to secondary bone diseases [16]. Therefore, PTH plays an important role in the functional mechanism of CKD-MBD. If PTH is monitored early, necessary measures might be taken on time to delay the occurrence of renal bone diseases.

β2-MG is a light chain protein of type I histocompatibility antigen on the membrane of all nucleated cells in the body [17]. It is mainly produced by lymphocytes, and its molecular weight is about 11.8 kD. Due to the small molecular weight and inability to bind with plasma protein, normal β2-MG can be freely filtered through the glomerulus. About 99.9% of β2-MG is reabsorbed in the renal proximal convoluted tubule, decomposed, and destroyed in the renal tubular epithelial cells, while only 0.1% is excreted from the body by urination [18]. The production rate of β2-MG was constant in vivo, and the β2-MG level in plasma was not affected by age, sex, the number of

muscle tissues, or other factors. Therefore, measuring the level of β2-MG in plasma is more sensitive than measuring the level of serum creatinine to evaluate renal function. Hence, β2-MG can be used as an early indicator to reflect renal damage as increased β2-MG levels in plasma can reflect the damage to glomerular filtration function or the increase of filtration load [19]. The determination of β2-MG level in plasma can also provide valuable data for the differential diagnosis, disease progression, and prognosis of renal diseases.

Urea nitrogen refers to "blood urea nitrogen" [20]. It is a nitrogen-containing compound in plasma other than proteins. It is the main end product of human protein metabolism and is filtered out of the body from the glomerulus [20]. Urea can be reabsorbed in all segments of tubules after glomerular filtration, but the faster the flow rate of urine in the tubules, the lesser the reabsorption; that is, the maximum clearance rate is reached easily [21]. BUN can be in the normal range in the early stages of renal function damage. When the eGFR drops below 50% of the normal value, the concentration of BUN increases rapidly; as the renal insufficiency is decompensated, BUN sequentially increases. Therefore, it is regarded as an index to judge glomerular filtration function in clinical practice [22]. When BUN is used alone for predicting CKD-MBD, the accuracy and predictive value becomes low and require the help of other indicators to make a joint judgment.

We analyzed the predictive value of β 2-MG, PTH, and BUN for the prognosis of CKD-MBD. The results indicate that the sensitivity, specificity, and accuracy of the β 2-MG+PTH+BUN combination are higher than the individual diagnostic ability of these

biomarkers. Our results indicate that the combined detection of β 2-MG, PTH, and BUN has higher value in predicting the prognosis of CKD-MBD. No other studies have attempted to analyze the diagnostic value of the combination of β 2-MG, PTH, and BUN for CKD-MBD. Our study indicates for the first time that the combined detection of β 2-MG+PTH+BUN has high diagnostic value for early CKD-MBD, which will help to accurately diagnose early CKD-MBD in the near future.

Ethics approval and consent to participate

This is an retrospective study, these data sourced from laboratory data in an electronic medical record (EMR) database and we are not directly in contact with patients. Before we conducted the study, the ethics committee gave its consent, confirming that the experiments were conducted in accordance with established ethical guidelines. The full name of the ethics committee: The Shanxi Provincial People's Hospital Medical Research Ethics Committee. The ethics approval number: ((2023) SYYKJL-No. 150) .

Patient and Public Involvement

None (The information we collect does not directly come from patients, but from case information.).

Consent for publication

All authors have read and approved the content, and agree to submit it for consideration for publication in your journal.

Availability of data and materials

The data presented in this study are available on request from the corresponding author.

Competing interests

All authors state that they have no conflicts of interest.

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Author Contributions

Z.Z., Y.G., B.H.: Conceptualization, Methodology, Software; Z.Z., Y.G., L.F., Z.W., D.Y., Z.T.Z., W.S.: Data curation, Writing- Original draft preparation. Z.Z., Y.G., Z.T.Z., Y.Z., R.L.: Visualization, Investigation; Y.Z., R.L., B.H.: Supervision; Y.G., B.H.: Software, Validation; B.H.: Writing-Reviewing and Editing.

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Table 1. Comparison of baseline information between the two groups (n = 864)

Variable	Total (n = 864)	CKD group (n = 716)	CKD-MBD group (n = 148)	*p*
Age, years	56.37 ±15.50	56.00 ±15.54	58.14 ±15.26	0.128
Male, n (%)	512(59.3)	434(60.6)	78(52.7)	0.075
Female, n (%)	352(40.7)	282(39.4)	70(47.3)	
Current smoking, n (%)				
Yes	247(28.6)	214(29.9)	33(22.3)	0.063
No	617(71.4)	502(70.1)	115(77.7)	
Alcohol drinking, n (%)				
Yes	169(19.6)	148(20.7)	21(14.2)	0.070
No	695(80.4)	568(79.3)	127(85.8)	
Body mass index, kg/m^2	24.49 ±3.90	24.69 ±3.94	23.52 ±3.54	**0.001**
CKD stage				
1	10(1.3)	8(1.1)	2(1.4)	
2	39(4.5)	35(4.9)	4(2.7)	
3	189(21.9)	186(26.0)	3(2.0)	**<0.001**
4	255(29.5)	235(32.8)	20(13.5)	
5	371(42.8)	252(35.2)	119(80.4)	

Table 2. Comparison of the diagnostic efficacy of single related indexes in the diagnosis of CKD-MBD

Variable	AUC	95%CI
TG*	0.552	0.500~0.603
IgA*	0.552	0.501~0.603
C3*	0.642	0.596~0.688
C4*	0.578	0.534~0.623
Na *	0.618	0.566~0.671
Cl *	0.671	0.622~0.720
Ca*	0.587	0.535~0.639
Mg	0.622	0.571~0.674
P	0.706	0.661~0.752
β2-MG	**0.763**	0.720~0.806
Uc*	0.586	0.534~0.638
BUN	**0.722**	0.678~0.766
Cys-C	0.702	0.657~0.747
Scr	**0.747**	0.705~0.788
eGFR*	**0.767**	0.728~0.807
PTH	**0.749**	0.703~0.801

BMI, body mass index; TG, triglycerides; Na, sodium; Cl, chlorine; Ca, calcium; Mg, magnesium; P, phosphate; β2-MG, β2-microglobulin; Uc, uric acid; BUN, blood urea nitrogen; Cys-C, cystatin c; Scr, serum creatinine; PTH, parathyroid hormone.

Figure legend

Figure 1. Flow chart of patient selection and inclusion.

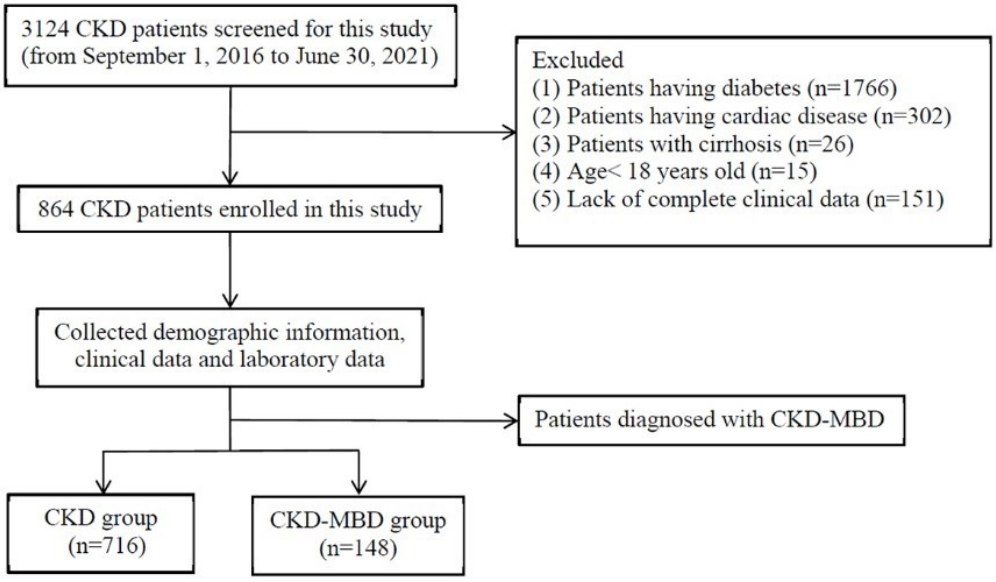

Figure 1-Flow chart of patient selection and inclusion

99x57mm (220 x 220 DPI)

A new triple therapy for the diagnosis of CKD-MBD: A Cross-sectional Study

Running title: Diagnostic Value in CKD-MBD

S-Table 1. Comparison of laboratory data between the two groups (n = 864)

Variable	Total (n = 864)	CKD group (n = 716)	CKD-MBD group (n = 148)	*p*
Cholesterol, mmol/L	4.68 ±1.63	4.72 ±1.63	4.52 ±1.64	0.175
target	412(47.7)	339(47.3)	73(49.3)	
low	198(22.9)	157(22.0)	41(27.7)	0.111
high	254(29.4)	220(30.7)	34(23.0)	
Triglycerides, mmol/L	4.50(3.62~5.42)	1.56(1.16~2.24)	1.47(1.03~2.01)	**0.047**
target	454(52.5)	370(51.7)	84(56.8)	0.260
high	410(47.5)	346(48.3)	64(43.2)	
HDL cholesterol, mmol/L	1.12 ±0.39	1.10 ±0.33	1.19 ±0.58	0.073
target	695(80.4)	580(81.0)	115(77.7)	
low	132(15.3)	114(15.9)	18(12.2)	**<0.001**
high	37(4.3)	22(3.1)	15(10.1)	
LDL cholesterol, mmol/L	2.98 ±1.21	2.99 ±1.14	2.93 ±1.47	0.658
target	374(43.3)	314(43.9)	60(40.5)	
low	250(28.9)	199(27.8)	51(34.5)	0.260
high	240(27.8)	203(28.4)	37(25.0)	
IgA, g/L	2.37(1.77~3.11)	2.39(1.81~3.16)	2.24(1.55~2.66)	**0.046**
target	777(89.9)	642(89.7)	135(91.2)	
low	12(1.4)	11(1.5)	1(0.7)	0.824
high	75(8.7)	63(8.8)	12(8.1)	
IgG, g/L	10.50(8.04~12.70)	10.50(8.16~12.75)	10.80(7.95~12.60)	0.463
target	672(77.8)	555(77.5)	117(79.1)	
low	136(15.7)	109(15.2)	27(18.2)	0.096
high	56(6.5)	52(7.3)	4(2.7)	
IgM, g/L	0.74(0.49~1.08)	0.74(0.50~1.06)	0.75(0.46~1.13)	0.973
target	754(87.3)	624(87.2)	130(87.8)	0.819
low	110(12.7)	92(12.8)	18(12.2)	
C3, g/L	0.93(0.80~1.05)	0.94(0.81~1.07)	0.82(0.76~0.97)	**<0.001**

target	484(56.0)	429(59.9)	55(37.2)	**<0.001**
low	380(44.0)	287(40.1)	93(62.8)	
C4, g/L	0.27(0.23~0.32)	0.27(0.23~0.33)	0.26(0.23~0.28)	**0.003**
target	783(90.6)	643(89.8)	140(94.6)	0.069
high	81(9.4)	73(10.2)	8(5.4)	
Potassium(K, mmol/L)	4.35(3.94~4.81)	4.33(3.94~4.78)	4.48(3.94~4.97)	0.066
target	722(83.6)	603(84.2)	119(80.4)	
low	80(9.3)	67(9.4)	13(8.8)	0.170
high	62(7.1)	46(6.4)	16(10.8)	
Sodium(Na, mmol/L)	139.33(137.38~140.95)	139.46(137.71~141.00)	138.06(135.53~140.21)	**<0.001**
target	838(97.0)	697(97.3)	141(95.3)	0.178
low	26(3.0)	19(2.7)	7(4.7)	
Chlorine(Cl, mmol/L)	107.22(105.05~110.70)	107.76(104.76~110.98)	104.42(100.38~108.08)	**<0.001**
target	581(67.2)	464(64.8)	117(79.1)	
low	30(3.5)	22(3.1)	8(5.4)	**<0.001**
high	253(29.3)	230(32.1)	23(15.5)	
Calcium(Ca, mmol/L)	2.12(2.00~2.26)	2.13(2.01~2.26)	2.09(1.94~2.19)	**0.001**
target	793(91.8)	666(93.0)	127(85.8)	
low	59(6.8)	42(5.9)	17(11.5)	**0.014**
high	12(1.4)	8(1.1)	4(2.7)	
Magnesium(Mg, mmol/L)	0.95(0.86~1.06)	0.94(0.86~1.04)	1.03(0.90~1.16)	**<0.001**
target	777(89.9)	660(92.2)	117(79.1)	**<0.001**
high	87(10.1)	56(7.8)	31(20.9)	
Phosphate(P, mmol/L)	1.40(1.18~1.69)	1.36(1.16~1.63)	1.68(1.45~2.05)	**<0.001**
target	501(58.0)	458(64.0)	43(29.1)	**<0.001**
high	363(42.0)	258(36.0)	105(70.9)	
β2-microglobulin, mg/mL	9.43(5.64~13.61)	8.49(5.22~12.20)	13.99(10.57~20.38)	**<0.001**
target	27(3.1)	24(3.4)	3(2.0)	0.603
high	837(96.9)	692(96.6)	145(98.0)	
Uric Acid, μmol/L	416.29(338.19~500.35)	420.63(346.60~508.76)	385.80(300.88~463.09)	**0.001**
target	412(47.7)	331(46.2)	81(54.7)	
low	9(1.0)	7(1.0)	2(1.4)	0.113
high	443(51.3)	378(52.8)	65(43.9)	
Blood urea nitrogen, mmol/L	16.10(10.78~22.60)	14.76(10.15~21.24)	21.60(16.89~31.03)	**<0.001**
target	75(8.7)	72(10.1)	3(2.0)	**0.002**

high	789(91.3)	644(89.9)	145(98.0)	
Cystatin C, mg/L	3.58(2.48~4.60)	3.34(2.34~4.33)	4.51(3.59~5.32)	**<0.001**
target	10(1.2)	10(1.4)	0(0)	0.226
high	854(98.8)	706(98.6)	148(100)	
Serum creatinine, μmol/L	331.06(179.29~510.70)	291.70(164.24~470.78)	495.52(369.62~734.34)	**<0.001**
Urinary protein, g/24 h	3.17 ±3.17	3.16 ±3.19	3.25 ±3.10	0.751
eGFR, mL/min/1.73 m^2	14.94(8.72~32.29)	18(10.02~35.39)	8.11(5.90~11.61)	**<0.001**
Parathyroid hormone, pg/mL	120.95(61.60~234.65)	102.70(55.85~203.30)	230.25(110.15~367.20)	**<0.001**
target	322(37.3)	306(42.7)	16(10.8)	
low	16(1.9)	11(1.5)	5(3.4)	**<0.001**
high	526(60.9)	399(55.7)	127(85.8)	

STable 2. Comparison of the diagnostic efficacy of the triple combination of related indexes in the diagnosis of CKD-MBD

Variable	AUC	95%CI
eGFR+β2-MG+PTH	0.796	0.758~0.834
eGFR+β2-MG+Scr	0.793	0.755~0.832
eGFR+β2-MG+BUN	0.800	0.762~0.837
eGFR+PTH+Scr	0.774	0.735~0.814
eGFR+PTH+BUN	0.777	0.738~0.816
eGFR+Scr+BUN	0.763	0.724~0.803
β2-MG+PTH+Scr	0.803	0.766~0.839
β2-MG+PTH+BUN	**0.804**	**0.767~0.841**
β2-MG+Scr+BUN	0.800	0.763~0.837
PTH+Scr+BUN	0.766	0.726~0.807

BUN, blood urea nitrogen; Cys-C, cystatin c; Scr, serum creatinine; PTH, parathyroid hormone; β2-MG, β2-microglobulin; eGFR, estimated glomerular filtration rate.

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
Outcome data	15*	Report numbers of outcome events or summary measures
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses

Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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A new triple therapy for the diagnosis of CKD-MBD: A cross-sectional study in Shanxi province

Running title: Diagnostic Value in CKD-MBD

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Abstract

Objectives: To seek a triple combination of biomarkers for early diagnosis of Chronic kidney disease-mineral and bone metabolic disorder and to explore the diagnostic efficacy of β2-microglobulin, parathyroid hormone, and blood urea nitrogen in Chronic kidney disease-mineral and bone metabolic disorder.

Participants: We collected medical records of 864 patients with chronic kidney disease (without direct contact with patients) and divided them into two groups based on the renal bone disease manifestations of all patients.

Primary and secondary outcome measures: There were 148 and 716 subjects in the Chronic kidney disease-mineral and bone metabolic disorder and the control groups, respectively. The aggregated data included basic information and various clinical laboratory indicators, such as blood lipid profile, antibody, and electrolyte levels, along

with renal function-related indicators.

Results: It was observed that most renal osteopathy occurs in the later stages of Chronic kidney disease. In the comparison of two clinical laboratory indicators, 16 factors were selected for curve analysis and compared. We discovered that factors with high diagnostic values were β2-microglobulin, parathyroid hormone and blood urea nitrogen.

Conclusions: The triple combination of β2-microglobulin +parathyroid hormone+blood urea nitrogen indicators can play the crucial role of a sensitive indicator for the early diagnosis of Chronic kidney disease-mineral and bone metabolic disorder and in preventing or delaying the progress of Chronic kidney disease-mineral and bone metabolic disorder.

Keywords: Blood urea nitrogen; β2-microglobulin; CKD-MBD; Parathyroid hormone; Urea nitrogen

Strengths of this study：

This is a cross-sectional study aimed at identifying detection methods for CKD-MBD. This study screened out other different detection criteria based on routine analysis data.

limitations of this study：

This study is limited and can only be analyzed based on existing medical record information. More medical record information needs to be added in the future to increase follow-up.

Introduction

Chronic kidney disease-mineral and bone metabolic disorder (CKD-MBD) is one of the common serious complications in patients with chronic kidney diseases (CKD) [1]. The incidence rate of MBD increases with the progression of CKD; more than 80% of MBD patients suffer from end-stage renal disease (ESRD) [2]. Recent epidemiologic studies have indicated that abnormalities in calcium and phosphorus serum levels and secondary hyperparathyroidism lead to the risk of death in maintenance dialysis patients [3]. Early detection of CKD-MBD can provide timely treatment as well as greatly improve their quality of life [4]. Therefore, prompt screening of CKD-MBD plays a very important role in the improvement of long-term prognosis and quality of life in such patients.

At present, bone biopsy remains the gold standard for diagnosing CKD-MBD. Since bone biopsy is invasive and causes trauma, it is not used as a routine examination method in clinical practice. Henceforth, medical staff must determine the need for conducting a bone biopsy for CKD stage 3–5 patients according to their health conditions [2]. Therefore, it is an urgent need to find the diagnostic and treatment markers of CKD-MBD for improved patient outcomes.

β2-microglobulin (β2-MG) is a small molecular weight protein that is clinically significant in the examination of kidney diseases [5]. If the blood plasma β2-MG level is increased, it indicates a reduction in the glomerular filtration function [6]. It is highly sensitive to serum creatinine, which can detect the decrease of glomerular filtration rate in the early stage. However, the diagnostic value of β2-MG is limited.

It is observed that parathyroid hormone (PTH) is closely related to the occurrence and development of CKD-MBD [7]. The main function of PTH is to regulate the metabolism of calcium and phosphorus in the body, relying on the actions of the main target organs: bone and kidney [8]. It mobilizes bone calcium into the blood, promotes the reabsorption of calcium ions by renal tubules and the excretion of phosphate, increases the blood calcium concentration, and decreases the blood phosphorus concentration. If PTH is detected early and found to be abnormal, timely correction may be able to control calcium and phosphorus metabolism disorders and alleviate and delay the development of renal bone diseases [9].

Blood urea nitrogen (BUN) is the end product of protein catabolism, and more than 90% of urea is eliminated via the kidneys [10]. When various pathological changes occur in the kidney, destroying the normal excretion function, the BUN concentration increases. Hence, the concentration of BUN in the blood is an important index of optimal renal function.

However, the evaluation accuracy of a single indicator is not as high as that of a multi-index joint evaluation. This study aimed to explore a better triple combination of diagnostic markers for the early diagnosis of CKD-MBD.

Materials and Methods

Study Design and Participants

As this was a cross-sectional study involving CKD patients, data were collected from patient records between September 1, 2016, and June 30, 2021. The study was based on medical record data obtained from the case information system containing demographic and laboratory data in an electronic medical record (EMR) database. Because data were de-identified, institutional review board review was not required.

For CKD patients, the inclusion criteria were: 1) patients diagnosed with CKD> 3 months; 2) patients diagnosed with CKD-MBD. Exclusion criteria included: 1) patients having other diseases which might influence related indexes of renal function, for example, diabetes, cirrhosis, etc.; 2) patients having incomplete clinical data; 3) patients<18 years old. After enrollment, we reviewed the medical records for demographic and clinical data as well as laboratory data, following which the patients were divided into two groups for CKD-MBD.

Patient and Public Involvement

None (The information we collect does not directly come from patients, but from case information.).

Collection of Clinical Parameters

The patients' medical records were retrospectively reviewed. Patient demographic information, medical history, and laboratory data were obtained. The indicators consisted of blood lipids, antibody and electrolyte levels, renal function indexes, and other related indicators. Blood lipid profile includes total cholesterol, triglycerides,

HDL cholesterol, and LDL cholesterol. The recommended reference values of the blood lipids were: total cholesterol, 3.5–5.2 mmol/L; triglycerides, 0.4–1.6 mmol/L; HDL-cholesterol, 0.8–1.8 mmol/L; and LDL-cholesterol, 2.3–3.4 mmol/L. Antibody testing included detection of serum IgA, IgG, IgM, C3, and C4 levels. The recommended reference values of the serum antibodies were: IgA, 0.7–4 g/L; IgG, 7–16 g/L; IgM, 0.4–2.3 g/L; C3, 0.9–1.8 g/L; and C4, 0.1–0.4 g/L. Serum electrolyte testing included K^+, Na^+, Cl^-, Ca^{2+}, Mg^{2+}, and P levels. The recommended reference values of the serum electrolyte levels were: K^+, 3.5–5.5 mmol/L; Na^+, 130–150 mmol/L; Cl^-, 96–110 mmol/L; Ca^{2+}, 1.8–2.6 mmol/L; Mg^{2+}, 0.6–1.2 mmol/L; P, 0.7–1.5 mmol/L. Renal function indexes included β2-microglobulin, uric acid, urea nitrogen, cystatin-C, serum creatinine, and eGFR levels. The eGFR level was estimated according to the CKD-EPI equation. In the present study, the CKD staging was done according to eGFR levels. If eGFR was >90 mL/min/1.73 m², it denoted CKD stage 1; If eGFR was 60~89 mL/min/1.73 m², CKD stage was 2; If eGFR was 30~59 mL/min/1.73 m², CKD stage was 3; If eGFR was 15~29 mL/min/1.73 m², CKD stage was 4; If eGFR was<15 mL/min/1.73 m², CKD stage was 5. The recommended reference values of the other renal function indexes were as follows: blood β2-microglobulin, 0.8–2.4 mg/L; uric acid, 150–410 μmol/L; BUN, 2.3–7 mmol/L; serum cystatin-C, 0.65–1.09 mg/L; serum creatinine, 50–132 μmol/L. Another important CKD-MBD-related indicator is blood parathyroid hormone (PTH). Its recommended reference value was 12–88 pg/mL.

Statistical Analysis

Continuous data were expressed as mean ±standard deviation (SD) or median and

interquartile range (IQR), according to variable distribution, while categorical variables were expressed as frequencies and percentages. Student's t-test, Mann-Whitney, and Chi-square tests were used to compare differences between the groups. Receiver operating characteristic curves (ROC) were developed to evaluate the biomarkers predictive of CKD-MBD. An area under the curve (AUC) was considered good if > 0.7 and excellent if > 0.8. Data analysis was carried out with SPSS version 25 (IBM SPSS Statistics, Chicago, IL, USA). p-values< 0.05 were considered statistically significant.

Results

Patient Selection

During the study period, a total of 3124 patients with CKD were initially selected, of which 1766 had diabetes, 302 had cardiac diseases, 26 had cirrhosis, 151 had incomplete data, and 15< 18 years old. After criteria fulfillment, 864 patients having complete data were included in this analysis. Patients were divided into two groups according to the diagnosis of CKD-MBD. There were 716 (82.9%) and 148 (17.1%) patients in the CKD and CKD-MBD groups, respectively (Figure 1).

Demographic Information

The demographic characteristics of the patients are shown in Table 1. A total of 864 patients, including 512 men and 352 women, participated in this analysis. The mean age of all patients was 56.37 ±15.50 (range, 19~93) years, while the age in the CKD and CKD-MBD groups were 56.00 ±15.54 years and 58.14 ±15.26 years, respectively. As shown in Table 1, there were no significant differences in age, gender, current smoking, and alcohol drinking between the two groups (all p>0.05). BMI was significantly higher in the CKD group (p<0.05). In the CKD group, stage 3, 4, and 5 patients were 26%, 32.8%, and 35.2%, respectively. But in the CKD-MBD group, stage 5 accounted for 80.4% of all patients. This result shows that CKD-MBD mostly occurs in stage 5 of CKD (Table 1).

Patients' Clinical Characteristics

S-Table 1 shows the comparison of clinical and laboratory data between the two groups. Our results showed that there was a significant difference in triglyceride levels between

the two groups; the triglyceride level in the CKD group [1.56(1.16~2.24)] was slightly higher than the triglyceride level in the CKD-MBD group [1.47(1.03~2.01)]. But from the perspective of classification indicators, no difference has been found in the distribution between the two groups. On the contrary, the HDL distribution was statistically significant between the two groups; in the CKD-MBD and CKD groups, the proportion was 10.1% and 3.1%, respectively. The antibody indicator analysis revealed that the IgA and C4 levels were lower in the CKD-MBD group, and the differences between the two groups were statistically significant, but in terms of category distribution, no difference was found between the groups. The distribution of C3 levels between the two groups was statistically significant both in terms of continuous variables and category distribution. The level of C3 in the CKD-MBD group was relatively low [0.82(0.76~0.97)] and accounted for a 62.8% distribution. In terms of electrolyte levels, except for K^+ ions, there were significant differences observed in Na^+, Cl^-, Ca^{2+}, Mg^{2+}, and P ion levels between the two groups. Compared with the CKD group, the levels of Na^+ [138.06(135.53~140.21)], Cl^- [104.42(100.38~108.08)] and Ca^{2+} [2.09(1.94~2.19)] ions in CKD-MBD group were lower, while the levels of Mg^{2+} [1.03(0.90~1.16)] and P [1.68(1.45~2.05)] ions were higher in the CKD-MBD group. From the aspect of antibody indicators, the results show that the β2-microglobulin and Cystatin-C levels were higher in the CKD-MBD group, and the difference between the two groups was statistically significant, but in terms of category distribution, no difference was found between the two groups. Compared with the CKD group, the uric acid level of the CKD-MBD group was lower while the BUN level was higher, which

was statistically significant. The serum creatinine level in the CKD-MBD group increased, and the eGFR level was reduced, but there was no significant change in urinary protein between the two groups when compared with the CKD group. It was revealed that PTH increased significantly in the CKD-MBD group; its levels in the "high" category of both the CKD-MBD and CKD groups accounted for 85.8% and 55.7%, respectively. Through the comparison of different clinical indicators between the two groups, we explored the efficacy of a few sensitive indicators for the early detection of CKD-MBD.

Comparison of the Diagnostic Efficacy of relevant indicators in the Diagnosis of CKD-MBD

Table 2 results showed that the diagnostic value of indicators related to renal function is relatively high. Among these indicators, the five indicators with the largest ROC: eGFR, β2-MG, PTH, Scr, and BUN, were selected. However, the AUC of these indicators is about 0.7. Therefore, considering the combined diagnosis of three indicators, the diagnostic value may be higher.

S-Table 2 displays that the AUC of the combined detection of β2-MG +PTH+BUN is significantly higher than those of their single detection and other combinations (AUC 0.804, 95% CI 0.767 to 0.841, $p< 0.05$).

Discussion

Chronic kidney disease-mineral and bone metabolic disorder (CKD-MBD), caused by disturbed calcium and phosphate homeostasis, is a major complication of CKD [11]. Although the current medical scenario has greatly improved, the early detection rate of CKD-MBD is still very low. In general, a timely and accurate diagnosis helps in improving patient prognosis. Findings of CKD-MBD in CKD are very common in clinical practice and are mostly attributed to a disorder of calcium and phosphorus metabolism caused by renal failure [12]. The late onset of CKD-MBD symptoms and the absence of noninvasive diagnostic indicators may lead to delayed diagnosis and obstruct prompt therapeutic interventions. Therefore, there is an urgent need for precise clinical approaches that can timely and accurately diagnose CKD-MBD [13]. This study aimed to explore the efficacy of a triple combination of β2-MG, PTH, and BUN as early biomarkers for CKD-MBD.

In order to observe the differences in these related indexes between the two groups, we used single-factor analysis to compare these indexes. The results showed significant differences between the two groups in terms of clinical laboratory indicators, which might provide clues for finding sensitive indicators of early diagnosis of CKD-MBD. We then used ROC curve analysis to examine the role of these indexes in the clinical prediction of CKD-MBD. There were five indexes with an AUC of 0.710. The AUC of β2-MG, BNU, Scr, eGFR, and PTH was 0.763, 0.722, 0.747, 0.767, and 0.749, respectively. Therefore, these five indexes might become potential biomarkers for the early diagnosis of CKD-MBD. Furthermore, joint index diagnoses were used to

improve the diagnostic value. We constructed 10 combinations and selected the triple combination with the highest AUC: β2-MG+PTH+BUN combination.

Studies have shown that calcium and phosphorus metabolism disorders and hyperparathyroidism can promote the occurrence of cardiovascular diseases and increase the mortality of patients [14]. PTH plays an important role in the regulation of calcium and phosphorus levels. For example, high blood phosphorus and low blood calcium can promote parathyroid hyperplasia and increase parathyroid hormone synthesis and secretion [15]. Moreover, PTH also plays a crucial part in regulating bone cell remodeling. A sequential increase in PTH will stimulate osteoblast receptors, increase the number and activity of osteoclasts, increase bone resorption, initiate the formation of bone cavities and new bones, and finally lead to secondary bone diseases [16]. Therefore, PTH plays an important role in the functional mechanism of CKD-MBD. If PTH is monitored early, necessary measures might be taken on time to delay the occurrence of renal bone diseases.

β2-MG is a light chain protein of type I histocompatibility antigen on the membrane of all nucleated cells in the body [17]. It is mainly produced by lymphocytes, and its molecular weight is about 11.8 kD. Due to the small molecular weight and inability to bind with plasma protein, normal β2-MG can be freely filtered through the glomerulus. About 99.9% of β2-MG is reabsorbed in the renal proximal convoluted tubule, decomposed, and destroyed in the renal tubular epithelial cells, while only 0.1% is excreted from the body by urination [18]. The production rate of β2-MG was constant in vivo, and the β2-MG level in plasma was not affected by age, sex, the number of

muscle tissues, or other factors. Therefore, measuring the level of β2-MG in plasma is more sensitive than measuring the level of serum creatinine to evaluate renal function. Hence, β2-MG can be used as an early indicator to reflect renal damage as increased β2-MG levels in plasma can reflect the damage to glomerular filtration function or the increase of filtration load [19]. The determination of β2-MG level in plasma can also provide valuable data for the differential diagnosis, disease progression, and prognosis of renal diseases.

Urea nitrogen refers to "blood urea nitrogen" [20]. It is a nitrogen-containing compound in plasma other than proteins. It is the main end product of human protein metabolism and is filtered out of the body from the glomerulus [20]. Urea can be reabsorbed in all segments of tubules after glomerular filtration, but the faster the flow rate of urine in the tubules, the lesser the reabsorption; that is, the maximum clearance rate is reached easily [21]. BUN can be in the normal range in the early stages of renal function damage. When the eGFR drops below 50% of the normal value, the concentration of BUN increases rapidly; as the renal insufficiency is decompensated, BUN sequentially increases. Therefore, it is regarded as an index to judge glomerular filtration function in clinical practice [22]. When BUN is used alone for predicting CKD-MBD, the accuracy and predictive value becomes low and require the help of other indicators to make a joint judgment.

Our data volume is limited, only recent data from one hospital. In the later stage, we will increase the number of hospitals and medical record data. The limitation of cross-sectional analysis is that it can only be compared with the testing information of patients

admitted during the same period. In the future, we will add follow-up records. On this data, as the data we can collect is based on a chronic kidney disease, so patients rarely undergo FGF-23 testing. This is also our insufficient information collection, which can only be supported by other information.

We analyzed the predictive value of β 2-MG, PTH, and BUN for the prognosis of CKD-MBD. The results indicate that the sensitivity, specificity, and accuracy of the β 2-MG+PTH+BUN combination are higher than the individual diagnostic ability of these biomarkers. Our results indicate that the combined detection of β 2-MG, PTH, and BUN has higher value in predicting the prognosis of CKD-MBD. No other studies have attempted to analyze the diagnostic value of the combination of β 2-MG, PTH, and BUN for CKD-MBD. Our study indicates for the first time that the combined detection of β 2-MG+PTH+BUN has high diagnostic value for early CKD-MBD, which will help to accurately diagnose early CKD-MBD in the near future.

Ethics approval and consent to participate

This is a retrospective study, these data sourced from laboratory data in an electronic medical record (EMR) database and we are not directly in contact with patients. Before we conducted the study, the ethics committee gave its consent, confirming that the experiments were conducted in accordance with established ethical guidelines. The full name of the ethics committee: The Shanxi Provincial People's Hospital Medical Research Ethics Committee. The ethics approval number: ((2023) SYYKJL-No. 150) .

Consent for publication

All authors have read and approved the content, and agree to submit it for consideration for publication in your journal.

Availability of data and materials

The data presented in this study are available on request from the corresponding author.

Competing interests

All authors state that they have no conflicts of interest.

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Author Contributions

B.H is the guarantor of this work and was responsible for conceptualisation, Methodology, Software, Supervision, Software, Validation, Writing-Reviewing and Editing, Responsible for the overall content. Z.Z was responsible for Conceptualization, Methodology, Software, Data curation, Writing- Original draft preparation, Visualization and Investigation. Y.G was responsible for Conceptualization, Methodology, Software, Data curation, Writing-Original draft preparation, Visualization, Investigation, Software and Validation. Z.T.Z was responsible for Data curation, Writing- Original draft preparation, Visualization, Investigation, Visualization, Investigation. W.S was responsible for Data curation, Writing- Original draft preparation. L.F was responsible for Data curation, Writing- Original draft preparation. Z.W was responsible for Data curation, Writing-Original draft preparation. D.Y was responsible for Data curation, Writing- Original draft preparation. Y.Z was responsible for Visualization, Investigation and Supervision. R.L was responsible for

Visualization, Investigation and Supervision. All authors reviewed and approved the final version and agreed to be accountable for all aspects of the work.

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Table 1. Comparison of baseline information between the two groups (n = 864)

Variable	Total (n = 864)	CKD group (n = 716)	CKD-MBD group (n = 148)	*p*
Age, years	56.37 ±15.50	56.00 ±15.54	58.14 ±15.26	0.128
Male, n (%)	512(59.3)	434(60.6)	78(52.7)	0.075
Female, n (%)	352(40.7)	282(39.4)	70(47.3)	
Current smoking, n (%)				
Yes	247(28.6)	214(29.9)	33(22.3)	0.063
No	617(71.4)	502(70.1)	115(77.7)	
Alcohol drinking, n (%)				
Yes	169(19.6)	148(20.7)	21(14.2)	0.070
No	695(80.4)	568(79.3)	127(85.8)	
Body mass index, kg/m^2	24.49 ±3.90	24.69 ±3.94	23.52 ±3.54	**0.001**
CKD stage				

1	10(1.3)	8(1.1)	2(1.4)	
2	39(4.5)	35(4.9)	4(2.7)	
3	189(21.9)	186(26.0)	3(2.0)	**<0.001**
4	255(29.5)	235(32.8)	20(13.5)	
5	371(42.8)	252(35.2)	119(80.4)	

Table 2. Comparison of the diagnostic efficacy of single related indexes in the diagnosis of CKD-MBD

Variable	AUC	95%CI
TG*	0.552	0.500~0.603
IgA*	0.552	0.501~0.603
C3*	0.642	0.596~0.688
C4*	0.578	0.534~0.623
Na *	0.618	0.566~0.671
Cl *	0.671	0.622~0.720
Ca*	0.587	0.535~0.639
Mg	0.622	0.571~0.674
P	0.706	0.661~0.752
β2-MG	**0.763**	0.720~0.806

Uc*	0.586	0.534~0.638
BUN	**0.722**	0.678~0.766
Cys-C	0.702	0.657~0.747
Scr	**0.747**	0.705~0.788
eGFR*	**0.767**	0.728~0.807
PTH	**0.749**	0.703~0.801

BMI, body mass index; TG, triglycerides; Na, sodium; Cl, chlorine; Ca, calcium; Mg, magnesium; P, phosphate; β2-MG, β2-microglobulin; Uc, uric acid; BUN, blood urea nitrogen; Cys-C, cystatin c; Scr, serum creatinine; PTH, parathyroid hormone.

Figure legend

Figure 1. Flow chart of patient selection and inclusion.

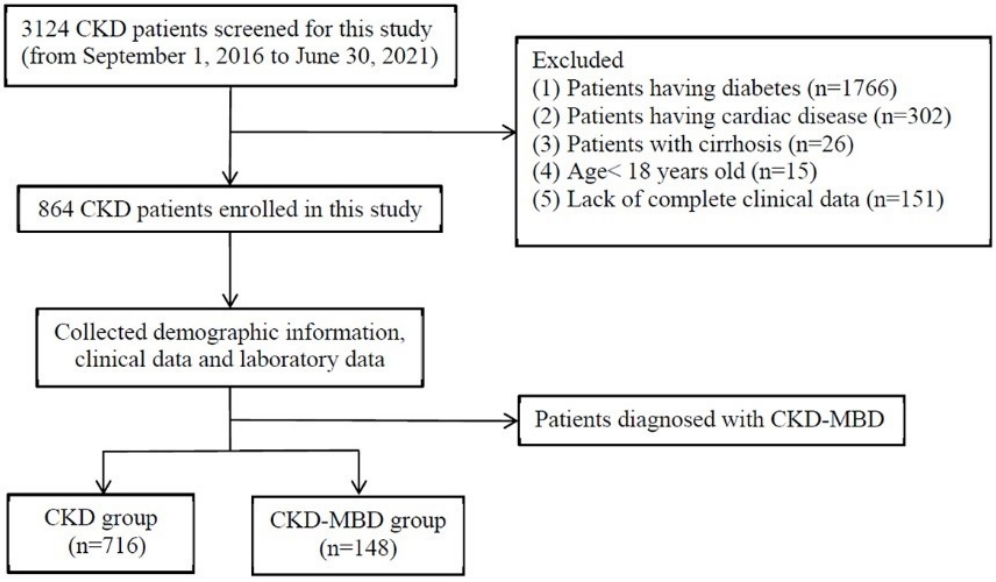

Figure 1-Flow chart of patient selection and inclusion

99x57mm (220 x 220 DPI)

A new triple therapy for the diagnosis of CKD-MBD: A Cross-sectional Study

Running title: Diagnostic Value in CKD-MBD

S-Table 1. Comparison of laboratory data between the two groups (n = 864)

Variable	Total (n = 864)	CKD group (n = 716)	CKD-MBD group (n = 148)	*p*
Cholesterol, mmol/L	4.68 ±1.63	4.72 ±1.63	4.52 ±1.64	0.175
target	412(47.7)	339(47.3)	73(49.3)	
low	198(22.9)	157(22.0)	41(27.7)	0.111
high	254(29.4)	220(30.7)	34(23.0)	
Triglycerides, mmol/L	4.50(3.62~5.42)	1.56(1.16~2.24)	1.47(1.03~2.01)	**0.047**
target	454(52.5)	370(51.7)	84(56.8)	0.260
high	410(47.5)	346(48.3)	64(43.2)	
HDL cholesterol, mmol/L	1.12 ±0.39	1.10 ±0.33	1.19 ±0.58	0.073
target	695(80.4)	580(81.0)	115(77.7)	
low	132(15.3)	114(15.9)	18(12.2)	**<0.001**
high	37(4.3)	22(3.1)	15(10.1)	
LDL cholesterol, mmol/L	2.98 ±1.21	2.99 ±1.14	2.93 ±1.47	0.658
target	374(43.3)	314(43.9)	60(40.5)	
low	250(28.9)	199(27.8)	51(34.5)	0.260
high	240(27.8)	203(28.4)	37(25.0)	
IgA, g/L	2.37(1.77~3.11)	2.39(1.81~3.16)	2.24(1.55~2.66)	**0.046**
target	777(89.9)	642(89.7)	135(91.2)	
low	12(1.4)	11(1.5)	1(0.7)	0.824
high	75(8.7)	63(8.8)	12(8.1)	
IgG, g/L	10.50(8.04~12.70)	10.50(8.16~12.75)	10.80(7.95~12.60)	0.463
target	672(77.8)	555(77.5)	117(79.1)	
low	136(15.7)	109(15.2)	27(18.2)	0.096
high	56(6.5)	52(7.3)	4(2.7)	
IgM, g/L	0.74(0.49~1.08)	0.74(0.50~1.06)	0.75(0.46~1.13)	0.973
target	754(87.3)	624(87.2)	130(87.8)	0.819
low	110(12.7)	92(12.8)	18(12.2)	
C3, g/L	0.93(0.80~1.05)	0.94(0.81~1.07)	0.82(0.76~0.97)	**<0.001**

target	484(56.0)	429(59.9)	55(37.2)	**<0.001**
low	380(44.0)	287(40.1)	93(62.8)	
C4, g/L	0.27(0.23~0.32)	0.27(0.23~0.33)	0.26(0.23~0.28)	**0.003**
target	783(90.6)	643(89.8)	140(94.6)	0.069
high	81(9.4)	73(10.2)	8(5.4)	
Potassium(K, mmol/L)	4.35(3.94~4.81)	4.33(3.94~4.78)	4.48(3.94~4.97)	0.066
target	722(83.6)	603(84.2)	119(80.4)	
low	80(9.3)	67(9.4)	13(8.8)	0.170
high	62(7.1)	46(6.4)	16(10.8)	
Sodium(Na, mmol/L)	139.33(137.38~140.95)	139.46(137.71~141.00)	138.06(135.53~140.21)	**<0.001**
target	838(97.0)	697(97.3)	141(95.3)	0.178
low	26(3.0)	19(2.7)	7(4.7)	
Chlorine(Cl, mmol/L)	107.22(105.05~110.70)	107.76(104.76~110.98)	104.42(100.38~108.08)	**<0.001**
target	581(67.2)	464(64.8)	117(79.1)	
low	30(3.5)	22(3.1)	8(5.4)	**<0.001**
high	253(29.3)	230(32.1)	23(15.5)	
Calcium(Ca, mmol/L)	2.12(2.00~2.26)	2.13(2.01~2.26)	2.09(1.94~2.19)	**0.001**
target	793(91.8)	666(93.0)	127(85.8)	
low	59(6.8)	42(5.9)	17(11.5)	**0.014**
high	12(1.4)	8(1.1)	4(2.7)	
Magnesium(Mg, mmol/L)	0.95(0.86~1.06)	0.94(0.86~1.04)	1.03(0.90~1.16)	**<0.001**
target	777(89.9)	660(92.2)	117(79.1)	**<0.001**
high	87(10.1)	56(7.8)	31(20.9)	
Phosphate(P, mmol/L)	1.40(1.18~1.69)	1.36(1.16~1.63)	1.68(1.45~2.05)	**<0.001**
target	501(58.0)	458(64.0)	43(29.1)	**<0.001**
high	363(42.0)	258(36.0)	105(70.9)	
β2-microglobulin, mg/mL	9.43(5.64~13.61)	8.49(5.22~12.20)	13.99(10.57~20.38)	**<0.001**
target	27(3.1)	24(3.4)	3(2.0)	0.603
high	837(96.9)	692(96.6)	145(98.0)	
Uric Acid, μmol/L	416.29(338.19~500.35)	420.63(346.60~508.76)	385.80(300.88~463.09)	**0.001**
target	412(47.7)	331(46.2)	81(54.7)	
low	9(1.0)	7(1.0)	2(1.4)	0.113
high	443(51.3)	378(52.8)	65(43.9)	
Blood urea nitrogen, mmol/L	16.10(10.78~22.60)	14.76(10.15~21.24)	21.60(16.89~31.03)	**<0.001**
target	75(8.7)	72(10.1)	3(2.0)	**0.002**

high	789(91.3)	644(89.9)	145(98.0)	
Cystatin C, mg/L	3.58(2.48~4.60)	3.34(2.34~4.33)	4.51(3.59~5.32)	**<0.001**
target	10(1.2)	10(1.4)	0(0)	0.226
high	854(98.8)	706(98.6)	148(100)	
Serum creatinine, μmol/L	331.06(179.29~510.70)	291.70(164.24~470.78)	495.52(369.62~734.34)	**<0.001**
Urinary protein, g/24 h	3.17 ±3.17	3.16 ±3.19	3.25 ±3.10	0.751
eGFR, mL/min/1.73 m^2	14.94(8.72~32.29)	18(10.02~35.39)	8.11(5.90~11.61)	**<0.001**
Parathyroid hormone, pg/mL	120.95(61.60~234.65)	102.70(55.85~203.30)	230.25(110.15~367.20)	**<0.001**
target	322(37.3)	306(42.7)	16(10.8)	
low	16(1.9)	11(1.5)	5(3.4)	**<0.001**
high	526(60.9)	399(55.7)	127(85.8)	

STable 2. Comparison of the diagnostic efficacy of the triple combination of related indexes in the diagnosis of CKD-MBD

Variable	AUC	95%CI
eGFR+β2-MG+PTH	0.796	0.758~0.834
eGFR+β2-MG+Scr	0.793	0.755~0.832
eGFR+β2-MG+BUN	0.800	0.762~0.837
eGFR+PTH+Scr	0.774	0.735~0.814
eGFR+PTH+BUN	0.777	0.738~0.816
eGFR+Scr+BUN	0.763	0.724~0.803
β2-MG+PTH+Scr	0.803	0.766~0.839
β2-MG+PTH+BUN	**0.804**	**0.767~0.841**
β2-MG+Scr+BUN	0.800	0.763~0.837
PTH+Scr+BUN	0.766	0.726~0.807

BUN, blood urea nitrogen; Cys-C, cystatin c; Scr, serum creatinine; PTH, parathyroid hormone; β2-MG, β2-microglobulin; eGFR, estimated glomerular filtration rate.

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
Outcome data	15*	Report numbers of outcome events or summary measures
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses

Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.