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# New triple therapy for the diagnosis of CKD-MBD: a cross-sectional study in Shanxi province

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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.

## STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This is a cross-sectional study aimed at identifying detection methods for chronic kidney disease-mineral and bone metabolic disorder.
⇒ This study screened out other different detection criteria based on routine analysis data.
⇒ This study is limited and can only be analysed 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 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.[2] Recent epidemiological 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, there 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 mobilises 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 patients with CKD, data were collected from patient records between 1 September 2016 and 30 June 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 database.

For patients with CKD, the inclusion criteria were (1) patients diagnosed with CKD>3 months and (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; (2) patients having incomplete clinical data and (3) patients <18 years old. After enrolment, 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 (high-density lipoprotein) cholesterol and LDL (Low-density lipoprotein) 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 the 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-MG, uric acid, urea nitrogen, cystatin-C, serum creatinine and eGFR levels. The eGFR level was estimated according to the CKD-EPI equation. In this 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-MG, 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 and serum creatinine, 50–132 µmol/L. Another important CKD-MBD-related indicator is blood PTH. Its recommended reference value was 12–88 pg/mL.

### Statistical analysis

Continuous data were expressed as mean±SD or median and IQR, according to variable distribution, while categorical variables were expressed as frequencies and percentages. Student's t-test, Mann-Whitney U test and $\chi^2$ 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 V.25 (IBM SPSS Statistics). 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 ( 1766 had diabetes, 302 had cardiac diseases, 26 had cirrhosis, 151 had incomplete data and 15 patients were <18 years old). After criteria fulfilment, 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)

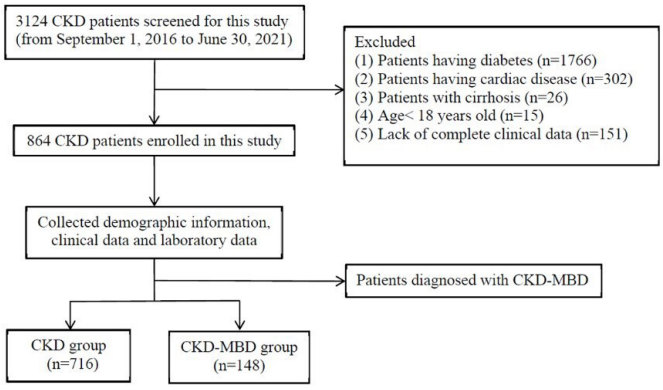


**Figure 1** Flow chart of patient selection and inclusion. CKD-MBD, chronic kidney disease-mineral and bone metabolic disorder.

years while the age in the CKD and CKD-MBD groups was 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

Online supplemental 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-MG 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

**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 value |
|---|---|---|---|---|
| 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) | **<0.001** |
| 2 | 39 (4.5) | 35 (4.9) | 4 (2.7) | |
| 3 | 189 (21.9) | 186 (26.0) | 3 (2.0) | |
| 4 | 255 (29.5) | 235 (32.8) | 20 (13.5) | |
| 5 | 371 (42.8) | 252 (35.2) | 119 (80.4) | |

The bold values indicate statistical significance.
CKD-MBD, chronic kidney disease-mineral and bone metabolic disorder.

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

The bold values indicate proximity to the mean and have statistical significance.
*We are further verifying and verifying the data in the supplementary materials.
AUC, area under the curve; BMI, body mass index; BUN, blood urea nitrogen; Ca, calcium; CKD-MBD, chronic kidney disease-mineral and bone metabolic disorder; Cl, chlorine; Cys-C, cystatin c; Mg, magnesium; Na, sodium; P, phosphate; PTH, parathyroid hormone; Scr, serum creatinine; TG, triglycerides; Uc, uric acid; β2-MG, β2-microglobulin.

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.

Online supplemental 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

CKD-MBD, caused by disturbed calcium and phosphate homoeostasis, 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 PTH synthesis and secretion.[15] Moreover, PTH also plays a crucial part in regulating bone cell remodelling. 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 'BUN'.[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 values become low and require the help of other indicators to make a joint judgement.

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 these data, as the data we can collect are based on a CKD so patients rarely undergo FGF-23 testing. This is also our insufficient information collection, which can only be supported by other information.

We analysed the predictive value of β 2MG, 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 a higher value in predicting the prognosis of CKD-MBD. No other studies have attempted to analyse the diagnostic value of the combination of β 2MG, 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 a diagnostic value for early CKD-MBD, which will help to accurately diagnose early CKD-MBD in the near future.

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**Contributors** BH 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. ZZhao was responsible for conceptualisation, methodology, software, data curation, writing–original draft preparation, visualisation and investigation. YG was responsible for conceptualisation, methodology, software, data curation, writing–original draft preparation, visualisation, investigation, software and validation. ZZhang was responsible for data curation, writing– original draft preparation, visualisation, investigation, visualisation, investigation. WS was responsible for data curation, writing– original draft preparation. LF was responsible for data curation, writing–original draft preparation. ZW was responsible for data curation, writing–original draft preparation. DY was responsible for data curation, writing–original draft preparation. YZ was responsible for visualisation, investigation and supervision. RL was responsible for visualisation, 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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**Competing interests** None declared.

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