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DEPARTMENT OF PHYSIOLOGY AND PATHOPHYSIOLOGY

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PATHOGENETIC SIGNIFICANCE OF SELECTED BIOMARKERS IN THE PROGRESSION OF CHRONIC KIDNEY DISEASE AND ITS ASSOCIATED CARDIOVASCULAR COMPLICATIONS

ABSTRACT

of a Dissertation for the Award of the PhD Degree

Doctoral program Pathophysiology

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The dissertation comprises 154 pages, including: Introduction, Purpose and Tasks – 4 pages; Literature Review – 45 pages; Clinical Contingent, Materials and Methods – 9 pages; Results – 29 pages; Discussion – 27 pages; Conclusions and Scientific Contributions – 3 pages. The dissertation is illustrated with 16 tables and 22 figures. The bibliography comprises 361 references.

The dissertation was discussed at a meeting of the Extended Departmental Council of the Department of Physiology and Pathophysiology at the Medical University – Pleven (Order No. 1916 of 23 June 2026 issued by the Rector) and was approved for public defense before a Scientific Jury.

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The dissertation materials are available for inspection at the Research Department and are published on the official website of the Medical University – Pleven: www.mu-pleven.bg

ABBREVIATIONS

ALP	Alkaline phosphatase
AUC	Area under the curve
BMI	Body Mass Index
BUN	Blood urea nitrogen
Ca	Serum calcium
Ca×P	Calcium phosphate product
CAD	Coronary artery disease
CRP	C-reactive protein
CKD	Chronic kidney disease
CKD-EPI	CKD Epidemiology Collaboration equation for calculating GFR
CKD-MBD	Chronic Kidney Disease-Mineral Bone Disorder
CRS	Cardiorenal syndrome
CVD	Cardiovascular diseases
dp-ucMGP	Dephosphorylated uncarboxylated matrix Gla-protein
eGFR	Estimated glomerular filtration rate
ECLIA	Electrochemiluminescent immunoassay
ELISA	Enzyme-linked immunosorbent assay
FGF23	Fibroblast growth factor 23
GFR	Glomerular filtration
hs-CRP	High-sensitivity C-reactive protein
IL-6	Interleukin-6
IQR	Interquartile range
KDIGO	Kidney Disease: Improving Global Outcomes
Klotho	Klotho protein
MGP	Matrix Gla-protein
NGAL	Neutrophil gelatinase-associated lipocalin
Pi	Inorganic phosphate
PTH	Parathyroid hormone
ROC	Receiver Operating Characteristics
SCr	Serum creatinine
UA	Uric acid
ucMGP	Uncarboxylated matrix Gla-protein
WBC	Leukocytes

CONTENTS

INTRODUCTION

Chronic kidney disease (CKD) is a progressive disorder characterized by persistent abnormalities in kidney structure and/or function lasting for more than three months. The disease is often asymptomatic in its early stages and progresses gradually, leading to a progressive decline in renal function and, ultimately, end-stage kidney disease. Owing to the absence of early clinical manifestations, many patients remain undiagnosed until the disease has reached advanced stages, thereby limiting opportunities for timely intervention and effective prevention.

Early diagnosis and appropriate monitoring of patients with CKD are essential for slowing disease progression and reducing the risk of complications. This is particularly important given the substantial socioeconomic burden associated with CKD. The disease is linked to reduced work capacity, impaired quality of life, and increased mortality. In advanced stages, patients require highly specialized and costly treatments, including dialysis and kidney transplantation, which impose a considerable financial burden on healthcare systems.

Despite significant advances in therapeutic strategies, predicting CKD progression and the development of cardiovascular complications in individual patients remains a major clinical challenge. Consequently, contemporary nephrology has focused increasingly on the identification and validation of reliable, sensitive, and minimally invasive biomarkers that enable not only the early detection of renal injury but also accurate risk stratification and prediction of disease progression, particularly in the context of cardiorenal interactions.

Particular attention has been directed toward biomarkers that are directly involved in the pathogenetic mechanisms underlying CKD and its associated cardiovascular complications. Among these, matrix Gla protein (MGP) is recognized as a major inhibitor of vascular calcification, whereas neutrophil gelatinase-associated lipocalin (NGAL) is a well-established biomarker of tubular injury and inflammation. Investigating these biomarkers may improve our understanding of the pathogenetic mechanisms linking renal dysfunction and cardiovascular disease and contribute to the development of novel diagnostic and therapeutic strategies. Ultimately, earlier diagnosis and personalized therapeutic approaches based on these biomarkers may improve the clinical outcomes and prognosis of patients with CKD.

PURPOSE

The aim of this dissertation is to investigate the serum levels of uncarboxylated matrix Gla protein (ucMGP), neutrophil gelatinase-associated lipocalin (NGAL), and parameters of calcium–phosphate metabolism, as well as to evaluate their diagnostic and prognostic value for predicting the progression of renal dysfunction and cardiovascular risk in patients with predialysis CKD.

TASKS

1. To recruit a study group of patients with predialysis CKD (stages G1 – G4) according to current classification criteria.

2. To examine the indicators of calcium–phosphorus metabolism by determining serum levels of calcium, phosphorus, parathyroid hormone, and alkaline phosphatase, and to analyze their relationship with renal function.

3. To determine serum levels of the uncarboxylated fraction of matrix Gla protein (ucMGP) and to assess their association with disorders of mineral metabolism and cardiovascular risk.

4. To evaluate serum concentrations of neutrophil gelatinase-associated lipocalin (NGAL), high-sensitivity C-reactive protein (hsCRP), and interleukin-6 (IL-6) as markers of tubular injury and chronic inflammation, and to assess their association with indicators of calcium–phosphorus metabolism.

5. To evaluate the diagnostic and prognostic value of MGP, NGAL, and calcium–phosphorus metabolism indicators in predicting the progression of renal dysfunction and cardiovascular risk.

CLINICAL CONTINGENT

Clinical contingent

To achieve the aim and objectives of the dissertation, 167 participants meeting the predefined criteria were enrolled in the study at Exacta Medica Medical Center, Pleven, between January 2020 and June 2024.

The research was carried out with funds from three scientific projects funded by the Medical University of Pleven:

- Project No.9/2020 "Study of serum levels of vitamin K2 in patients with chronic kidney disease in the pre-dialysis period"
- Project No.15/2023 "Mineral metabolism, biomarkers of inflammation and their relationship with vitamin K2 status in patients with chronic kidney disease"
- Project No.12/2024 "Investigating the role of lipocalin-2 (LCN-2), matrix metalloproteinases-3, -8 (MMP-3, -8) and their tissue inhibitors-2, -3 (TIMP-2, -3), as biomarkers for assessing renal function in patients with chronic kidney disease"

The studies were approved by the Ethics Committee of the Medical University of Pleven.

Criteria for inclusion of participants in the study groups:

- Age 20–85 years;
- Established CKD stages G1–G4 according to KDIGO criteria;
- Signed informed consent form.

Exclusion criteria:

- Pregnancy;
- Acute kidney injury;
- CKD stage G5 according to KDIGO criteria;
- Kidney transplantation;
- Acute infectious diseases;
- Severe congestive heart failure;
- Liver failure;
- Malignant diseases;
- Systemic connective tissue diseases;
- Refusal to provide written informed consent.

Inclusion criteria for control participants:

- Participants without signs of kidney disease between the ages of 20 and 85;
- Signed informed consent form.

Forming the groups

CKD was classified according to the KDIGO guidelines, and patients were assigned to stages G1–G4 based on eGFR values and the presence of markers of kidney damage (albuminuria and/or proteinuria). eGFR was calculated using the CKD-EPI equation and normalized to a body surface area of 1.73 m² (mL/min/1.73 m²).

To evaluate the association between ucMGP levels, mineral metabolism, and cardiovascular risk (Tasks 2 and 3), a total of 84 participants were included and divided into three groups:

- Group I – controls: participants without evidence of kidney disease, (eGFR ≥ 90 ml/min; n = 15)
- Group II – patients with CKD with mild to moderately decreased GFR, (G2 + G3a, eGFR 45 – 89 mL/min; n = 42)
- Group III – CKD patients with moderately to severely decreased GFR, (G3b + G4, eGFR 15 – 44 mL/min; n = 27)

To evaluate NGAL as a marker of CKD progression and an early biomarker of kidney injury (Tasks 4 and 5), a total of 83 participants were included: 12 healthy controls and 71 patients with CKD stages G1–G4 according to KDIGO criteria.

Patients with CKD were divided into three study groups:

- Group I (G1, eGFR ≥ 90 mL/min; n = 16) – classified based on the presence of microalbuminuria (n = 14) or proteinuria (n = 2)
- Group II (G2 + G3a, eGFR 89 – 45 mL/min; n = 33)
- Group III (G3b + G4, eGFR 44 – 15 mL/min; n = 22)

MATERIALS AND METHODS

1. Clinical methods: medical history and physical examination

A detailed medical history was obtained from all participants regarding symptoms, cardiovascular risk factors, comorbid conditions, and current treatment. Data were collected on the duration of chronic kidney disease, blood pressure values, and ongoing therapy. In patients with arterial hypertension and type 2 diabetes mellitus, additional information was collected regarding disease duration, treatment regimen, and the presence of vascular complications. Available medical records were reviewed. In some participants, an additional cardiological consultation was performed to further assess cardiovascular status and associated comorbidities.

2. Anthropometry

Body mass was measured according to standardized procedures using a calibrated electronic scale, while height was measured using a stadiometer. Body mass index (BMI) was calculated based on body mass (kg) and height (m) using the formula: BMI = body mass (kg) / height² (m^2). The obtained BMI values were interpreted according to the World Health Organization (WHO) classification, which is also adopted in Bulgaria:

- underweight: < 18.5 kg/m^2
- normal body weight: 18.5 – 24.99 kg/m^2
- overweight: 25.0 – 29.99 kg/m^2
- obesity: ≥ 30 kg/m^2

3. Sociological methods

3.1. Documentary method

A standardized questionnaire was used for all study groups, collecting information on participants' age, anthropometric parameters, medical history, comorbidities, and current pharmacological treatment.

4. Laboratory tests

For the assessment of biochemical and immunological parameters (BUN, SCr, UA, Ca, Pi, ALP, PTH, hs-CRP, IL-6, ucMGP, and NGAL), venous blood samples were collected in the morning (8:00–9:30 AM) following a 12-hour overnight fast. Serum was separated by centrifugation at 2500 rpm for 10 minutes, and all analyses were performed on the same day as sample collection in an accredited medical diagnostic laboratory according to standard laboratory procedures. Complete blood count was performed on EDTA-anticoagulated venous blood using a Sysmex XP automated hematology analyzer.

4.1. Biochemical studies

- **Renal function parameters:** Serum creatinine (SCr) was determined by the kinetic compensated Jaffe method and blood urea nitrogen (BUN) by the enzymatic method on a Cobas C 311 automated biochemical analyzer (Roche Diagnostics GmbH, Basel, Switzerland). Glomerular filtration rate (eGFR) was calculated using the CKD-EPI equation and standardized to a body surface area of 1.73 m^2.
- **Biochemical and metabolic markers:** Serum concentrations of calcium (Ca), inorganic phosphate (Pi), alkaline phosphatase (ALP), and uric acid (UA) were determined by standardized methods on a Cobas C 311 automated biochemical analyzer (Roche Diagnostics GmbH, Basel, Switzerland).
- **Inflammatory markers:** High-sensitivity C-reactive protein (hs-CRP) was assayed by turbidimetric immunoassay, and interleukin-6 (IL-6) by electrochemiluminescent immunoassay (ECLIA) with a Cobas E 411 automated immunoanalyzer (Roche Diagnostics GmbH, Basel, Switzerland), according to the manufacturer's instructions.

4.2. Immunological studies

4.2.1. PTH

- Parathyroid hormone (PTH) was measured by electrochemiluminescent immunoassay (ECLIA) on a Cobas E 411 automated immunoanalyzer (Roche Diagnostics GmbH, Basel, Switzerland). The Elecsys PTH kit (catalog number: 11972103122) was used, according to the manufacturer's instructions.

The reference values of the measured parameters, according to the laboratory-specific reference ranges, are presented in Table 1.

Table 1. Reference ranges of the measured parameters.

Indicator	Reference values
SCr	Men: 62 – 106 umol/L; Women: 44 – 80 umol/L
BUN	Men: 3.2 – 7.4 mmol/L; Women: 2.5 – 6.7 mmol/L
UA	Men: 202 – 416 umol/L; Women: 143 – 339 umol/L
Ca	2.15 – 2.55 mmol/L
Pi	0.87 – 1.45 mmol/L
ALP	≤ 128 U/L
PTH	15 – 65 pg/mL
hs-CRP	≤ 3.0 mmol/L
IL-6	< 7 pg/mL
WBC	3.2 – 10.5 G/L

4.2.2. ELISA methods

Venous blood samples were collected in the morning between 8:00 and 9:30 AM following a 12-hour overnight fast. A 5-mL blood sample was collected into sterile serum tubes without anticoagulant. After centrifugation at 1500 rpm, the obtained serum was divided into smaller volumes and stored at −80°C until immunological analyses were performed.

- **Determination of ucMGP by ELISA**

Serum ucMGP concentrations were determined using a quantitative sandwich ELISA method with a commercially available Human Undercarboxylated Matrix Gla Protein ELISA Kit (Abbexa Ltd., Cambridge, UK; cat. no. abx257511). The assay was performed according to the manufacturer's instructions. Absorbance was measured at 450 nm, and ucMGP concentrations were calculated from a standard calibration curve.

- **Determination of NGAL by ELISA**

Serum NGAL concentrations were determined using a quantitative sandwich ELISA method with a commercially available Human Lipocalin-2/NGAL Immunoassay ELISA Kit (R&D Systems, USA; cat. no. DLCN20). The assay was performed according to the manufacturer's instructions. Absorbance was measured at 450 nm, and NGAL concentrations were calculated from a standard calibration curve.

5. Mathematical method

- Calculation of the Ca×P/eGFR ratio.

$$\text{Ca} \times \text{P/eGFR Ratio} = \frac{\text{Ca} \times \text{P}}{\text{eGFR}} = \frac{(\text{mmol/L})^2}{(\text{mL/min/1.73 m}^2)} \quad (1)$$

$$\text{Ca} \times \text{P/eGFR Ratio (Percentage)} = \frac{\text{Ca} \times \text{P}}{\text{eGFR}} \times 100 = \frac{(\text{mmol/L})^2 \times 100}{(\text{mL/min/1.73 m}^2)} \quad (2)$$

- Due to the low numerical values of the Ca×P/eGFR ratio, expressing this parameter as a percentage (as shown in the second formula) facilitates interpretation of the results and improves data presentation.

6. Statistical methods

The experimental data were analyzed using the statistical software package IBM SPSS Statistics v27.0. The results are presented in tables, figures, and descriptive statistical summaries. Continuous variables are presented as mean ± standard deviation (SD) or median with interquartile range (IQR), as appropriate. Categorical variables are presented as frequencies (n) and percentages (%).

Normality of data distribution was assessed using the Kolmogorov–Smirnov and Shapiro–Wilk tests. A p-value < 0.05 was considered statistically significant.

Statistical analyses included descriptive statistics, comparative analysis, correlation analysis, regression analysis, and ROC analysis.

Descriptive analysis:

- Frequency analysis of categorical variables, including absolute frequencies and relative frequencies expressed as percentages.
- Descriptive analysis of quantitative variables, including arithmetic mean, standard deviation, standard error of the mean, and median.

Comparative analysis: Normally distributed continuous variables are expressed as mean ± standard deviation (X ± SD), whereas non-normally distributed variables are presented as median and interquartile range (IQR).

- For normally distributed data, differences between groups were assessed using one-way analysis of variance (ANOVA) with Tukey's post-hoc test for comparisons among multiple groups, as well as independent-samples and paired-samples t-tests.
- For variables with non-normal distribution, non-parametric tests were applied: the Kruskal–Wallis test for comparisons among more than two groups and the Mann–Whitney U test for comparisons between two independent groups.

Correlation analysis:

- Pearson correlation analysis was used to assess the association between two continuous variables with normal distribution.
- Spearman's rank correlation analysis (Spearman's rho) was applied for variables with non-normal distribution and/or ordinal variables.

The strength of correlations was assessed based on the correlation coefficient (r) using the following classification: weak correlation ($r < 0.3$), moderate correlation ($0.3 \leq r < 0.7$), and strong correlation ($0.7 \leq r \leq 1$).

Regression analysis:

Binary logistic regression analysis was performed to identify independent predictors of CVD risk. After assessment of multicollinearity, statistically significant variables were included in a multivariable logistic regression model using forward stepwise selection. The results are presented as odds ratios (ORs) with 95% confidence intervals (CIs) and p-values.

ROC analysis:

Receiver operating characteristic (ROC) curve analysis was performed to assess the predictive performance of ucMGP and the Ca×P/eGFR and ucMGP/eGFR ratios for CVD risk, as well as the ability of NGAL to detect early kidney injury. The results are reported as area under the curve (AUC) values with 95% confidence intervals (CIs) and p-values. The optimal cut-off value was determined using the Youden index.

RESULTS

1. 1. Mineral metabolism and cardiovascular risk

The study included 84 participants aged 31–87 years (32 men and 52 women). Of these, 15 participants had preserved renal function, whereas 69 had predialysis stages of chronic kidney disease (CKD G2–G4) according to the KDIGO classification. CKD staging was performed according to estimated glomerular filtration rate (eGFR) values. Due to the limited number of patients in each CKD stage, patients with CKD were categorized into two groups for statistical analysis: those with eGFR values of 89–45 mL/min and those with eGFR values of 44–15 mL/min, representing lower and higher degrees of renal impairment, respectively.

The distribution of diagnoses across the groups was as follows:

- Group I (eGFR ≥ 90 mL/min/1.73 m²; n = 15): participants with no evidence of kidney disease.
- Group II (eGFR 89–45 mL/min/1.73 m²; n = 42): hypertensive nephropathy (n = 16), diabetic nephropathy (n = 13), chronic pyelonephritis (n = 5), chronic glomerulonephritis (n = 2), chronic tubulointerstitial nephritis (n = 2), autosomal dominant polycystic kidney disease (n = 2), and obstructive uropathy (n = 2).
- Group III (eGFR 44–15 mL/min/1.73 m²; n = 27): hypertensive nephropathy (n = 10), diabetic nephropathy (n = 8), chronic pyelonephritis (n = 4), chronic glomerulonephritis (n = 2), chronic tubulointerstitial nephritis (n = 2), and autosomal dominant polycystic kidney disease (n = 1).

The demographic, clinical, laboratory, and immunological characteristics of the study participants are presented in Table 2.

Table 2. Comparative analysis of clinical, laboratory, and immunological parameters among the three study groups according to eGFR categories.

Parameters	**Group I (eGFR ≥ 90) (n = 15)**	**Group II (eGFR 89 - 45) (n = 42)**	**Group III (eGFR 44 - 15) (n = 27)**	**p =**
Men/Women (n/n)	4/11	15/27	13/14	
Age (years)[1]	58.20 ± 15.49	65.38 ± 12.89	67.29 ± 10.57	0.012
Ca (mmol/L)[2]	2.47 (2.43 - 2.54)	2.47 (2.42 - 2.53)	2.49 (2.45 - 2.57)	0.494
Pi (mmol/L) [1]	1.15 ± 0.14	1.12 ± 0.15	1.22 ± 0.19	0.080
Ca×P (mmol /L)[1]	2.86 ± 0.41	2.76 ± 0.40	3.11 ± 0.63	0.018
SCr (µmol/L) [2]	60 (56 - 67)	95 (70 - 109)	169 (150 - 211)	< 0.001
BUN (mmol/L) [1]	5.87 ± 1.32	6.60 ± 1.74	12.22 ± 3.99	< 0.001
eGFR (mL/min) [2]	102 (92 - 106)	67 (52 - 82)	31 (25 - 41)	< 0.001
ALP (U/L)[1]	62.00 ± 15,25	71.79 ± 16	79.26 ± 24.06	0.020
PTH (pg/mL)[2]	73.6 (28 - 87)	65.2 (51.9 - 100)	84.3 (56.2 - 132.65)	0.493
ucMGP (ng/mL) [2]	2.37 (1.86 - 3.70)	2.14 (1.74 - 2.44)	2.14 (1.75 - 3.71)	0.948
CVD (No/Yes)	12/3	23/19	6/21	

[1] Mean ± SD; [2] Median and IQR. Abbreviations: Ca – serum calcium; Pi – inorganic phosphate; CaxP – calcium phosphate product; SCr – serum creatinine; BUN – blood urea nitrogen, eGFR – estimated glomerular filtration rate; uc-MGP – uncarboxylated matrix Gla-protein. $p < 0.05$ – statistical significance.

There was a statistically significant difference in age, with patients in groups II and III being older than the control group ($p = 0.012$). No significant differences were observed for Ca ($p = 0.494$) and Pi ($p = 0.080$), despite a trend towards higher Pi values in group III. Ca×P and ALP increased with worsening renal function ($p = 0.018$ and $p = 0.020$, respectively). SCr, BUN and eGFR showed a progressive change between groups (all $p < 0.001$), while PTH and ucMGP did not differ significantly ($p = 0.493$ and $p = 0.948$). The proportion of patients with CVD increased with worsening renal function (group I: 3/15; group II: 19/42; group III: 21/27).

2. Evaluation of individual cardiovascular risk in patients with CKD using the Ca×P/eGFR ratio

To investigate the relationship between mineral metabolism and cardiovascular disease (CVD), participants were stratified into two groups according to the presence or absence of CVD. Arterial hypertension was present in all patients with CVD (100%), followed by coronary artery disease (44%), valvular calcifications (42%), heart failure (30%), peripheral arterial disease (19%), and arrhythmias (9%). Due to the presence of multiple comorbidities, these categories were not mutually exclusive.

Table 3 presents the main characteristics of the study participants, divided into two groups according to CVD status.

Table 3. Demographic, clinical and laboratory characteristics of patients divided according to the presence or absence of CVD.

Parameters	**All patients (n = 84)**	**Group without CVD (n = 41)**	**CVD group (n = 43)**	**p =**
Men/Women (n/n)	32/52	11/30	21/22	–
Age (years) [1]	64.71 ± 12.95	64.70 ± 13.82	64.72 ± 12.22	0.996
SCr (μmol/L) [1]	114.65 ± 52.92	93.73 ± 52.03	134.60 ± 46.06	< 0.001
BUN (mmol/L) [1]	8.27 ± 3.78	6.79 ± 2.79	9.69 ± 4.08	< 0.001
UA (μmol/L) [1]	313.22 ± 97.23	283.43 ± 87.15	332.25 ± 99.68	0.059
eGFR (mL/min/1.73 m²) [1]	61.60 ± 27.79	74.09 ± 27.42	49.69 ± 22.63	< 0.001
Ca (mmol/L) [1]	2.49 ± 0.21	2.51 ± 0.29	2.48 ± 0.10	0.444
Pi (mmol/L) [1]	1.16 ± 0.17	1.13 ± 0.15	1.18 ± 0.18	0.202
Ca×P ($mmol^2/L^2$) [1]	2.90 ± 0.51	2.86 ± 0.53	2.94 ± 0.50	0.480
PTH (pg/mL) [1]	84.39 ± 50.39	89.07 ± 67.41	82.13 ± 41.02	0.677
ALP (U/L) [1]	72.44 ± 19.54	70.53 ± 18.98	74.25 ± 20.11	0.387

[1] Mean ± SD; Abbreviations: SCr – serum creatinine; BUN – blood urea nitrogen; UA – uric acid; eGFR – estimated glomerular filtration rate; Ca – serum calcium; Pi – inorganic phosphate; Ca×P – calcium-phosphate product; PTH – parathyroid hormone; ALP – alkaline phosphatase. $p < 0.05$ – statistically significant.

There was no statistically significant difference in age between patients with and without CVD ($p = 0.996$). Patients with CVD had significantly higher SCr and BUN values and lower eGFR values (all $p < 0.001$), while UA showed a trend towards an increase without statistical significance ($p = 0.059$). No significant differences were found for Ca, Pi, Ca×P, PTH and ALP (all $p > 0.05$).

The Ca×P/eGFR ratio in patients with and without CVD is shown in Figure 1.

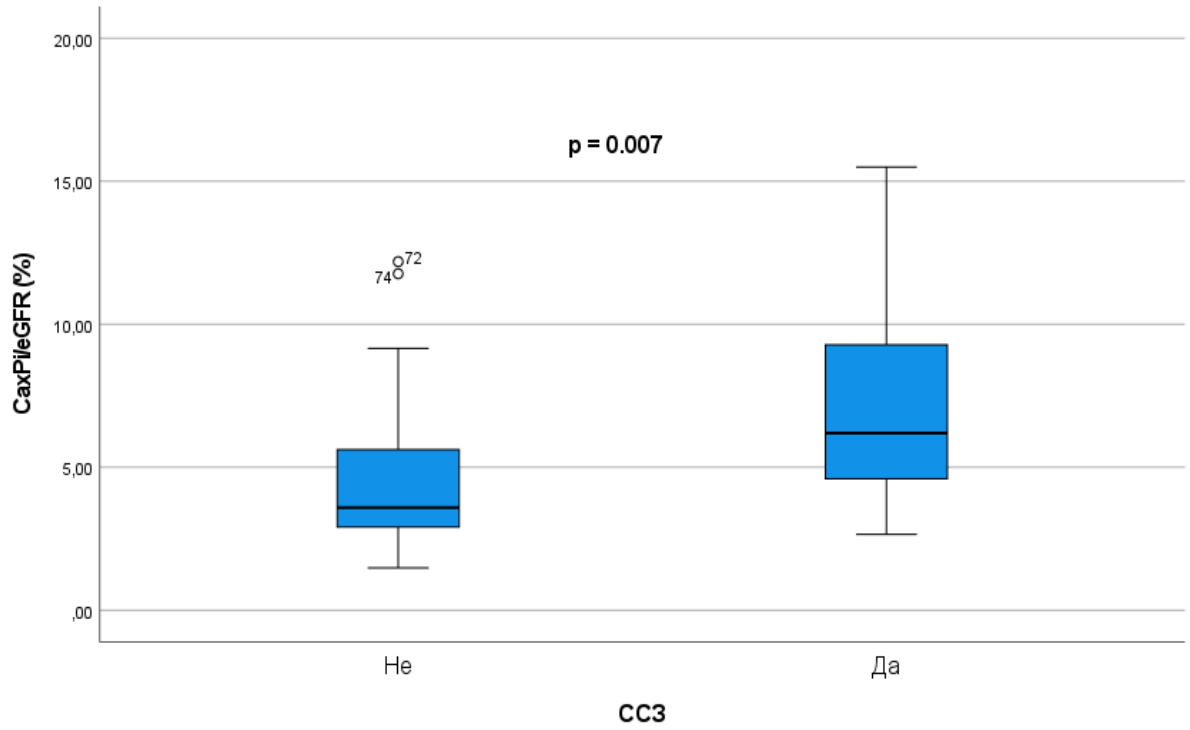

Figure 1. *Boxplot diagram comparing the Ca×P/eGFR ratio between patients with and without CVD in the entire CKD cohort. Ca×P/eGFR is presented in (mmol/L)2 /(ml/min/1.73 m2). No – group without CVD, Yes – group with CVD, p < 0.05 – statistical significance.*

The Ca×P/eGFR ratio was statistically significantly higher in patients with CVD compared to those without CVD (0.07 ± 0.04 vs. 0.05 ± 0.04 (mmol/L)²/(ml/min/1.73 m²); p = 0.007), suggesting a potential association between an increased Ca×P/eGFR ratio and increased cardiovascular risk.

In order to identify predictors of cardiovascular risk in the studied patients, a binary logistic regression analysis was performed, presented in Table 4.

Table 4. Binary logistic regression analysis of predictors of cardiovascular risk in the overall CKD cohort.

Variables	Univariate analysis * (Single predictors)			Multivariate analysis ** (Predictors in the model)		
	Коефициент (B)	**OR (95% CI)**	**p =**	**Коефициент (B)**	**OR (95% CI)**	**p =**
BUN (mmol/L)	0.275	1.317 (1.109 – 1.563)	0.002	–	–	–
SCr (μmol/L)	0.018	1.018 (1.007 – 1.029)	0.001	–	–	–
eGFR (mL/min/1.73 m²)	– 0.038	0.963 (0.944 – 0.982)	< 0.001	– 0.038	0.963 (0.944 – 0.982)	< 0.001
Ca×P/eGFR $(mmol/L)^2$ /(мл/мин/1.73 $м^2$)	0.187	1.206 (1.042 – 1.395)	0.012	–	–	–

* Method = Enter, ** Method = forward stepwise (likelihood ratio). Abbreviations: OR – odds ratio; CI – confidence interval; BUN – blood urea nitrogen; SCr – serum creatinine; eGFR – estimated glomerular filtration rate; Ca×P – calcium-phosphorus product. $p < 0.05$ – statistical significance.

In the univariate analysis, statistically significant associations were identified between the risk of cardiovascular disease (CVD) and several clinical parameters. Elevated levels of blood urea nitrogen (BUN) ($p = 0.002$, OR = 1.317; 95% CI: 1.109–1.563) and serum creatinine (SCr) ($p = 0.001$, OR = 1.018; 95% CI: 1.007–1.029) were associated with an increased risk of CVD. Conversely, lower estimated glomerular filtration rate (eGFR) values were associated with a higher risk of CVD ($p < 0.001$, OR = 0.963; 95% CI: 0.944–0.982). Additionally, the Ca×P/eGFR ratio demonstrated a statistically significant association with CVD risk ($p = 0.012$; OR = 1.206; 95% CI: 1.042–1.395).

In the multivariate analysis, only eGFR remained an independent predictor of CVD risk ($p < 0.001$, OR = 0.963; 95% CI: 0.944–0.982), with lower eGFR values being associated with a higher probability of CVD presence.

To assess the diagnostic ability of the Ca×P/eGFR ratio for predicting cardiovascular risk, ROC analysis was applied (Figure 2).

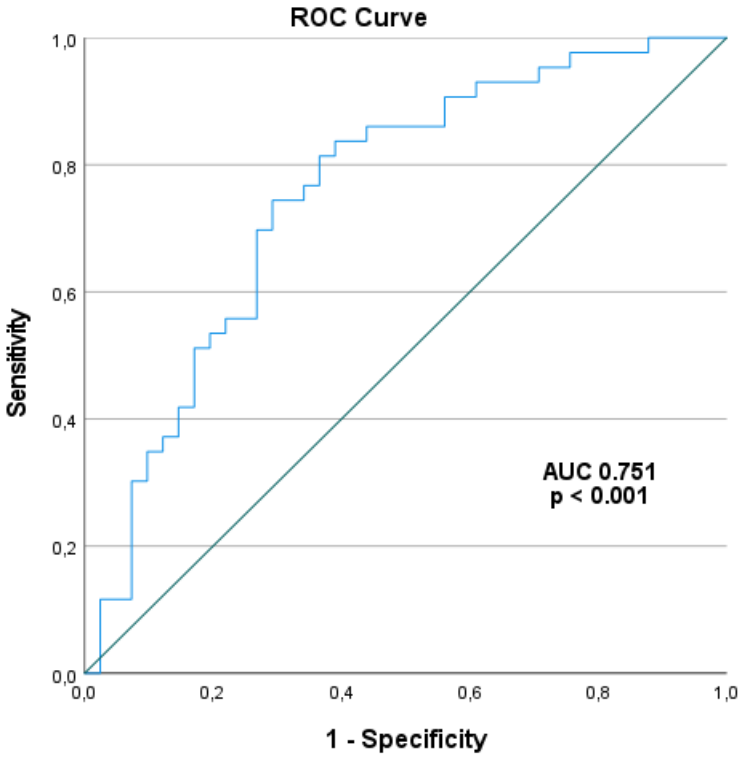

Figure 2. *ROC curve illustrating the diagnostic ability of the Ca×P/eGFR ratio for assessing cardiovascular risk in patients in the predialysis stage of CKD. Abbreviations: ROC - Receiver Operating Characteristic; AUC - area under the curve; p - probability value.*

ROC analysis showed good discriminatory ability of the Ca×P/eGFR ratio to distinguish patients with high and low CVD risk (AUC = 0.751; $p < 0.001$; 95% CI: 0.645–0.857). The optimal cutoff value was 0.048 (mmol/L)/(ml/min/1.73 m²), with a sensitivity of 74.4% and specificity of 70.7%, supporting the potential clinical applicability of the indicator for cardiovascular risk stratification in patients with predialysis CKD.

Table 5 presents the results of the correlation analysis assessing the relationship of the Ca×P/GFR ratio with renal function.

Table 5. Correlation analysis between the Ca×P/eGFR ratio and parameters of renal function.

Correlations	Pearson Correlation Coefficient (r)	p =
Ca×P/eGFR и SCr	0.841 **	< 0.001
Ca×P/eGFR и eGFR	– 0.796 **	< 0.001
Ca×P/eGFR и BUN	0.723 **	< 0.001
Ca×P/eGFR и UA	0.264 *	0.043
Ca×P/eGFR и Ca	0.491 **	< 0.001
Ca×P/eGFR и Pi	0.458 **	< 0.001
Ca×P/eGFR и PTH	0.366 *	0.016
Ca×P/eGFR и ALP	0.399 **	< 0.001

* The correlation is significant at the 0.05 level, ** The correlation is significant at the 0.01 level. Abbreviations: Ca×P/eGFR – ratio of calcium-phosphate product to estimated glomerular filtration rate; SCr – serum creatinine; BUN – blood urea nitrogen; UA – uric acid; Ca – serum calcium; Pi – inorganic phosphate; PTH – parathyroid hormone; ALP – alkaline phosphatase.

Pearson correlation analysis revealed several significant correlations. The strongest positive correlation was observed between the Ca×P/eGFR ratio and serum creatinine (SCr) ($r = 0.841$, $p < 0.001$). A strong negative correlation was identified between the Ca×P/eGFR ratio and estimated glomerular filtration rate (eGFR) ($r = -0.796$, $p < 0.001$). Significant positive correlations were also observed between the Ca×P/eGFR ratio and blood urea nitrogen (BUN) ($r = 0.723$, $p < 0.001$), calcium (Ca) ($r = 0.491$, $p < 0.001$), inorganic phosphate (Pi) ($r = 0.458$, $p < 0.001$), and alkaline phosphatase (ALP) ($r = 0.399$, $p < 0.001$). Moderate positive correlations were found between the Ca×P/eGFR ratio and uric acid (UA) ($r = 0.264$, $p = 0.043$) and parathyroid hormone (PTH) ($r = 0.366$, $p = 0.016$).

The observed correlations between the Ca×P/eGFR ratio and various parameters of renal function highlight the potential of this ratio as a relevant indicator for assessing kidney function and its impact on cardiovascular health in patients with chronic kidney disease (CKD).

3. Uncarboxylated MGP as a biomarker for cardiovascular risk

To investigate the association between ucMGP, chronic kidney disease (CKD), and cardiovascular disease, participants were divided into two groups according to eGFR categories based on KDIGO criteria:

- Group I (GFR ≥ 90 ml/min; n = 15) – control group, comprising participants without evidence of kidney disease.
- Group II (GFR 15 – 89 ml/min; n = 69) – patients with established CKD.

Table 6 presents the clinical, laboratory, and immunological characteristics of the two study groups.

Table 6. Comparative analysis of the main indicators in the studied groups.

Parameters	**Group I (GFR ≥ 90 ml/min) (n = 15)**	**Group II (GFR 15–89 ml/min) (n = 69)**	**p =**
Age (years)	58.2 ± 15.5	66.13 ± 11.2	
Ca (mmol/L)	2.48 ± 0.10	2.5 ± 0.24	0.806
Pi (mmol/L)	1.15 ± 0.15	1.16 ± 0.18	0.902
BUN (mmol/L)	5.87 ± 1.32	8.80 ± 3.95	0.002
SCr (μmol/L)	62.8 ± 12.28	125.93 ± 51.63	< 0.001
eGFR (ml/min)	102.87 ± 15.05	52.64 ± 20.99	< 0.001
ucMGP (ng/mL)	2.31 ± 1.13	2.29 ± 0.9	0.888

Results are presented as mean ± SD. Ca – serum calcium, Pi – inorganic phosphate, BUN – blood urea nitrogen, SCr – serum creatinine, eGFR – estimated glomerular filtration rate, ucMGP – uncarboxylated MGP. $p < 0.05$ – statistical significance

The mean age of patients in group II was higher compared with the control group (66.13 ± 11.2 vs. 58.2 ± 15.5 years). Serum calcium (Ca) and inorganic phosphate (Pi) levels did not show statistically significant differences between the two groups. Patients in group II exhibited statistically significantly higher levels of blood urea nitrogen (BUN) and serum creatinine (SCr) ($p = 0.002$ and $p < 0.001$, respectively), as well as significantly lower estimated glomerular filtration rate (eGFR) values ($p < 0.001$), reflecting impaired renal function in patients with chronic kidney disease (CKD).

Mean serum levels of uncarboxylated matrix Gla protein (ucMGP) did not differ significantly between patients with CKD and the control group (2.29 ± 0.9 vs. 2.31 ± 1.13 ng/mL, $p = 0.888$). No association was observed between serum ucMGP levels and the degree of renal function impairment in the studied population.

The results of the comparison of clinical and laboratory indicators according to the presence of concomitant CVDs in the two groups are presented in Table 7.

Table 7. Distribution of laboratory parameters and ucMGP according to cardiovascular disease status in the two study groups.

Parameters	**Group I (eGFR ≥ 90 ml/min)**			**Group II (eGFR 15 – 89 ml/min)**		
CVD (%)	No (80%) (n = 12)	Yes (20%) (n = 3)	p =	No (42%) (n = 29)	Yes (58%) (n = 40)	p =
Ca (mmol/L)	2.48 ± 0.12	2.5 ± 0.05	0.784	2.53 ± 0.34	2.48 ± 0.11	0.469
Pi (mmol/L)	1.11 ± 0.12	1.31 ± 0.17	0.043	1.15 ± 0.16	1.17 ± 0.18	0.761
BUN (mmol/L)	5.85 ± 1.48	5.97 ± 0.38	0.665	7.18 ± 3.13	9.98 ± 4.1	0.003
SCr (µmol/L)	59.92 ± 11.6	74.33 ± 8.02	0.001	107.72 ± 55.88	139.13 ± 44.51	0.043
eGFR (ml/min)	102.67 ± 16.24	103.67 ± 11.6	0.612	62.28 ± 21.8	45.65 ± 17.44	0.002
ucMGP (ng/mL)	2.07 ± 1.1	3,28 ± 0.79	0.060	1.98 ± 0.73	2.52 ± 0.96	0.009

Results are presented as mean ± SD. CVD – cardiovascular disease, Ca – serum calcium, Pi – inorganic phosphate, BUN – blood urea nitrogen, SCr – serum creatinine, eGFR – estimated glomerular filtration rate, ucMGP – uncarboxylated MGP. $p < 0.05$ – statistical significance

In group I (eGFR ≥ 90 mL/min), 20% of participants had cardiovascular disease (CVD), whereas 80% did not. No statistically significant differences were observed between the two subgroups regarding serum calcium (Ca) levels and eGFR ($p = 0.784$ and $p = 0.612$, respectively). Participants with CVD demonstrated statistically significantly higher levels of inorganic phosphate (Pi) ($p = 0.043$) and serum creatinine (SCr) ($p = 0.001$). Serum uncarboxylated matrix Gla protein (ucMGP) levels were higher in participants with CVD, although the difference did not reach statistical significance ($p = 0.060$).

In group II (eGFR 15–89 mL/min), 58% of participants had CVD, while 42% did not. No statistically significant differences were observed between the two subgroups regarding Ca and Pi levels ($p = 0.469$ and $p = 0.761$, respectively). Participants with CVD showed statistically significantly higher levels of blood urea nitrogen (BUN) ($p = 0.003$) and SCr ($p = 0.043$), as well as significantly lower eGFR values ($p = 0.002$). Serum ucMGP levels were also significantly higher in participants with CVD ($p = 0.009$).

Elevated levels of ucMGP are associated with the presence of cardiovascular disease, both in the general population and in groups with and without CKD (Figures 3 and 4).

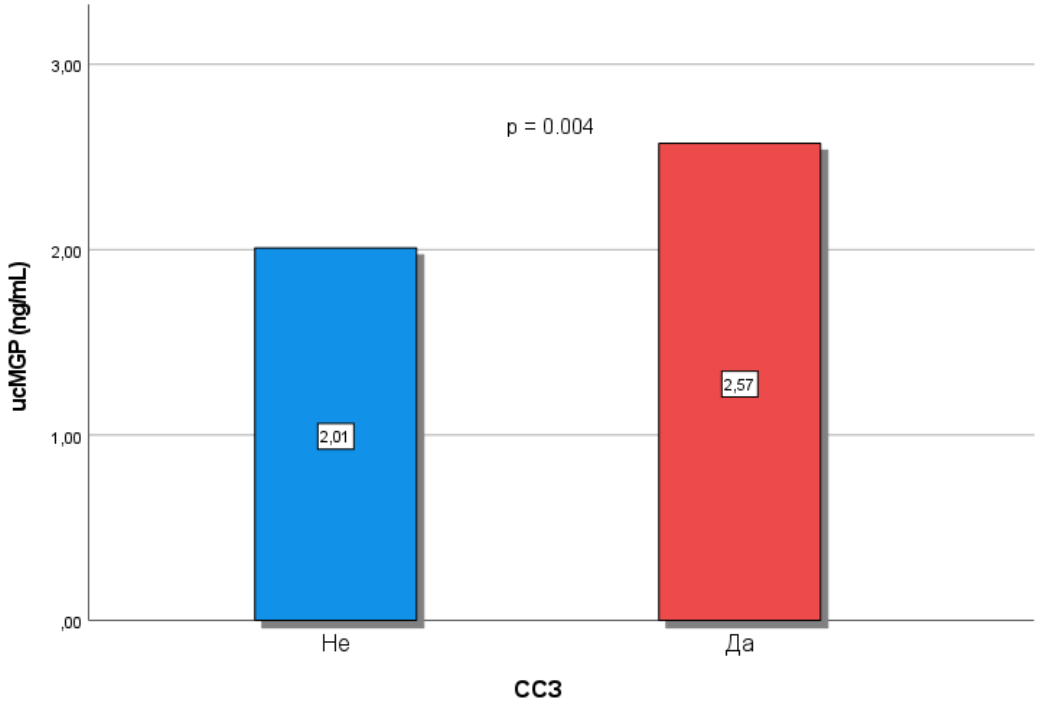

Figure 3. *Bar chart illustrating the mean ucMGP concentration in the general patient population according to the presence or absence of CVD.*

Participants in the CVD group exhibited statistically significantly higher serum ucMGP levels compared with those without CVD (p = 0.004).

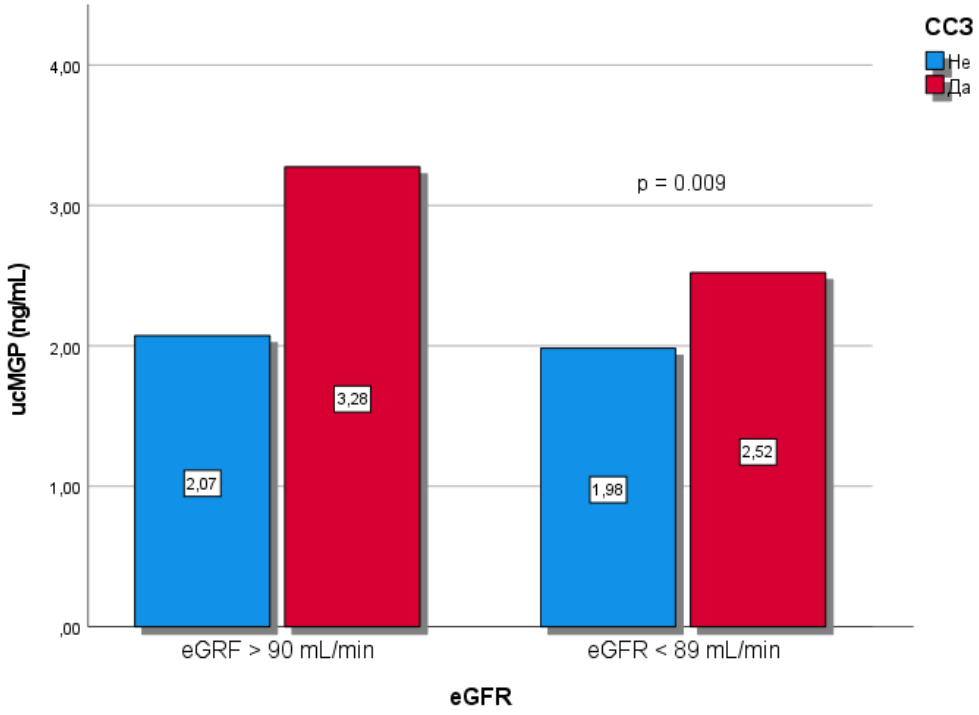

Figure 4. *Bar chart illustrating the mean ucMGP concentration in subjects with preserved and reduced renal function in both groups according to the presence or absence of CVD.*

In the group with preserved renal function, mean serum ucMGP levels were significantly higher in patients with CVD (3.28 ng/mL) compared with those without CVD

(2.07 ng/mL). Similarly, in group II, increased ucMGP levels were observed in patients with CVD (2.52 ng/mL) compared with those without CVD (1.98 ng/mL), with the differences reaching statistical significance (p = 0.009).

The results of the comparative analysis of clinical and laboratory parameters according to sex in the two study groups are presented in Table 8.

Table 8. Comparison of clinical and laboratory parameters and ucMGP levels between men and women in the two study groups.

Parameters	**Group I (eGFR ≥ 90 ml/min)**			**Group II (eGFR 15 – 89 ml/min)**		
	Men (n = 4)	Women (n = 11)	p =	Men (n = 28)	Women (n = 41)	p =
Ca (mmol/L)	2.54 ± 0.08	2.46 ± 0.11	0.089	2.44 ± 0.1	2.53 ± 0.29	0.131
Pi (mmol/L)	1.13 ± 0.15	1.19 ± 0.19	0.005	1.31 ± 0.14	1.1 ± 0.19	0.054
BUN (mmol/L)	5.78 ± 0.65	5.91 ± 1.52	0.948	9.05 ± 3.98	8.63 ± 3.98	0.582
SCr (µmol/L)	74.0 ± 10.17	58.73 ± 10.56	0.013	149 ± 53.34	110.17 ± 44.55	0.002
eGFR (mL/min)	103.25 ± 10.7	102.73 ± 16.8	0.844	49.21 ± 18.73	54.98 ± 22.26	0.346
ucMGP (ng/mL)	3.53 ± 0.4	1,87 ± 0.97	0.006	2.28 ± 0.85	2.09 ± 0.89	0.017

Results are presented as mean ± SD. Ca – serum calcium, Pi – inorganic phosphate, BUN – blood urea nitrogen, SCr – serum creatinine, eGFR – estimated glomerular filtration rate, ucMGP – uncarboxylated MGP. $p < 0.05$ – statistical significance

In group I (eGFR ≥ 90 mL/min), no statistically significant differences between sexes were observed regarding serum levels of Ca, BUN, and eGFR (p = 0.089, p = 0.948, and p = 0.844, respectively). Statistically significantly higher Pi levels were observed in women compared with men (p = 0.005), whereas SCr levels were significantly higher in men (p = 0.013). Serum ucMGP concentrations were also significantly higher in men compared with women (p = 0.006).

In group II (eGFR 15–89 mL/min), no statistically significant differences were identified between men and women regarding serum levels of Ca, Pi, BUN, and eGFR (p = 0.131, p = 0.054, p = 0.582, and p = 0.346, respectively). Serum creatinine was significantly higher in men compared with women (p = 0.002). Serum ucMGP levels also demonstrated a statistically significant difference between sexes, with higher values observed in men (p = 0.017).

The obtained results indicate the presence of sex-related differences in several biochemical parameters, with the most pronounced differences observed for SCr and ucMGP. Mean serum ucMGP concentrations (ng/mL) in women and men according to the degree of renal function are presented in Figures 5 and 6.

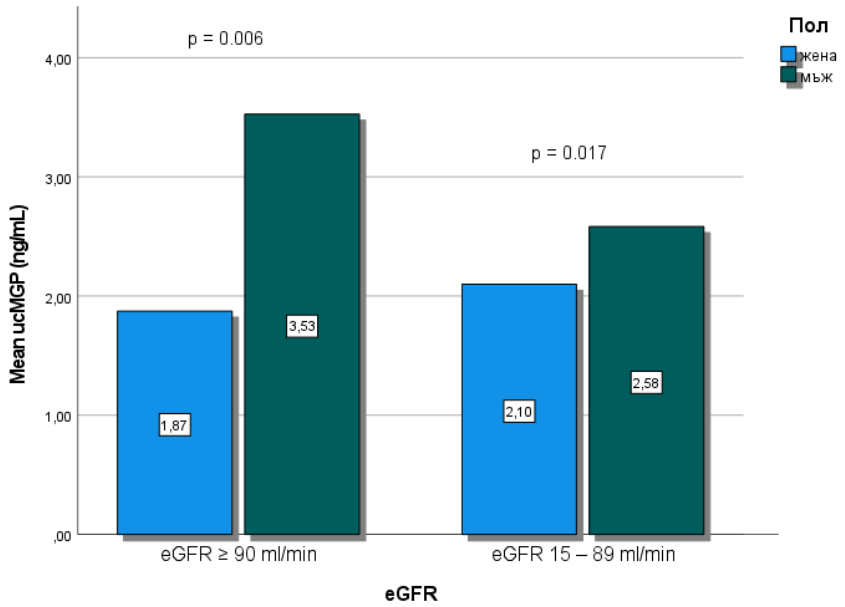

Figure 5. *Bar chart illustrating mean ucMGP levels (ng/mL) in women and men, distributed according to the degree of renal dysfunction.*

In the group with preserved renal function, a statistically significant difference between sexes was observed, with higher serum ucMGP levels in men (3.53 ng/mL) compared with women (1.87 ng/mL, p = 0.006). Similarly, in the CKD group, a significant difference between sexes was identified, with higher ucMGP levels again observed in men (2.58 ng/mL) compared with women (2.10 ng/mL, p = 0.017).

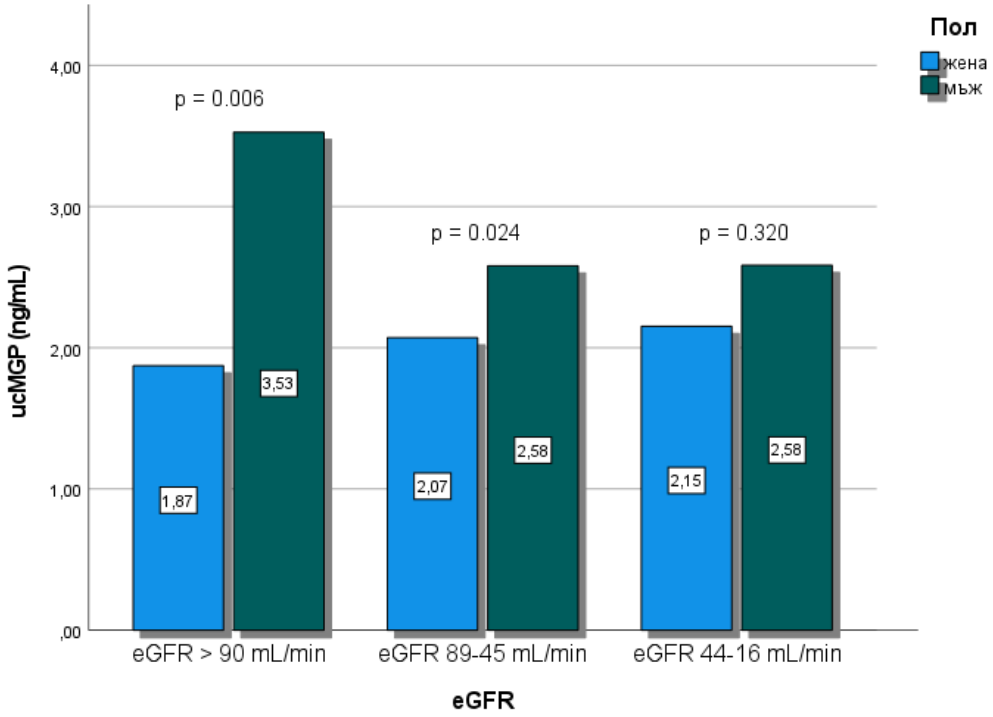

Figure 6. *Bar chart illustrating the mean ucMGP levels (ng/mL) in women and men, depending on the degree of renal dysfunction in the main cohort divided into three groups (Table 2).*

In the group with preserved renal function, a statistically significant difference between sexes was observed (p = 0.006). Men exhibited significantly higher mean ucMGP levels (3.53 ng/mL) compared with women (1.87 ng/mL). These findings suggest a pronounced sex-related dimorphism in ucMGP levels in individuals with preserved renal function, potentially associated with differences in hormonal status or vascular metabolism.

In the group with eGFR 45–89 mL/min, the difference between sexes was also statistically significant (p = 0.024), although with a lower magnitude of significance. Serum ucMGP levels were higher in men (2.58 ng/mL) compared with women (2.07 ng/mL). Among patients with advanced renal impairment (eGFR 16–44 mL/min), ucMGP levels remained higher in men (2.58 ng/mL) than in women (2.15 ng/mL); however, the difference did not reach statistical significance (p = 0.320).

The results of the correlation analyses examining the association between ucMGP levels and demographic and clinical parameters are presented in Table 9.

Table 9. Correlations between ucMGP levels and demographic and clinical parameters in groups stratified by eGFR.

Group	Correlations	Spearman's rho	p =
Group I (eGFR ≥ 90 ml/min)	ucMGP / Gender	0.734**	0.002
Group II (eGFR 89 – 15 ml/min)	ucMGP / CVD	0.315**	0.008
	ucMGP / Age	0.265*	0.028
	ucMGP / Gender	0.290*	0.016

* The correlation is significant at the 0.05 level, ** The correlation is significant at the 0.01 level. Abbreviations: CVD – cardiovascular disease, eGFR – estimated glomerular filtration rate, ucMGP – uncarboxylated MGP.

Correlation analysis revealed statistically significant correlations between serum ucMGP levels and the studied clinical parameters. In group I (eGFR ≥ 90 ml/min), a significant positive correlation was found between ucMGP and gender (p = 0.002). In group II (eGFR 89–15 ml/min), ucMGP showed significant positive correlations with the presence of CVD (p = 0.008), age (p = 0.028) and gender (p = 0.016). The results show that in case of reduced renal function, ucMGP levels are associated with demographic factors and cardiovascular diseases, while in case of preserved renal function, a significant correlation is observed only with gender.

Given the established correlations between ucMGP and CVD, its diagnostic value as a marker of cardiovascular risk was further tested using ROC analyses. Both ucMGP as a stand-alone marker and its ratio to eGFR (ucMGP/eGFR) were evaluated (Figures 7 and 8).

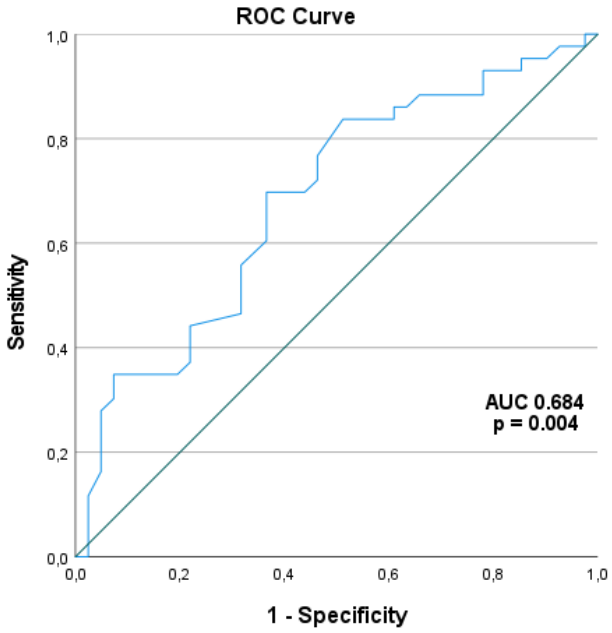

Figure 7. *ROC analysis of ucMGP as a biomarker for cardiovascular risk in patients with chronic kidney disease.*

ROC analysis of ucMGP as a biomarker for cardiovascular risk in patients with chronic kidney disease (CKD) demonstrated statistically significant discriminatory ability. The area under the curve (AUC) was 0.684 (95% CI: 0.569–0.798; p = 0.004), corresponding to moderate diagnostic accuracy. These findings indicate that ucMGP has a statistically significant, but moderate, predictive value for cardiovascular risk. The practical interpretation of these results suggests that ucMGP has limited value as a standalone diagnostic marker for cardiovascular risk assessment in patients with CKD.

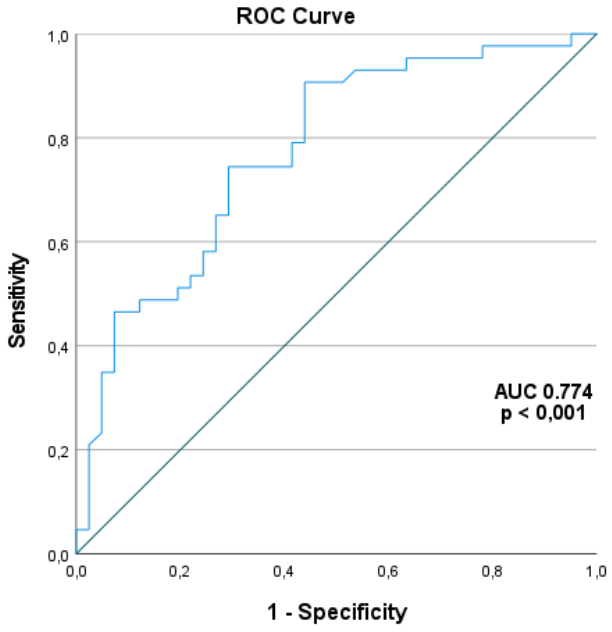

Figure 8. *ROC analysis of the ucMGP/eGFR ratio as a biomarker for cardiovascular risk in patients with CKD.*

The ucMGP/eGFR ratio showed better diagnostic value for cardiovascular risk (AUC = 0.774; p < 0.001). The results highlight its superiority over ucMGP as a stand-alone biomarker.

Binary logistic regressionan alysis was performed to identify independent cardiovascular risk factors in the overall CKD cohort. The results are presented in Tables 10 and 11.

Table 10. Binary logistic regression analysis evaluating ucMGP as a predictor of CVD in the overall CKD cohort.

Variables	**Single predictive factors ***			**Predictive factors in the model ** (Nagelkerke R^2 = 0.504)**		
	Coefficient (B)	OR (95% CI)	p =	Coefficient (B)	OR (95% CI)	p =
Age (years)	0.000	1.000 (0.967 - 1.034)	0.996	- 0.067	0.935 (0.888 - 0.984)	0.010
Gender	- 0.957	0.384 (0.154 - 0.958)	0.040	- 1.899	0.150 (0.031 - 0.720)	0.018
SCr (µmol/L)	0.018	1.018 (1.007 - 1.029)	0.001	- 0.050	0.951 (0.918 - 0.985)	0.005
eGFR, (mL/min/1.73 m^2)	- 0,038	0.963 (0.944 - 0.982)	< 0.001	- 0,109	0.896 (0.841 - 0.955)	0.001
BUN (mmol/L)	0.275	1.317 (1.109 - 1.563)	0.002	0.332	1.393 (1.066 - 1.821)	0.015
ucMGP (ng/mL)	0.699	2.012 (1.201 - 3.371)	0.008	1.071	2.919 (1.344 - 6.342)	0.007

* Method = Enter, ** Method = forward stepwise (likelihood ratio). Abbreviations: OR – odds ratio; CI – confidence interval; SCr – serum creatinine; eGFR – estimated glomerular filtration rate; BUN – blood urea nitrogen; ucMGP – uncarboxylated matrix Gla-protein. p < 0.05 – statistical significance.

In univariate analysis, statistically significant associations with CVD were found for gender (p = 0.040), SCr (p = 0.001), eGFR (p < 0.001), BUN (p = 0.002) and ucMGP (p = 0.008), while age did not show a significant relationship (p = 0.996). In the multivariate model (Nagelkerke R^2 = 0.504), age, gender, SCr, eGFR, BUN and ucMGP remained independent predictors of CVD, with ucMGP showing the highest predictive value (OR = 2.919; 95% CI: 1.344–6.342; p = 0.007). Significant associations were also found for age (p = 0.010), gender (p = 0.018), SCr (p = 0.005), eGFR (p = 0.001) and BUN (p = 0.015).

Table 11. Binary logistic regression analysis evaluating the ucMGP/eGFR ratio as a predictor of CVD in the overall CKD cohort.

Variables	Single predictive factors *			Predictive factors in the model ** (Nagelkerke $R^2 = 0.464$)		
	Coefficient (B)	OR (95% CI)	p =	Coefficient (B)	OR (95% CI)	p =
Age (years)	0.000	1.000 (0.967 - 1.034)	0.996	- 0.063	0.939 (0.894 - 0.987)	0.012
Gender	- 0.957	0.384 (0.154 - 0.958)	0.040	- 2.289	0.101 (0.022 - 0.469)	0.003
SCr (µmol/L)	0.018	1.018 (1.007 - 1.029)	0.001	- 0.057	0.945 (0.910 - 0.980)	0.002
GFR, (mL/min)	- 0,038	0.963 (0.944 - 0.982)	< 0.001	- 0.090	0.914 (0.862 - 0.969)	0.002
BUN (mmol/L)	0.275	1.317 (1.109 - 1.563)	0.002	0.324	1.383 (1.064 - 1.797)	0.015
ucMGP/eGFR	0.357	1.429 (1.159 - 1.762)	0.001	0.319	1.375 (1.007 - 1.877)	0.045

* Method = Enter, ** Method = forward stepwise (likelihood ratio). Abbreviations: OR – odds ratio; CI – confidence interval; SCr – serum creatinine; eGFR – estimated glomerular filtration rate; BUN – blood urea nitrogen; ucMGP/eGFR – ratio of uncarboxylated matrix Gla-protein/estimated glomerular filtration rate. $p < 0.05$ – statistical significance.

In univariate analysis, significant associations with CVD were found for gender ($p = 0.040$), SCr ($p = 0.001$), eGFR ($p < 0.001$), BUN ($p = 0.002$) and the ucMGP/eGFR ratio ($p = 0.001$), while age did not show a significant relationship ($p = 0.996$). In the multivariate model (Nagelkerke $R^2 = 0.464$) independent predictors of CVD remained age, gender, SCr, eGFR, BUN and the ucMGP/eGFR ratio, which showed a significant association (OR = 1.375; 95% CI: 1.007–1.877; $p = 0.045$). Significant relationships were also found for age ($p = 0.012$), gender ($p = 0.003$), SCr ($p = 0.002$), eGFR ($p = 0.002$) and BUN ($p = 0.015$).

4. NGAL as a marker of tubular damage, chronic inflammation and progression of CKD

To assess the association between NGAL levels, mineral metabolism parameters, inflammation, and CKD severity, data from 71 patients with CKD stages G1–G4 according to the KDIGO classification were analyzed. The following biochemical and immunological parameters were measured in all patients: Ca, Pi, SCr, BUN, UA, hsCRP, IL-6, WBC, PTH, ALP, and NGAL.

The distribution of patients with CKD according to groups and diagnoses was as follows:

- Group I (eGFR ≥ 90 mL/min; n = 16): diabetic nephropathy (n = 11), hypertensive nephropathy (n = 3), and chronic pyelonephritis (n = 2).
- Group II (eGFR 45–89 mL/min; n = 33): hypertensive nephropathy (n = 12), diabetic nephropathy (n = 9), chronic pyelonephritis (n = 5), chronic glomerulonephritis (n = 3), chronic interstitial nephritis (n = 2), autosomal dominant polycystic kidney disease (n = 1), and renal amyloidosis (n = 1).
- Group III (eGFR 15–44 mL/min; n = 22): hypertensive nephropathy (n = 6), diabetic nephropathy (n = 7), chronic pyelonephritis (n = 3), chronic glomerulonephritis (n = 2), chronic interstitial nephritis (n = 2), autosomal dominant polycystic kidney disease (n = 1), and contrast-induced nephropathy (n = 1).

The demographic, clinical, laboratory, and immunological characteristics of patients in the three study groups are presented in Table 12.

Table 12. Demographic, clinical, laboratory and immunological indicators of patients in the study groups.

Parameters	**Group I (eGFR ≥ 90) (n = 16)**	**Group II (eGFR 89-45) (n = 33)**	**Group III (eGFR 44-15) (n = 22)**	**p =**
Men/Women (n/n)	5/11	23/10	11/11	
Age (years) [1]	48.81 ± 16.97	67.03 ± 9.69	68.09 ± 11.48	< 0.001
eGFR (mL/min)[1]	102.31 ± 11.75	60.94 ± 11.61	25.82 ± 9.71	< 0.001
SCr (µmol/L) [2]	61.00 (59.25 - 78.75)	105.00 (91.50 - 131.50)	244.00 (164.50 - 281.25)	< 0.001
UA (µmol/L) [1]	275.00 ± 80.73	340.15 ± 71.44	351.86 ± 78.62	0.010
Ca (mmol/L) [1]	2.42 ± 0.11	2.39 ± 0.09	2.32 ± 0.15	0.029
Pi (mmol/L) [1]	1.11 ± 0.15	1.15 ± 0.20	1.28 ± 0.27	0.041
PTH (pg/mL) [2]	21.77 (17.89 - 34.92)	29.10 (20.38 - 41.92)	61.73 (32.80 - 130.80)	< 0.001
ALP (U/L) [1]	74.38 ± 25.51	73.58 ± 19.79	94.32 ± 33.06	0.011
hs-CRP (mg/L) [2]	1.69 (1.13 - 2.96)	2.97 (2.31 - 5.60)	3.72 (2.47 - 10.55)	0.009
IL-6 (pg/mL) [2]	1.50 (1.50 - 1.50)	1.50 (1.50 - 3.21)	3.08 (1.50 - 9.46)	0.007
WBC (G/L) [2]	7.40 (5.70 - 10.40)	7.85 (6.73 - 8.67)	8.40 (7.10 - 10.15)	0.340
NGAL (ng/mL) [1]	113.40 ± 46.47	155.35 ± 50.18	267.80 ± 72.50	< 0.001

[1] Mean ± SD; [2] Median and IQR. Abbreviations: eGFR – estimated glomerular filtration rate; SCr – serum creatinine; UA – uric acid; Ca – serum calcium; Pi – inorganic phosphate; PTH – parathyroid hormone; ALP – alkaline phosphatase; hs-CRP – high-sensitivity C-reactive protein; IL-6 – interleukin-6; WBC – white blood cells; NGAL – neutrophil gelatinase-associated lipocalin; $p < 0.05$ – statistical significance.

Statistically significant differences were found between the groups in terms of indicators reflecting the progression of CKD. With the progression of the disease, eGFR significantly decreased ($p < 0.001$), while SCr and UA increased ($p < 0.001$ and $p = 0.010$). A decrease in Ca ($p = 0.029$) and an increase in Pi ($p = 0.041$), PTH ($p < 0.001$) and ALP ($p = 0.011$) were observed, with the highest PTH values being recorded in patients with advanced stage of CKD. The inflammatory markers hs-CRP and IL-6 showed a significant increase ($p = 0.009$ and $p = 0.007$, respectively), with IL-6 increasing most markedly in the third group. The leukocyte count showed a trend towards an increase without statistical significance ($p = 0.340$).

NGAL levels increased significantly with the progression of renal dysfunction ($p < 0.001$), with the highest values being found in group III (Figure 9).

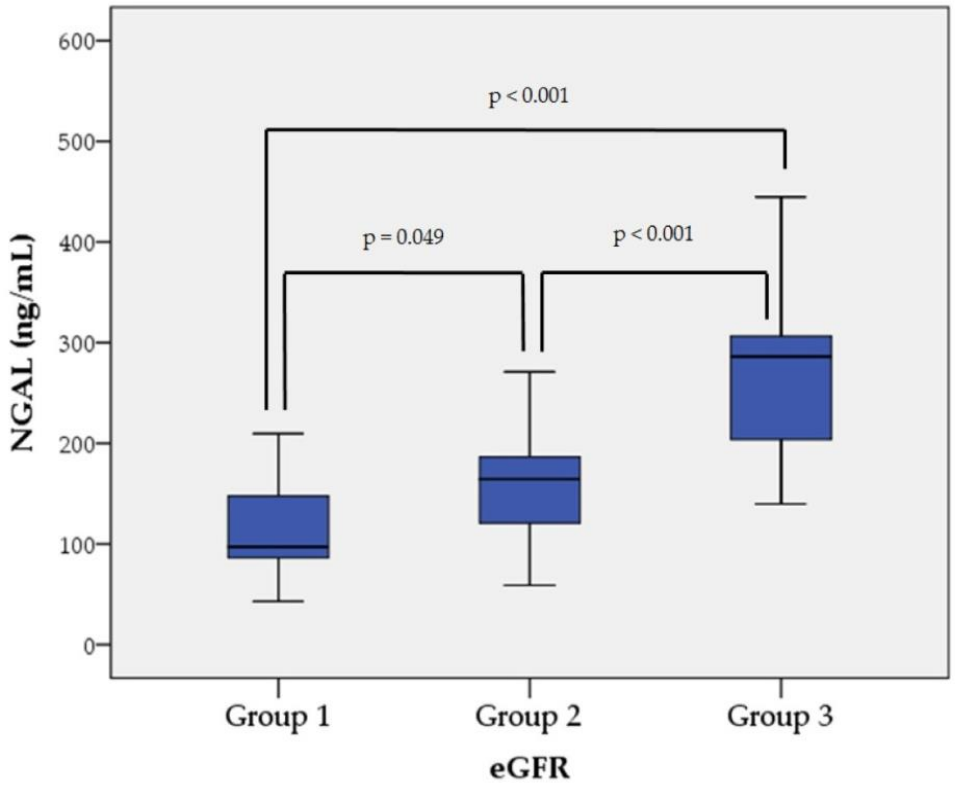

Figure 9. *Comparative analysis of serum NGAL levels in the three study groups depending on eGFR.*

NGAL concentrations showed a progressive increase with decreasing eGFR, with the lowest values being found in the control group and the highest in patients with advanced CKD. Statistically significant differences were found between groups I and II ($p = 0.049$), groups II and III ($p < 0.001$), and groups I and III ($p < 0.001$). The increase in NGAL correlated with the progression of renal damage, with the greater variability in group III probably reflecting differences in the degree of tubular damage.

The results of the correlation analyses conducted to assess the relationship between serum NGAL and the studied clinical and laboratory parameters are presented in Table 13.

Table 13. Correlations between NGAL levels and clinical and biochemical parameters in the total study cohort.

Correlations	Spearman's rho	p =
NGAL and Age	0.284 *	0.016
NGAL and eGFR	– 0.730 **	< 0.001
NGAL and SCr	0.736 **	< 0.001
NGAL and UA	0.260 *	0.029
NGAL and Ca	– 0.334 **	0.004
NGAL and Pi	0.265 *	0.025
NGAL and PTH	0.494 **	< 0.001
NGAL and ALP	0.370 **	0.001
NGAL and hs-CRP	0.334 **	0.004
NGAL and IL-6	0.509 **	< 0.001
NGAL and WBC	0.362 **	0.005

* The correlation is significant at the 0.05 level, ** The correlation is significant at the 0.01 level. Abbreviations: NGAL – neutrophil gelatinase associated lipocalin; eGFR – estimated glomerular filtration rate; SCr – serum creatinine; UA – uric acid; Ca – serum calcium; Pi – inorganic phosphate; PTH – parathyroid hormone; ALP – alkaline phosphatase; hs-CRP – high-sensitivity C-reactive protein; IL-6 – interleukin-6; WBC – leukocytes.

Correlation analysis revealed significant relationships between serum NGAL levels and the studied clinical and biochemical parameters. NGAL showed a strong negative correlation with eGFR (rho = –0.730, $p < 0.001$) and a positive correlation with SCr (rho = 0.736, $p < 0.001$). A negative correlation with Ca (rho = –0.334, $p = 0.004$) and positive correlations with age, UA, Pi, PTH and ALP were found. Among the inflammatory markers, NGAL correlated positively with hs-CRP, IL-6 and leukocyte count ($p = 0.004$, $p < 0.001$ and $p = 0.005$, respectively). The obtained results indicate a relationship between increased NGAL levels, renal dysfunction and the activity of the inflammatory process.

5. NGAL като биомаркер за ранна бъбречна увреда

A total of 28 subjects, including 12 healthy controls and 16 patients with CKD stage G1 and preserved renal function, were included to evaluate NGAL as a marker of early renal injury. Renal injury was defined by the presence of microalbuminuria (n = 14) or proteinuria (n = 2). The underlying diagnoses were diabetic nephropathy (n = 11), hypertensive nephropathy (n = 3), and chronic pyelonephritis (n = 2).

The comparative analysis of clinical and laboratory parameters between healthy controls and patients with CKD stage G1 is presented in Table 14.

Table 14. Clinical and laboratory characteristics of individuals in the two study groups.

Parameters	**Controls (n = 12)**	**Group G1 (eGFR ≥ 90 ml/min) (n = 16)**	**p =**
Men/Women (n/n)	4/8	5/11	
Age (years) [1]	51.17 ± 10.49	48.81 ± 16.97	0.676
eGFR (mL/min) [2]	103 (93 - 117)	98 (92 - 116)	0.607
SCr (µmol/L) [1]	77.50 ± 10.42	68.75 ± 13.91	0.080
UA (µmol/L) [1]	315.00 ± 82.00	275.00 ± 80.73	0.209
Ca (mmol/L) [1]	2.50 ± 0.09	2.42 ± 0.11	0.044
Pi (mmol/L) [1]	1.18 (1.16 - 1.26)	1.15 (0.97 - 1.22)	0.056
PTH (pg/mL) [1]	26.82 ± 6.09	26.32 ± 14.33	0.911
ALP (U/L) [1]	60.50 ± 21.17	74.38 ± 25.51	0.138
hs-CRP (mg/L) [2]	1.67 (1.23 - 2.45)	1.69 (1.13 - 2.96)	0.576
IL-6 (pg/mL) [2]	1.50 (1.50 - 1.50)	1.50 (1.50 - 1.50)	0.212
NGAL (ng/mL) [1]	76.29 ± 20.12	113.41 ± 46.46	0.016

[1] Mean ± SD; [2] Median and IQR. Abbreviations: eGFR – estimated glomerular filtration rate; SCr – serum creatinine; UA – uric acid; Ca – serum calcium; Pi – inorganic phosphate; PTH – parathyroid hormone; ALP – alkaline phosphatase; hs-CRP – high-sensitivity C-reactive protein; IL-6 – interleukin-6; NGAL – neutrophil gelatinase-associated lipocalin; $p < 0.05$ – statistical significance.

The control group and group G1 were comparable in age, gender distribution and basic clinical and laboratory parameters, including renal function, mineral metabolism and inflammatory markers. No significant differences were found in eGFR, SCr, UA, Ca, Pi, PTH, ALP, hs-CRP and IL-6. Only NGAL levels were statistically significantly higher in group G1 compared to the control group ($p = 0.016$).

To assess the potential prognostic value of NGAL as an early biomarker of renal injury, univariable binary logistic regression analysis was performed (Table 15).

Table 15. Univariate binary logistic regression analysis.

Variables	Single predictive factors		
	Coefficient (B)	OR (95% CI)	p =
NGAL (ng/mL)	0.119	1.126 (1.002 - 1.265)	0.045

Abbreviations: OR – odds ratio; CI – confidence interval; NGAL – neutrophil gelatinase associated lipocalin. $p < 0.05$ – statistical significance.

Univariate analysis showed a significant association of the biomarker with G1 stage (OR = 1.126, p = 0.045). OR = 1.126 indicates that with a one-unit increase in NGAL, the chance of the event occurring increases by about 12.6%.

ROC analysis was performed to evaluate the diagnostic performance of NGAL as a marker of early renal injury in patients with CKD stage G1 (Figure 10).

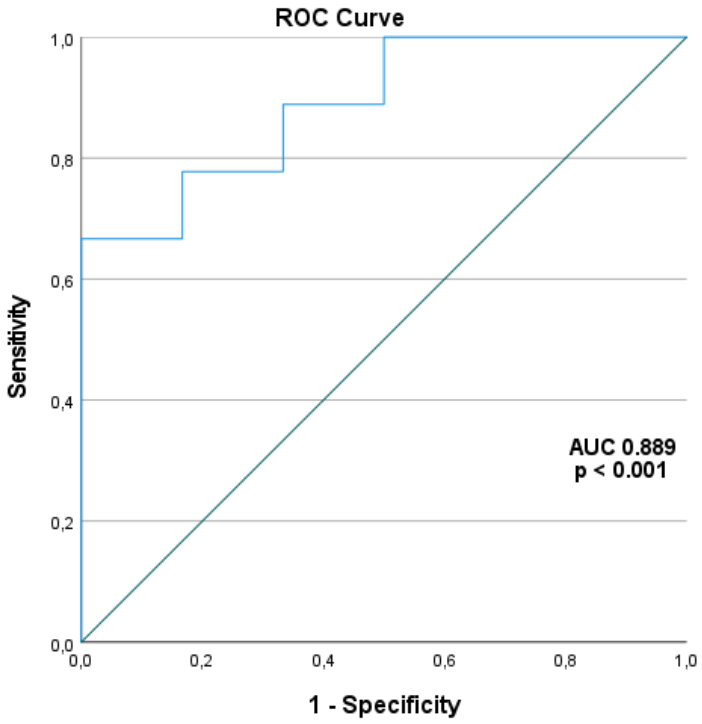

Figure 10. *ROC analysis of NGAL as an ultra-early biomarker for tubular damage in patients with CKD.*

ROC analysis also demonstrated excellent discriminative ability of NGAL to distinguish patients with CKD stage G1 from healthy controls (AUC = 0.889, $p < 0.001$).

DISCUSSION

Cardiovascular disease (CVD) represents the leading cause of morbidity and mortality among patients with chronic kidney disease (CKD), accounting for approximately half of all deaths in the advanced stages of the disease. The incidence of ischemic heart disease, heart failure, arrhythmias, and sudden cardiac death is substantially increased in this population and demonstrates a progressive rise with the deterioration of renal function. Beyond the contribution of traditional cardiovascular risk factors, CKD-specific disturbances in mineral and bone metabolism, which constitute the basis of the CKD-mineral and bone disorder (CKD-MBD) syndrome, play a crucial role in accelerating vascular calcification and further increasing cardiovascular risk.

Vascular calcification represents one of the major pathogenetic links between CKD and the heightened cardiovascular burden observed in these patients. In CKD, vascular calcification occurs predominantly in two forms: medial and intimal calcification. Medial calcification is particularly prevalent in advanced stages of renal impairment and may develop independently of conventional atherosclerotic risk factors, being strongly associated with disturbances in calcium-phosphate homeostasis. This process results in increased arterial stiffness, impaired vascular compliance, and elevated cardiac afterload, thereby contributing to progressive cardiovascular dysfunction. In contrast, intimal calcification is closely associated with atherosclerotic plaque formation and inflammatory mechanisms, increasing the risk of acute cardiovascular events. Recent evidence has demonstrated an inverse relationship between the extent of vascular calcification and bone mineral density, suggesting the presence of a complex interplay between vascular and skeletal pathology. This relationship contributes to the simultaneous increase in fracture risk and cardiovascular complications among patients with CKD.

The analysis of the results presented in Table 2 demonstrates a progressive deterioration of renal function with advancing CKD stage. This is evidenced by a statistically significant increase in serum creatinine (SCr) and blood urea nitrogen (BUN) levels, accompanied by a gradual decline in glomerular filtration rate ($p < 0.001$). A significant increase in mean age was also observed across the groups ($p = 0.012$).

With regard to mineral metabolism, serum calcium concentrations remained relatively stable between groups, whereas phosphate levels demonstrated an increasing trend with CKD progression. A significant elevation in the Ca×P product ($p = 0.018$) and alkaline phosphatase (ALP) activity ($p = 0.020$) was observed, reflecting the progressive impairment of mineral homeostasis associated with advancing CKD and being consistent with established mechanisms involved in the development of vascular calcification.

No significant differences were detected in serum parathyroid hormone (PTH) or uncarboxylated matrix Gla protein (ucMGP) concentrations between the groups.

Particularly noteworthy is the absence of variation in ucMGP levels ($p = 0.948$), indicating that this marker may not directly correspond to the severity of renal dysfunction. Its expression appears to be more closely associated with local vascular processes than with the degree of renal impairment.

Regarding cardiovascular status, there was a clear progressive increase in the prevalence of cardiovascular disease with advancing CKD stage. The incidence increased from 20% in the control group to 45% among patients with CKD G2-G3a and reached 78% in patients with CKD G3b-G4. These findings further support the close relationship between renal dysfunction and cardiovascular pathology.

The results demonstrate a typical pattern of CKD progression, characterized by a significant decline in renal function, accompanied by early and partially compensated disturbances in mineral metabolism, occurring in parallel with an increase in cardiovascular risk.

Mineral metabolism disorders in CKD represent not merely biochemical manifestations of the disease but also independent pathogenetic drivers contributing to the development of cardiovascular complications. They promote vascular calcification, endothelial dysfunction, increased arterial stiffness, and adverse myocardial remodeling, thereby playing a central role in the pathogenesis of cardiorenal syndrome (CRS) type 4. In this condition, chronic renal dysfunction induces progressive structural and functional alterations within the cardiovascular system, ultimately contributing to the increased cardiovascular morbidity and mortality observed in patients with CKD.

The development of CRS type 4 is most commonly associated with four major pathophysiological mechanisms: (1) disturbances in calcium-phosphorus metabolism, resulting in medial vascular calcification and valvular calcification; (2) chronic pro-inflammatory and pro-fibrotic processes that contribute to the development of atherosclerosis, coronary artery disease, myocardial fibrosis, heart failure, and arrhythmias; (3) cardiovascular complications associated with renal hypertension, including myocardial infarction, stroke, and heart failure; and (4) additional comorbid conditions, such as anemia, volume overload, and electrolyte disturbances, which increase cardiac workload and predispose to arrhythmogenesis.

Due to the complex nature of these processes, the identification of reliable biomarkers remains a major challenge. Currently used biomarkers reflect individual components of the pathophysiology of CRS type 4, including neurohormonal activation, chronic inflammation, oxidative stress, endothelial dysfunction, and myocardial fibrosis. Among the most extensively studied are BNP, NT-proBNP, cardiac troponins, galectin-3, urotensin II, cystatin C, microalbuminuria, uric acid, homocysteine, and aldosterone. Despite their established diagnostic and prognostic value, none of these biomarkers simultaneously provides integrated information on renal function and disturbances in mineral metabolism, both of which are key determinants in the development of CKD–MBD.

In assessing cardiovascular risk in patients with CKD, traditional biomarkers include SCr, eGFR, proteinuria, lipid profile, CRP, homocysteine, and other indicators of renal

function, metabolic disorders, and systemic inflammation. Each of these markers provides valuable information about specific aspects of the disease. However, none simultaneously reflects the disturbances in mineral metabolism and the progressive decline in renal function, which are the two main mechanisms underlying vascular calcification in CKD. In recent years, considerable attention has been paid to biomarkers such as FGF-23 and Klotho, which play complementary roles in the regulation of phosphate homeostasis and the development of vascular calcification. Despite their established pathophysiological importance, their application in routine clinical practice remains limited due to high costs, lack of standardized measurement methods, and limited availability. This highlights the need to identify more accessible and easily applicable biomarkers that can provide integrated information regarding the risk of cardiovascular complications.

In this context, the proposed Ca×P/eGFR ratio represents a novel integrative biomarker that combines information on calcium-phosphorus metabolism disorders with the degree of renal dysfunction. The rationale for this index is based on well-established pathophysiological mechanisms associated with CKD. An elevated Ca×P product reflects a condition that promotes the development of vascular and valvular calcification, while a reduced eGFR is a well-established independent predictor of adverse cardiovascular outcomes. The combination of these two parameters allows for a more comprehensive assessment of cardiovascular risk compared to the assessment of each of the two parameters separately.

The results of the present study support this hypothesis. When comparing patients with and without CVD, statistically significant differences were identified only in parameters reflecting renal function: eGFR ($p < 0.001$), SCr ($p < 0.001$), and BUN ($p < 0.001$). In contrast, no significant differences were observed in the serum levels of Ca, Pi, Ca×P, UA, PTH, and ALP (Table 3). These findings highlight the predominant role of progressive renal impairment in the development of cardiovascular complications. Multivariate logistic regression analysis confirmed that impaired renal function, assessed by eGFR, was the strongest independent predictor of the presence of CVD in patients with CKD ($p < 0.001$; OR = 0.963) (Table 4). However, the analysis also demonstrated that the Ca×P/eGFR ratio provides significant prognostic information. The ratio was significantly higher in patients with CVD compared with those without CVD ($p = 0.007$) (Figure 1). The mean value of the index was 0.05 ± 0.04 $(mmol/L)^2/(ml/min/1.73\ m^2)$ in patients without CVD and 0.07 ± 0.04 $(mmol/L)^2/(ml/min/1.73\ m^2)$ in patients with CVD. Univariate logistic regression analysis identified the Ca×P/eGFR ratio as a statistically significant predictor of the presence of CVD ($p = 0.012$; OR = 1.206), supporting its potential role as a novel integrative biomarker for cardiovascular risk assessment (Table 4). Although eGFR remains the strongest independent predictor, the incorporation of mineral metabolism-related information through the Ca×P component enables a more comprehensive characterization of the pathophysiological processes underlying vascular calcification. Further evidence supporting the diagnostic potential of this index is provided by ROC analysis. The obtained

area under the ROC curve (AUC = 0.751; $p < 0.001$) demonstrates good discriminatory ability for distinguishing patients with and without CVD. At the optimal cutoff value of 0.048 $(mmol/L)^2/(ml/min/1.73\ m^2)$, the sensitivity was 74.4% and the specificity was 70.7% (Figure 2). These findings suggest that the Ca×P/eGFR ratio may serve as a useful tool for early cardiovascular risk stratification in patients with non-dialysis stages of CKD.

Correlation analysis demonstrated that the Ca×P/eGFR index is closely associated with both renal function parameters and disturbances in mineral-bone metabolism (Table 5). The strongest correlations were observed with SCr ($r = 0.841$; $p < 0.001$), eGFR ($r = -0.796$; $p < 0.001$), and BUN ($r = 0.723$; $p < 0.001$). These findings indicate that as renal dysfunction progresses, the values of the index increase accordingly, with a substantial proportion of its variability being determined by the severity of renal impairment.

Beyond renal function parameters, the index showed statistically significant positive correlations with key markers of mineral-bone metabolism, including Ca ($r = 0.491$; $p < 0.001$), Pi ($r = 0.458$; $p < 0.001$), ALP ($r = 0.399$; $p < 0.001$), and PTH ($r = 0.366$; $p = 0.016$). These associations are consistent with the established pathophysiological alterations observed in CKD-related mineral and bone disorder (CKD-MBD) and suggest that the index reflects not only the degree of renal dysfunction but also disturbances in calcium-phosphate homeostasis. Uric acid demonstrated only a weak, although statistically significant, correlation with the index ($r = 0.264$; $p = 0.043$), indicating a limited contribution to its variability.

The findings of this study demonstrate that the Ca×P/eGFR ratio represents an integrated indicator that reflects two major pathophysiological processes in CKD: progressive deterioration of renal function and disturbances in mineral metabolism. This integrated approach may explain its superior ability to characterize cardiovascular risk compared with the assessment of traditional laboratory parameters individually.

Based on the obtained results, we developed a risk stratification scale using the Ca×P/eGFR ratio for the assessment of cardiovascular risk in patients with predialysis stages of CKD. The proposed scale comprises six risk categories, ranging from “very low risk” to “extremely high risk” (Table 16).

Table 16: Cardiovascular risk assessment scale in predialysis CKD patients based on the Ca×P/eGFR ratio.

Risk classification	Ca×P/eGFR Ratio *	Ca×P/eGFR Ratio (%) *	Risk description
Very low risk	< 0.03	< 3	Very low probability of cardiovascular complications
Low risk	0.03 – 0.05	3 – 5	Low probability of cardiovascular complications
Moderate risk	0.05 – 0.10	5 – 10	Moderate likelihood of cardiovascular complications
High risk	0.10 – 0.15	10 – 15	Increased likelihood of cardiovascular complications
Very high risk	0.15 – 0.20	15 – 20	Significant likelihood of serious cardiovascular events
Extremely high risk	> 0.20	> 20	Critical probability of severe cardiovascular events

* The Ca×P/eGFR ratio and the Ca×P/eGFR ratio (percentage) are presented in (mmol/L)2 /(ml/min/1.73 m2) and (mmol/L)2 × 100/(ml/min/1.73 m2), respectively.

The proposed scale should be regarded as an initial risk stratification model developed based on the findings of the present study. Although it demonstrates potential clinical applicability, its diagnostic and prognostic performance requires validation in prospective studies involving larger and independent patient cohorts. Further evaluation is also needed to determine its calibration, reproducibility, and incremental prognostic value compared with established cardiovascular risk assessment models. Following successful validation, the proposed scale may serve as a simple and accessible tool for early cardiovascular risk stratification in patients with predialysis CKD. Its implementation could facilitate the identification of high-risk individuals, support individualized therapeutic strategies, optimize clinical monitoring, and enable the timely initiation of preventive interventions. Furthermore, serial assessment of the Ca×P/eGFR ratio may provide additional information regarding disease progression and response to therapeutic interventions.

To our knowledge, this study is the first to propose the Ca×P/eGFR ratio as an integrative biomarker for cardiovascular risk assessment in patients with predialysis CKD. The main advantage of this indicator is that it is based on widely available laboratory parameters routinely measured in clinical practice, without requiring additional specialized investigations. This feature provides the potential for its straightforward implementation in everyday clinical practice, pending confirmation of its prognostic value in future validation studies.

In addition, the results of the present study should be interpreted in light of some limitations. First, the relatively small size of the study population limits the statistical

significance and generalizability of the results. In addition, the cross -sectional design of the study does not allow for the establishment of causal relationships and assessment of the prognostic value of the index with respect to future cardiovascular events. It should also be noted that multivariate analysis identified eGFR as the main independent predictor of the presence of CVD. Therefore, additional prospective studies are needed to determine the independent contribution of the Ca×P/eGFR ratio and its added value to existing risk prediction models. Despite these limitations, the results obtained indicate that the Ca×P/eGFR ratio is a promising integrative indicator for assessing cardiovascular risk in patients with predialysis stages of CKD. By combining information on renal function and disorders in mineral-bone metabolism, the index reflects two interconnected pathophysiological mechanisms determining the development of cardio-renal complications. The obtained data show good diagnostic ability and support its potential as a complementary tool for early risk stratification.

The present study provides a foundation for future prospective investigations aimed at validating the Ca×P/eGFR ratio in larger, independent patient populations and defining its potential role within clinical algorithms for the assessment and monitoring of cardiovascular risk in patients with CKD.

After assessing cardiovascular risk using the integrative index Ca×P/eGFR, we focused on investigating molecular biomarkers reflecting the processes of vascular calcification and mineral imbalance in patients with CKD. Within the same study, serum levels of ucMGP were analyzed using clinical, laboratory, correlation, ROC and regression analyses. The aim was to assess the relationship of the protein with renal function and CVD, as well as its potential contribution to the comprehensive risk assessment in CRS type 4.

Cardiovascular complications develop early in the course of CKD and represent a major contributor to increased morbidity and mortality. Disturbances in calcium-phosphate metabolism, secondary hyperparathyroidism, and activation of pathways promoting vascular calcification play a key role in their pathogenesis. Matrix Gla protein (MGP) is one of the principal endogenous inhibitors of vascular calcification, and its biological activity depends on vitamin K-dependent carboxylation. Uncarboxylated MGP (ucMGP) represents the functionally inactive form of the protein, and elevated serum levels are considered an indirect marker of impaired carboxylation and an increased propensity toward vascular calcification.

Our results did not demonstrate statistically significant differences in serum ucMGP levels between patients with preserved and impaired renal function (Tables 2 and 6), suggesting that ucMGP should not be considered a marker of the severity of renal dysfunction. In contrast, the analysis stratified according to the presence of CVD (Table 7, Figures 3 and 4) revealed higher ucMGP levels in patients with cardiovascular disease, with the difference reaching statistical significance among patients with CKD ($p = 0.009$).

Analysis of the data presented in Table 7 demonstrated a significant association between serum ucMGP levels and the presence of CVD in both patients with preserved renal

function and those with CKD. In the control group, patients with CVD exhibited statistically higher Pi ($p = 0.043$) and SCr ($p = 0.001$) levels, as well as a trend toward increased ucMGP concentrations ($p = 0.060$), suggesting a potential role of this biomarker in the earliest stages of vascular injury. Among patients with CKD (Group II), the presence of CVD was associated with higher BUN ($p = 0.003$) and SCr ($p = 0.043$) levels, lower eGFR values ($p = 0.002$), and a statistically significant increase in serum ucMGP concentrations ($p = 0.009$). Correlation analysis (Table 9) revealed distinct patterns depending on the presence of renal impairment. Among participants with preserved renal function, ucMGP demonstrated a significant correlation only with gender (rho = 0.734; $p = 0.002$). In contrast, in patients with CKD, positive associations were observed between ucMGP levels and the presence of CVD (rho = 0.315; $p = 0.008$), age (rho = 0.265; $p = 0.028$), and gender (rho = 0.290; $p = 0.016$).

Overall, the results indicate that in patients with preserved renal function, ucMGP levels are predominantly associated with gender, whereas with the progression of CKD, additional associations emerge, including age and the presence of CVD. These findings suggest that renal dysfunction modifies the clinical context in which ucMGP should be interpreted and may strengthen its association with cardiovascular risk.

The obtained results are consistent with published literature data indicating that ucMGP levels are associated with male sex, advanced age, increased BMI, reduced eGFR, arterial stiffness, endothelial dysfunction, arterial hypertension, coronary artery disease, and adverse cardiac remodeling.

In support of these findings, ROC analysis (Figures 7 and 8) demonstrated a moderate discriminative ability of ucMGP for differentiating patients with and without CVD (AUC = 0.684; $p = 0.004$). Binary logistic regression analyses (Tables 10 and 11) confirmed that both ucMGP and the ucMGP/eGFR ratio were significant predictors of CVD. Following adjustment for age, sex, and renal function, both parameters remained statistically significant, supporting their independent association with cardiovascular risk. However, given the cross-sectional design of the study, these findings should be interpreted as evidence of association rather than causation.

Of particular interest is the combined ucMGP/eGFR parameter, which may provide additional information by integrating biomarker status with renal function. ROC analysis (Figures 7 and 8) demonstrated higher diagnostic accuracy and sensitivity of the integrated ucMGP/eGFR index (AUC = 0.774; $p < 0.001$) compared with ucMGP alone. These findings indicate that combining a marker of vascular calcification with an indicator of renal function may provide more accurate cardiovascular risk stratification.

An important finding of the present study is the marked gender difference in serum ucMGP levels, with men demonstrating significantly higher concentrations in all groups studied (Table 8, Figures 5 and 6). These differences may be explained by the higher incidence of vascular calcification in men, potentially related to increased MGP synthesis, variations in dietary vitamin K intake, differences in vitamin K-dependent carboxylation, and the influence of sex hormones on calcium-phosphate metabolism in the vascular wall.

In summary, serum ucMGP levels are significantly associated with cardiovascular disease (CVD) in patients with CKD, regardless of CKD stage and conventional measures of renal function. The lack of significant differences between CKD stages (Tables 2 and 6), together with the observed associations of ucMGP with cardiovascular status, age, and gender (Table 9), supports its potential role as a marker of systemic vascular changes. ROC analysis (Figures 7 and 8) and regression models (Tables 10 and 11) demonstrated moderate discriminatory capacity of ucMGP and suggested its potential contribution to cardiovascular risk stratification. These findings indicate that ucMGP should not be considered as a stand-alone diagnostic biomarker, but rather as a component of an integrated model reflecting vascular calcification, mineral metabolism disorders, and cardio-renal interactions in CKD. Combining ucMGP with eGFR may improve cardiovascular risk assessment. However, the clinical utility of this approach requires confirmation in larger prospective studies.

While markers of mineral metabolism and vascular calcification (Ca×P/eGFR and ucMGP) primarily reflect systemic and vascular components of CRS, tubulointerstitial injury represents a key and often earlier component of CKD progression. Its assessment remains challenging because traditional markers of renal function, including SCr, eGFR, and proteinuria, do not reliably reflect early structural alterations in the renal parenchyma. The limitations of these markers, which are influenced by age, sex, muscle mass, hydration status, and other factors, highlight the need for biomarkers capable of detecting subclinical tubular injury before significant decline in glomerular function occurs.

Among these biomarkers, NGAL is recognized as a sensitive indicator of tubular stress and inflammatory activation. Although initially introduced as a biomarker of acute kidney injury, accumulating evidence in recent years supports its role in the assessment of CKD. Serum and urinary NGAL concentrations increase significantly earlier than SCr, making it a promising biomarker for the early detection of tubular injury. However, despite its high sensitivity, NGAL is not entirely organ-specific, as its levels may also be elevated in systemic inflammation, infections, and other extrarenal conditions. Therefore, its interpretation should be individualized according to the clinical context and characteristics of the studied population.

In the present study, serum NGAL levels differed significantly among the studied groups ($p < 0.001$) (Table 12). A progressive increase was observed from group I (113.40 ng/mL) through group II (155.35 ng/mL) to group III (267.80 ng/mL), paralleling the decline in eGFR and the increase in SCr levels. In group III, NGAL concentrations were nearly two-fold higher than those observed in group I, indicating the high sensitivity of this biomarker to the progression of renal injury.

Correlation analysis demonstrated a strong negative association between NGAL and eGFR (rho = –0.730; $p < 0.001$) and a positive correlation with SCr (rho = 0.736; $p < 0.001$), confirming its relationship with the deterioration of renal function. Furthermore, NGAL levels correlated positively with hs-CRP (rho = 0.334; $p = 0.004$), IL-6 (rho = 0.509; $p < 0.001$), and leukocyte count (rho = 0.362; $p = 0.005$), supporting the involvement of chronic

inflammatory processes in CKD progression. Significant associations were also observed with Pi (rho = 0.265; p = 0.025), PTH (rho = 0.494; p < 0.001), and ALP (rho = 0.370; p = 0.001), together with an inverse correlation with serum Ca (rho = –0.334; p = 0.004), suggesting a potential role of NGAL in the disturbances of mineral and bone metabolism. The obtained findings support the potential of NGAL as an integrative biomarker reflecting multiple interconnected processes in CKD, including renal injury, inflammatory activation, and disturbances in mineral metabolism (Table 13).

It cannot be excluded that part of the observed increase in NGAL levels reflects systemic inflammatory activation rather than exclusively kidney-specific injury. However, the presence of a significant negative correlation with eGFR and a positive correlation with SCr, a classical marker of renal function, suggests that NGAL also reflects, at least partially, kidney-specific processes.

Tubular injury plays a crucial pathogenic role in the progression of CKD. This mechanism is also observed in diseases characterized by primary glomerular injury. Published data indicate that NGAL levels are elevated from the early stages of diabetic nephropathy, IgA nephropathy, hypertensive nephropathy, and other forms of chronic kidney injury, with serum concentrations correlating with the severity of tubulointerstitial alterations and interstitial fibrosis.

Our study also identified significant associations between NGAL and PTH, Ca, and Pi, suggesting a potential link between tubular injury and disturbances in mineral and bone metabolism in CKD. Current experimental evidence indicates a possible role of NGAL in the regulation of bone metabolism through interactions with FGF23-dependent pathways, as well as through effects on phosphate homeostasis and vitamin D activation. Although these mechanisms remain incompletely understood, the findings of the present study extend the concept of NGAL beyond its classical role as a marker of tubular injury.

A positive correlation between NGAL and age was also observed (rho = 0.284; p = 0.016), which may reflect the age-related decline in renal function and the increased prevalence of chronic inflammatory processes (Table 13).

Subsequent analysis comparing G1 patients with healthy controls provided additional insights into the potential role of NGAL as an early diagnostic biomarker (Table 14). The two groups did not differ significantly in age, eGFR, or SCr levels, confirming preserved glomerular function in patients with G1 CKD. Serum calcium was the only parameter that showed a statistically significant difference, with lower levels observed in the G1 group, whereas Pi demonstrated a trend toward lower values without reaching statistical significance. No significant differences were observed in PTH, ALP, hs-CRP, or IL-6 levels, suggesting the absence of pronounced systemic inflammation or secondary hyperparathyroidism at this early stage of disease. The most notable finding was the statistically significant increase in serum NGAL concentrations in G1 patients compared with controls (113.41 ± 46.46 ng/mL vs. 76.29 ± 20.12 ng/mL; p = 0.016). These results

suggest the presence of early tubular injury that is not detectable by conventional markers of renal function.

The findings support the concept that functional parameters and structural renal injury do not always progress in parallel. Due to the substantial functional reserve and compensatory capacity of the kidneys, early tubulointerstitial injury may remain undetected when relying solely on SCr and eGFR.

Binary logistic regression demonstrated a significant association between NGAL levels and the presence of G1-stage CKD (OR = 1.126; p = 0.045), while ROC analysis showed excellent discriminatory performance of the biomarker (AUC = 0.889; p < 0.001) (Table 15, Figure 10). Although multivariate analysis could not be performed due to the limited sample size, these findings support the potential utility of NGAL for the early detection of CKD.

The combined analysis of all studied groups demonstrates a consistent increase in NGAL levels from the subclinical phase of the disease, with a progressive rise accompanying CKD progression. In the early stages, NGAL primarily reflects tubular epithelial activation and injury, whereas in advanced stages it may reflect persistent inflammation, interstitial fibrosis, and progressive loss of nephron mass. This pattern supports the concept that tubulointerstitial injury represents a central mechanism in the pathogenesis of CKD and suggests that NGAL is not merely a marker of disease progression, but also a potential early indicator of an emerging pathological process.

The practical implications of these findings are considerable. While SCr and eGFR primarily reflect the decline in glomerular function, NGAL provides an opportunity for earlier detection of tubular injury, more accurate assessment of disease activity, and improved risk stratification. This is particularly relevant in the follow-up of patients with immune-mediated glomerular diseases, in which control of glomerular inflammation does not always prevent the development of tubular atrophy and interstitial fibrosis.

The practical significance of this integrated approach is considerable. While traditional markers, such as SCr and eGFR, primarily reflect the decline in glomerular function at more advanced stages of CKD, NGAL provides opportunities for early detection of tubular injury, more precise assessment of disease activity, and improved risk stratification for disease progression. Therefore, the systematic use of NGAL across different stages of CKD may facilitate a transition from a reactive to a proactive diagnostic approach, enabling the identification of renal injury before the development of irreversible functional alterations.

In summary, the present dissertation demonstrates that CKD is characterized by a close interplay between renal dysfunction, disturbances in mineral metabolism, and cardiovascular risk, which can be more comprehensively assessed through an integrative approach incorporating both functional and molecular biomarkers. In this context, the Ca×P/eGFR ratio reflects the combined impact of impaired mineral homeostasis and reduced renal function and demonstrates good discriminatory capacity for cardiovascular

risk stratification. Serum ucMGP, in turn, is associated with CVD independently of the degree of renal dysfunction and may reflect the activity of vitamin K-dependent processes involved in vascular calcification and vascular remodeling. Concurrently, NGAL enables sensitive assessment of tubulointerstitial injury from the early stages of CKD and demonstrates associations with inflammatory activity and the progression of renal dysfunction.

Taken together, these indicators define a multifactorial pathophysiological model of CRS type 4, in which glomerular injury, tubular dysfunction, and vascular calcification do not occur as isolated processes, but rather interact and mutually amplify one another. The findings of the present study support the concept that the integration of functional (eGFR), metabolic (Ca, Pi), and molecular (ucMGP, NGAL) biomarkers may facilitate more accurate early identification and risk stratification of patients with increased cardiovascular risk. However, these observations should be interpreted with caution and require validation in prospective and multicenter studies before potential implementation in clinical practice.

CONCLUSIONS

1. In patients with predialysis CKD stages G1–G4, progressive disturbances in calcium-phosphate metabolism were observed, correlating with the degree of renal dysfunction and cardiovascular risk.

2. The Ca×P/eGFR ratio integrates information on disturbances in mineral metabolism and demonstrates good discriminatory capacity for cardiovascular risk stratification according to the degree of renal dysfunction.

3. Serum ucMGP levels were associated with cardiovascular disease independently of the severity of renal dysfunction, supporting the potential role of this biomarker in cardiovascular risk assessment.

4. Serum NGAL levels increased with the progression of renal injury and demonstrated significant associations with renal function parameters, confirming its value as a sensitive biomarker of tubulointerstitial injury.

5. The observed associations between NGAL, inflammatory markers, and disturbances in mineral metabolism support the involvement of chronic inflammation in the pathogenesis and progression of CKD.

CONTRIBUTIONS

ORIGINAL CONTRIBUTIONS

1. A comprehensive study was performed integrating biomarkers of vascular calcification (ucMGP), tubular injury (NGAL), inflammation (hs-CRP, IL-6), and disturbances in mineral metabolism in patients with predialysis CKD stages G1–G4.

2. The Ca×P/eGFR ratio was introduced as an integrative indicator reflecting mineral metabolism disturbances and renal dysfunction, and its direct association with cardiovascular risk was established.

3. A six-level cardiovascular risk stratification scale based on the Ca×P/eGFR ratio was developed, enabling the categorization of patients from very low to extremely high risk.

4. The findings demonstrated that ucMGP is a marker primarily associated with vascular pathology, sex, and age, rather than with the extent of renal damage.

5. It was demonstrated that NGAL levels are elevated even in the early (subclinical) stages of CKD, despite preserved renal function (eGFR ≥ 90 mL/min), defining NGAL as an ultra-early biomarker of renal injury.

6. The combined use of the ucMGP/eGFR and Ca×P/eGFR biomarker ratios demonstrated higher accuracy in cardiovascular risk stratification compared with the use of either biomarker alone.

CONFIRMATORY CONTRIBUTIONS

1. The association between disturbances in calcium–phosphorus metabolism and increased cardiovascular risk in patients with CKD was confirmed.

2. The association between elevated serum ucMGP levels and the presence of cardiovascular disease was confirmed, supporting its role as a biomarker of cardiovascular risk.

3. It was confirmed that serum NGAL levels increase with the progression of renal impairment and correlate with established indicators of renal function.

4. The association between NGAL, systemic inflammation, and disturbances in mineral metabolism in CKD was confirmed.

PUBLICATIONS AND PARTICIPATIONS

PUBLICATIONS

1. **Borislav Ignatov**, Tatyana Simeonova, Tsvetelina Eftimova, Anelia Dimitrova, Krasimir Kostov. The relationship between undercarboxylated matrix Gla protein and cardiovascular diseases in pre-dialysis chronic kidney disease patients.
   Journal of Biomedical and Clinical Research 18(1): 81–89 (2025), ISSN 1313-9053

   Borislav Ignatov, Krasimir Kostov. The role of Lipocalin-2 and Galectin-3 in the pathogenesis of chronic kidney disease.
   First National Conference of Pathophysiology, Tsigov Chark/25-27.04.2025, ISBN: 978-619-237-151-7

2. Tatyana Simeonova, **Borislav Ignatov**, Tsvetelina Eftimova, Krasimir Kostov. Is Undercarboxylated Matrix Gla Protein a Reliable Biomarker of Vitamin K2 Status in Patients with Chronic Kidney Disease? Journal of IMAB - Annual Proceeding (Scientific Papers) 31(2):6179-6184, ISSN: 1312-773X,
   IF: 0.2, Q4 (Web of Science, Scopus)

PARTICIPATIONS

1. **Borislav Ignatov**, Tsvetelina Eftimova, Tatyana Simeonova, Petya Dragomirova, Pavlina Yordanova-Laleva, Sergey Kostadinov, Anelia Dimitrova. Study of circulating uncarboxylated matrix Gla-protein as a biomarker for vitamin K2 status in patients with chronic kidney disease.
   Anniversary scientific conference "60 years of Pathophysiology", Medical University - Varna, September 24 - 25, 2021.

2. **Borislav Ignatov**, Tsvetelina Eftimova, Tatyana Simeonova, Petya Dragomirova, Pavlina Yordanova-Laleva, Anelia Dimitrova. Vitamin K2 status in patients with Chronic Kidney Disease.
   12-th South-East European Conference of chemotherapy, infections and cancer & 32-st Annual Assembly of International Medical Association Bulgaria 20-23 October, 2022, Trakia University – Stara Zagora
3. **Borislav Ignatov**, Tsvetelina Eftimova, Tatyana Simeonova, Petya Dragomirova, Pavlina Yordanova-Laleva, Sergey Kostadinov, Aneliya Dimitrova. Is

undercarboxylated MGP a potential marker for vitamin K2 status or vascular calcification in patients with CKD?
National Congress of Physiology with international participation, Stara Zagora Mineral Baths, Spa Hotel "Armira", 30.10–01.11.2022.

4. **B. Ignatov**, T. Eftimova, T. Simeonova, P. Dragomirova, A. Dimitrova, K. Kostov. Relationship between levels of uncarboxylated matrix Gla-protein, gender, age and cardiovascular risk in patients with chronic kidney disease in the predialysis period. National Conference on Nephrology, October 11-13, 2024, Albena Resort.

5. **B. Ignatov**, T. Simeonova, V. Raykova, G. Ignatov, T. Eftimova, K. Kostov. Circulating uncarboxylated matrix Gla protein as a potential biomarker for cardiovascular risk in patients with chronic kidney disease.
Anniversary scientific conference "50 years of medical education and science in Pleven", November 1-3, 2024.

6. **B. Ignatov**, K. Kostov. The role of Lipocalin 2 and Galectin 3 in the pathogenesis of chronic kidney disease.
First National Conference on Pathophysiology, 25 - 27 April 2025, Tsigov Chark.

7. Alexander Blazhev, Krasimir Kostov, **Borislav Ignatov**, Velizar Mitev, Svetla Blazheva, Tatyana Simeonova. Characterizing endothelin isoforms, uric acid, and systemic inflammation in progressive chronic kidney disease.
Balkan.bio 2025 – 6th Balkan Conference on BioSciences, October 30–31, 2025, Plovdiv

8. **B. Ignatov**, T. Eftimova, T. Simeonova, A. Blazhev, K. Kostov. Vitamin K2 status in patients with chronic kidney disease in the predialysis period.
National Conference on Nephrology, 17 - 19 October 2025, Albena Resort.

9. **Borislav Ignatov**, Krasimir Kostov. Matrix Gla Protein as a Key Regulator of Vascular Biology: Molecular Forms, Functional Activity, and Role as a Biomarker.
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