

# Assessment of kidney function in diabetic patients. Is there a role for new biomarkers NGAL, cystatin C and KIM-1?

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## ABSTRACT

**Purpose:** Assessment of kidney injury early detection in diabetic patients has great importance for therapy and prognosis. The aim of this study was to assess whether neutrophil gelatinase-associated lipocalin (NGAL), cystatin C, and kidney injury molecule-1 (KIM-1) could represent sensitive markers of kidney function/injury in patients with coronary heart disease and diabetes.

**Materials and Methods:** The study comprised 121 consecutive patients with diabetes referred for coronary angiography due to coronary heart disease and a reference group consisting of 64 patients without diabetes.

**Results:** Cystatin C, serum and urinary NGAL values were significantly higher in diabetics than in non-diabetics. There was no significant difference in KIM-1 levels in both groups. Serum NGAL in diabetic group was associated with serum creatinine, fibrinogen, urinary NGAL, cystatin C and inversely related to kidney function assessed with 4 equations. After analysing levels of studied biomarkers in both groups, no significant difference in patients with estimated glomerular filtration rate (eGFR) below 60 ml/min/1.73m<sup>2</sup> was found. The analysis of patients with eGFR over 60 ml/min/1.73m<sup>2</sup> showed significant differences in cystatin C and urinary NGAL levels. The area under the curve for serum NGAL, urinary NGAL and cystatin C was 0.60 (95% CI, 0.51 to 0.69), 0.59 (95% CI, 0.5 to 0.68), 0.62 (95% CI, 0.54 to 0.71), respectively, good cut-off values of studied biomarkers to detect diabetes were not found.

**Conclusion:** NGAL, cystatin C and KIM-1 are not more useful than eGFR in the assessment of kidney function in diabetic patients with coronary heart disease.

**Key words:** Diabetes, eGFR, KIM-1, NGAL, cystatin C.

## INTRODUCTION

Cardiovascular diseases, especially ischemic heart disease, are still the leading cause of death in Poland [1]. The K/DOQI guidelines state that individuals with a reduced glomerular filtration rate (GFR) below 60 ml/min are at greater risk for cardiovascular disease and cardiac death [2]. Problem of proper assessment of renal function is of crucial importance in Invasive Cardiology Departments. Diabetes and impaired renal function are the strongest predictors of incidence of

contrast induced nephropathy [3]. At the moment there are a few methods assessing kidney function. The most common method is still serum creatinine concentration. Nevertheless, serum creatinine concentration is affected by factors other than GFR. More accurate and precise assessment of renal function is possible with equations based upon serum creatinine. However, these formulae also have several limitations and can provide widely various results. The aforementioned considerations raise questions on new methods of renal function assessment. Recent studies suggest that neutrophil

gelatinase-associated lipocalin (NGAL), cystatin C, and kidney injury molecule-1 (KIM-1) are the most promising potential biomarkers for early identification of CKD.

NGAL is a member of the lipocalin family of proteins and is expressed only at very low levels in several human tissues, including kidney [4]. Therefore NGAL protein has been extensively studied in different models of acute kidney injury (AKI). In numerous studies, the increase of NGAL in urine and serum has been a good predictor of a brief-term onset of AKI, especially anticipating the increase in serum creatinine [5-10]. There is also a growing number of publications suggesting that NGAL could be a marker of CKD and its severity [11,12].

Cystatin C is synthesized at a relatively constant rate and released into the plasma by all nucleated cells [13]. This protein is freely filtered by the glomerulus, completely reabsorbed and metabolized by the proximal tubules, therefore the serum cystatin C concentration correlates closely with GFR [13]. In a number of studies, serum cystatin C was more sensitive in identifying reduction of kidney function than serum creatinine, especially in patients with mild renal dysfunction [14-18].

KIM-1 is a novel, lately described renal tubular cell injury marker [19]. High urinary KIM-1 levels predict the onset of AKI and are also associated with adverse clinical outcomes in patients with AKI [20,21]. Recent publications also suggest that KIM-1 may be involved in the pathophysiology of CKD [22].

These biomarkers have been regarded as factors enabling the detection of kidney damage earlier than it is currently possible with traditional markers such as serum creatinine. Since in our previous study [23], we found high incidence of CKD (defined as GFR below 60 ml/min) in diabetic patients referred for percutaneous coronary interventions despite normal creatinine concentration, therefore the aim of this study was to assess kidney function with traditional methods (GFR equations) and new biomarkers (NGAL, cystatin C, KIM-1) in the same population of patients with stable angina and diabetes.

## MATERIAL AND METHODS

### Patients and the reference group

The study comprised 121 consecutive patients with diabetes referred for coronary angiography to the Department of Invasive Cardiology of the Medical University Hospital in Bialystok in 2008 due to coronary heart disease. The reference group consisted of 64 patients also referred for coronary angiography without diabetes. Lack of diabetes was confirmed in the oral glucose tolerance test (OGTT).

Medical records of study participants were collected, including baseline demographic characteristics, concomitant diseases, previous myocardial infarction (MI), previous

percutaneous coronary intervention (PCI), coronary artery bypass grafting surgery (CABG), pharmacotherapy and presence of cardiovascular risk factors. After percutaneous intervention study database was completed with angiographic characteristics and therapy (PCI or CABG).

We excluded patients with acute coronary syndrome.

All the patients were instructed on the aim of the study and gave their informed consent. The study protocol was approved by the local Ethics Committee at the Medical University of Bialystok, Poland (approval number: R-I-002/207/2008)

### Biochemical analyses

Venous blood samples were collected in the morning, following an overnight fasting, before the procedure and before any food intake. The first urine micturition of the day was also collected before percutaneous coronary intervention. Common biochemical parameters, including hematological parameters, cholesterol, high-density lipoprotein, low-density lipoprotein, triglycerides, creatinine, glucose, HbA1c, fibrinogen were assessed by the standard laboratory methods in the central laboratory at the Medical University Hospital in Bialystok. Serum and urinary NGAL, serum cystatin C and urinary KIM-1 concentrations were measured using commercially available kits. Serum and urinary NGAL was evaluated using enzyme-linked immunosorbent assay (ELISA) kits (AntibodyShop, Gentofte, Denmark). Serum cystatin C was measured using commercially available kits from BioVendor R&D based on ELISA method (Modrice, Czech Republic). Urinary KIM-1 measurements were performed using polyclonal antibodies raised against the human KIM-1 ectodomain (Uscn Life Science & Technology Company, Wuhan, China). All the tests were performed according to the manufacturers' instructions by the same person. All biomarkers' levels were expressed as ng/ml.

Kidney function in the study was assessed according to 4 formulae: Cockcroft-Gault equation [24], lean body mass-adjusted Cockcroft-Gault equation [25], simplified MDRD equation [26] and CKD-EPI study equation [27].

### Statistical analysis

Data given was analyzed using Statistica 6.0 PL computer software (Tulsa, OK, USA). Data was expressed as means  $\pm$  SD or a percentage frequency, as appropriate. Differences between groups were established by an unpaired t-test for normally distributed values and by Mann-Whitney test for nonparametric values. Pearson or Spearman correlation coefficient was employed to test correlations between variables. Afterwards, we performed multiple regression analyses to assess independent relationships. Values of  $p < 0.05$  were considered statistically significant. ROC analysis was employed to calculate the area under the curve for studied biomarkers to find best cut-off values able to detect diabetes.

## RESULTS

### Clinical characteristics of the studied patients

Diabetic patients' mean age was  $66.5 \pm 8.31$  while mean age of patients without diabetes was  $64.14 \pm 9.91$  years (NS). In the diabetes group there were 41 (33.9%) females and 80 (66.1%) males. In the reference group there were 21 (32.8%) females and 43 (67.2%) males.

Body mass index for diabetic patients was  $30.64 \pm 4.84$  kg/m<sup>2</sup>, and for non-diabetic it was  $27.94 \pm 4.73$  kg/m<sup>2</sup> ( $p < 0.001$ ). Arterial hypertension was equally frequent in diabetic and non-diabetic groups (95.9% vs. 90.6%). Systolic, as well as, diastolic blood pressure was similar in both groups i.e. with diabetes RRs  $132.27 \pm 20.05$  mmHg, RRr  $75.45 \pm 10.61$  mmHg, and without diabetes RRs  $128.48 \pm 18.57$  mmHg, RRr  $73.63 \pm 10.31$  mmHg (NS). Previous myocardial infarction was found more often in diabetic patients (46.3% vs. 32.8%;  $p < 0.05$ ). Peripheral artery disease was also detected more frequently in diabetic patients (16.5% vs. 3.1%;  $p < 0.01$ ). The proportion of previous PCI (32.2% vs. 32.8%), as well as CABG (5.0% vs. 6.3%) was similar in the study and control groups. There were few currently smoking patients in the diabetic and non-diabetic groups (10.7% vs. 15.6%).

Coronary angiography performed in the diabetic group revealed multivessel disease in 40 patients (33.05%), one- or two-vessel disease in 56 patients (46.29%), and no significant lesions in 25 patients (20.66%). In the non-diabetic group, angiographic analysis revealed multivessel disease in 15 patients (23.4%), one- or two-vessel disease in 25 patients (39.1%), and insignificant coronary artery disease in 24 patients (37.5%). The duration of percutaneous coronary intervention ( $52 \pm 20$  min in group with diabetes (DM) vs.  $55 \pm 28$  min in control group), the volume of contrast agent administered ( $158 \pm 77$  ml in DM group vs.  $144 \pm 88$  ml control group) and the time of hospitalization ( $3 \pm 2$  days DM group vs.  $3 \pm 1$  days control group) were similar in both groups.

Evaluation of heart failure according to The New York Heart Association (NYHA) functional classification revealed the following results in diabetic patients: class I-49.6%, class II-33.9%, class III- 15.7%, class IV- 0.8%, and in non-diabetic patients: I class - 76.5%, class II-17.2%, class III-6.3%, class IV- 0.

Mean left-ventricular ejection fraction was significantly lower in diabetic patients ( $47.38\% \pm 9.69$  vs.  $50.24\% \pm 8.61$ ;  $p < 0.05$ ). Pharmacotherapy was not different between both groups; all patients were taking angiotensin-converting enzyme inhibitors or angiotensin II receptor antagonists. ASA were being taken by 95.9% of diabetic patients and 89.1% non-diabetic. Statins by 92.6% of diabetic and 90.6% non-diabetic, fibrates (4.1%) only by diabetic patients. Beta-blockers by 96.7% of diabetic and 90.6% non-diabetic.

### Biochemical characteristics of studied patients

Higher proportion of HbA1c was found in diabetic patients compared with non-diabetic ( $7.20 \pm 1.65\%$  vs.  $5.70 \pm 0.27\%$ ;  $p < 0.001$ ). Glucose concentration at admission was higher in diabetic patients ( $141.01 \pm 57.92$  mg/dL vs.  $97.84 \pm 14.50$  mg/dL;  $p < 0.001$ ). Serum creatinine concentration was  $1.17 \pm 0.55$  mg/dL and in non-diabetic patients  $1.11 \pm 1.01$  mg/dL ( $p < 0.05$ ). In diabetic patients, we noticed higher fibrinogen concentration ( $428.57 \pm 101.48$  mg/dL vs.  $395.09 \pm 69.84$  mg/dL;  $p < 0.05$ ) and white blood cells (WBC) count ( $7.89 \pm 2.26$   $10^3/\mu\text{L}$  vs.  $7.11 \pm 1.72$   $10^3/\mu\text{L}$ ,  $p < 0.05$ ). No difference was found between both groups (diabetic vs. non-diabetic) in total cholesterol concentration ( $163.92 \pm 46.79$  mg/dL vs.  $166.64 \pm 42.24$  mg/dL), LDL cholesterol ( $93.93 \pm 41.10$  mg/dL vs.  $96.75 \pm 34.89$  mg/dL), HDL cholesterol ( $40.75 \pm 10.02$  mg/dL vs.  $45.12 \pm 12.83$  mg/dL), triglycerides ( $148.81 \pm 84.60$  mg/dL vs.  $123.95 \pm 62.26$  mg/dL), RBC ( $4.57 \pm 0.53$  mln/ $\mu\text{L}$  vs.  $4.54 \pm 0.47$  mln/ $\mu\text{L}$ ), HbG ( $14.12 \pm 3.46$  vs.  $13.75 \pm 1.35$  g/dL) or HCT ( $40.32 \pm 4.44$  vs.  $40.82 \pm 3.91\%$ ).

### Assessment kidney function

Kidney function in diabetic and non-diabetic patients was assessed using either creatinine clearance or estimated glomerular filtration rate (eGFR) by 4 formulae and the results were presented in our previous study [23]. In the present study, we focused on the possible role of the new biomarkers: NGAL, cystatin C and KIM-1 in the assessment of kidney function in diabetic patients.

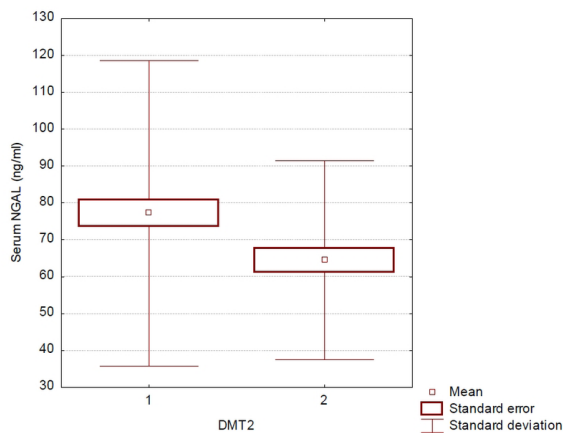
Normal serum creatinine (less than 1.5 mg/dl in males and less than 1.2 mg/dl in females) was observed in 91 (75.2%) diabetic and in 53 (82.8%) non-diabetic patients. Mean eGFR in diabetic patients was  $81.33 \pm 31.84$  ml/min (Cockcroft-Gault equation),  $59.07 \pm 21.40$  ml/min (lean body mass-adjusted Cockcroft-Gault equation),  $69.23 \pm 21.52$  ml/min/1.73m<sup>2</sup> (simplified MDRD equation),  $68.20 \pm 22.32$  ml/min/1.73m<sup>2</sup> (CKD-EPI study equation) which was significantly lower than in non-diabetic patients:  $84.09 \pm 28.98$  ml/min (Cockcroft-Gault equation),  $66.78 \pm 20.65$  ml/min,  $p < 0.05$  (lean body mass-adjusted Cockcroft-Gault equation),  $76.73 \pm 19.36$  ml/min/1.73m<sup>2</sup>,  $p < 0.01$  (simplified MDRD equation),  $76.49 \pm 20.85$  ml/min/1.73m<sup>2</sup>,  $p < 0.01$  (CKD-EPI study equation). According to Cockcroft-Gault equation, the presence of CKD (defined as eGFR below 60 ml/min was diagnosed in 31 (25.6%) diabetic patients and in 11 (17.2%) non-diabetic patients. Using lean body mass-adjusted Cockcroft-Gault equation, the presence of CKD was diagnosed in 58 (47.9%) diabetic patients and in 23 (35.9%) non-diabetic patients, while using simplified MDRD equation, it was diagnosed in 36 (29.8%) diabetic patients and in 13 (20.3%) non-diabetic patients. According to CKD-EPI study equation, we observed stage 3 CKD in 39 (32.2%) diabetic patients and in 14 (21.9%) non-diabetic patients. Dividing the study population into two subgroups according

to eGFR  $<60$  ml/min/1.73m<sup>2</sup> (recommended simplified MDRD equation) and eGFR  $>60$  ml/min/1.73m<sup>2</sup>, mean GFR in diabetic group was  $43.36 \pm 13.94$  ml/min/1.73m<sup>2</sup> and  $80.18 \pm 13.12$  ml/min/1.73m<sup>2</sup>, respectively, and in non-diabetic group  $46.83 \pm 14.73$  ml/min/1.73m<sup>2</sup> and  $84.35 \pm 11.33$  ml/min/1.73m<sup>2</sup>, respectively.

### Serum and urinary NGAL

Serum NGAL values were significantly higher in diabetics than in non-diabetics ( $77.15 \pm 41.41$  ng/ml vs.  $64.43 \pm 26.99$  ng/ml,  $p < 0.05$ ) (Fig. 1). Urinary NGAL levels were significantly higher in diabetic compared to non-diabetic patients ( $27.47 \pm 28.91$  ng/ml vs.  $13.63 \pm 11.91$  ng/ml;  $p < 0.05$ ) (Fig. 2). In diabetic group, serum NGAL was related to serum creatinine ( $r = 0.697$ ;  $p < 0.001$ ), WBC count ( $r = 0.213$ ;  $p < 0.05$ ), platelet count ( $r = 0.217$ ;  $p < 0.05$ ), fibrinogen ( $r = 0.362$ ;  $p < 0.001$ ), urinary NGAL ( $r = 0.535$ ;  $p < 0.001$ ) and serum cystatin C ( $r = 0.726$ ;  $p < 0.001$ ). Significant correlations were also found between serum NGAL and eGFR by Cockcroft-Gault equation ( $r = -0.480$ ;  $p < 0.001$ ), eGFR by lean body mass-adjusted Cockcroft-Gault equation ( $r = -0.541$ ;  $p < 0.001$ ), eGFR by simplified MDRD equation ( $r = -0.629$ ;  $p < 0.001$ ) and eGFR by CKD-EPI equation ( $r = -0.527$ ;  $p < 0.001$ ). In non-diabetic group, serum NGAL correlated with serum creatinine ( $r = 0.427$ ;  $p < 0.001$ ), urinary NGAL ( $r = 0.305$ ;  $p < 0.05$ ), serum cystatin C ( $r = 0.446$ ;  $p < 0.001$ ), fibrinogen ( $r = 0.247$ ;  $p < 0.05$ ), eGFR by Cockcroft-Gault equation ( $r = -0.341$ ;  $p < 0.01$ ), eGFR by lean body mass-adjusted Cockcroft-Gault equation ( $r = -0.408$ ;  $p < 0.001$ ), eGFR by simplified MDRD equation ( $r = -0.440$ ;  $p < 0.001$ ) and eGFR by CKD-EPI equation ( $r = -0.4$ ;  $p < 0.001$ ). In diabetic patients, urinary NGAL levels directly correlated with serum creatinine ( $r = 0.289$ ;  $p < 0.01$ ), serum NGAL ( $r = 0.535$ ;  $p < 0.001$ ), serum cystatin C ( $r = 0.353$ ;  $p < 0.001$ ), urinary KIM-1 ( $r = 0.242$ ;  $p < 0.05$ ), an inverse correlation was found between urinary NGAL and eGFR by lean body mass-adjusted Cockcroft-Gault equation ( $r = -0.189$ ;  $p < 0.05$ ), eGFR by simplified MDRD equation ( $r = -0.198$ ;  $p < 0.05$ ). In non-diabetic patients, urinary NGAL

**Figure 1.** Serum NGAL in diabetic (1) and non-diabetic (2) group ( $p < 0.05$ ).



was found to be correlated to serum creatinine ( $r = 0.458$ ;  $p < 0.001$ ), serum NGAL ( $r = 0.305$ ;  $p < 0.05$ ), serum cystatin C ( $r = 0.452$ ;  $p < 0.001$ ), fibrinogen ( $r = 0.280$ ;  $p < 0.05$ ), whereas an inverse correlation was found with eGFR by Cockcroft-Gault equation ( $r = -0.251$ ;  $p < 0.05$ ), eGFR by lean body mass-adjusted Cockcroft-Gault equation ( $r = -0.306$ ;  $p < 0.05$ ) and eGFR by simplified MDRD equation ( $r = -0.394$ ;  $p < 0.001$ ).

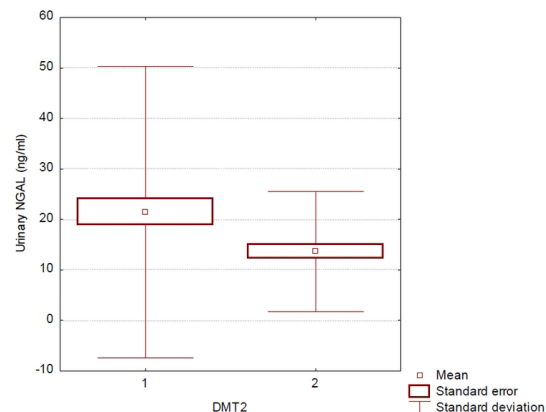
When we analyzed creatinine and cystatin C in the tertiles of serum and urinary NGAL, we found that only in the first tertiles of serum NGAL both creatinine and cystatin C differed significantly ( $p = 0.21$  and  $p = 0.15$ , respectively), in the case of urinary NGAL only in the first tertile serum creatinine was different ( $p = 0.043$ ).

In the model of a multiple regression analysis in diabetic patients, the strongest predictors of serum NGAL were serum creatinine ( $\beta = 0.647$ ;  $p = 0.001$ ), platelet count ( $\beta = 0.331$ ;  $p = 0.001$ ) and fibrinogen ( $\beta = 0.189$ ;  $p = 0.004$ ). The equation explained 60% of the variation of serum NGAL in this group. In non-diabetic patients, the only predictors of serum NGAL in multiple regression analysis were serum creatinine ( $\beta = 0.605$ ;  $p = 0.001$ ) and age ( $\beta = 0.279$ ;  $p = 0.006$ ). The equation explained 45% of the variation of serum NGAL in this group.

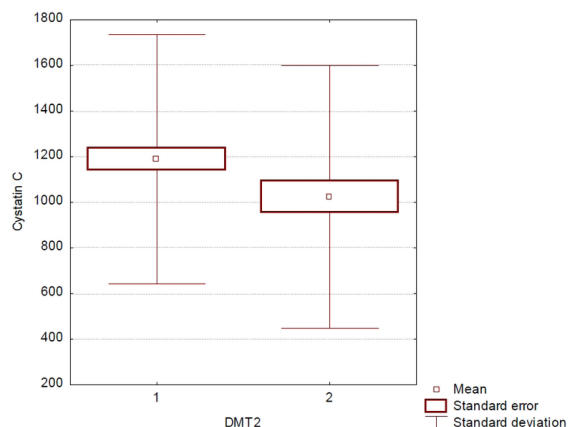
### Cystatin C

Cystatin C levels were significantly higher in diabetics than in non-diabetics ( $1188.39 \pm 546.41$  ng/ml vs.  $1023.69 \pm 574.98$  ng/ml;  $p < 0.01$ ) (Fig. 3). In diabetic group, cystatin C was related to serum creatinine ( $r = 0.713$ ;  $p < 0.001$ ), fibrinogen ( $r = 0.461$ ;  $p < 0.001$ ), age ( $r = 0.289$ ;  $p < 0.001$ ), serum NGAL ( $r = 0.726$ ;  $p < 0.001$ ), urinary NGAL ( $r = 0.353$ ;  $p < 0.001$ ), urinary KIM-1 ( $r = 0.247$ ;  $p < 0.05$ ) and an inverse correlation was shown with eGFR by Cockcroft-Gault equation ( $r = -0.502$ ;  $p < 0.001$ ), eGFR by lean body mass-adjusted Cockcroft-Gault equation ( $r = -0.593$ ;  $p < 0.001$ ), eGFR by simplified MDRD equation ( $r = -0.644$ ;  $p < 0.001$ ), eGFR by CKD-EPI equation ( $r = -0.645$ ;  $p < 0.001$ ). In non-diabetic group, cystatin C correlated with serum creatinine ( $r = 0.888$ ;  $p < 0.001$ ), serum NGAL ( $r = 0.446$ ;

**Figure 2.** Urinary NGAL in diabetic (1) and non-diabetic (2) group ( $p < 0.05$ ).



**Figure 3. Cystatin C in diabetic (1) and non-diabetic (2) group (p<0.01).**



p<0.001), urinary NGAL (r=0.452; p<0.001), urinary KIM-1 (r=0.532; p<0.05), age (r=0.257; p<0.05), whereas an inverse correlation was found with eGFR by Cockcroft-Gault equation (r=-0.613; p<0.001), eGFR by lean body mass-adjusted Cockcroft-Gault equation (r=-0.653; p<0.001), eGFR by simplified MDRD equation (r=-0.753; p<0.001) and eGFR by CKD-EPI equation (r=-0.659, p<0.001).

In multivariate analysis in diabetic patients, predictors of serum cystatin C were serum creatinine (beta=0.561; p=0.01) and fibrinogen (beta=0.341; p=0.001). The equation explained 55% of the variation of serum cystatin C in this group. In the model of multiple regression analysis in non-diabetic patients, the strongest predictors of serum cystatin C were serum creatinine (beta=0.882; p=0.001) and age (beta=0.221; p=0.001). The equation explained 83% of the variation of serum cystatin C in this group.

### KIM-1

No statistical differences were found in urinary KIM-1 values between both groups (49.26 ± 41.39 ng/ml DM patients vs. 45.42 ± 45.93 ng/ml non DM patients). In diabetic group, urinary KIM-1 was related to serum creatinine (r=0.275; p<0.01), urinary NGAL (r=0.242; p<0.05) and serum cystatin

C (r=0.247; p<0.05). In non-diabetic group, significant correlations were shown between urinary KIM-1 and serum creatinine (r=0.614; p<0.01), serum cystatin C (r=0.532; p<0.05), eGFR by Cockcroft-Gault equation (r=-0.531; p<0.05), eGFR by lean body mass-adjusted Cockcroft-Gault equation (r=-0.475; p<0.05), eGFR by simplified MDRD equation (r=-0.482; p<0.05).

### Impact of diabetes on studied biomarkers (serum and urinary NGAL, serum cystatin C, urinary KIM-1)

The serum and urinary NGAL, cystatin C values were significantly higher in diabetics than in non-diabetics. Urinary KIM-1 levels did not differ significantly between both groups. In diabetic patients none of the three studied biomarkers was found to correlate with the duration of diabetes and the type of treatment of diabetes. Studied biomarkers were similar in both groups of patients with eGFR below 60 ml/min/1.73m<sup>2</sup> (presence of CKD by K/DOQI) (Tab. 1). However, in patients with eGFR over 60 ml/min/1.73m<sup>2</sup> cystatin C and urinary NGAL showed significant differences between diabetic and non-diabetic subjects (Tab. 2).

### ROC analysis of studied biomarkers (serum and urinary NGAL, serum cystatin C)

The area under the curve for serum NGAL, urinary NGAL and cystatin C was 0.60 (95% CI, 0.51 to 0.69), 0.59 (95% CI, 0.5 to 0.68), 0.62 (95% CI, 0.54 to 0.71), respectively (Fig. 4). We did not find good cut-off values of the studied biomarkers to detect diabetes.

## DISCUSSION

In the present study, in both patient groups, the values of mean serum creatinine concentration were within the reference ranges (1.17 ± 0.55 mg/dL in diabetic group vs. 1.11 ± 1.01 mg/dL in non-diabetic). However, after the assessment of eGFR using recommended simplified MDRD equation, it appeared

**Table 1. Serum and urinary NGAL, Cystatin C and KIM-1 in both study groups with eGFR <60ml/min/1.73m<sup>2</sup>.**

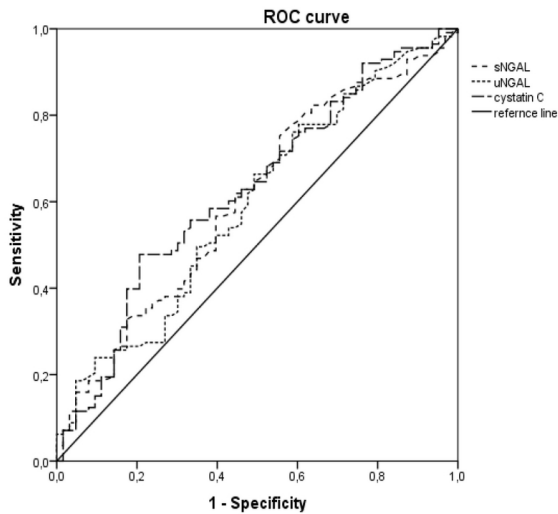
	Diabetic patients with eGFR <60ml/min/1.73m <sup>2</sup> (mean GFR 43.36±13.94)	Non-diabetic patients with eGFR <60ml/min/1.73m <sup>2</sup> (mean GFR 46.83±14.73)
Serum NGAL (ng/ml)	110.73±55.94	94.71±35.91
Urinary NGAL (ng/ml)	30.32±47.39	20.07±20.19
Cystatin C (ng/ml)	1676.81±696.09	1699.00±960.07
KIM-1 (ng/ml)	47.51±42.60	76.88±72.75

**Table 2. Serum and urinary NGAL, Cystatin C and KIM-1 in both study groups with eGFR >60ml/min/1.73m<sup>2</sup> (\*p<0.05).**

	Diabetic patients with eGFR >60ml/min/1.73m <sup>2</sup> (mean GFR 80.18±13.12)	Non-diabetic patients with eGFR >60ml/min/1.73m <sup>2</sup> (mean GFR 84.35±11.33)
Serum NGAL (ng/ml)	62.75±20.97	56.71±17.57
Urinary NGAL (ng/ml)	17.90±15.62	11.94±8.09*
Cystatin C (ng/ml)	979.07±275.54	851.55±215.72*
KIM-1 (ng/ml)	49.98±41.15	39.51±39.88



**Figure 4. ROC Analysis of studied biomarkers (serum and urinary NGAL, serum cystatin C).**



that 30% of diabetic patients and 20% of non-diabetic patients can be diagnosed with CKD only on the basis of the value of  $eGFR < 60 \text{ ml/min/1.73m}^2$ . As we previously show [23], the incidence of CKD (defined as GFR below 60 ml/min), in diabetic patients referred for percutaneous coronary interventions, is high despite normal creatinine concentration. Therefore, in the present study, we tried to answer the question whether new biomarkers of kidney function/injury may be useful to assess kidney function, particularly in diabetic patients. This is of utmost importance as patients admitted for either coronary angiography or percutaneous coronary interventions are discharged from the hospital shortly after the procedure and are prone to contrast-induced acute kidney injury (usually undetected due to short stay in the hospital before the significant rise in serum creatinine is seen) with all its complications. In addition, diagnosis of CKD is extremely important especially at the Department of Invasive Cardiology since reduced GFR in patients undergoing PCI has important clinical implications and involves, as already mentioned above, the increase in mortality, bleeding and restenosis in the follow-up, as well as adjustment of contrast volume or dose of certain drugs [28].

In the present study, serum and urinary NGAL values were significantly higher in diabetics than in non-diabetics. In addition, NGAL levels strongly correlated with parameters of renal function: positive correlation with serum creatinine and inverse with the values of  $eGFR$  using the four formulae. Therefore, the serum concentration of NGAL (which is freely filtered by the glomerulus) should increase with decreasing GFR. In our present study, at baseline diabetic group presented with significantly lower  $eGFR$  compared with patients without diabetes. Thus, there is a decrease of renal clearance of NGAL and increased accumulation of this protein in systemic circulation in these patients. In addition, higher NGAL concentration in serum in diabetic patients

may result from the increased pool of the protein produced by neutrophils and other inflammatory cells (statistically significant difference in WBC between both groups,  $p < 0.05$ ).

Currently, atherosclerosis is considered a chronic inflammatory condition. The relationship of elevated levels of fibrinogen and other markers of the acute phase such as CRP and leukocytosis with the development of coronary heart disease is emphasized [29]. In our present study, diabetic group more often than non-diabetic patients had multivessel disease, underwent myocardial infarction, or had a concomitant peripheral artery disease, hence the presence of strongly marked acute phase parameters in this population, which could additionally be a source of NGAL protein (observed positive correlations in diabetic patients between serum NGAL and fibrinogen, WBC).

The statistically significant differences in the urinary NGAL concentrations between both groups of patients observed in this study might be explained by decreasing GFR, but also by two hypotheses. Firstly, higher urinary NGAL concentrations in diabetic patients may result from the reduced protein reabsorption through the damaged cells of renal proximal tubules. Secondly, NGAL secretion into the urine may additionally result from the increased de novo synthesis of the protein by the distal tubule cells, the concept of protective role of NGAL protein, which is said to stimulate the repair of damaged epithelium [30]. Dividing the study population into two subgroups according to  $eGFR < 60 \text{ ml/min/1.73m}^2$  and  $eGFR > 60 \text{ ml/min/1.73m}^2$  (NFK cut-off point for the diagnosis of CKD), in diabetic and non-diabetic group with  $eGFR < 60 \text{ ml/min/1.73m}^2$  no differences in urinary NGAL concentrations were observed. Analyzing subgroup of patients with  $eGFR > 60 \text{ ml/min/1.73m}^2$  significantly higher urinary NGAL concentrations were found only in diabetic patients. This may suggest, that these patients present the damage of proximal tubular cells already at early stages of CKD.

In the recent study by Bolignano et al. [31], serum and urinary NGAL levels were evaluated in patients with type 2 diabetes, depending on the severity of albuminuria. Patients with type 2 diabetes were divided into three groups with normal albuminuria, microalbuminuria and macroalbuminuria. All groups showed higher urinary NGAL values compared with well matched control group. Urinary NGAL levels increased with the severity of albuminuria. Interestingly, increased NGAL values were already found in diabetic patients without early signs of glomerular damage (normoalbuminuric with mean  $eGFR 118 \text{ ml/min}$ ). On this basis, the authors of the study [31] concluded, that damage to proximal tubular cells in diabetic patients occurs very early, even in the absence of clinical signs of glomerular injury. From this perspective, urinary NGAL concentration may reflect the degree of early kidney damage (especially renal tubules). In addition, this protein may be somehow related to the pathophysiological processes of renal adaptation to

diabetes, probably as a protective mechanism stimulating repair of damaged cells.

As in our present study, Bolignano et al. [32] confirmed a close relationship between urinary NGAL concentrations and classical parameters of renal function (a positive correlation with serum creatinine, inverse with eGFR). In addition, Bolignano et al. [32] have recently reported that NGAL protein may have predictive value for the progression of CKD. Macroalbuminuria in patients with higher baseline NGAL shows a considerably increased risk of worsening residual renal function within 1 year in comparison to those with lower baseline NGAL values. In conclusion, NGAL might be a promising biomarker of CKD [11], but further studies are necessary.

In the present study, serum cystatin C values were significantly higher in diabetics than in non-diabetics. As cystatin C is physiologically freely filtered by the glomerulus and then reabsorbed and metabolized in the tubules, accumulation of this protein in serum is mainly the result of a decreasing glomerular filtration. On this basis, we can speculate that higher cystatin C concentration in diabetic patients results from a significantly lower baseline eGFR in this group. In the present study, cystatin C closely correlated with classical parameters of renal function (serum creatinine, eGFR). In addition, in our study a positive correlation with new biomarkers (serum and urinary NGAL) was found.

However, dividing study population into two subgroups with  $eGFR > 60 \text{ ml/min/1.73m}^2$  and  $eGFR < 60 \text{ ml/min/1.73m}^2$  (NFK cut-off point for diagnosis of CKD), in diabetic and non-diabetic patients with  $eGFR < 60 \text{ ml/min/1.73m}^2$  there was no difference in serum cystatin C concentrations. Analyzing the subgroup of patients with  $eGFR > 60 \text{ ml/min/1.73m}^2$  showed significantly higher levels of cystatin C in patients with type 2 diabetes. Borges et al. [33] evaluated cystatin C concentration in 243 patients with hypertension, of whom 61 were also diagnosed with type 2 diabetes. The study inclusion criteria were:  $eGFR > 60 \text{ ml/min/1.73m}^2$  and normoalbuminuria. Serum cystatin C was found to be statistically higher in patients with type 2 diabetes, whereas eGFR calculated using the MDRD formula in both groups was comparable ( $69.7 \text{ ml/min/1.73m}^2$  diabetic patients vs.  $67.8 \text{ ml/min/1.73m}^2$  non-diabetic patients). Observational studies show how important it is to identify individuals with elevated cystatin C levels. Perkins et al. [34] in a 4-year observational study of patients with type 2 diabetes with normal and elevated values of eGFR demonstrated that serially estimated cystatin C as compared to traditional methods of assessing renal function (serum creatinine, equations to calculate eGFR) most accurately correlated with renal function deteriorating with the time [34]. In another prospective study of older people (aged 60 and over) with a  $eGFR > 60 \text{ ml/min/1.73m}^2$ , Shlipak et al. [35] observed that elevated baseline concentrations of cystatin C were associated with a four-fold higher risk of progression to CKD in more than 4 years of

follow-up. Cystatin C concentration identifies “preclinical” state of kidney dysfunction.

Elevated cystatin C appears to be an important risk factor for adverse cardiovascular outcomes [36,37]. Analyzing all these relationships and correlations we can only assume that in our present study, increased cystatin C concentrations in patients with type 2 diabetes were the result of both greater accumulation of risk factors for CVD as well as more advanced athero-inflammatory process in this subgroup of patients. However, we are fully aware that the limitation of our work was the lack of albuminuria assessment in both groups of patients. The strength of our study was simultaneous assessment of different kidney injury molecules in patients at risk (coronary artery disease, diabetes) but with normal serum creatinine.

In our study, no significant differences in urinary KIM-1 concentrations between both patient groups were found. Because this protein physiologically is undetectable in the urine, the concentration of  $49.26 \pm 41.36 \text{ ng/ml}$  in the diabetic group and  $45.42 \pm 45.93 \text{ ng/ml}$  in non-diabetic group may present evidence for tubular cells injury, where KIM-1 is expressed only after toxic stimuli [38,39].

Up to date, there is no comprehensive data on KIM-1 in the diagnosis and/or prognosis of CKD. In 2009, Waanders et al. [22] published a randomized, double-blind study, which analyzed the hypothesis that reduction of proteinuria by various therapeutic fields may decrease the concentration of KIM-1 in urine. The authors demonstrated on group of 34 patients with proteinuria (without diabetes) that reducing urinary protein excretion through the use of losartan/losartan with hydrochlorothiazide, low-sodium diet or a combination of both, may reduce secretion of KIM-1 protein in the urine in parallel to the decrease in proteinuria. Since continuous proteinuria causes renal tubular damage and interstitial fibrosis of renal parenchyma, researchers concluded that the urine KIM-1 molecule may be a biomarker of chronic tubulo-interstitial damage, demonstrating the progression of the underlying disease [22]. It should be emphasized that currently there are no guidelines on the KIM-1 indications in the diagnosis of CKD therefore the results could be reproducible. The question on how to analyze urine samples i.e. morning urine sample, random, after night rest or of urine collection, remains open [40].

## CONCLUSIONS

Concluding, concentrations of early biomarkers of kidney dysfunction (urinary and serum NGAL, cystatin C) are higher in diabetic patients in comparison with non-diabetics referred for percutaneous coronary interventions, especially at  $eGFR > 60 \text{ ml/min}$  suggesting early kidney impairment in this group of patients, although levels of these biomarker might be affected by diabetes itself (protein breakdown,

binding to circulating proteins). The analyzed markers such as NGAL, cystatin C and KIM-1 are not more useful than eGFR calculated on the basis of serum creatinine in early diagnosis of CKD, however further studies are necessary, particularly with validation of these new biomarkers against measured creatinine clearance or GFR.

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