ORIGINAL ARTICLE—STUDENT AWARD WINNER 2016*

Serum levels of 12 renal function and injury markers in patients with glomerulonephritis

Natalia M. Serwin[1], Magda Wiśniewska[2], Anna Jesionowska[3], Edyta Skwirczyńska[4], Zuzanna Marcinowska[5], Barbara Dołęgowska[1]

1 Department of Microbiology and Immunology, Pomeranian Medical University in Szczecin, Szczecin, Poland
2 Department of Nephrology, Transplantology and Internal Diseases, Pomeranian Medical University in Szczecin, Szczecin, Poland
3 Department of Biochemistry and Medical Chemistry, Pomeranian Medical University in Szczecin, Szczecin, Poland
4 Department of History of Medicine and Medical Ethics, Pomeranian Medical University in Szczecin, Szczecin, Poland
5 Department of Medical Analytics, Pomeranian Medical University in Szczecin, Szczecin, Poland

KEY WORDS

biomarkers of renal function, calbindin, glomerulonephritis, osteopontin, renalase

ABSTRACT

INTRODUCTION Glomerulonephritis (GN) is a complex disease that affects the function of the whole nephron. There are few data on the serum levels of the most common biomarkers of kidney function and injury in GN, or the studies provide ambiguous results.

OBJECTIVES The aim of the study was to evaluate the levels of known kidney-specific and nonspecific markers of renal function or injury in the serum of patients with diagnosed primary or secondary GN, with or without the presence of nephrotic syndrome (NS) and arterial hypertension (AH).

PATIENTS AND METHODS The study included 58 patients with diagnosed GN and 6 patients with congenital defects (CD) of the kidney and AH (CD+AH). The serum levels of β_2-microglobulin (β_2M), neutrophil-gelatinase associated lipocalin (NGAL), osteopontin, trefoil factor 3 (TFF-3), calbindin, glutathione-S-transferase-π (GST-π), interleukin 18 (IL-18), kidney injury molecule 1 (KIM-1), and monocyte chemoattractant protein 1 (MCP-1) were measured with Kidney Toxicity Panels 1 and 2 using the Bio-Plex method. Renalase levels were measured using an enzyme-linked immunosorbent assay.

RESULTS In the whole group and in the subgroups (GN, GN+AH, GN+NS, CD+AH), the levels of NGAL, KIM-1, TFF-3, IL-18, β_2M, and calbindin correlated with estimated glomerular filtration rate (eGFR). In patients with NS, this correlation for calbindin was reversed. Renalase, MCP-1, GST-π, and osteopontin levels were independent of eGFR. Increase in IL-18 levels in the group with GN was assiociated with lower odds of the kidney disease. When this group was divided according to eGFR into subgroups G1–G5, TFF-3, NGAL, and β_2M levels increased with the stage of the disease.

CONCLUSIONS In patients with NS, renalase and MCP-1 might regulate each other's levels. Further studies are needed to investigate associations between renalase, MCP-1, and osteopontin as factors unrelated to eGFR in GN. NS may contribute to the loss of calbindin from serum. NGAL, KIM-1, TFF-3, IL-18, β_2M, and calbindin are good indicators of kidney function loss in patients with GN.

Correspondence to:
Natalia M. Serwin, MSc, Zakład Mikrobiologii i Diagnostyki Immunologicznej, Pomorski Uniwersytet Medyczny w Szczecinie, al. Powstańców Wlkp 72, 70-111 Szczecin, Poland, phone: +48 91 466 16 52, e-mail: natmat@pum.edu.pl
Received: April 30, 2016.
Revision accepted: May 6, 2016.
Published online: August 9, 2016.
Conflict of interest: none declared.
Pol Arch Med Wewn. 2016; 126 (7-8): 483-493
doi:10.20452/pamw.3477

* The authors won the second award of the Editor-in-Chief for the best student paper in 2016. For more information, go to www.pamw.pl.

INTRODUCTION Glomerulonephritis (GN) is a complex kidney disease with varied etiological, pathological, and clinical symptoms. It is an immune-mediated glomerulus disease, which is often, but not always, inflammatory in nature. Malfunction of glomeruli as the first barrier in blood and urine formation results in impairment of absorption, excretion, and metabolism of certain physiological and pathophysiological components. GN may be also a primary kidney disease that leads to numerous complications, such as nephrotic syndrome (NS), nephritic syndrome, and arterial hypertension (AH), but may also result from numerous other conditions, such as autoimmune and metabolic diseases, cancer, or ultrastructural defects of the kidney; it may also develop during infections, intoxication, or be caused by genetic predispositions or physical injuries.[1]

Kidney injury biomarkers such as renalase, β_2-microglobulin (β_2M), neutrophil gelatinase-

-associated lipocalin (NGAL), osteopontin, trefoil factor 3 (TFF-3), calbindin (calbindin-1, CALB1, calbindin-D28k), glutathione-S-transferase-π (GST-π, GST-pi), interleukin 18 (IL-18), kidney injury molecule 1 (KIM-1), and monocyte chemoattractant protein 1 (MCP-1), are predominantly found in urine and serve as good indicators of numerous kidney dysfunctions. Moreover, some of them are exclusively related to certain parts of the nephron, providing information on the site of the injury, and their blood levels may serve as a valuable indicator or predictor of certain kidney dysfunction. Together with the indicators of kidney function, creatinine (Cr) and cystatin C, the above biomarkers yield useful information on kidney function.

So far, not many researchers have focused on the serum levels of known kidney-specific and nonspecific markers of renal function or injury. Therefore, the aim of our study was to evaluate the levels of these markers in the serum of patients with diagnosed primary or secondary GN, with or without the presence of NS or AH. Our results may fill in the gap in the current literature data on serum levels of these factors, as well as help determine the potential future directions of research.

PATIENTS AND METHODS

Study design Most of the factors described and evaluated in this study were predominantly measured in urine and normalized using urine Cr. Considering that changes in the serum levels of the analyzed factors can result from numerous other physiological and pathophysiological conditions not considered in this publication, such as cancer or inflammatory diseases (including autoimmune ones), the major study exclusion criterion was the presence of any diseases or dysfunctions that could affect the results. The inclusion criterion was the presence of diseases underlying the development and progression of GN classified as either nephrotic GN (GN+NS) or nonnephrotic GN (GN). As hypertension is strongly related to renal function, patients with GN with associated AH constituted the next subgroup: GN and AH without NS (GN+AH). AH was not observed in patients from the GN+NS subgroup. The design of our study allowed us to include a small group of patients with congenital defects (CD) of the kidneys as a group of kidney diseases of a completely different etiology. All patients with CDs had AH (the CD+AH subgroup).

All patients were also independently divided into subgroups based on estimated glomerular filtration rate (eGFR) calculated using the Modification of Diet in Renal Disease formula: G1 included patients with eGFR exceeding 90 ml/min/1.73 m^2; G2, those with eGFR between 60 and 89 ml/min/1.73 m^2; G3, those with eGFR between 30 and 59 ml/min/1.73 m^2; G4, those with eGFR between 15 and 29 ml/min/1.73 m^2; and G5, those with eGFR of less than 15 ml/min/1.73 m^2. This classification was introduced mainly to compare the obtained results with reference ranges reported in literature for chronic kidney disease stages, and these subgroups were not analyzed in such detail as the disease-based subgroups.

Patients and methods All participants were patients of the Department of Nephrology, Transplantology and Internal Diseases, Pomeranian Medical University in Szczecin, Poland. Serum samples were obtained from venous blood collected in the morning using S-Monovette tubes (Sarstedt, Nümbrecht, Germany) with a clotting activator, centrifuged for 10 minutes at 1000 × *g*, and freezed until use. All patients gave written informed consent to participate in the study.

Renalase concentrations were measured using an enzyme-linked immunosorbent assay kit specific for human renalase (WuHan EIAab, Wuhan, China). The serum levels of the basic biochemical parameters such as albumin, total protein, glucose, creatinine, uric acid, and calcium were measured using commercially available reagent kits (BioMaxima, Lublin, Poland). The levels of kidney toxicity markers were measured using 2 Bio-Plex Pro™ RBM Kidney Toxicity Assays (Bio-Rad, Hercules, California, United States). Kidney Toxicity Panel 1 allows to measure urinary levels of calbindin, clusterin, GST-π, IL-18, KIM-1, and MCP-1, while Kidney Toxicity Panel 2 is used to evaluate urinary levels of albumin, β_2M, cystatin C, NGAL, osteopontin, and TFF-3. Both assays were primarily designed and validated for urine measurements, so the dilution of serum samples was calculated based on literature data on physiological and pathophysiological serum levels of the above parameters and on recommendations of the manufacturer. Each sample dilution was the same for the entire panel; therefore, 2 abundant serum proteins, albumin and clusterin, were beyond the range of standard curves and were excluded from further analysis. The levels of mentioned biomarkers were then read using Bio-Plex 200 System, and analyzed using Bio-Plex Manager™ (Bio-Rad).

Four known equations were used to calculate eGFRs (ml/min/1.73 m^2): 1) Modification of Diet in Renal Disease (MDRD) equation: $175 \times (S_{Cr})^{-1.154} \times (\text{age})^{-0.203} \times 0.742$ [if female] × 1.210 [if black], where S_{Cr} (standardized serum creatinine) = mg/dl, age = years; 2) The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation: $141 \times \min(S_{Cr}/\kappa, 1)^{\alpha} \times \max(S_{Cr}/\kappa, 1)^{-1.209} \times 0.993^{\text{age}} \times 1.018$ [if female] × 1.159 [if black], where S_{Cr} (standardized serum creatinine) = mg/dl, κ = 0.7 [females] or 0.9 [males], α = –0.329 [females] or –0.411 [males], min = indicates the minimum of S_{Cr}/κ or 1, max = indicates the maximum of S_{Cr}/κ or 1, age = years; 3) CKD-EPI-cystatin C equation: $133 \times \min(S_{cys}/0.8, 1)^{-0.499} \times \max(S_{cys}/0.8, 1)^{-1.328} \times 0.996^{\text{age}} \times 0.932$ [if female], where S_{cys} (standardized serum cystatin C) = mg/l, min = indicates the minimum of $S_{cys}/0.8$ or 1, max = indicates the maximum of $S_{cys}/0.8$ or 1, age = years; 4) CKD-EPI creatinine-cystatin C equation: $135 \times \min(S_{Cr}/\kappa, 1)^{\alpha} \times \max(S_{Cr}/\kappa,$

$1)^{-0.601} \times \min(S_{cys}/0.8, 1)^{-0.375} \times \max(S_{cys}/0.8, 1)^{-0.711} \times 0.995^{age} \times 0.969$ [if female] × 1.08 [if black], where S_{Cr} (serum creatinine) = mg/dl, S_{cys} (standardized serum cystatin C) = mg/l, κ = 0.7 [females] or 0.9 [males], α = –0.248 [females] or –0.207 [males], $\min(S_{Cr}/\kappa \text{ or } 1)$ = indicates the minimum of S_{Cr}/κ or 1, $\max(S_{Cr}/\kappa \text{ or } 1)$ = indicates the maximum of S_{Cr}/κ or 1, $\min(S_{cys}/0.8, 1)$ = indicates the minimum of $S_{cys}/0.8$, 1, $\max(S_{cys}/0.8, 1)$ = indicates the maximum of $S_{cys}/0.8$, 1, age = years.[2]

The study was approved by a local ethics committee.

Statistical analysis Data were analyzed using the STASTISTICA 12.5 software (StatSoft, Tulsa, Oklahoma, United States). The descriptive statistics involved calculation of the mean value, standard deviation, and minimum and maximum values of quantitative variables. The assumption of normality was verified using the Shapiro–Wilk test. The analyzed parameters were compared within the distinguished subgroups using the *t* test. In the absence of the homogeneity of variance, the Welch test (modification of the *t* test) was applied. In the absence of normality, the Mann–Whitney test was used. When the parameters were compared within more than 2 groups, the Kruskal–Wallis analysis of variance and post hoc tests were applied. Correlations between selected parameters were analyzed using the Pearson correlation coefficient.

In order to further explore the predictive properties of the tested biomarkers, we constructed 4 logistic multiple regression models. The dependent (explained) variables were GN and its derivatives (GN + NS and GN + AH), as well as CD + AH, whereas the 12 analyzed serum biomarkers were considered as independent variables (explanatory variables, predictors). The goodness of fit of the model was assessed using the Hosmer–Lemeshow test. The receiver-operating characteristic curve (ROC) was constructed and the area under the curve (AUC) was calculated. The level of statistical significance was set at an α value of 0.05.

RESULTS

Characteristics of the study group and the subgroups divided according to disease The study involved patients at a mean age of 46.3 ±16.2 years, including 28 women and 36 men. TABLE 1 presents the levels of the analyzed markers, as reported in the available literature for healthy individuals and those with kidney and heart diseases. TABLE 2 presents the characteristics of the analyzed biomarkers, together with any significant differences between the subgroups of patients divided according to disease.

Correlations in the subgroups divided according to disease The analysis of correlations between the analyzed biomarkers and indicators of kidney function (Cr, cystatin C, and eGFR) in the whole study group (n = 64) showed a moderate to strong negative correlation between KIM-1, NGAL, and IL-18 and with eGFR calculated using all the above equations, as well as a positive correlation between KIM-1, NGAL, and IL-18 and cystatin C levels. Weak to moderate correlations with eGFR were also observed for β_2M, TFF-3, and calbindin. Interestingly, in the GN+NS subgroup, the above trend for calbindin was reversed: it showed a strong positive correlation with eGFR evaluated using the MDRD equation, as well as a similar trend, although without statistical significance, with the other 3 eGFR determinants. There were no correlations between serum levels of renalase, osteopontin, MCP-1, and GST-π and eGFR evaluated using the 4 equations. Only in the CD+AH subgroup, GST-π showed a significant negative correlation with eGFR. Detailed correlation coefficients between studied factors and Cr, cystatin C, and eGFR are presented in supplementary material online (Supplementary material online, *Table S1*).

Some of the analyzed biomarkers correlated positively with one another. MCP-1 was the only marker that showed negative correlations: with renalase in the GN+NS group ($r = -0.81$; $P < 0.05$) and with KIM-1 in the CD+AH group ($r = -0.86$, $P < 0.05$). As mentioned above, NGAL, KIM-1, IL-18, TFF-3, and β_2M showed a strong to moderate correlation with eGFR; these markers generally showed more correlations than the other ones. Coefficients are presented in Supplementary material online (*Table S2*), and the more important ones are explained in more detail in the Discussion section.

Characteristics of the subgroups divided according to estimated glomerular filtration rate Detailed characteristics of biochemical parameters as well as significant differences between the subgroups divided according to eGFR are shown in TABLE 3. Significant differences between the markers of renal injury were found for MCP-1, KIM-1, IL-18, TFF-3, NGAL, and β_2M. This was not surprising considering their strong to moderate correlations (except for MCP-1) with eGFR, as shown for the whole study group and disease based subgroups. The levels of TFF-3, NGAL, β_2M, and cystatin C (as not included in the MDRD eGFR subgroups) gradually increased with the stages of the disease.

The most interesting correlations between biomarkers that were not correlated with eGFR were observed in the G1 and G2 subgroups. In the G1 subgroup, we found a negative correlation between renalase and MCP-1 ($r = -0.53$, $P < 0.05$) and a positive one between GST-π and calbindin ($r = 0.48$, $P < 0.05$); in the G2 subgroup, we found a correlation between renalase and osteopontin ($r = 0.49$, $P < 0.05$) and between MCP-1 and osteopontin ($r = 0.54$, $P < 0.05$). Detailed correlations between all markers in the eGFR subgroups are presented in Supplementary material online, *Table S3*.

Multiple logistic regression The multiple logistic regression showed that when GN was the dependent variable, IL-18 was a significant biomarker in

TABLE 1 Physiological and pathological serum (or plasma) levels of biomarkers involved in the pathogenesis or complications of chronic kidney disease and/or cardiovascular disorders

Serum protein fraction	Healthy individuals	Chronic kidney disease (stages)	Acute kidney injury	Other renal dysfunctions	Coronary artery disease	Other cardiovascular disorders
calbindin	20 pg/ml[9]	–	–	–	–	–
KIM-1	64.4 pg/ml (plasma)[11] 126.2 pg/ml[12]	–	1458 pg/ml (plasma)[11]	pretransplant kidney recipients, 165.3 pg/ml; acute rejection group, 221.2 pg/ml; non-rejection group, 125.5 pg/ml[12]	–	–
NGAL	132 ng/ml[35] 80 ng/ml[36] 2.5 ng/ml[6]	–	–	acute renal failure, >50 ng/ml[6] contrast-induced nephropathy, 151 ng/ml (plasma)[7]	–	acute decompensated heart failure with worsening renal function: 194 ng/ml acute decompensated heart failure without worsening renal function: 128 ng/ml[8]
GST-π	9.2 ng/ml[10]	–	–	–	–	–
IL-18	51.22 pg/ml[5] 185.75 pg/ml[3]	1–2: 818.1 pg/ml 3–4: 1207.46 pg/ml 5: 1454.75 pg/ml[3]		IgA nephropathy, 360.26 pg/ml[5]	–	–
MCP-1	239 pg/ml[37] 52.5 pg/ml[38] 73.6 pg/ml[39] 70 pg/ml (plasma)[40] 142.2 pg/ml (plasma)[41]	–	–	diabetic nephropathy, 250–300 pg/ml;[42] 74.0 pg/ml;[38] 69–91 pg/ml (plasma);[40] subclinical kidney disease, 262.9 pg/ml[22]	125.7 pg/ml[39]	essential hypertension: 95.4 pg/ml (plasma)[41]
osteopontin	33 (25–50) ng/ml[17] 1.71 ng/ml[18] 36 ng/ml[19] 53.9 ng/ml[20] 8.8 ng/ml[16]	9.3 ng/ml[16]	–	–	1.81 ng/ml[18]	–
TFF-3	17.8 ng/ml[4]	1: 23.6 ng/ml 2: 29.9 ng/ml 3: 54.9 ng/ml 4: 85.0 ng/ml 5: 176.6 ng/ml[4]	–	–	–	–
β_2M	<1.8mg/l[13] 2.0 mg/l[14]	1: 5.77 mg/l (M), 5.08 mg/l (F) 2: 6.24 mg/l (M), 5.84 mg/l (F) 3: 6.29 mg/l (M), 5.88 mg/l (F) 4: 9.63 mg/l (M), 6.19 mg/l (F)[15a]	end-stage renal disease (on dialysis): 92.6 mg/l[14]	–	–	–
renalase	251.0 ng/ml[16] 120.87 ±16.31 ng/ml (plasma)[43]	316.1 ng/ml[16] 1–2: 162.1 ng/l; 3–5: 217.4 ng/l[44]	–	–	single-branch stenosis, 116.54 ng/ml two-branch stenosis, 111.18 ng/ml multiple-branch stenosis, 109.21 ng/ml (plasma)[43]	–

a stages of chronic kidney disease based on creatinine clearance

Abbreviations: β_2M, β_2-microglobulin; F, female; GST-π, glutatione-S-transferase-π; IL-18, interleukin 18; KIM-1, kidney injury molecule 1; M, male; MCP-1, monocyte chemoattractant protein 1; NGAL, neutrophil gelatinase-associated lipocalin; TFF-3, trefoil factor 3

TABLE 2 Analysis of biomarker levels and other biochemical parameters in the subgroups of patients with various diseases

Parameter	All patients (n = 64)	GN (n = 20)	GN+NS (n = 12)	GN+AH (n = 26)	CD+AH (n = 6)	*P* value
creatinine, mg/dl	1.2 ±0.7 (0.6–4.9)	0.98 ±0.43 (0.6–2.6)	1.08 ±0.36 (0.7–1.9)	1.28 ±0.88 (0.7–4.9)	1.58 ±1.06 (0.9–3.7)	NS
MDRD eGFR	74.7 ±30.8 (12.0–159.0)	89.15 ±31.19 (25.0–159.0)[a]	75.42 ±29.13 (42.0–140.0)	70.54 ±27.94 (14.0–113.0)	43.50 ±19.12 (12.0–66.0)[a]	0.012
CKD-EPI eGFR	80.3 ±31.0 (12.0–141.0)	95.15 ±26.87 (26.0–141.0)[a]	83.33 ±30.12 (45.0–132.0)	75.15 ±29.94 (14.0–115.0)	46.50 ±20.99 (12.0–72.0)[a]	0.0076
CKD-EPI cystatin C eGFR	94.5 ±35.1 (23.0–164.0)	103.30 ±32.97 (40.0–164.0)[a]	98.33 ±37.54 (45.0–157.0)[a]	96.65 ±31.79 (23.0–138.0)[a]	48.33 ±16.54 (24.0–69.0)[a]	0.014
CKD-EPI creatinine--cystatin eGFR	88.4 ±33.8 (17.0–161.0)	99.85 ±29.46 (32.0–161.0)[a]	92.33 ±35.29 (44.0–143.0)	87.42 ±32.94 (17.0–132.0)[a]	46.17 ±16.17 (17.0–63.0)[a]	0.013
Hb, mmol/l	8.7 ±1.0 (6.5–11.1)	8.62 ±0.94 (6.5–10.1)	8.70 ±1.11 (7.3–10.2)	8.77 ±1.03 (7.3–11.1)	8.30 ±0.75 (7.5–9.3)	NS
RBC, T/l	4.8 ±0.5 (3.5–6.0)	4.76 ±0.56 (3.5–5.5)	4.71 ±0.54 (3.7–5.5)	4.78 ±0.58 (3.6–6.0)	4.71 ±0.28 (4.4–5.2)	NS
MCHC, mmol/l	20.4 ±0.8 (17.9–22.5)	20.84 ±0.91 (19.5–22.5)[a]	20.55 ±0.91 (19.0–22.2)	20.13 ±0.58 (17.9–21.0)	19.80 ±0.28 (19.5–20.3)[a]	0.0071
uric acid, mg/dl	7.3 ±1.7 (3.5–12.6)	6.72 ±1.27 (3.7–8.5)	7.70 ±1.66 (5.3–10.9)	7.56 ±2.05 (3.5–12.6)	7.10 ±1.41 (4.9–8.4)	NS
total protein, g/dl	6.4 ±0.8 (4.9–9.8)	6.39 ±0.72 (5.1–7.6)	6.37 ±0.27 (5.8–6.7)	6.33 ±1.04 (4.9–9.8)	6.47 ±0.35 (6.1–7.1)	NS
albumin, g/dl	4.0 ±0.4 (2.9–5.4)	4.03 ±0.31 (3.3–4.8)	3.92 ±0.16 (3.7–4.2)	4.19 ±0.53 (2.9–5.4)[a]	3.63 ±0.24 (3.4–4.0)[a]	0.0028
glucose, mg/dl	83.3 ±16.1 (36.0–135.0)	80.80 ±9.06 (65.0–102.0)	77.42 ±13.61 (54.0–98.0)	88.35 ±20.67 (36.0–135.0)	81.17 ±13.44 (66.0–105.0)	NS
calcium, mg/dl	9.6 ±0.5 (8.6–10.8)	9.55 ±0.32 (9.1–10.2)	9.37 ±0.38 (8.7–10.2)	9.63 ±0.56 (8.6–10.5)	9.91 ±0.6 (9.0–10.8)	NS
renalase, ng/ml	108.4 ±76.8 (18.0–386.0)	98.89 ±67.9 (24.9–310.7)	99.69 ±39.77 (23.6–158.6)	125.44 ±100.18 (18.0–386.0)	83.19 ±16.38 (56.7–106.3)	NS
β_2M, ng/ml	1124.0 ±341.6 (508.4–2460.0)	1029.50 ±258.72 (508.4–1487.7)	1037.73 ±210.15 (709.5–1374.3)	1170.88 ±368.42 (585.0–2386.0)	1408.02 ±527.79 (1077.1–2460.0)	NS
cystatin C, mg/l	0.9 ±0.4 (0.4–2.7)	0.86 ±0.29 (0.5–1.6)[a]	0.91 ±0.37 (0.4–1.6)	0.91 ±0.47 (0.5–2.7)[a]	1.45 ±0.44 (1.0–2.3)[a]	0.026
NGAL, ng/ml	285.7 ±154.8 (72.6–1053.1)	283.06 ±104.72 (121.9–478.6)	283.07 ±139.95 (72.6–545.8)	259.25 ±133.58 (110.7–641.4)	414.00 ±323.08 (217.6–1053.1)	NS
osteopontin, ng/ml	614.2 ±246.8 (258.1–1869.2)	560.55 ±128.06 (258.1–718.1)	613.53 ±178.5 (323.3–918.3)	691.27 ±332.32 (323.3–1869.2)	460.59 ±110.25 (299.1–578.2)	NS
TFF-3, ng/ml	188.1 ±128.5 (55.7–797.1)	159.01 ±70.16 (55.7–383.2)	167.38 ±54.19 (87.6–275.2)	188.10 ±140.86 (83.8–797.1)	326.08 ±237.83 (75.8–764.0)	NS
albindin, ng/ml	28.8 ±14.4 (10.5–116.5)	25.05 ±8.47 (10.5–41.4)	28.46 ±9.96 (16.9–56.7)	28.22 ±9.56 (12.6–51.9)	44.69 ±35.88 (23.6–116.5)	NS
GST-π, ng/ml	49.9 ±18.4 (21.0–115.3)	43.50 ±20.75 (21.0–115.3)[a]	52.53 ±12.41 (31.7–71.7)	54.91 ±16.57 (22.9–90.7)[a]	44.33 ±23.52 (24.0–79.4)	0.032
IL-18, pg/ml	466.6 ±134.9 (80.0–860.0)	405.00 ±72.51 (270.0–540.0)[a]	490.00 ±193.53 (80.0–860.0)	479.15 ±131.54 (280.0–840.0)	570.67 ±100.98 (406.0–666.0)[a]	0.013
KIM-1, pg/ml	65.9 ±44.4 (10.0–250.0)	49.50 ±26.05 (10.0–130.0)[a]	69.17 ±25.75 (10.0–110.0)	61.31 ±41.04 (10.0–180.0)[a]	133.50 ±75.26 (72.0–250.0)[a]	0.033
MCP-1, pg/ml	185.9 ±77.9 (64.0–400.0)	186.50 ±70.58 (80.0–330.0)	180.00 ±73.24 (80.0–300.0)	188.08 ±84.85 (80.0–400.0)	185.83 ±97.59 (64.0–314.0)	NS

Data are presented as mean ± SD (minimum–maximum).

a significant differences

Abbreviations: CD+AH, congenital defect and arterial hypertension; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; eGFR, estimated glomerular filtration rate; GN, glomerulonephritis; GN+AH, glomerulonephritis and arterial hypertension; GN+NS, glomerulonephritis and nephritic syndrome; Hb, hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MDRD, Modification of Diet in Renal Disease; NS, nonsignificant; RBC, red blood cells; others, see **TABLE 1**

TABLE 3 Analysis of biomarker levels and other biochemical parameters in the subgroups of patients divided according to estimated glomerular filtration rate calculated using the Modification of Diet in Renal Disease formula

Parameter	G1, n = 23	G2, n = 23	G3, n = 11	G4, n = 4	G5, n = 3	*P* value
creatinine, mg/dl	0.80 ±0.12 (0.63–1.11)[a]	1.0 ±0.18 (0.75–1.30)	1.47 ±0.33 (1.00–2.15)[a]	2.23 ±0.34 (1.84–2.59)	3.09 ±2.17 (0.68–4.88)[a]	<0.001
MDRD eGFR	105.09 ±20.94 (79.00–159.00)	71.4 ±7.23 (60.00–85.00)	45.09 ±7.19 (34.00–55.00)	26.75 ±1.71 (25.00–29.00)	40.00 ±46.78 (12.00–94.00)	NA
CKD-EPI eGFR	110.39 ±14.59 (87.00–141.00)	79.3 ±9.87 (61.00–100.00)	48.09 ±7.4 (37.00–58.00)	28.00 ±3.56 (25.00–33.00)	44.33 ±54.28 (12.00–107.00)	NA
CKD-EPI cystatin C eGFR	124.48 ±21.14 (60.00–164.00)	94.4 ±22.48 (45.00–131.00)	56.91 ±12.70 (41.00–85.00)	53.50 ±15.46 (40.00–71.00)	58.33 ±60.34 (23.00–128.00)	NA
CKD-EPI creatinine--cystatin eGFR	120.91 ±17.55 (84.00–161.00)	86.7 ±13.23 (56.00–111.00)	51.64 ±7.74 (40.00–64.00)	38.25 ±7.23 (32.00–45.00)	52.67 ±61.78 (17.00–124.00)	NA
Hb, mmol/l	9.00 ±0.87 (7.5–10.2)	8.4 ±1.02 (6.50–11.10)	8.99 ±0.88 (7.50–10.00)	8.40 ±1.01 (7.30–9.60)	7.43 ±0.06 (7.4–7.5)	NS
RBC, T/l	4.92 ±0.47 (4.11–5.55)	4.6 ±0.62 (3.52–5.95)	4.92 ±0.37 (4.35–5.42)	4.67 ±0.57 (4.14–5.36)	4.42 ±0.42 (3.94–4.73)	NS
MCHC, mmol/l	20.73 ±0.83 (19.60–22.50)[a]	20.4 ±0.75 (19.00–22.10)	20.19 ±0.58 (19.50–21.70)	20.23 ±0.43 (19.90–20.80)	19.17 ±1.10 (17.90–19.80)[a]	0.035
uric acid, mg/dl	7.02 ±1.88 (3.7–12.63)	6.8 ±1.31 (3.46–8.79)	8.61 ±1.96 (4.90–11.73)	7.90 ±0.83 (6.86–8.84)	7.00 ±1.16 (6.00–8.27)	NS
total protein, g/dL	6.39 ±0.67 (5.00–7.70)	6.3 ±1.03 (4.90–9.80)	6.25 ±0.37 (5.50–6.70)	6.93 ±0.63 (6.20–7.70)	6.20 ±0.70 (5.40–6.70)	NS
albumin, g/dl	4.10 ±0.48 (2.90–5.40)	4.0 ±0.44 (3.30–5.40)	3.83 ±0.27 (3.40–4.30)	4.05 ±0.21 (3.80–4.30)	4.23 ±0.40 (4.00–4.70)	NS
glucose, mg/dl	82.04 ±16.30 (54.00–129.00)	85.3 ±16.32 (65.00–135.00)	88.73 ±11.20 (66.00–105.00)	78.75 ±14.10 (63.00–93.00)	63.33 ±23.76 (36.00–79.00)	NS
calcium, mg/dl	9.52 ±0.4 (8.58–10.29)	9.6 ±0.49 (8.74–10.53)	9.66 ±0.62 (8.68–10.83)	9.42 ±0.27 (9.05–9.64)	9.55 ±0.71 (8.96–10.34)	NS
renalase, ng/ml	115.08 ±74.66 (18.02–376.60)	98.7 ±75.73 (23.60–310.70)	90.85 ±24.87 (42.00–123.30)	190.53 ±163.81 (30.20–386.00)	85.47 ±19.27 (64.26–101.90)	NS
β_2M , ng/ml	928.95 ±201.38 (508.37–1374.34)[a]	1181.1 ±263. (651.35–1820.81)[a]	1299.99 ±422.68 (928.68–2459.95)[a]	1417.76 ±702.77 (8833.75–2385.98)	1143.61 ±118.13 (1016.12–1249.35)	0.0018
cystatin C, mg/l	0.66 ±0.19 (0.38–1.34)[a]	0.9 ±0.21 (0.60–1.42)[a]	1.29 ±0.22 (0.93–1.64)[a]	1.36 ±0.29 (1.00–1.63)	1.84 ±1.16 (0.53–2.74)	<0.001
NGAL, ng/ml	228.82 ±116.20 (72.60–545.78)[a]	249.3 ±85.34)	354.35 ±87.62 (217.55–471.16)[a]	390.66 ±107.31 (238.25–483.73)	608.14 ±462.48 (129.92–1053.08)	0.0037
osteopontin, ng/ml	588.03 ±189.92 (323.34–1195.22)	622.6 ±300.80 (258.11–1869.24)	563.31 ±139.50 (363.25–833.26)	908.68 ±346.96 (600.51–1370.80)	544.54 ±212.49 (323.34–747.09)	NS
TFF-3, ng/ml	137.68 ±63.96 (55.70–375.25)[a]	153.7 ±39.42 (70.80–217.05)	267.29 ±172.86 (144.82–764.01)[a]	278.34 ±92.82 (160.46–383.18)[a]	426.49 ±342.93 (121.93–797.09)	0.0001
calbindin, ng/ml	25.37 ±9.78 (10.53–56.67)	28.7 ±9.16 (13.05–51.94)	26.25 ±6.95 (16.85–43.54)	36.53 ±8.13 (25.14–43.87)	55.30 ±53.22 (20.33–116.54)	NS
GST-π, ng/ml	46.06 ±17.96 (21.03–80.47)	53.9 ±21.02 (26.73–115.29)	47.18 ±14.89 (23.97–68.24)	47.68 ±10.14 (34.81–59.60)	62.05 ±18.09 (43.31–79.42)	NS
IL-18, pg/ml	396.52 ±101.25 (80.00–530.00)[a]	454.7 ±99.29 (310.00–750.00)	609.64 ±154.84 (360.00–860.00)[a]	492.50 ±51.88 (430.00–540.00)	536.00 ±221.78 (280.00–670.00)	0.0026
KIM-1, pg/ml	45.65 ±25.19 (10.00–110.00)[a]	60.8 ±32.61 (10.00–130.00)	98.36 ±62.42 (40.00–250.00)[a]	72.50 ±17.08 (50.00–90.00)	131.67 ±77.84 (50.00–205.00)	0.005
MCP-1, pg/ml	159.57 ±67.05 (80.00–300.00)[a]	229.2 ±76.51 (110.00–400.00)[a]	170.36 ±76.47 (64.00–314.00)	165.00 ±88.13 (90–290.00)	139.67 ±34.93 (119.00–180.00)	0.021

Data are shown as mean ± SD (minimum–maximum). Ranges of eGFR for the G1–G5 subgroups are given in the Patients and Methods section.

a significant differences

Abbreviations: NA, not applicable; others, see TABLES 1 and 2

the model (odds ratio [OR], 0.994; 95% confidence interval [CI], 0.988–0.999; likelihood ratio test, $P = 0.008$). The result of the Hosmer–Lemeshow test allowed us to accept the hypothesis of the goodness of fit of the model ($P = 0.73$). The AUC reached 0.714 (FIGURE 1).

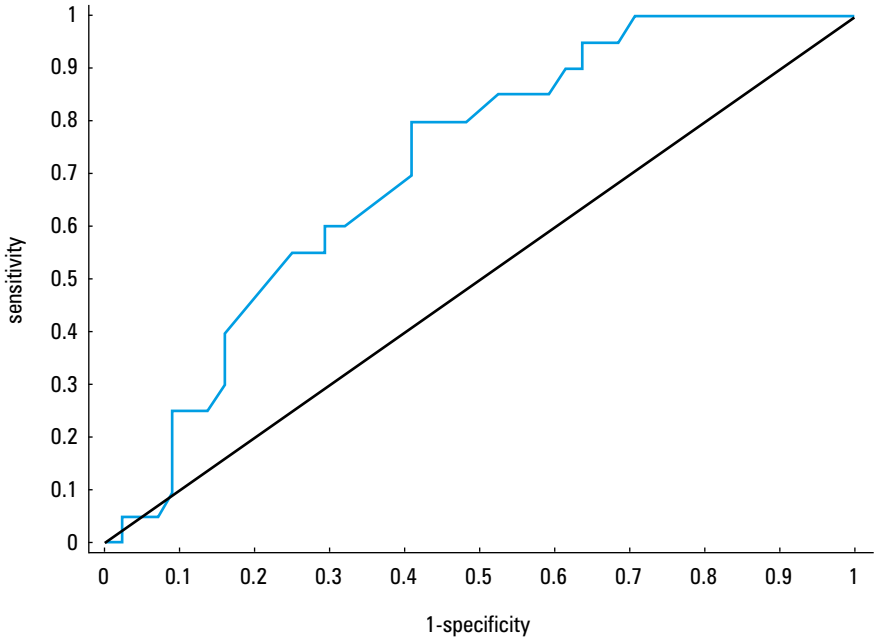

FIGURE 1 Receiver--operating characteristic (ROC) curve for logistic regression model for glomerulonephritis as dependent variable; significant independent variable was interleukin 18 (area under the ROC curve, 0.714)

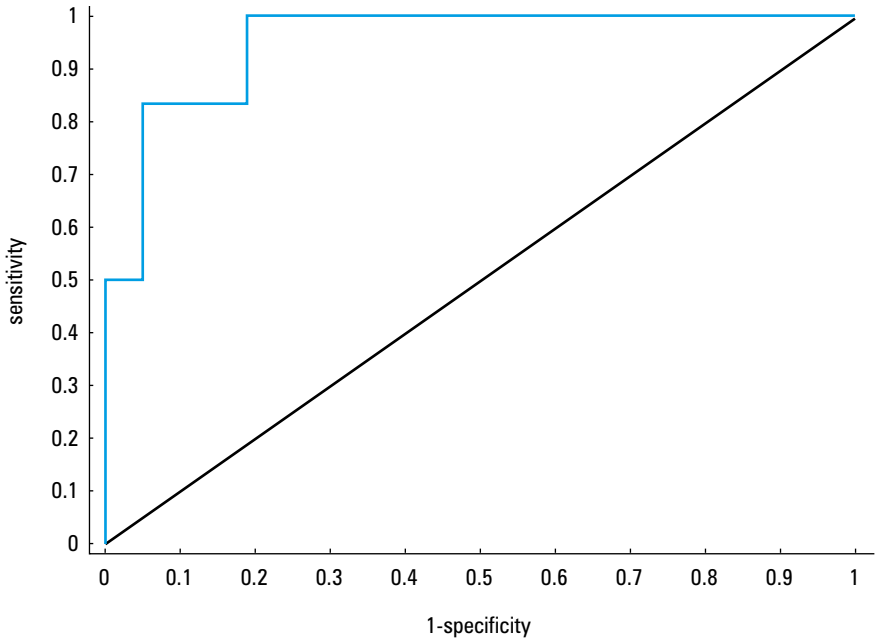

FIGURE 2 Receiver--operating characteristic curve (ROC) for logistic regression model for the subgroup of patients with congenital defects of the kidney and arterial hypertension as dependent variable; significant independent variables were ostoepontin and kidney injury molecule 1 (area under the ROC curve, 0.951).

When the dependent variables were GN+NS and GN+AH, none of the parameters studied were significant in explaining the dependent variable. When the dependent variable was CD+AH, significant predictors in the logistic regression model were osteopontin (OR, 0.987; 95% CI, 0.976–0.998; $P = 0.024$) and KIM-1 (OR, 1.039; 95% CI, 1.011–1.068; $P = 0.0002$). The Hosmer–Lemeshow test allowed us to accept the hypothesis of the goodness of fit of the model ($P = 0.84$). The AUC was 0.951 (FIGURE 2). The ROC curve was also calculated seperately for KIM-1 and osteopontin, and the AUC for those biomarkers was 0.875 and 0.77, respectively (data not shown).

The detailed results of the different eGFR subgroups are shown in TABLE 3. Since diversity of this group is based on MDRD eGFR, only the most important differences and correlations are presented in the Discussion section.

DISCUSSION To the best of our knowledge, we were the first to analyze the serum levels of kidney toxicity markers using the Bio-Plex method (Kidney Toxicity Panels 1 and 2). Most of similar studies were performed using immunoenzymatic tests; therefore, we are aware that the use of a different method may have provided discrepant results.

We found numerous similarities between our results and those obtained by other investigators, mainly with regards to MCP-1. IL-18 and TFF-3 showed about 2- to 4-fold higher levels than those reported in other studies for different stages of chronic kidney disease,[3,4] but similarly to those data, the IL-18 and TFF-3 levels increased together with a decrease in eGFR. IL-18 levels were similar to those observed for patients with IgA nephropathy (466.6 pg/ml vs 360.26 pg/ml, respectively).[5] NGAL levels were also similar, although

slightly higher in our study than in other studies on patients with kidney diseases.[6-8]

As for calbindin, no studies have reported its levels in renal patients so far. In healthy individuals, calbindin levels were found to be lower than 20 pg/ml and were undetectable using a commercially available enzyme-linked immunosorbent assay,[9] while our study showed that the mean value in patients with kidney diseases was 28.8 ng/ml. Similarly, serum levels had not been studied before in renal patients. The mean GST-π levels were reported to be 9.2 ng/ml in healthy people,[10] while in our study, they reached 49.9 ng/ml. The mean levels of KIM-1 (65.9 pg/ml) and β_2M (1.124 μg/ml) in our study were comparable to those described for healthy people in other studies, and were much lower than those in patients with kidney diseases.[11-15] As for osteopontin, its mean level in our study was 614.2 ng/ml, while in the literature, its levels in patients with chronic kidney disease were reported to be, depending on the study, in the range between 1.71 and 53 ng/ml.[16-20]

Of note, cystatin C concentrations were in the range between 0.4 and 2.7 mg/l in our study, which is consistent with existing literature data. Therefore, we were able to calculate eGFR using formulas containing cystatin C concentrations.

The serum levels of renalase, MCP-1, GST-π, and osteopontin seem to be eGFR-independent factors involved in kidney damage. Correlations between renalase and osteopontin in the whole study group and in the GN+AH subgroup suggest that there might be a common mechanism underlying their release, mechanism of action, or degradation. Together with osteoprotegerin, matrix γ-carboxyglutamic acid protein, and fetuin A, osteopontin is involved in the inhibition of atherosclerotic calcification.[16] In blood, osteopontin plays a role of a proinflammatory protein and potentially chemotactic molecule for neutrophils and macrophages, and similarly to KIM-1, plays an important role in immune response.[12] In our study, increase in osteopontin levels was associated with a small, but significant reduction in the odds of kidney disease (while KIM-1 had an opposite effect) in the CD+AH subgroup, which probably resulted from an inhibitory effect of this molecule on vascular calcification and nephrocalcinosis, or from much more complicated relations associated with the mechanism regulating the process of bone destruction in kidney disease. It cannot be also excluded that this protective role is related to antiapoptic and immunostimulatory properties of osteopontin.[21]

MCP-1 levels were similar in all analyzed subgroups, except for the CD+AH subgroup, in which 2-fold higher concentrations were observed. Two strong negative correlations, namely, with renalase in the GN+NA subgroup and with KIM-1 in the CA+AH subgroup, are particularly interesting. While a negative correlation with KIM-1 probably results from the elevated levels of this molecule, a negative correlation with renalase levels seems to be more complex. The serum levels of MCP-1, a molecule considered to be a product of a wide range of cells and tissues (especially monocytes and macrophages), may indicate numerous inflammatory processes because MCP-1 acts mainly as a proinflammatory chemokine.[22] Macrophages are the principal kidney infiltrating population of leukocytes in type 2 diabetes mellitus, and the MCP-1 expression in glomerular and interstitial compartments of the kidney correlates with albuminuria and outcome of kidney function, due to high infiltration and kidney damage caused by macrophages.[23,24] Also in a study by Morii et al,[25] the urinary levels of MCP-1 correlated positively and significantly with urinary albumin levels, which was caused by an increased leakage of plasma protein from the glomerular capillary to tubular fluid. This also explains why significant outcome was limited only to the NS subgroup in our study. A negative correlation between MCP-1 and renalase levels is not completely clear due to still unclear properties of renalase; most theories suggest that renalase is an enzyme with high affinity to biogenic amines. However, recent findings have shown that it might be also an anti-inflammatory cytokine that increases cell survival, and a single nucleotide polymorphism in the renalase gene is a high-risk factor for developing diabetes mellitus.[26] Also, in the animal model of acute kidney injury, administration of renalase correlated with renal protection and lower macrophage infiltration of the kidney,[26] which taken together with our results can be the basis for a hypothesis that renalase might inhibit the activity of MCP-1 in patients with NS. Therefore, the correlation between MCP-1 and renalase levels should be further studied in patients with other primary and secondary kidney diseases, especially diabetic nephropathy.

Similarly to MCP-1, renalase, and osteopontin, GST-π levels in our study did not correlate with kidney function, as assessed using the Cr, cystatin C, and eGFR equations. The only exception was the CA+AH subgroup, in which it was significantly correlated with KIM-1 and NGAL levels. The discrepancy in the results between the subgroups with and without GN might have been caused by the fact that GN is accompanied by chronic oxidative stress, which may lead to further oxidative injury, and that high levels of reactive oxygen species upregulate the expression of GST-π. This might have hidden potential correlations.[10,27,28]

The remaining biomarkers, β_2M, NGAL, TFF-3, calbindin, IL-18, and KIM-1, in most cases correlated linearly and negatively with kidney function and injury measured by all 4 eGFR equations. A decrease in eGFR may generally be associated with increased serum levels of the above factors by stimulating their production and release in response to damage, or by modification or even inhibition of their catabolism, or by increased resorption in different parts of the nephron. Considering that the majority of those factors are

bound to or produced by immune cells, the increase of their serum levels is caused by inflammation, and increased levels of leukocytes and antibodies during immune response contribute to disease progression.

IL-18 is a cytokine with a wide variety of functions. Its levels correlate with the severity of numerous diseases, including circulatory disorders, kidney injury, autoimmune diseases.[5,29,30] IL-18 is not only a marker of tissue damage but is also actively involved in the damage process of numerous other organs.[30] It has been shown that an increase in serum IL-18 levels may result in worse renal outcome. In addition, it may be a marker of kidney disease progression in patients diagnosed with IgA nephropathy or ischemic acute kidney injury.[31] This results from the proinflammatory properties of IL-18, the activity of caspase 1 (which processes the inactive form of IL-18), the presence of IL-18-binding protein, and the regulatory role of IL-18 in the production of interferon γ.[30] In some situations, IL-18 may also play a protective role; it probably prevents the development of metabolic syndrome or macular degeneration.[30] Our study showed that in contrast to the majority of the reports published, IL-18 lowers the odd of kidney disease in patients with GN.

TFF-3 is a less well-analyzed marker of kidney function. Nonetheless, at least one large study showed that its concentration increases with the progression of kidney disease.[26] Our study, although performed on a much smaller group of patients and therefore analyzed using nonparametric tests, showed the same tendency but with about 4-fold higher levels than those reported in literature for each stage. The most recent data show that this molecule was underestimated in epithelial restitution and regeneration in injured kidneys, and that TFF-3 release is stimulated by nutrient starvation and hypoxia.[4] As the TFF-3 level correlated with eGFR in most subgroups, its correlation with the most common and possibly the most accurate markers, NGAL, KIM-1, IL-18, and β_2M, is not a surprising finding.

The negative correlation between calbindin and eGFR in patients with GN and NS, as observed in our study, suggests that NS is not just a consequence of kidney damage but also a factor that should guide the decision on the type of therapy for NS. Calbindin is a crucial protein involved in bone remodeling, with antiapoptotic properties shown for neuronal cells as a result of its calcium-buffering and caspase-3 inhibiting properties.[32] Of the biomarkers analyzed in this study, calbindin is the only indicator of damage to kidney collecting ducts; it is also related to the condition of distal tubules.[33] Therefore, it is considered to be a good biomarker of distal nephron segment injuries, which cause its decrease, and unlike the other biomarkers, renal damage with proteinuria is diagnosed on the basis of increased calbindin expressions, rather than decreased one.[34]

Conclusions Taken together, it should be noted that renalase and MCP-1 in NS might have an opposite effect and regulate each others' levels. Further studies should investigate the relations between renalase, MCP-1, and osteopontin, as factors unrelated to eGFR in patients with GN. As renalase and MCP-1 were frequently reported to be associated with diabetes mellitus, a group of diabetes patients should be also included. NS may contribute to loss of calbindin from serum, but it cannot be excluded that accumulation of calbindin may stimulate the gradual loss of kidney function. NGAL, KIM-1, TFF-3, IL-18, β_2M, and calbindin are good indicators of kidney function loss evaluated using the 4 eGFR equations in patients with GN, which confirms the findings from single previous studies.

Our study describes numeorus factors measured at the same time and in the same conditions, many of which have never been described in detail or even described at all in the serum of patients with kidney disease, including glomerulonephtiris and congenital kidney defects. Some of the observations are obviously not accidental and can be the basis for further research.

Limitations Due to the pioneering design of the study, which required experimental determination of the optimal dilution of serum samples to obtain reliable results, we had to perform it in duplicates. Therefore, in order to perform the assays in the largest possible group of patients, we decided not to enroll the control group. To refer to normal, physiological levels of the analyzed biomarkers, we used literature data. Nevertheless, we believe that the results are interesting enough to be published.

We are aware that due to a small size of our subgroups, some of the differences and correlations may be accidental and some of the results might not have reached statistical significance. Furthermore, not all our results may be compared to existing literature data because of different methods used.

Contribution statement NMS designed the study and performed the immunoenzymatic assays. MW drafted the clinical part of article, and analysed the data. AJ, ES, and ZM collected material and data, and performed biochemical assays. BD supervised the study, as well as edited and corrected the draft of the article.

Acknowledgments We would like to thank Maciej Mularczyk from the Department of Anatomy, Pomeranian Medical University in Szczecin, Poland, for his invaluable help and understanding during the study development and analysis of the results. We are also grateful to Dariusz Pianka, the Field Application Specialist from Bio-Rad, Warsaw, Poland, for assistance in experimental settings during tbe development of the method.

This study was funded by the National Science Centre, Poland (project No. 2014/13/N/

NZ5/03670, granted to NMS, entitled "Renalase – a potential marker of early kidney damage and risk of cardiovascular complications among patients with chronic kidney disease and its complication").

Supplementary material online Supplementary material online is available with the online version of the article at www.pamw.pl.

REFERENCES

1 Chadban SJ, Atkins RC. Glomerulonephritis. Lancet. 2005; 365: 1797-1806.

2 The National Kidney Foundation 2016. Available from: https://http://www.kidney.org/home. Accessed April 10, 2016.

3 Formanowicz D, Wanic-Kossowska M, Pawliczak E, et al. Usefulness of serum interleukin-18 in predicting cardiovascular mortality in patients with chronic kidney disease - systems and clinical approach. Sci Rep. 2015; 5: 18332.

4 Du TY, Luo HM, Qin HC, et al. Circulating serum trefoil factor 3 (TFF3) is dramatically increased in chronic kidney disease. PLoS One. 2013; 8: e80271.

5 B, Ni Z, Cao L, et al. Serum IL-18 is closely associated with renal tubulointerstitial injury and predicts renal prognosis in IgA nephropathy. Mediators Inflamm. 2012; 2012: 728417.

6 Mishra J, Dent C, Tarabishi R, et al. Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery. Lancet. 2005; 365: 1231-1238.

7 Hirsch R, Dent C, Pfriem H, et al. NGAL is an early predictive biomarker of contrast-induced nephropathy in children. Pediatr Nephrol. 2007; 22: 2089-2095.

8 Aghel A, Shrestha K, Mullens W, et al. Serum Neutrophil Gelatinase-Associated Lipocalin (NGAL) in Predicting Worsening Renal Function in Acute Decompensated Heart Failure. J Card Fail. 2010; 16: 49-54

9 Hasegawa S, Kato K, Takashi M, et al. Increased levels of calbindin-D in serum and urine from patients treated by extracorporeal shock wave lithotripsy. J Urol. 1993; 149: 1414-1418.

10 Niitsu Y, Takahashi Y, Saito T, et al. Serum glutathione-S-transferase-pi as a tumor marker for gastrointestinal malignancies. Cancer. 1989; 63: 317-323.

11 Sabbisetti VS, Waikar SS, Antoine DJ, et al. Blood kidney injury molecule-1 is a biomarker of acute and chronic kidney injury and predicts progression to ESRD in type I diabetes. J Am Soc Nephrol. 2014; 25: 2177-2186.

12 Jin ZK, Tian PX, Wang XZ, et al. Kidney injury molecule-1 and osteopontin: new markers for prediction of early kidney transplant rejection. Mol Immunol. 2013; 54: 457-464.

13 Kim MK, Yun KJ, Chun HJ, et al. Clinical utility of serum beta-2-microglobulin as a predictor of diabetic complications in patients with type 2 diabetes without renal impairment. Diabetes Metab. 2014; 40: 459-465.

14 Mumtaz A, Anees M, Bilal M, Ibrahim M. Beta-2 microglobulin levels in hemodialysis patients. Saudi J Kidney Dis Transpl. 2010; 21:701-706.

15 Shahjahan, Yasmin R, Mahsud MAJ, et al. Correlation of Beta-2 Microglobulin with serum creatinine and creatinine clearance in patients with different levels of renal function. Gomal J Med Sci. 2011; 9: 178-182.

16 Gluba-Brzozka A, Michalska-Kasiczak M, Franczyk-Skora B, et al. Markers of increased cardiovascular risk in patients with chronic kidney disease. Lipids Health Dis. 2014; 13: 135.

17 Yilmaz Y, Ozturk O, Alahdab YO, et al. Serum osteopontin levels as a predictor of portal inflammation in patients with nonalcoholic fatty liver disease. Dig Liver Dis. 2013; 45: 58-62.

18 Tousoulis D, Siasos G, Maniatis K, et al. Serum osteoprotegerin and osteopontin levels are associated with arterial stiffness and the presence and severity of coronary artery disease. Int J Cardiol. 2013; 167: 1924-1928.

19 Sfiridaki A, Miyakis S, Pappa C, et al. Circulating osteopontin: a dual marker of bone destruction and angiogenesis in patients with multiple myeloma. Journal of Hematology & Oncology. 2011; 4:1.

20 M, Andisheh-Tadbir A, et al. Serum levels of osteopontin as a prognostic factor in patients with oral squamous cell carcinoma. Tumour Biol. 2014; 35: 3827-3829.

21 Xie Y, Sakatsume M, Nishi S, et al. Expression, roles, receptors, and regulation of osteopontin in the kidney. Kidney Int. 2001; 60: 1645-1657.

22 Murea M, Register TC, Divers J, et al. Relationships between serum MCP-1 and subclinical kidney disease: African American-Diabetes Heart Study. BMC Nephrology. 2012; 13: 148.

23 Chow FY, Nikolic-Paterson DJ, Ozols E, et al. Monocyte chemoattractant protein-1 promotes the development of diabetic renal injury in streptozotocin-treated mice. Kidney Int. 2006; 69: 73-80.

24 Tesch GH. MCP-1/CCL2: a new diagnostic marker and therapeutic target for progressive renal injury in diabetic nephropathy. Am J Physiol Renal Physiol. 2008; 294: 697-701.

25 Morii T, Fujita H, Narita T, et al. Association of monocyte chemoattractant protein-1 with renal tubular damage in diabetic nephropathy. J Diabetes Complications. 2003; 17: 11-15.

26 Guo X, Wang L, Velazquez H, et al. Renalase: its role as a cytokine, and an update on its association with type 1 diabetes and ischemic stroke. Curr Opin Nephrol Hypertens. 2014; 23: 513-518.

27 Huang J, Tan PH, Tan BK, Bay BH. GST-pi expression correlates with oxidative stress and apoptosis in breast cancer. Oncol Rep. 2004; 12: 921-925.

28 Gwinner W, Gröne HJ. Role of reactive oxygen species in glomerulonephritis. Nephrol. Dial. Transplant. 2000; 15: 1127-1132.

29 Krishnan SM, Sobey CG, Latz E, et al. IL-1beta and IL-18: inflammatory markers or mediators of hypertension? Br J Pharmacol. 2014; 171: 5589-5602.

30 Dinarello CA, Novick D, Kim S, Kaplanski G. Interleukin-18 and IL-18 Binding Protein. Front Immunol. 2013; 4: 289.

31 Melnikov VY, Ecder T, Fantuzzi G, et al. Impaired IL-18 processing protects caspase-1-deficient mice from ischemic acute renal failure. J Clin Invest. 2001; 107: 1145-1152.

32 Bellido T, Huening M, Raval-Pandya M, et al. Calbindin-D28k is expressed in osteoblastic cells and suppresses their apoptosis by inhibiting caspase-3 activity. J Biol Chem. 2000; 275: 26328-26332.

33 Bonventre JV, Vaidya VS, Schmouder R, et al. Next-generation biomarkers for detecting kidney toxicity. Nat Biotechnol. 2010; 28: 436-440.

34 Iida T, Fujinaka H, Xu B, et al. Decreased urinary calbindin 1 levels in proteinuric rats and humans with distal nephron segment injuries. Clin Exp Nephrol. 2014; 18: 432-443.

35 Gala-Bladzinska A, Kuzniewski M. [Performance neutrophil gelatinase-associated lipocalin in clinical settings]. Przegl Lek. 2013; 70: 400-403.

36 Wheeler DS, Devarajan P, Ma Q, et al. Serum Neutrophil Gelatinase-associated Lipocalin (NGAL) as a Marker of Acute Kidney Injury in Critically Ill Children with Septic Shock. Crit Care Med. 2008; 36: 1297-1303.

37 Arakelyan A, Petrkova J, Hermanova Z, et al. Serum Levels of the MCP-1 Chemokine in Patients With Ischemic Stroke and Myocardial Infarction. Mediators Inflamm. 2005; 2005: 175-179.

38 Wada T, Furuichi K, Sakai N, et al. Up-regulation of monocyte chemoattractant protein-1 in tubulointerstitial lesions of human diabetic nephropathy. Kidney Int. 2000; 58: 1492-1499.

39 Martinovic I, Abegunewardene N, Seul M, et al. Elevated monocyte chemoattractant protein-1 serum levels in patients at risk for coronary artery disease. Circ J. 2005; 69: 1484-1489.

40 Chiarelli F, Cipollone F, Mohn A, et al. Circulating Monocyte Chemoattractant Protein-1 and Early Development of Nephropathy in Type 1 Diabetes. 2002; 25: 1829-1834.

41 Madej A, Okopien B, Kowalski J, et al. Plasma concentrations of adhesion molecules and chemokines in patients with essential hypertension. Pharmacol Rep. 2005; 57: 878-881.

42 Banba N, Nakamura T, Matsumura M, et al. Possible relationship of monocyte chemoattractant protein-1 with diabetic nephropathy. Kidney International. 2000; 58: 684-690.

43 He B, Hao J, Sheng W, et al. Correlation between plasma renalase level and coronary artery disease. Pak J Med Sci. 2014; 30: 863-967.

44 Wang F, Li J, Xing T, Xie Y, Wang N. Serum renalase is related to catecholamine levels and renal function. Clin Exp Nephrol. 2015; 19: 92-98.

Stężenie dwunastu markerów czynności i uszkodzenia nerek w surowicy pacjentów z kłębuszkowym zapaleniem nerek

Natalia M. Serwin[1], Magda Wiśniewska[2], Anna Jesionowska[3], Edyta Skwirczyńska[4], Zuzanna Marcinowska[5], Barbara Dołęgowska[1]

1 Zakład Mikrobiologii i Diagnostyki Immunologicznej, Pomorski Uniwersytet Medyczny w Szczecinie, Szczecin
2 Klinika Nefrologii, Transplantologii i Chorób Wewnętrznych, Pomorski Uniwersytet Medyczny w Szczecinie, Szczecin
3 Katedra Biochemii i Chemii Medycznej, Pomorski Uniwersytet Medyczny w Szczecinie, Szczecin
4 Zakład Historii Medycyny i Etyki Lekarskiej, Pomorski Uniwersytet Medyczny w Szczecinie, Szczecin
5 Zakład Analityki Medycznej, Pomorski Uniwersytet Medyczny w Szczecinie, Szczecin

SŁOWA KLUCZOWE

biomarkery funkcji nerek, kalbindyna, kłębuszkowe zapalenie nerek, osteopontyna, renalaza

STRESZCZENIE

WPROWADZENIE Kłębuszkowe zapalenie nerek (*glomerulonephritis* – GN) to złożona choroba wpływająca na funkcjonowanie całego nefronu. Niewiele jest danych dotyczących stężenia w surowicy najbardziej popularnych biomarkerów czynności i uszkodzenia nerek u pacjentów z GN lub badania takie prezentują niejednoznaczne wyniki.

CELE Celem badania było określenie poziomu znanych specyficznych i niespecyficznych dla nerki markerów czynności lub uszkodzenia nerek w surowicy pacjentów z rozpoznaniem pierwotnego lub wtórnego GN z uwzględnieniem obecności zespołu nerczycowego (*nephrotic syndrome* – NS) i nadciśnienia tętniczego (*arterial hypertension* – AH) lub ich braku.

PACJENCI I METODY Badaniem objęto 58 pacjentów z rozpoznaniem GN i 6 pacjentów z wrodzonymi wadami nerek (*congenital defects* – CD) i nadciśnieniem (CD+AH). Stężenia β_2-mikroglobuliny (β_2M), lipokaliny związanej z żelatynazą neutrofili (NGAL), osteopontyny, czynnika TFF-3 (*trefoil factor 3*), kalbindyny, S-transferazy glutationowej-π (GST-π), interleukiny 18 (IL-18), cząsteczki uszkodzenia nerek 1 (KIM-1), białka chemotaktycznego dla monocytów 1 (MCP-1) określono za pomocą dwóch zestawów: Kidney Toxicity Panel 1 i 2 za pomocą metody Bio-Plex. Stężenie renalazy określono za pomocą testu immunoenzymatycznego.

WYNIKI U wszystkich pacjentów oraz w podgrupach (GN, GN+AH, GN+NS, CD+AH) stężenia NGAL, KIM-1, TFF-3, IL-18, β_2M i kalbindyny korelowały z szacowanym współczynnikiem przesączania kłębuszkowego (*estimated glomerular filtration rate* – eGFR). U pacjentów z NS zależność ta dla kalbindyny była odwrotna. Stężenia renalazy, MCP-1, GST-π i osteopontyny były niezależne od eGFR. Zwiększenie stężenia IL-18 w grupie pacjentów z samym GN zmniejsza szansę wystąpienia choroby. Po dokonaniu podziału tej grupy ze względu na eGFR na podgrupy G1–G5, stężenia TFF-3, NGAL i β_2M zwiększały się wraz ze stadium choroby.

WNIOSKI U pacjentów z NS renalaza i MCP-1 w NS mogą wzajemnie regulować swoje stężenia. Należy przeprowadzić dalsze badania nad korelacjami między renalazą, MCP-1 i osteopontyną jako czynnikami niezwiązanymi z eGFR w GN. NS może się przyczynić do utraty kalbindyny z surowicy. NGAL, KIM-1, TFF-3, IL-18, β_2M i kalbindyna są dobrymi wskaźnikami utraty funkcji nerek u pacjentów z GN.

Adres do korespondencji:
mgr Natalia M. Serwin, Zakład Mikrobiologii i Diagnostyki Immunologicznej, Pomorski Uniwersytet Medyczny w Szczecinie, al. Powstańców Wlkp. 72, 70-111 Szczecin; tel.: 91 466 16 52, e-mail: natmat@pum.edu.pl
Praca wpłynęła: 30.04.2016.
Przyjęta do druku: 06.05.2016.
Publikacja *online*: 09.08.2016.
Nie zgłoszono sprzeczności interesów.
Pol Arch Med Wewn. 2016; 126 (7-8): 483-493
doi:10.20452/pamw.3477

* Autorzy pracy otrzymali II nagrodę Redaktor Naczelnej w konkursie na najlepszą studencką pracę oryginalną w 2016 r. Regulamin konkursu jest dostępny na stronie www.pamw.pl.