Algorithm for Early Diagnosis of Contrast-Induced Nephropathy Using Biomarkers of Renal Damage

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The aim of the study was to develop an algorithm for early diagnosis of contrast-induced nephropathy using renal damage biomarkers cystatin C, nephrin, and lipocalin 2.

Materials and Methods. Eighty-four patients, who for the first time underwent transcutaneous coronary intervention with radiocontrast agents, were included in the study. Group 1 was composed of patients with diabetes mellitus type 2 (n=44), group 2 consisted of patients without carbohydrate metabolism impairment (n=40). All patients were determined the concentration of renal damage biomarkers before and after the procedure.

Results. Cystatin C, nephrin, and lipocalin 2 have been established to have high sensitivity and specificity. Determining them for 3 days enables objective assessment of the contrast-induced nephropathy dynamics. The proposed algorithm with biomarker application makes it possible to predict the development of acute renal insufficiency during operative interventions on the coronary arteries with introduction of contrast agents, and to carry out preventive therapy and intensive rehydration.

Key words: contrast-induced nephropathy; diabetes mellitus type 2; cystatin C; nephrin; lipocalin 2.

In recent years, application of radiocontrast agents increased in urography, angiography, computed tomography and operative procedures. Over 80 million doses are used annually. Despite the development of new and less toxic agents, the risk of contrast-induced nephropathy (CIN) development remains significant [1–5]. In patients with initially damaged renal function the risk of this pathology development is essentially higher than in patients with the acquired one, and in those suffering diabetes mellitus type 2 (DM 2) the rate of CIN development can reach 50% and more [6]. CIN is the cause of acute kidney damage occurring after intravascular introduction of an iodine-containing radiocontrast media [7–10].

At present, the level of blood serum creatinine is recognized to be a golden standard in CIN diagnosis. According to KDIGO (2012) recommendations, this pathology is diagnosed at a creatinine level exceeding the initial one by 26 μmol/L during 48–72 h following the introduction of the contrast agent excluding other causes [11–13]. However, creatinine itself only relatively reflects the state of the renal function. For example, it is established that about 50% of the renal function can be lost prior to its level increase [14]. Besides, nonspecificity of serum creatinine in toxic kidney damage and its dependence on a number of nonrenal factors motivate the search of new CIN markers [15, 16].

The aim of the study was to develop an algorithm for early diagnosis of contrast-induced nephropathy using renal damage biomarkers cystatin C, nephrin, and lipocalin 2.

Materials and Methods. Eighty four patients, who for the first time underwent transcutaneous coronary intervention (TCI) with radiocontrast agents, were included in the study. The study complies with the Declaration of Helsinki (the Declaration was passed in June 1964, Helsinki, Finland and revised in October 2000, Edinburgh, Scotland) and was performed following approval by the Ethics Committee of Nizhny Novgorod Regional Clinical Hospital named after N.A. Semashko. Written informed consent was obtained from every patient. Patients were divided into two groups: group 1 (the main group) comprised DM 2 patients (n=44); group 2 (control) (n=40) consisted of patients without impairments of carbohydrate metabolism. DM 2 was established in compliance with the national standards for diagnosing and treatment of diabetes mellitus [17].

All patients underwent clinical, laboratory and instrumental examination. Glycated hemoglobin HbA1c was tested using D-10 analyzer with standard kits (Bio-Rad, France). Lipid spectrum indices and creatinine level were determined with the help of diagnostic systems supplied by Olvex Diagnosticum (Saint Petersburg,

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Russia), urea was investigated with Mochevina FS diagnostic kit (Diakon-DS, Russia) using StatFax 3300 analyzer (Awareness Technology Inc., USA). The level of cystatin C in blood serum, nephrin in urine, and lipocalin 2/NGAL in blood plasma were determined before and after TCI.

Cystatin C is a nonglycated protein, which is used as a biomarker of acute renal insufficiency. This protein can indicate CIN development earlier than creatinine — already on the first day. Cystatin C was determined in the blood serum by an immunoturbidimetric test using Cystatin C FS diagnostic kit (DiaSys Diagnostic Systems, Germany). Its normal values are 0.58–1.02 mg/ml [18, 19].

Nephrine is a pore-forming protein, which participates in formation of a renal filter playing an important role in contrast substance elimination. Nephrin was determined in a middle portion of morning urine by enzyme immunoassay method using an automated EVOLIS Twin Plus system (Bio-Rad, France). The normal values for nephrin in urine are 0.118–20.0 ng/ml [19–21].

Lipocalin 2 is a 25 kDa protein monomer. It is the earliest biomarker of an acute damage, as it is accumulated in blood during the first hours of CIN development. Lipocalin 2 was determined on biochemical automatic Siemens analyzer with the help of NGAL Test Reagent Kit (BioPorto Diagnostics, Denmark) using an immunoturbidimetric method. The normal values of lipocalin 2 in blood plasma are 37–106 ng/ml. Its level after TCI exceeding 150 ng/ml was considered as CIN [20, 22].

Glomerular filtration rate (GFR) was estimated based on creatinine using the Cockcroft–Gault formula normalized to the body surface area, MDRD (Modification of Diet in Renal Disease), and CKD-EPI (the Chronic Kidney Disease Epidemiology Collaboration) equations [23].

The risk of CIN was assessed in all patients based on Mehran risk score prior to TCI. A score of 5 was considered low risk of CIN development, 6–10 moderate risk, 11–16 high risk, over 16 very high risk [24].

The results were processed using Statistica 7.0 program. Methods of nonparametric statistics such as computing median, the 25th and 75th percentiles (Me [25p; 75p]) were used for data analysis. The qualitative attributes are presented by absolute values, percentage is indicated by decimal fractions. Significance of differences of independent groups by one attribute was determined by Kruskal–Wallis ANOVA test. Differences were considered statistically significant at $p<0.05$.

Results and Discussion. The main group was comparable with the control in age, gender, duration of cardiovascular diseases, kidney pathology, body mass index, hematocrit level, heart failure (class III–IV according to NYHA Functional Classification), left ventricular ejection fraction, the volume of the contrast medium introduced, the character of interventional therapy. However, in patients with DM 2 systolic and diastolic arterial pressure, HbA1c, uric acid level, and lipid metabolism indices were statistically significantly higher. Multiple coronary vessel damage, defined as hemodynamically significant stenosis of two or more coronary vessels, occurred in those patients more frequently (Table 1).

The group of very high risk of CIN development according to Mehran scale included 18 (21.5%) patients; high risk 32 (38%) patients, moderate and low risk 26 (30.9%) and 8 (9.6%), respectively.

Functional condition of kidneys was studied in patients of the main (Table 2) and control (Table 3) groups prior to TCI and during 3 days after the contrast procedures using standard indices of CIN diagnosis (urea, blood serum creatinine and GFR [11, 12, 25], and new biomarkers (cystatin C, nephrin, and lipocalin 2).

Taking into account KDIGO criteria (2012) and increased levels of new biomarkers, CIN was diagnosed in all patients of the main group and in 35 of 40 (87.5%) patients of the control group. Patients of both groups were noted to have an increased level of blood serum creatinine, urea, new biomarkers and GFR reduction after contrast medium introduction. Adequate hydration resulted in normalizing urea and creatinine indices on day 2–3 after the contrast procedures. GFR remained reduced despite the conducted therapy.

In the main group the levels of cystatin C and nephrin remained increased compared with the initial data ($p=0.01$ and $p=1\cdot10^{-5}$, respectively), there appeared a tendency to lipocalin 2 decrease, though this marker was also higher compared with the initial level for 2–3 days after TCI ($p=1\cdot10^{-5}$). Similar results were obtained in the patients of the control group. The initial level of the new biomarkers was in norm, designating the fact that kidneys had not been compromised prior to the introduction of the contrast agents. This is the evidence of a specificity and sensitivity of these biomarkers in CIN diagnosis. According to the data of several studies, the specificity is found on average to be 74%, and the sensitivity 90% [11, 15, 16, 18–21].

Of special interest was to trace a 24-hour dynamics of the given markers after TCI (Table 4).

Analyzing the data of the 24-hour dynamics of blood serum creatinine and cystatin C, nephrin, and lipocalin 2 in patients with DM 2 after contrast agent introduction, increase of creatinine 24 h after TCI can be stated. There was noted statistically significant increase of lipocalin 2 level in blood plasma 2 h, cystatin C 6 h, and nephrin in the urine 8 h after contrast medium introduction.

Considering the data obtained, an algorithm for early CIN diagnosis in patients with planned contrast procedures can be proposed (see the Figure). This algorithm makes it possible 1) to identify the group of individual risk of CIN development, 2) to timely diagnose CIN with the help of proposed biomarkers, and 3) to effectively conduct prophylactic procedures to prevent the development of acute renal insufficiency caused by toxic effect of radiocontrast agents.

Table 1

Characteristics of patients included into the study (Me [25p; 75p]; abs. value/%)

Parameters	Group 1 — diabetes mellitus type 2 (n=44)	Group 2 — without carbohydrate metabolism impairment (n=40)	p
Age (years)	60.92 [51.2; 65.0]	59.9 [49.7; 66.0]	0.4
Gender:			
men	28/63.6	26/65	0.85
women	16/36.4	14/35	0.85
Diabetes mellitus type 2 duration (years)	6.2 [0.5; 13.2]	—	—
Hypertensive disease duration (years)	20.6 [11.0; 22.4]	19.8 [10.8; 20.9]	0.07
Ischemic heart disease duration (years)	9.54 [4.50; 15.0]	8.86 [5.20; 14.20]	0.05
Kidney disease duration (years)	5.6 [3.2; 6.4]	4.9 [2.8; 5.9]	0.07
Body mass index	29.54 [27.35; 31.49]	28.49 [25.30; 30.80]	0.38
Systolic arterial pressure (mm Hg)	150.2 [140.0; 160.0]	142.2 [140.0; 150.0]	0.04
Diastolic arterial pressure (mm Hg)	92.8 [90.0; 100.0]	87.5 [80.0; 90.0]	0.01
HbA1c (%)	9.5 [6.8; 11.3]	5.7 [5.2; 6.1]	0.004
Uric acid (μmol/L)	405.1 [320.0; 477.0]	302 [246; 392]	0.01
Hematocrit (L/L)	0.41 [0.38; 0.49]	0.42 [0.39; 0.44]	0.06
Total cholesterol (mmol/L)	5.96 [5.60; 6.80]	5.14 [4.90; 6.90]	0.04
Triglycerides (mmol/L)	2.97 [1.63; 2.95]	1.83 [1.25; 2.34]	0.01
Atherogenic index	3.61 [3.0; 4.20]	3.57 [2.70; 4.40]	0.08
Left ventricular ejection fraction (%)	51.96 [51.0; 59.0]	52.0 [50.0; 58.7]	0.2
Heart failure, class III–IV according to NYHA	16/36.3	12/30	0.08
Multiple damage of coronary vessels	24/54.5	13/32.5	0.001
Contrast volume (ml)	174.9 [50.0; 220.0]	167.7 [55.0; 220.0]	0.2
Selective coronarography with stenting	33/75	29/72.5	0.08

Table 2

Indices of functional condition of kidneys before and after transcutaneous coronary intervention in patients with diabetes mellitus type 2 (n=44) (Me [25p; 75p])

Parameters	Initially — before transcutaneous coronary intervention	After transcutaneous coronary intervention					
		Day 1	Day 2	Day 3	p^*	p^+	p^v
Urea (mmol/L)	6.8 [5.4; 7.9]	10.4 [5.8; 16.5]	9.9 [5.9; 10.1]	8.1 [5.8; 10.2]	0.02	0.03	0.02
Creatinine (μmol/L)	95.2 [83.0; 108.0]	127.8 [105.9; 140.4]	113.8 [91.2; 135.4]	86.45 [69.40; 103.50]	0.001	0.02	0.02
Cystatin C (mg/ml)	1.28 [1.05; 1.29]	1.48 [1.48; 1.80]	1.49 [1.47; 1.72]	1.32 [1.28; 1.37]	$1{\cdot}10^{-5}$	0.01	0.02
Nephrin (ng/ml)	21.6 [18.9; 23.2]	55.8 [47.2; 62.3]	53.6 [48.3; 56.0]	53.4 [49.2; 55.3]	$1{\cdot}10^{-5}$	$1{\cdot}10^{-5}$	$1{\cdot}10^{-5}$
Lipocalin 2 (ng/ml)	72.6 [68.9; 92.0]	330 [220; 368]	270 [225; 348]	220 [169; 280]	$1{\cdot}10^{-5}$	$1{\cdot}10^{-5}$	$1{\cdot}10^{-5}$
Glomerular filtration rate using Cockcroft–Gault formula (ml/min)	79.37 [78.0; 88.50]	67.0 [62.0; 69.8]	69.0 [67.0; 69.9]	68.0 [65.0; 72.8]	0.01	0.02	0.04
Glomerular filtration rate using MDRD formula (ml/min/1.73 m^2)	77.8 [68.0; 87.6]	50.7 [48.9; 62.9]	56.2 [50.2; 68.9]	64.8 [56.0; 72.0]	0.01	0.01	0.04
Glomerular filtration rate using CKD-EPI formula (ml/min/1.73 m^2)	76.1 [57.1; 82.8]	49.7 [47.1; 59.8]	55.3 [50.2; 62.7]	62.2 [54.0; 70.7]	0.01	0.02	0.04

Note. p^*, p^+, p^v — statistically significant difference of indices between initial values and those on day 1, 2, 3, respectively.

Table 3

Indices of functional condition of kidneys before and after transcutaneous coronary intervention in patients without carbohydrate metabolism impairments (n=40) (Me [25p; 75p])

Parameters	Initially — before transcutaneous coronary intervention	After transcutaneous coronary intervention					
		Day 1	Day 2	Day 3	p*	p+	pv
Urea (mmol/L)	6.52 [5.30; 7.60]	6.8 [5.8; 7.1]	6.08 [5.9; 7.0]	6.12 [5.80; 7.10]	0.1	0.2	0.1
Creatinine (μmol/L)	92.1 [83.0; 110.0]	118.7 [105.9; 140.4]	98.2 [96.7; 104.0]	94.4 [89.0; 99.7]	0.01	0.02	0.1
Cystatin C (mg/ml)	1.01 [0.98; 1.03]	1.28 [1.22; 1.62]	1.31 [1.28; 1.65]	1.29 [1.20; 1.32]	$1 \cdot 10^{-5}$	0.01	0.02
Nephrin (ng/ml)	15.6 [11.2; 17.9]	42.6 [32.8; 58.9]	41.8 [38.2; 56.9]	40.9 [38.3; 55.4]	$1 \cdot 10^{-5}$	$1 \cdot 10^{-5}$	$1 \cdot 10^{-5}$
Lipocalin 2 (ng/ml)	52.3 [38.0; 102.0]	296 [190; 312]	282 [206; 303]	273 [180; 283]	$1 \cdot 10^{-5}$	$1 \cdot 10^{-5}$	$1 \cdot 10^{-5}$
Glomerular filtration rate using Cockcroft–Gault formula (ml/min)	81.35 [72.0; 84.50]	72.0 [63.8; 71.2]	74.8 [67.0; 76.2]	78.2 [68.0; 81.2]	0.03	0.2	0.4
Glomerular filtration rate using MDRD formula (ml/min/1.73 m^2)	74.9 [62.6; 87.4]	67.85 [49.80; 63.30]	71.4 [52.8; 72.1]	73.8 [61.0; 78.0]	$1 \cdot 10^{-5}$	0.05	0.1
Glomerular filtration rate using CKD-EPI formula (ml/min/1.73 m^2)	75.7 [59.1; 86.2]	62.7 [46.8; 61.2]	63.8 [50.8; 65.6]	64.3 [53.0; 65.7]	0.001	0.02	0.04

Note. p*, p+, pv — statistically significant difference of indices between initial values and those on day 1, 2, 3, respectively.

Table 4

Dynamics of biomarkers before and after transcutaneous coronary intervention in patients with diabetes mellitus type 2 (n=44) (Me [25p; 75p])

Parameters	Initially — before transcutaneous coronary intervention	After transcutaneous coronary intervention				
		In 2 hours	In 4 hours	In 6 hours	In 8 hours	In 24 hours
Creatinine (μmol/L)	95.2 [83.0;108.0]	94.6 [82.0; 106.0]	96.7 [86.0; 108.0]	95.8 [92.0; 110.0]	102.3 [97.0; 116.0]	127.8 [105.9; 140.4]
Cystatin C (mg/ml)	1.28 [1.05; 1.29]	1.29 [1.04; 1.29]	1.29 [1.05; 1.30]	1.44 [1.07; 1.48]	1.45 [1.27; 1.50]	1.48 [1.48; 1.80]
Nephrin (ng/ml)	21.6 [18.9; 23.2]	23.6 [19.8; 28.8]	28.6 [22.3; 29.9]	29.8 [25.8; 32.6]	48.9 [33.9; 52.1]	55.8 [47.2; 62.3]
Lipocalin 2 (ng/ml)	72.6 [68.9; 92.0]	296 [223; 302]	304 [289; 323]	310 [298; 324]	328 [304; 334]	330 [220; 368]

Note. p<0.01 — statistically significant difference of indices between initial values and 24-hour dynamic.

STAGES OF DIAGNOSING CONTRAST-INDUCES NEPHROPATHY

Before contrast procedures

Determination of CIN risk factors by means of standard indices:
- history-taking, complete blood count (hemoglobin, hematocrit);
- clinical urine analysis, creatinine in blood serum;
- estimation of glomerular filtration rate, standardized to the body surface area;
- estimation of CIN development risk based on Mehran risk score

CIN diagnosis using new biomarkers:
- lipocalin 2 in blood plasma;
- nephrin in urine;
- cystatin C in blood

After contrast procedures

Determination of new biomarkers in 2, 24, 48 hours:
- lipocalin 2 in blood plasma;
- nephrin in urine;
- cystatin C in blood

Note. When diagnosing CIN, preventive therapy is conducted before the contrast procedure, and intensive rehydration after the procedure.

Algorithm for early diagnosis of contrast-induced nephropathy

Conclusion. The proposed algorithm with biomarker application makes it possible to predict the development of acute renal insufficiency during operative interventions on the coronary arteries with introduction of contrast agents, and to carry out preventive therapy and intensive rehydration. Renal injury biomarkers (cystatin C, nephrin, and lipocalin 2) owing to their high sensitivity and specificity allow diagnosing contrast-induced nephropathy during the first 24-hours after transcutaneous coronary intervention being especially important for patients with diabetes mellitus type 2.

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Conflicts of Interest. The authors declare no conflicts of interest related to this work.

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