Early biomarkers of nephrotoxicity associated with the use of anti-VEGF drugs

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Abstract. Anti-angiogenic anticancer drugs that block vascular endothelial growth factor (VEGF) can cause kidney damage. An early assessment of the risk of nephrotoxicity would allow the development of optimal treatment approaches and allow for relatively safer therapeutic regimens. The aim of this study was to assess the utility of neutrophilic gelatinase-associated lipocalin (NGAL), kidney injury molecule 1 (KIM-1), hypoxia inducible factor-1α (HIF-1α) and nephrin levels in urine as early biomarkers for the nephrotoxicity of anti-VEGF drugs. The study included 50 patients who received anti-VEGF drugs (aflibercept, bevacizumab or ramucirumab) for 8 weeks. The levels of KIM-1, NGAL, HIF-1α and nephrin in urine samples were determined by ELISA before treatment and after 1, 2, 4 and 8 weeks of treatment. To assess risk factors for nephrotoxicity, a logistic regression analysis was performed with the inclusion of the primary clinical and laboratory parameters. The primary outcome measure was a decrease in glomerular filtration rate (GFR) to <60 ml/min/1.73 m^2 at 8 weeks, and nephrotoxicity resulting in discontinuation within 9 months. The primary outcome goal was achieved in 21 (42%) patients treated with anti-VEGF drugs. Increased NGAL, KIM-1, HIF-1α and nephrin levels in urine at 1 week of treatment predicted the development of nephrotoxicity. High sensitivity and specificity of these urinary biomarkers were established by ROC analysis: KIM-1 [area under the curve (AUC) 0.69], NGAL (AUC 0.7), HIF-1α (AUC 0.7) and nephrin (AUC 0.7). The unfavorable predictors of nephrotoxicity were an initial decrease in estimated GFR, a history of arterial hypertension, and an increase in urinary concentration KIM-1 OR of 1.1 [CI 95% 1.02-1.183], and HIF-1α OR of 5.6 [CI 95% 3.601-8.949] (P<0,05) at 1 and 2 weeks of treatment. Urinary NGAL, KIM-1, HIF-1α and nephrin are early biomarkers of nephrotoxicity following treatment with anti-VEGF anticancer drugs. The independent risk factors for nephrotoxicity are the initial decrease in GFR, arterial hypertension, and an increase in the concentration of KIM-1 and HIF-1α in the urine in the early stages of therapy.

Introduction

Antiangiogenic drugs have an antitumor effect through suppressing neoangiogenesis in tumors and reducing vascularization, which leads to inhibition of the proliferation of tumor cells and their metastasis. The vascular endothelial growth factor (VEGF) A isoform is secreted by tumors to stimulate the proliferation, migration and survival of endothelial cells by binding and activating VEGF receptors expressed on endothelial cells (1). Anti-VEGF drugs are aimed at blocking VEGF itself or its receptors (VEGFR). Indications for the use of antiangiogenic drugs are metastatic forms of various malignant tumors, including the following: Colorectal, ovarian, breast, stomach and non-small-cell lung cancer (2,3). The most common adverse effects of anti-VEGF therapy are hypertension, proteinuria and thrombotic microangiopathy (4-6). The result of these pathological processes can be a decrease in the glomerular filtration rate up to the development of acute kidney injury, even in patients with intravitreal administration (7,8). In some cases, therapy with anti-VEGF drugs can lead to gradual but irreversible changes in renal function, up to end-stage renal failure (9). Assessment of the risk of deterioration of renal function and factors that can increase the nephrotoxicity of anti-VEGF drugs and their compensation can improve renal prognosis (10). The identification of biomarkers that will allow the recognition of renal interstitial ischemia and glomerular damage in the early stages can help reduce the risk of renal dysfunction when optimizing treatment and concomitant therapy. Currently, neutrophilic gelatinase-associated lipocalin (NGAL) and KIM-1 are known early markers present in the urine for detection of acute renal injury in ischemic and toxic tubular injury, for example, as a result of cisplatin therapy (11-14). NGAL, also known as lipocalin 2, is the best-studied biomarker for acute kidney injury (9-11). NGAL expression is significantly increased in the kidney,

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namely, in the distal segments of the nephron, especially in the thick ascending part of Henle's loop and collecting ducts after exposure to a damaging factor, such as ischemic or toxic insults (15-17). NGAL participates in the suppression of tubular cell apoptosis (18). However, an increase in NGAL can also predict the development of chronic kidney disease, up to end-stage renal failure (19).

KIM-1 is a proximal tubule transmembrane protein that is virtually absent in the urine under normal conditions (20). For ischemic or direct toxic damage to the proximal tubule, the ectodomain is cleaved by matrix metalloproteases (MMPs), and the soluble form of KIM-1 is shed into the urine (21,22). Urine KIM-1 levels are associated with KIM-1 protein expression in experimental and clinical renal disease (23,24). KIM-1 and NGAL are considered biomarkers of tubular injury in the development of acute renal injury associated with the administration of nephrotoxic drugs, such as cisplatin (12,25). To date, there are no predictive biomarkers of kidney dysfunction for patients receiving antiangiogenic drugs. The aim of this study was to evaluate the possibility of using NGAL, KIM-1, hypoxia inducible factor-1α (HIF-1α) and nephrin in urine as early biomarkers of nephrotoxicity following treatment with anti-VEGF drugs.

Materials and methods

Study subjects. The study included 50 patients who received chemotherapy with antiangiogenic drugs (bevacizumab, aflibercept, or ramucirumab) either as monotherapy or in combination regimens that did not result in nephrotoxicity. The median age of the patients was 46 [interquartile range (IQR) 34-57] years, with an age range of 24-80 years. The study included 22 men (44%) and 28 (56%) women.

Of the 50 patients, 17 (34%) received monotherapy with antiangiogenic drugs, and in the other 33 (66%), antiangiogenic drugs were used as part of combined treatment regimens (5-fluorouracil + irinotecan; irinotecan, capecitabine, paclitaxel or eribulin). A total of 11 patients (22%) received bevacizumab, 29 (58%) received ramucirumab, and 10 (20%) received aflibercept.

The present study was approved by Local Ethics Committee A.S. Loginov Moscow Clinical Scientific Center of Moscow Healthcare Department (approval no. 2/2020, 17th Feb 2020). All subjects provided informed written consent before participation, and the study conformed with the guidelines described in the Declaration of Helsinki (26).

Patients received the recommended doses of anti-VEGF drugs: Aflibercept 4 mg/kg every 2 weeks, bevacizumab 5 mg/kg every 2 weeks, 10 mg/kg every 3 weeks, ramucirumab 8 mg/kg every 2 weeks. The study included patients with cancers of different sites: Colorectal cancer 52% (n=26), ovarian cancer 20% (n=10), breast cancer 20% (n=10) and stomach cancer 8% (n=4). Patient characteristics are presented in Table I. The exclusion criteria for the appointment of therapy was a decrease in GFR to <60 ml/min/1.73 m^2 according to the CKD-EPI formula (27), other chronic diseases or kidney and urinary tract tumors, heart failure, uncontrolled arterial hypertension, decompensated diabetes mellitus, extensive atherosclerosis with renal arteries damage or prolonged hospitalization. Acute kidney injury was defined according to the KDIGO-Clinical Practice Guideline for AKI (28).

Among the clinical characteristics, we assessed sex, age, body mass index, the presence of arterial hypertension before treatment, levels of systolic and diastolic blood pressure, the type of antiangiogenic drug (bevacizumab, aflibercept, ramucirumab), the use of anticoagulants (low molecular weight heparin, Xa inhibitors), and nonsteroidal anti-inflammatory drugs. Among the laboratory parameters, the hemoglobin level, the number of platelets and schistocytes, D-dimer, serum lactate dehydrogenase (LDH) levels, serum creatinine levels and the calculated estimated (e)GFR according to the CKD-EPI formula, and the levels of 24 h albuminuria were assessed. These parameters were entered into the database before treatment and during the course of administration at the end of weeks 1, 2, 4 and 8.

ELISA for NGAL, KIM-1, nephrin and HIF-1α quantification. A total of 10 ml morning urine was collected into dry plastic sterile tubes. The urine was centrifuged to remove cellular and crystalline sediment at room temperature for 15 min at a speed of 1,027 x g. The resulting supernatant was transferred to Eppendorf tubes for subsequent freezing. Frozen samples were stored at -20°C until required. Urine samples for research were taken before the start of treatment and over time at the following points: At the end of 1, 2, 4 and 8 weeks from the start of therapy. The concentrations of biomarkers in urine samples were determined by ELISA using specific kits: NGAL Human ELISA (BioVendor, cat. no. RD191102200R), Human KIM-1 ELISA Kit (cat. no. ELH-TIM1, RayBio), Human HIF-1α ELISA Kit (cat. no. ELH-HIF1a, RayBio) and Human ELISA Kit for Nephrin (Cloud Clone, cat. no. SEA937Hu), according to the manufacturer's protocol. Each measurement of standard and experimental samples was performed in duplicate. Urine levels of the biomarkers were standardized to urine creatinine levels.

Statistical analysis. Statistical analysis was performed using Jamovi version 2.2.2 (Jamovi team). Data are presented as the median and IQR. When analyzing the sample, in connection with the abnormal distribution of indicators, the nonparametric Friedman's test was used for statistical processing. To determine the significance of the increase in the levels of biomarkers, a Friedman test with a post hoc pairwise comparison using the Durbin-Conover test was performed. To show that there were no differences between subgroups of patients treated with different chemotherapeutic regimens, a Kruskal-Wallis test with a Bonferroni post hoc test was used.

As an endpoint, the risk of nephrotoxicity was assessed as a decrease in the glomerular filtration rate to <60 ml/min/1.73 m^2 at 8 weeks of treatment with antiangiogenic drugs was used.

A receiver operating characteristic curve was used for analysis of urinary biomarkers after 1 and 2 weeks.

The results after a 9-month follow-up were also assessed. To assess risk factors for nephrotoxicity, logistic regression analysis was performed with the inclusion of the following factors: Age, sex, body mass index, smoking status, the presence of concomitant cardiovascular diseases (myocardial

Table I. Characteristics of the recruited cohort.

Characteristics	Value
Age, median (range)	46 (24-80)
Sex, n (%)	
Female	28 (56)
Male	22 (44)
Diagnosis, n (%)	
Gastric cancer	4 (8)
Colon cancer	14 (28)
Sigmoid cancer	6 (12)
Rectal cancer	6 (12)
Breast cancer	10 (20)
Ovarian cancer	10 (20)
Body mass index, median (range)	27.3 (16.4-40.3)
Smoking, n (%)	
Smokers	9 (18)
Non-smokers	41 (82)
Concomitant diseases, n (%)	
Cerebral vascular accident	1 (2)
Myocardial infarction	2 (4)
Ischemic heart disease	14 (28)
Arterial hypertension	15 (30)
Diabetes mellitus	6 (12)
Chemotherapy, n (%)	
Monotherapy	17 (34)
Polychemotherapy	33 (66)
5-fluorouracil + irinotecan	14 (28)
Irinotecan	7 (14)
Capecitabine	7 (14)
Paclitaxel	3 (6)
Eribulin	2 (4)
Anti-VEGF-drug, n (%)	
Aflibercept	10 (20)
Bevacizumab	29 (58)
Ramucirumab	11 (22)
Antihypertensive, n (%)	15 (30)
Angiotensin converting enzyme inhibitors	5 (10)
Angiotensin receptor blockers	7 (14)
β-blockers	1 (2)
Diuretics	2 (4)
No treatment	35 (70)
Anticoagulants, n (%)	13 (26)
Low molecular heparin	6 (12)
Xa inhibitors	7 (14)
No treatment	37 (74)

VEGF, vascular endothelial growth factor.

infarction, coronary artery disease, or cerebrovascular accident), arterial hypertension status, presence of diabetes mellitus, and the type of antiangiogenic drug, type of cancer, eGFR before treatment, achievement of blood pressure target ≤130/80 mmHg, as well as the concentration of biomarkers in the urine before treatment and after the 1st and 2nd weeks of therapy. The odds ratio (OR) was estimated with a 95% CI (confidence interval). Indicators with a significance level of P<0.05 was considered to indicate a statistically significant difference.

Results

Clinical and laboratory parameters during treatment. Arterial hypertension with an increase in systolic blood pressure >130 mmHg and diastolic pressure >80 mmHg developed in 26 (52%) of the 50 patients. The median systolic blood pressure in the entire sample of patients was 128 (120-137) mmHg before the start of therapy, while a statistically significant increase in blood pressure to 143 (132-153) mmHg, was noted 4 weeks after the start of therapy (P<0.001).

An increase in creatinine levels >26.5 mmol/l according to the KDIGO criteria (acute kidney injury 1 stage) was observed in only 3 (6%) patients. A gradual decrease in eGFR to <60 ml/min/1.73 m^2 at 8 weeks of treatment was observed in 21 (42%) patients. The median eGFR in the group was 90 (76-95) ml/min/1.73 m^2 before the start of treatment and 65 (57-74) ml/min/1.73 m^2 at 8 weeks after the start of therapy. We noted a statistically significant decrease in GFR within the 8-week follow-up regardless of the chemotherapy used (monotherapy with an antiangiogenic drug; FOLFIRI; irinotecan, capecitabine; paclitaxel; eribulin). Using a Friedman's test, the analysis showed that all comparisons were P<0.05. However, when performing a Kruskal-Wallis test with pairwise comparisons, it showed no statistically significant differences between the groups of patients who received chemotherapy in combination with antiangiogenic drugs based on the chemotherapeutic regimen administered (χ^2 Kruskal-Wallis test=6.77, P=0.238, Fig. 1).

Anemia was detected in all 50 patients after 8 weeks of treatment with antiangiogenic drugs, while a decrease in hemoglobin <105 g/l was observed in 24 (48%) patients. The median hemoglobin level in the entire sample of patients was 132 (129-136) g/l before treatment and 104 (99-107) g/l after 8 weeks of treatment; a significant decrease in hemoglobin level was observed after 2 weeks of therapy (Fig. 1A). In parallel with the decrease in hemoglobin, an increase in the number of schistocytes was observed by the 8th week of treatment, although the increase was ≤1%. There was also a slight increase in LDH; 260 (202-296) U/l before the start of treatment and 319 (259-390) U/l 8 weeks after the first injection of the antiangiogenic drug. The changes became significant 2 weeks after the start of therapy. The median platelet count in patients was 347 (292-429) x10^3/μl before the start of treatment and 256 (202-257) x10^3/μl 8 weeks after the first dose of the antiangiogenic drug; that is, it decreased by 26% from the initial levels (Fig. 1B).

Along with a decrease in GFR, an increase in urinary biomarkers of renal damage was also observed. Associations were revealed between the following factors: Hemoglobin and LDH levels after 4 weeks of treatment, and hemoglobin and LDH with the number of schistocytes after 8 weeks of treatment (Table II).
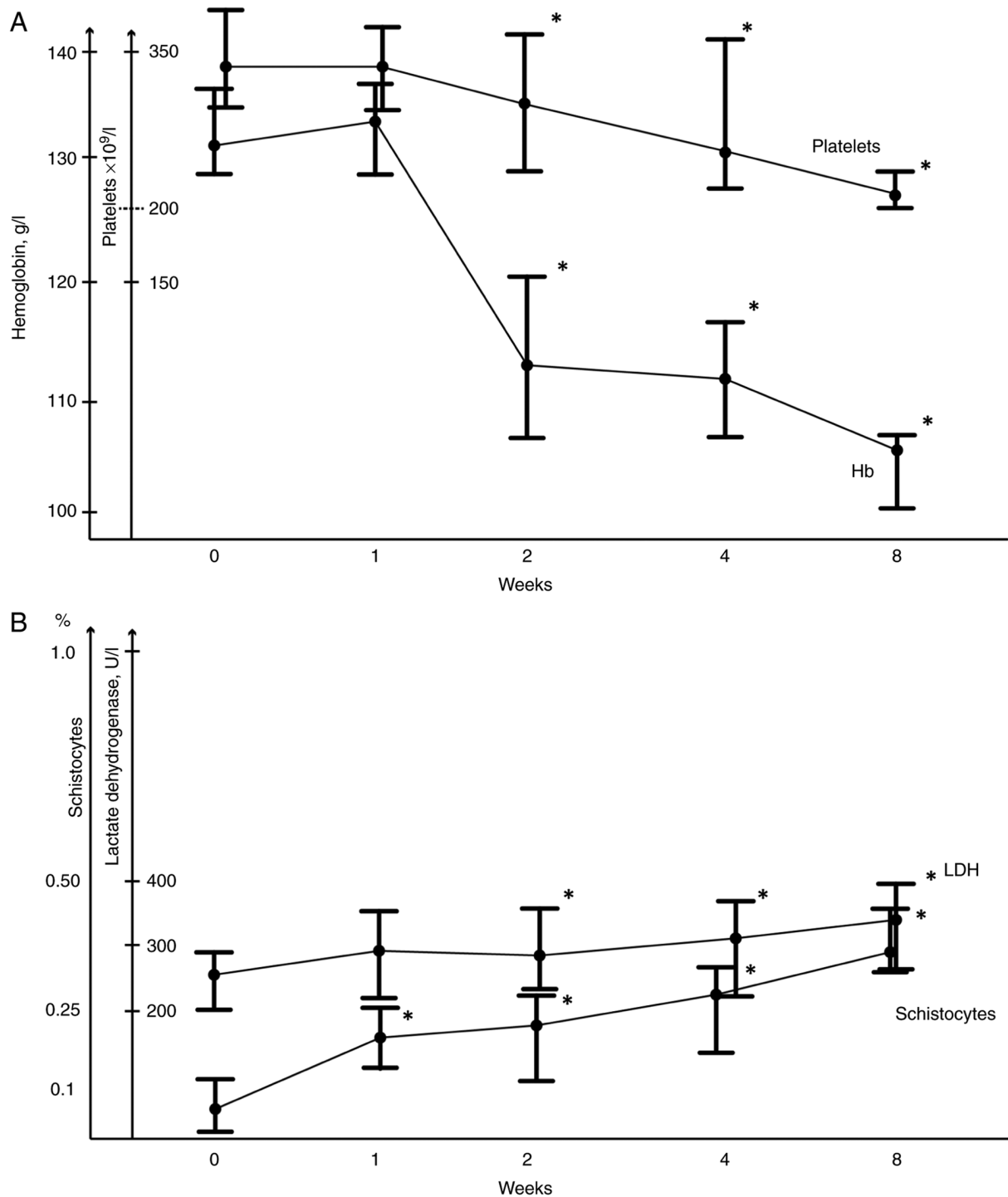
Figure 1. Laboratory parameters before and after anti-VEGF therapy. (A) A decrease in hemoglobin and platelet count by 20 and 26%, respectively, from the initial level was observed. (B) An increase in the number of schistocytes and a slight increase in LDH levels were observed by the 8th week of treatment. LDH, lactate dehydrogenase. *P<0.001 vs. 0 weeks. VEGF, vascular endothelial growth factor; LDH, lactate dehydrogenase.

NGAL and KIM-1, HIF-1α and nephrin in urine before and after 1, 2, 4 and 8 weeks of treatment. A significant increase in NGAL and KIM-1 levels in the urine was noted after 1 week of therapy, and HIF-1α and nephrin after 2 weeks (Fig. 2). A significant change in the levels of LDH, the number of schistocytes and the platelet counts were detected later (2-8 weeks) (Fig. 1).

An increase in biomarkers after 1 week of treatment preceded a subsequent decrease in GFR. A high sensitivity

and specificity of the studied biomarkers compared to creatinine, urea levels and GFR, assessed after a week of treatment, for predicting the nephrotoxicity of antiangiogenic drugs was established by receiver operating characteristic curve analysis (Table III).

Risk factors of nephrotoxicity associated with anti-VEGF drugs. Multivariate logistic regression analysis with the inclusion of the following factors (age, sex, body mass index, the

Table II. Association between routine laboratory parameters and urinary biomarkers after 8 weeks of treatment.

Urinary markers	Hemoglobin	Schistocytes	Platelets	Lactate dehydrogenase	Urine Albumin	D-dimer
NGAL	P=0.009[b]	P=0.003[b]	P<0.001[c]	P=0.030[a]	P=0.002[c]	P=0.026[a]
	Rs=-0.367	Rs=0.416	Rs=-0.453	Rs=0.306	Rs=0.433	Rs=0.423
KIM-1	P=0.010[b]	P=0.003[b]	P<0.001[c]	P=0.030[a]	P=0.002[c]	P=0.028[a]
	Rs=-0.360	Rs=0.418	Rs=-0.452	Rs=0.304	Rs=0.432	Rs=0.414
HIF-1A	P=0.050[a]	P=0.002[b]	P<0.001[c]	P=0.038[a]	P=0.002[c]	P=0.026[a]
	Rs=-0.367	Rs=0.435	Rs=-0.460	Rs=0.294	Rs=0.432	Rs=0.421
Nephrin	P=0.008[b]	P=0.003[b]	P=0.001[c]	P=0.030[a]	P=0.002[c]	P=0.024[a]
	Rs=-0.372	Rs=0.418	Rs=-0.448	Rs=0.307	Rs=0.435	Rs=0.426

[a]$P \leq 0.05$, [b]$P \leq 0.01$, [c]$P \leq 0.001$. NGAL, Neutrophilic gelatinase-associated lipocalin; HIF-1α, hypoxia inducible factor-1α; KIM-1, kidney injury molecule 1.
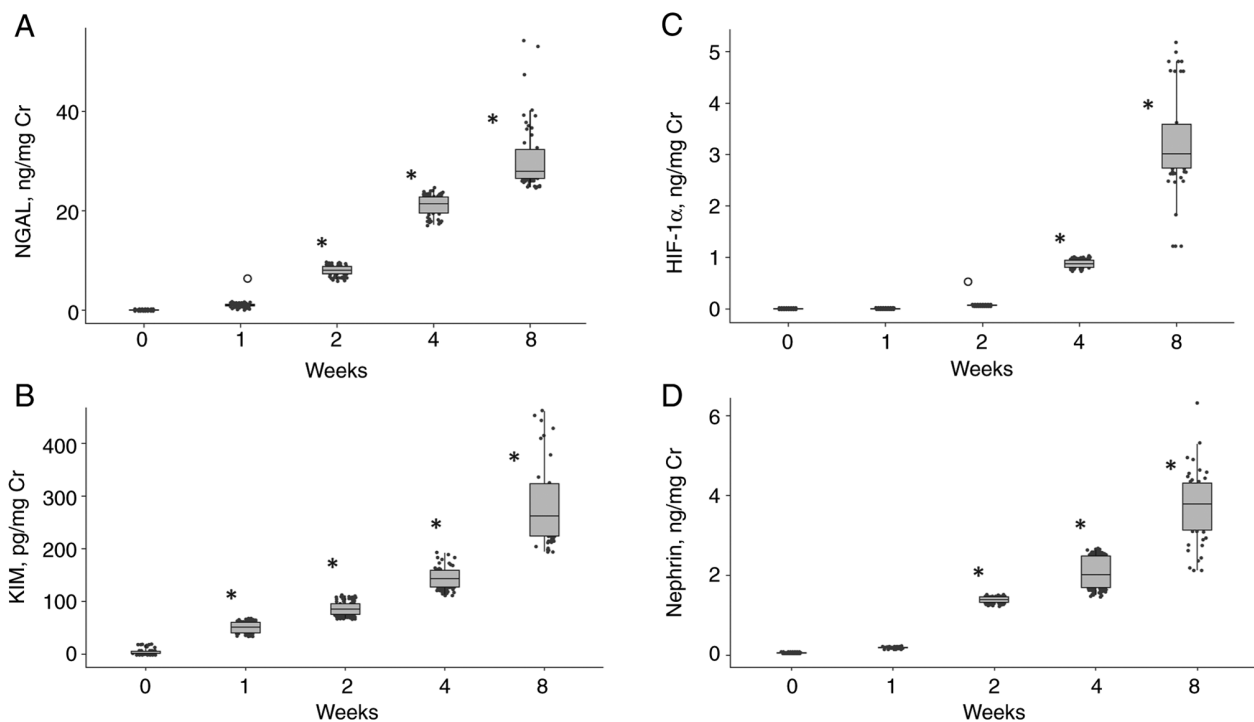

Figure 2. The levels of the assessed biomarkers in the urine before and after anti-VEGF therapy. A significant increase was observed in the (A) NGAL and (B) KIM-1 levels in the urine after 1 week, and an increase in (C) HIF-1α and (D) nephrin levels were noted after 2 weeks of anti-VEGF therapy. °P<0.01, *P<0.001 vs. 0 weeks. VEGF, vascular endothelial growth factor; NGAL, neutrophilic gelatinase-associated lipocalin; HIF-1α, hypoxia inducible factor-1α; KIM-1, kidney injury molecule 1.

presence of cardiovascular diseases (coronary artery disease, myocardial infarction or stroke), presence of arterial hypertension, presence of diabetes mellitus, smoking status, the type of antiangiogenic drug, GFR rates before treatment, and concentrations of NGAL, KIM-1, nephrin and HIF-1α in the urine after 1 and 2 weeks from the start of therapy showed an initial decrease in the eGFR to <80 ml/min/1.73 m^2 [OR, 3.250 (1.060-9.967), P=0.039], which was associated with a history of arterial hypertension [OR, 1.503 (1.135-1.990), P=0.013]. Additionally, the risk (OR) of nephrotoxicity of antiangiogenic drugs was higher in patients with an increase in KIM-1 in urine (odds ratio 1.1, 95% CI 1.02-1.183) after 1 week and with an increase in HIF-1α in urine (odds ratio

5.7, 95% CI 3.601-8.949) after 2 weeks of treatment (P<0.05; Table IV).

Outcomes after 9-months of follow-up. After a 9-month follow-up, the anti-angiogenic therapy was ended in all patients. A total of 22 (40%) patients showed signs of nephrotoxicity; of these, 17 patients demonstrated a decrease in GFR to <60 ml/min 1.73 m^2 by the 8th week of treatment (primary endpoint). Anti-VEGF therapy was terminated in 14 (28%) patients due to its nephrotoxicity (proteinuria or decreased eGFR), in 7 (14%) patients due to the uncontrolled arterial hypertension, in 5 (10%) patients due to the thrombosis, and in 1 (2%) patient due to the bleeding. In 16 (32%) patients

Table III. Receiver operating characteristic curve analyses of urinary biomarkers after 1 and 2 weeks.

A, After 1 week

Urinary markers	Cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC	Juden index
NGAL	1.045 ng/mg Cr	68.75	67.65	50.00	82.14	0.70	0.364
KIM-1	54.07 pg/mg Cr	68.75	61.76	45.83	80.77	0.69	0.305
HIF-1α	6.02×10^{-5} ng/mg Cr	68.75	67.65	50.00	82.14	0.70	0.364
Nephrin	0.184 ng/mg Cr	68.75	67.65	50.00	82.14	0.70	0.364

B, After 2 weeks

Urinary markers	Cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC	Juden index
NGAL	8.11 ng/mg Cr	68.75	64.71	47.83	81.48	0.70	0.340
KIM-1	80.0 pg/mg Cr	69.57	48.15	53.33	85.50	0.60	0.180
HIF-1α	0.85 ng/mg Cr	73.91	48.15	58.84	68.42	0.59	0.220
Nephrin	1.37 ng/mg Cr	69.57	48.15	53.33	65.00	0.59	0.180

NGAL, Neutrophilic gelatinase-associated lipocalin; HIF-1α, hypoxia inducible factor-1α; KIM-1, kidney injury molecule 1; PPV, positive predictive value; NPV, negative predictive value; AUC, area under the curve.

Table IV. Logistic regression model of nephrotoxicity associated with anti-VEGF treatment.

Variable	OR unadjusted	CI 95% Lower	CI 95% Upper	P-value	OR adjusted	CI 95% Lower	CI 95% Upper	P-value
Age	1.061	1.007	1.119	0.026[a]	1.018	0.965	1.075	0.51
Female sex	4.875	0.875	24.15	0.071	2.631	0.157	44.123	0.501
Arterial hypertension	3.5	1.405	31.314	0.042[a]	1.503	1.135	1.99	0.013[a]
Diabetes mellitus	0.316	0.047	2.118	0.235	0.472	0.055	4.081	0.495
Body mass index	1.009	0.892	1.14	0.892	1.016	0.846	1.22	0.864
Cardiovascular disease	1.5	0.267	8.411	0.645	0.374	0.01	13.68	0.592
Smoking status	6.782	1.08	42.57	0.041[a]	0.654	0.049	8.808	0.749
Decrease in baseline GFR	7.474	1.033	59.856	0.048[a]	3.25	1.06	9.967	0.039[a]
Type of anti-VEGF drug	0.974	0.385	2.464	0.955	0.35	0.082	1.493	0.156
Type of cancer	1.269	0.795	2.062	0.318	0.519	0.114	2.366	0.397
KIM-1 (week 1)	1.492	1.121	1.986	0.006[b]	1.1	1.02	1.183	0.023[a]
NGAL (week 1)	7.18	0.245	21.023	0.253	9.48	0.007	12.088	0.253
HIF-1α (week 2)	6.944	5.374	8.974	0.027[a]	5.677	3.601	8.949	0.017[a]

[a]P<0.05, [b]P<0.01. GFR, glomerular filtration rate; KIM-1, kidney injury molecule-1; NGAL, neutrophil gelatinase-associated lipocalin; HIF-1α, hypoxia-inducible factor 1-α.

anti-VEGF therapy was terminated due to the progression of underlying diseases, in 7 (14%) patients the reason for canceling the drug remains unknown.

Discussion

Our study included patients treated with one of three antiangiogenic drugs: Bevacizumab, aflibercept or ramucirumab. An increase in blood pressure >130/80 mmHg during the treatment was observed in half of the patients, higher than the rates previously reported in the literature. For example, a frequent (42.4%) cause of arterial hypertension is aflibercept; other drugs show a lower frequency of arterial hypertension, including ramucirumab (21%) and bevacizumab (23.6%), while severe hypertension of 3-4 degrees was observed in 7.9% of patients. A target level of <130/80 mmHg was more stringent than what was used in other studies (29-31). By the 8th week of treatment, a decrease in GFR to <60 ml/min in 42% of the examined patients was observed. According to the logistic regression analysis, the degree of GFR reduction did

not significantly depend on the specific antiangiogenic drug. Despite the fact that anti-VEGF-antiangiogenic drugs were prescribed to patients with initially normal renal function, even a small decrease in eGFR to <80 ml/min, turned out to be a risk factor for nephrotoxicity. Another risk factor for nephrotoxicity of anti-VEGF drugs was arterial hypertension before the initiation of therapy and lack of sufficient correction during the treatment. However, the achievement of a target pressure ≤130/80 mmHg was a favorable factor for a stable GFR during treatment with anti-VEGF. Normal D-dimer levels during the treatment were also beneficial in maintaining GFR.

In the mechanisms of GFR reduction, endothelial dysfunction due to blockade of the effects of VEGF with an increase in vascular tone, a decrease in natriuresis, a regression of fenestrated capillaries, and ultimately a decrease in intrarenal blood flow have been reported (32-34). Thrombotic microangiopathy (TMA) is a serious complication of therapy. According to several studies evaluating morphological changes with anti-VEGF drug treatment, TMA of the microvasculature of the kidneys is most often observed when there is oedema of endothelial cells with detachment from the basement membrane and microthrombi in the vessels of the kidneys (35-37). With prolonged use of anti-VEGF drugs, chronic irreversible processes are revealed, including thickening of the vascular wall, fibrous hyperplasia of the intima, arterio- and arteriolosclerosis and organized blood clots with recanalization, which ultimately ends with ischemic atrophy of the renal cortex (38). The pathogenesis of anemia is complex in those patients who receive anti-VEGF drug treatment. Decreased hemoglobin levels may be due to the concomitant use of anti-VEGF drugs and other chemotherapeutic drugs with myelosuppressive effects, such as irinotecan, fluoropyrimidines (5-fluorouracil, capecitabine), paclitaxel and eribulin. However, we also noted decreased hemoglobin levels in patients who received antiangiogenic drugs as a monotherapy. We suggest the contribution of microangiopathic hemolysis in the development of anemia, due to excessive schistocytes, as well as increased LDH levels and a low platelet count. Acute TMA with kidney damage manifests as a result of microangiopathic hemolytic anemia, thrombocytopenia, hypertension, moderate urinary syndrome and impaired renal function (39).

In the present study, cases of acute TMA were not observed; however, a gradual decrease in GFR was accompanied by a decrease in hemoglobin and platelet count, as well as increases in LDH levels, the number of schistocytes and the D-dimer levels, which indicated the occurrence of endothelial dysfunction and microangiopathic processes, and the development of microthrombosis in the renal vessels. There was a significant increase in the parameters of microangiopathic hemolysis by the 8th week of treatment; however, in the early stages, a significant increase in urine markers of hypoxia, tubular damage and podocytic damage were recorded. It has been hypothesized that the TMA process appears to be renally localized rather than part of a systemic process in which thrombocytopenia, schistocytes on peripheral smear and hemolytic anemia occur (5).

Currently, biomarkers of tubular injury have been studied in acute toxic injury, for example, in treatment with cisplatin (9-12). Markers that predict damage when treated with anti-VEGF drugs have not been studied. We assessed the levels of NGAL, KIM-1, HIF-1α and nephrin in urine as factors that may reveal acute or chronic hypoxic renal injury. In our study, the urinary NGAL levels were significantly increased 1 week after the start of anti-VEGF therapy and gradually increased over time, while the eGFR rate remained normal. An early increase in urinary NGAL reflected tubular damage and predicted further deterioration in renal function. KIM-1 levels in urine were also evaluated, and this analysis revealed an early increase in its levels after drug treatment, and this early increase was predictive of a gradual deterioration in renal function.

Considering the role of endothelial dysfunction and thrombotic microangiopathy in the progression of renal damage during treatment with antiangiogenic drugs, we assessed the levels of HIF-1α in the urine. HIF-1α is a protein produced in the cell in response to a decrease in oxygen concentration (40). Elevated serum HIF-1α levels are detected in chronic kidney disease, reflecting a loss of peritubular capillaries and renal tissue hypoxia (41,42). In the present study, logistic regression analysis revealed that HIF-1α was a factor involved in nephrotoxicity. The relationship between HIF-1α indicators and the levels of markers of microangiopathic hemolysis and GFR suggests the importance of chronic ischemia/hypoxia in the development of renal dysfunction during treatment with anti-VEGF drugs. Several experimental studies on patients with chronic kidney disease have shown that in the kidney, HIF-1α is the dominant form expressed in tubular epithelial cells and it acts as the major regulator of hypoxic adaptation (43-45). A reno-protective role of HIF against ischemic injury in ischemia/reperfusion models and toxic nephropathies have been described. In these cases HIF induction protects tubular cells from ischemic injury (46-48). Hypoxia-inducible gene expression in primary renal proximal tubular epithelial cells is almost completely blocked by inactivation of HIF-1α, suggesting that their response to hypoxia is largely dependent on HIF-1α (49). HIF-1α has been shown to play a role in the pathogenesis of renal interstitial fibrosis in patients with chronic kidney disease (45).

Kimura *et al* (50) showed that hypoxia and the resultant stabilization of HIF-1α play pivotal roles in the development of tubulointerstitial fibrosis. HIF-1 induces expression of profibrogenic genes, including tissue inhibitor of metalloproteinase 1, connective tissue growth factor and plasminogen activator inhibitor 1 (49,51,52).

Kidneys are one of the most vascularized organs, and considerably susceptible to ischemia in patients who take anti-VEGF drugs. Ischemia clinically manifests as arterial hypertension and renal vascular disease with a decrease in GFR. Histologically, there are signs of thrombotic microangiopathy (35-38). Based on previous studies, it is hypothesized that the primary source of HIF-1 α in the urine of patients who take anti-VEGF drugs are renal tissue cells undergoing hypoxia due to the loss of peritubular capillaries (53,54). The increased expression of HIF-1α has been suggested to promote the nephrotoxicity and the decrease in GFR through profibrotic effects and inflammatory processes (55).

The occurrence of proteinuria during treatment with anti-VEGF drugs is associated with impaired expression of nephrin, a transmembrane podocytic protein that forms the basis of the gap diaphragm and is regulated by VEGF. When

anti-VEGF drugs are used, nephrin molecules are cleaved from the podocyte, and the slit diaphragm and the glomerular filter is destroyed (56,57). Proteinuria ranks second amongst the most common side effects of anti-VEGF drugs, but its values rarely exceed 2 g/day. Proteinuria >3.5 g per day and nephrotic syndrome are detected on average in 6.5% of patients (58,59). In the present study, an increase in nephrinuria was observed. However, by the 8th week of treatment, there were no cases of high proteinuria or nephrotic syndrome. According to logistic regression models, it was noted that arterial hypertension, an early decline in GFR, and increased KIM-1 and HIF-1α levels had a significant impact on the decrease in GFR within the first 8 weeks of treatment. Age, sex, presence/absence of CVD, smoking status, type of chemotherapy and type of tumor had no significant impact on nephrotoxicity development.

The limitations of our study were the relatively small cohort and the short follow-up period (8 weeks); the limitations did not allow us to establish the frequency of a pronounced decrease in GFR to <30 ml/min/1.73 m^2, cases of nephrotic syndrome or high proteinuria, which would require discontinuation of the drug treatment. Additionally, the levels of biomarkers in the early stage of kidney damage in patients who took anti-VEGF drugs were defined by us. Further studies are required to ascertain more informative values of urine biomarkers and their concentration changes.

In conclusion, increased levels of NGAL, KIM-1, HIF-1α and nephrin in the urine reflect the processes occurring during renal tissue damage; these markers have high sensitivity and specificity for predicting the nephrotoxicity of anti-VEGF drugs. The independent risk factors for nephrotoxicity are a decrease in the GFR and arterial hypertension before the start of therapy, as well as an early increase in the concentrations of KIM-1 and HIF-1α in the urine.

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

NC conceived and designed the study, and drafted the manuscript. KG analyzed and interpretated the data, and was involved in drafting the manuscript. VM analyzed data and drafted the manuscript. LZ acquired and analyzed the data. TK participated in data collection and revised the manuscript critically for important intellectual content. KG and NC confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by Local Ethics Committee A.S. Loginov Moscow Clinical Scientific Center of Moscow Healthcare Department (approval no. 2/2020, 17th Feb 2020). All subjects provided informed written consent before participation, and the study conformed with the guidelines described in the Declaration of Helsinki.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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