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Urinary biomarkers in prediction of subclinical acute kidney injury in pediatric oncology patients treated with nephrotoxic agents

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Abstract

Background Acute kidney injury (AKI) is a common complication in pediatric oncology patients, most often caused by nephrotoxic drugs. We aimed to assess whether levels of urinary kidney injury molecule-1 (uKIM-1), neutrophil gelatinase-associated lipocalin (uNGAL), liver fatty acid binding protein (uL-FABP) and Vanin-1 (uVNN-1), individually and in combination-integrated could be early markers for cytotoxic treatment induced AKI.

Methods Children with different malignant diseases treated with cisplatin (CIS) or ifosfamide (IFO) were included. AKI was defined using pediatric KDIGO (Kidney Disease Improving Global Outcomes) criteria by comparing pretreatment serum creatinine (sCr) values with those acquired at 48 h after the first or second chemotherapy cycle. Five serum (at baseline, 2, 6, 24 and 48 h after treatment) and four urine samples (at baseline, 2, 6 and 24 h after treatment) were obtained. Urinary biomarkers (uBm) were normalized to urine creatinine.

Results Thirty-eight patients were assessed. Within 48 h following chemotherapy 6 (15.79%) patients experienced AKI. Patients with AKI were younger and tend to have lower baseline sCr values than patients without AKI, but these differences were not statistically significant. Compared to baselines, all uBm were significantly increased during the first 6 h while sCr concentrations did not change significantly during the study period. The median increases in uBm during the first 6 h after treatment were 529.8% (interquartile range – IQR, 63.9-1835.2%) – 2194.0% (IQR, 255.3-4695.5%) in AKI vs. 302.2% (IQR 114.6-561.2%) -429.8% (156.5–1467.0%) in non-AKI group depending of tested uBm. The magnitude of these changes over time didn't differ significantly between groups. The area under receiver operator curve (AUC) for uL-FABP and uNGAL at 24 h after chemotherapy were 0.81 and 0.72, respectively. The ROC analysis revealed that the other individual biomarkers' performance at any time-point wasn't statistically significant (AUC < 0.7). A model of integrated-combined uBm, 2 h (AUC 0.78), 6 h (AUC 0.85) and 24 h after (AUC 0.92) treatment with CIS and/or IFO showed good utility for early AKI prediction.

Conclusions The results of this study support that the use of the uBm to improves early AKI prediction in patients receiving CIS and/or IFO containing chemotherapy. Further studies on larger comparable groups of patients are needed.

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Keywords Acute kidney injury, Chemotherapy, Children, Urinary biomarkers

Introduction

Acute kidney injury (AKI) is a common complication in pediatric cancer patients and can be caused by various factors, including infiltration and/or compression by tumor mass, chemotherapy, abdominal radiotherapy, immunotherapy, surgery (especially nephrectomy), supportive treatment, dehydration and infections. Administration of some antineoplastic agents, including cisplatin (CIS) and ifosfamide (IFO), may cause AKI via renal tubular damage by involvement of several pathways such as oxidative stress, inflammation and vasoconstriction [1]. Literature reports show a wide range of AKI incidence, following cytotoxic treatment, between 16.9% and 52.6% [2, 3]. Initially, reduction in renal function may manifest without clinical symptoms, but over time, it could progress, potentially leading to the development of renal failure and higher mortality rate [4]. The conventional approach of assessing kidney function by using serum creatinine does not detect very mild renal deterioration and therefore is not a reliable predictor of early AKI. In addition, the concentration of serum creatinine can be influenced by factors such as lean body mass, age, sex, ethnicity, hydration status, dietary protein intake, tubular secretion, and extrarenal elimination of creatinine [5]. It is important to note that muscle wasting is common among cancer patients and that body weight and nutritional status can vary significantly within a single patient over the course of their treatment [6].

Researchers have been looking for biomarkers that allow detection of AKI development prior to rise of serum creatinine. Over the past years, urinary biomarkers such as kidney injury molecule-1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL), liver fatty acid binding protein (L-FABP) and Vanin-1 (VNN-1) have been introduced as potentially promising indicators for the early detection of kidney damage [7, 8]. The aim of our study was to examine the diagnostic utility of these urinary biomarkers separately and in combination for the early detection of cytotoxic-induced AKI in children treated by CIS and/or IFO anticancer treatment. We monitored time-dependent changes in urinary NGAL, KIM-1, L-FABP and VNN-1 levels as well as in serum creatinine concentration in patients treated with CIS and/or IFO.

Methods

Patients and study design

We performed a cross-sectional study during 5.5 years, enrolling 38 pediatric patients treated with CIS or IFO - based chemotherapy for various types of malignancies. Clinical data, serum and urine specimens were collected at early CIS and/or IFO infusions (13 patients in the first and 25 patients in the second chemotherapy cycle). Exclusion criteria for this study were one or more of the following: pre-existing AKI, chronic kidney disease (CKD), previous/concurrent use of other nephrotoxic drugs, and/or administration of contrast medium in the previous 14 days. The study was approved by the Institutional Ethic Committee. Prior to study enrollment, written informed consent was obtained from the parents or legal guardians of each participant.

Chemotherapy regimens

Chemotherapy regimens that include two investigated cytotoxic drugs, CIS and IFO, differ accordingly to the cancer diagnosis and its stage/risk stratification. CIS-based protocols consist of 4 to 8 cycles given every 2 to 3 weeks, with a varying dose: e.g. single high-dose (HD) IV infusion [80–100 mg/m^2 (body surface area - BSA)] vs. five days of low-dose (LD) IV infusion [20 mg/m^2 daily]. All patients were hydrated with 3 l/m^2/d, 12 h prior and during the chemotherapy administration. The cumulative dose of CIS per cycle was 50–100 mg/m^2. IFO (with a dose range of 0.8–3 g/m^2/day) was administered intravenously over two to five consecutive days per course, accompanied by simultaneous 24-hour continuous hydration (3 l/m^2/d) and Uromitexan (Mesna) at a dose of 120% of the equivalent IFO dose. The cumulative dose of IFO per cycle was 3 to 9 g/m^2.

Sample acquisition and analysis

For each patient, five serum (immediately before, 2 h, 6 h, 24 h and 48 h after chemotherapy) and four urine samples (immediately before, 2 h, 6 h and 24 h after chemotherapy) were obtained. All serum and urine samples were centrifuged (3000 rpm for 10 min) and sample supernatants were frozen at − 80 °C prior to analysis. Serum creatinine levels were measured with Dimension autoanalyzer (Siemens Healthcare GmbH, Germany), using a modification of Jaffe method. The estimated glomerular filtration rate (eGFR) in ml/min/1.73m^2 was calculated using Schwartz's formula [9].

AKI was defined using KDIGO (Kidney Disease Improving Global Outcomes) [10] criteria. Three stages of AKI were included according to relative changes in serum creatinine (sCr). Stage 1 AKI was defined as increase in sCr by ≥ 26.5 μmol/L from baseline within 48 h; stage 2 AKI increase in sCr to 2–2.9 times from baseline value and stage 3 AKI increase in sCr to > 3 times from baseline. For the diagnosis of AKI, only the sCr criteria were used. The urine output criterion was

not used because oliguria is not typical feature of CIS or IFO-induced AKI.

Biomarker measurements

Urinary KIM-1, NGAL, L-FABP and VNN-1 from all four urine samples were assayed using commercially available enzyme-linked immunoassays (ELISA) (R&D Systems, Inc., Minneapolis, MN, USA), according to the manufacturer's recommendations. The levels of urinary (u) KIM-1, NGAL, L-FABP and VNN-1 were adjusted according to urine creatinine (uCr) concentrations in order to minimize hydration effects and were expressed in ng/mg.

Statistical analysis

Data are presented as median values and 25th -75th percentile values after the Shapiro-Wilks test showed significant deviation from normal distribution. Accordingly, two independent groups were compared by Fisher's exact test and Mann-Whitney U test, while serial measurements in the same patients were compared by using repeated measures ANOVA after the logarithmic data transformation. Diagnostic accuracy of the parameters was tested by receiver operating characteristic curve (ROC) analysis, followed by binary logistic regression analysis for several parameters' modeling and thus generated models were subjected to the subsequent ROC analysis in order to get the best predictor of AKI status. Statistical significance was set at the $P < 0.05$ value.

Table 1 Demographic, clinical and baseline laboratory characteristics of the AKI and non-AKI groups

Parameter	KDIGO		
	Non-AKI ($n=32$)	AKI ($n=6$)	*P*
Male/female (n; (%))	20/12 (62.50/37.50)	5/1 (83.30/16.70)	0.643
Age (years)	6.00 (2.25-14.00)	1.50 (1.00-6.75)	0.062
BMI (kg/m^2)	16.20 (13.90-18.39)	15.30 (14.88–15.83)	0.519
Baseline sCr (umol/l)	45.50 (30.50-56.25)	26.00 (19.00-46.25)	0.062
sCr at 48 h post-CT (umol/l)	49.00 (38.25-69.00)	41.00 (28.75-97.00)	0.682
Baseline eGFR (ml/min/1.73m^2)	142.50 (124.00-157.13)	158.00 (118.90-198.75)	0.279
eGFR at 48 h post-CT (ml/min/1.73m^2)	119.56 (110.57-141.58)	104.69 (77.44-121.04)	0.116
Chemotherapy regimens; n (%)			
CIS ($n=13$, 34.21%)	11 (37.37)	2 (33.33)	0.421
IFO ($n=23$, 60.53%)	20 (62.50)	3 (50.00)	
CIS + IFO ($n=2$, 5.26%)	1 (3.13)	1 (16.67)	
Types of cancer; n (%)			
RMS + ES ($n=12$, 31.58)	10 (31.25)	2 (33.33)	0.947
NB ($n=7$, 18.42)	5 (15.62)	2 (33.33)	
ALL ($n=4$. 10.53)	4 (12.50)	0 (0.00)	
BL ($n=4$, 10.53)	4 (12.50)	0 (0.00)	
GCT ($n=4$, 10.53)	3 (9.38)	1 (16.67)	
HB + HCC ($n=4$, 10.53)	3 (9.38)	1 (16.67)	
NPC ($n=2$, 5.26)	2 (6.25)	0 (0.00)	
PPB ($n=1$, 2.63)	1 (3.12)	0 (0.00)	

AKI – acute kidney injury; BMI – body mass index; sCr – creatinine; CT-Chemotherapy eGFR – estimated glomerular filtration rate; CIS – cisplatin; IFO – ifosfamide, STS - soft tissue sarcomas; RMS – rhabdomyosarcoma; ES - Ewing sarcoma; NB – neuroblastoma; ALL - acute lymphoblastic leukemia; BL - Burkitt lymphoma; GCT - germ cell tumors; HB – hepatoblastoma; HCC - hepatocellular carcinoma; NPC – nasopharyngeal carcinoma; PPB - pleuropulmonary blastoma

Results

Patient characteristics

A total of 38 patients receiving CIS or IFO-based regimens were enrolled in the study. Age ranged from 1 to 17 years, median 5 years (IQR 2–14 years). Majority of study participants were of male gender (25/38, 65.79%). Cancer diagnoses included: soft tissue sarcomas (STS), namely rhabdomyosarcoma (RMS) and Ewing sarcoma (ES) ($n=12$, 31.57%), neuroblastoma (NB) ($n=7$, 18.42%), acute lymphoblastic leukemia (ALL, high risk - HR) ($n=4$, 10.53%), Burkitt lymphoma (BL) ($n=4$, 10.53%), germ cell tumors (GCT) ($n=4$, 10.53%), liver tumors, concretely hepatoblastoma (HB) or hepatocellular carcinoma (HCC) ($n=4$, 10.53%), and other, rare types of pediatric tumors (pleuropulmonary blastoma - PPB and nasopharyngeal carcinoma - NPC) ($n=3$, 7.89%). IFO was used in 23 (60.53%) patients and CIS in 13 (34.21%), while 2 (5.26%) received combined treatment with both drugs. All patients had normal eGFR prior to treatment.

Chemotherapy induced AKI

Among 38 study patients, 6 patients (15.79%) experienced CIS or IFO-related AKI, within 2 days following the chemotherapy infusion. Demographic, clinical and baseline laboratory characteristics of the AKI and non-AKI groups are presented in Table 1. According to KDIGO criteria, 4/38 (10.53%) patients experienced stage 1 AKI and 2/38 (5.26%) patients had criteria for stage 2 AKI.

Median age of patients who experienced chemotherapy related AKI was 1.50 years (IQR 1.00-6.75 years) and they were younger then patients who did not developed CIS or IFO induced AKI (median age 6.00 years; IQR 2.25-14.00 years), but that difference did not reach statistical significance (Table 1). Predominance of male gender was seen in both groups. Children who developed AKI tend to have lower BSA, compared with those who did not develop AKI, but neither statistically significant.

There was no significant difference in eGFR at 48 h after chemotherapy among the 2 study groups (Table 1).

In all patients, each of the four tested urinary biomarker levels rises gradually after chemotherapy and were higher at each time point compared to basal levels (Fig. 1a). The median increases in uNGAL/uCr ratio were 517.3% (IQR 25.6-264287.2%) in the patients with

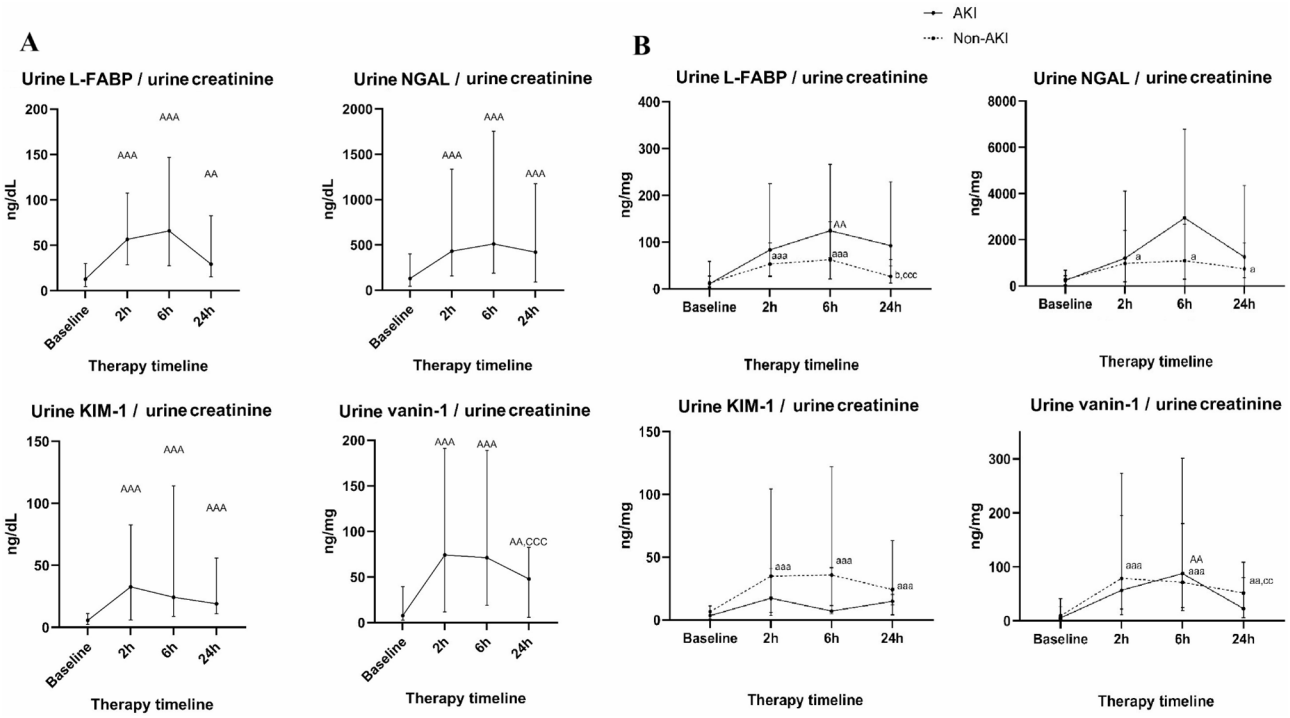

Fig. 1 AKI urinary biomarkers' change during the 24 h after the administration of chemotherapy All patients ($n=38$). $^{AA,\ AAA}p<0.01$, *0.001*, respectively, vs. baseline value; $^{CCC}p<0.001$, respectively vs. 6 h post-CT value AKI status (AKI: $n=6$, Non-AKI: $n=32$). $^{a,\ aa,\ aaa}p<0.05$, *0.01*, *0.001*, respectively, vs. baseline value in non-AKI group; $^{b}p<0.05$ vs. 2 h post-CT value in non-AKI group; $^{cc,\ ccc}p<0.01$, *0.001*, respectively, vs. 6 h post-CT value in non-AKI group; $^{AA}p<0.01$ vs. baseline value in AKI group

AKI and 84.1% (IQR 17.4-580.8%) in the patients without AKI during the first 24 h after CT ($p=0.261$) (Fig. 1b). The highest increases of 1782.4% (IQR 244.7-19451.4%) in the patients with AKI and 321.1% (IQR 36.7-1222.5%) in the patients without AKI were observed 6 h after CT ($p=0.136$) (Fig. 1b).

The median increases in uKIM-1/uCr ratio were 336.3% (IQR 71.8-1102.4%) in the patients with AKI and 214.4% (IQR 68.9-551.1%) in the patients without AKI during the first 24 h after CT ($p=0.740$) (Fig. 1b). The highest increases of 529.8% (IQR 63.9-1835.2%) in the patients with AKI and 347.6% (IQR 98.2-1412.9%) in the patients without AKI were observed 6 h after CT ($p=0.953$) (Fig. 1b).

The median increases in uL-FABP/uCr ratio were 732.9% (IQR 35.3–3476.0%) in the patients with AKI and 128.5% (IQR 1.6-738.8%) in the patients without AKI during the first 24 h after CT ($p=0.229$) (Fig. 1b). The highest increase of 1086.1% (IQR 279.8-2544.1%) in the patients with AKI and of 302.2% (IQR 114.6-561.2%) in the patients without AKI was observed 6 h after CT ($p=0.279$) (Fig. 1b).

The median increases in uVanin-1/uCr ratio were 463.7% (IQR 68.8-819.5%) in the patients with AKI and 111.6% (IQR 12.8-1127.5%) in the patients without AKI during the first 24 h after CT ($p=0.469$) (Fig. 1b). The highest increases of 2194.0% (IQR 255.3-4695.5%) in the patients with AKI and 429.8% (IQR 156.5–1467.0%) in the patients without AKI were observed 6 h after CT ($p=0.097$) (Fig. 1b).

A significant difference between the AKI and non-AKI group of patients was reached only for uLFABP/uCr ratio 24 h after chemo therapy ($p=0.014$) (Table 2).

In order to estimate diagnostic potency of the measured urinary biomarkers regarding AKI status, we performed ROC analysis and presented detailed results in the Table 3. The largest AUCs for predicting the onset of AKI were uL-FABP/uCr measured 24 h after chemotherapy (AUC 0.833; 95% confidence interval, 0.66–0.96; $p<0.001$) (Fig. 2a) and uNGAL/uCr measured 24 h after chemotherapy (AUC 0.719; 95% confidence interval, 0.513–0.925; $p=0.037$) (Fig. 2b). A uL-FABP/uCr ratio of 31.78 ng/mg 24 h after chemotherapy infusion had a sensitivity of 1 and a specificity of 0.6 to predict chemotherapy associated AKI (Fig. 2a). For a cut-off concentration of 434.53 ng/mg, uNGAL/uCr at 24 h after chemotherapy had sensitivity of 0.8 and specificity of 0.6 for AKI prediction (Fig. 2b). These values indicate that 24 h uL-FABP and uNGAL are good predictors for early detection of AKI.

To create a new biomarker with significant diagnostic accuracy towards AKI status prediction, which will be of utmost significance for clinical practice, we constructed a model of integrated-combined parameters (calculated

Table 2 Urinary KIM-1, NGAL, L-FABP and VNN-1/uCr ratio before and after chemotherapy administration in participants with and without chemotherapy-associated AKI

Parameter	KDIGO		*p*
	Non-AKI (*n* = 30)	AKI (*n* = 6)	
Baseline-NGAL/uCr-(ng/mg)	125.42 (40.66-390.69)	242.50 (37.03-442.85)	0.770
uNGAL/uCr at 2 h post-CT (ng/mg)	405.63 (151.83-1199.19)	1200.31 (181.63-4100.21)	0.297
uNGAL/uCr at 6 h post-CT (ng/mg)	509.33 (172.31–1211.00)	2940.67 (285.29-6786.42)	0.185
uNGAL/uCr at 24 h post-CT (ng/mg)	383.03 (82.59-844.84)	1255.89 (358.95-4352.41)	0.097
Baseline uKIM-1/uCr (ng/mg)	6.60 (2.77–11.10)	3.48 (0.78–11.23)	0.336
uKIM-1/uCr at 2 h post-CT (ng/mg)	34.99 (6.18-104.25)	17.25 (3.44–41.11)	0.316
uKIM-1/uCr at 6 h post-CT (ng/mg)	35.86 (11.74-122.17)	7.00 (4.67–41.75)	0.056
uKIM-1/uCr at 24 h post-CT (ng/mg)	24.18 (11.79–63.21)	14.81 (3.90–20.20)	0.116
Baseline uL-FABP/uCr (ng/mg)	12.83 (4.64–27.37)	10.75 (3.78–58.48)	0.953
uL-FABP/uCr at 2 h post-CT (ng/mg)	53.33 (27.38–97.69)	83.61 (26.52-224.75)	0.469
uL-FABP/uCr at 6 h post-CT (ng/mg)	62.67 (21.11–143.50)	124.34 (66.67–266.00)	0.199
uL-FABP /uCr at 24 h post-CT (ng/mg)	26.58 (12.51–62.72)	92.35 (49.33-228.91)	0.014
Baseline uVNN-1/uCr (ng/mg)	9.15 (2.93–41.37)	5.35 (1.12–26.19)	0.336
uVNN-1/uCr at 2 h post-CT (ng/mg)	78.89 (11.56-195.32)	56.59 (21.92-273.29)	0.800
uVNN-1/uCr at 6 h post-CT (ng/mg)	71.29 (18.47–180.20)	87.50 (24.58–301.00)	0.800
uVNN-1/uCr at 24 h post-CT (ng/mg)	51.65 (5.65–80.43)	22.69 (5.68-108.69)	0.891

AK I– acute kidney injury; uNGAL – urinary neutrophil gelatinase-associated lipocalin; uCr - urine creatinine; CT – chemotherapy; uKIM-1 – urinary kidney injury molecule-1; uL-FABP – urinary liver fatty acid binding protein; uVNN-1 – urinary Vanin-1.

Table 3 Diagnostic potency of urinary biomarkers' for AKI status by using ROC curve analysis

Parameter	AUC (95th CI)	SE	Cut-off Value	Sensitivity	Sp	*P*
uNGAL/uCr baseline (ng/mg)	0.542 (0.276–0.807)	0.135	355.99	0.5	0.3	0.758
uNGAL/uCr at 2 h post-CT (ng/mg)	0.641 (0.367–0.914)	0.140	1373.50	0.5	0.2	0.313
uNGAL/uCr at 6 h post-CT (ng/mg)	0.677 (0.405–0.949)	0.139	4840.50	0.5	0.1	0.202
uNGAL/uCr at 24 h post-CT (ng/mg)	0.719 (0.513–0.925)	0.105	434.53	0.8	0.6	0.037
uKIM-1/uCr baseline (ng/mg)	0.628 (0.376–0.879)	0.128	4.62	0.7	0.6	0.320
uKIM-1/uCr at 2 h post-CT (ng/mg)	0.635 (0.432–0.838)	0.104	34.40	0.8	0.5	0.191
uKIM-1/uCr at 6 h post-CT (ng/mg)	0.750 (0.541–0.959)	0.107	9.17	0.7	0.8	0.019
uKIM-1/uCr at 24 h post-CT (ng/mg)	0.708 (0.521–0.896)	0.096	21.59	1.0	0.5	0.029
uL-FABP/uCr baseline (ng/mg)	0.510 (0.230–0.791)	0.143	34.27	0.3	0.8	0.942
uL-FABP/uCr at 2 h post-CT (ng/mg)	0.583 (0.328–0.838)	0.130	66.12	0.7	0.6	0.522
uL-FABP/uCr at 6 h post-CT (ng/mg)	0.672 (0.468–0.876)	0.104	75.84	0.8	0.6	0.099
uL-FABP /uCr at 24 h post-CT (ng/mg)	0.813 (0.665–0.960)	0.075	31.78	1.0	0.6	< 0.001
uVNN-1/uCr baseline (ng/mg)	0.630 (0.391–0.870)	0.122	6.48	0.8	0.4	0.287
uVNN-1/uCr at 2 h post-CT (ng/mg)	0.464 (0.231–0.696)	0.119	74.31	0.7	0.5	0.758
uVNN-1/uCr at 6 h post-CT (ng/mg)	0.464 (0.213–0.714)	0.128	42.67	0.5	0.6	0.775
uVNN-1/uCr at 24 h post-CT (ng/mg)	0.521 (0.258–0.783)	0.134	39.72	0.7	0.6	0.876

AKI – acute kidney injury, AUC – area under the curve, CI – confidence interval, SE – standard error; Sn - sensitivity; Sp - specificity; uNGAL – urinary neutrophil gelatinase-associated lipocalin; uCr – urine creatinine; CT – chemotherapy; uKIM-1 – urinary kidney injury molecule-1; uL-FABP – urinary liver fatty acid binding protein; uVNN-1 – urinary Vanin-1

probabilities for the group of parameters) by using binary logistic regression analysis. We got a set of 4 new variables, consisted of all parameters measured in urine at same time points, and those variables were subsequently subjected to ROC analysis. Results are presented at the Table 4.

The highest AUC of the combined biomarkers was 0.917 (95% CI 0.816–1.017), for urinary biomarkers measured 24 h after chemotherapy (Fig. 3). Urinary biomarkers measured at 2 and 6 h after chemotherapy showed significant diagnostic accuracy for AKI prediction in patients treated with CIS and/or IFO (Table 4). Other modelled variables, according to this analysis, didn't show significant diagnostic accuracy for AKI prediction in this group of patients.

Discussion

CIS and IFO are effective and widely used antineoplastic drugs for treatment of various solid tumors in children [11, 12]. Acute kidney injury (AKI) represents a significant dose-limiting complication of chemotherapy protocols involving CIS and IFO. The serum creatinine

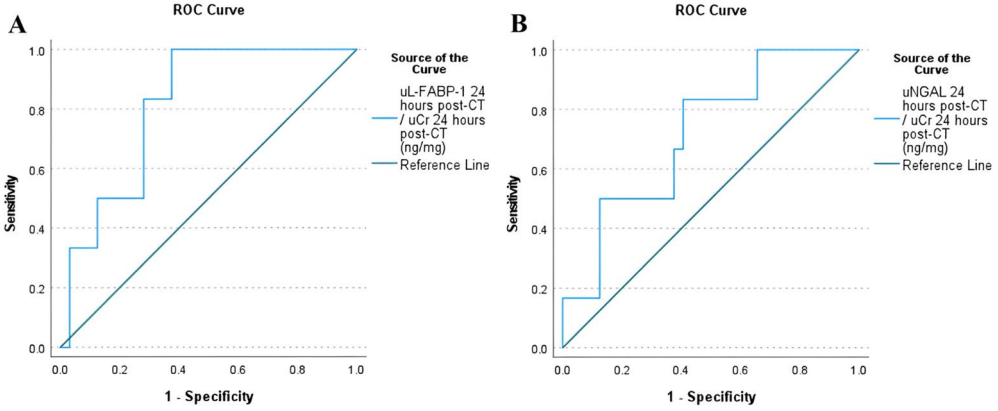

Fig. 2 ROC analysis for diagnostic accuracy in AKI prediction of urinary L-FABP/urine creatinine and urinary NGAL/urine creatinine 24 h after treatment. Urinary L-FABP/urine creatinine 24 h after treatment. Urinary NGAL/urine creatinine 24 h after treatment

Table 4 Diagnostic accuracy of the models of integrated urinary AKI biomarkers' by ROC analysis

Variable(s)	AUC (95th CI)	SE	*P*
Basal urinary biomarkers	0.729 (0.493–0.965)	0.121	0.057
2 h urinary biomarkers	0.776 (0.594–0.958)	0.093	0.003
6 h urinary biomarkers	0.854 (0.698–1.011)	0.080	< 0.001
24 h urinary biomarkers	0.917 (0.816–1.017)	0.051	< 0.001

AUC - area under curve; CI – confidence interval; SE – standard error

increases 48 h to 72 h after CIS administration, therefore, toxicity is not apparent in the first hours of injury [13]. Majority of patients receiving CIS/IFO-based chemotherapy are discharged from hospital within 48–72 h after CT and usually routine biochemistry or urine analysis are not performed at these time points. Hence, an AKI episode may be missed. In addition to slow rise of this traditionally used functional biomarker, it should be kept in mind that serum creatinine concentrations may be artificially low especially in young ones with low body weight and due to cachexia and loss of muscle mass in critically ill patients such as oncology patients. Also, it should be noted that chemotherapy protocols with CIS and or IFO include hyperhydration 12 h prior and during the chemotherapy administration as a prevention measure for kidney protection. Fluid overload could be an additional reason for lower serum creatinine values. Taking into account above mentioned, the current definitions of pediatric AKI based on serum creatinine and urinary output in this group of patients lack precision.

In contrast to serum creatinine, damage-associated biomarkers specific to tubular injury which occurs in patients treated with CIS and IFO, can potentially identify patients in whom tubular damage has already occurred, before a rise in serum creatinine occurs. Increasing evidence suggests that a biomarker-based approach could be promising for identifying patients at

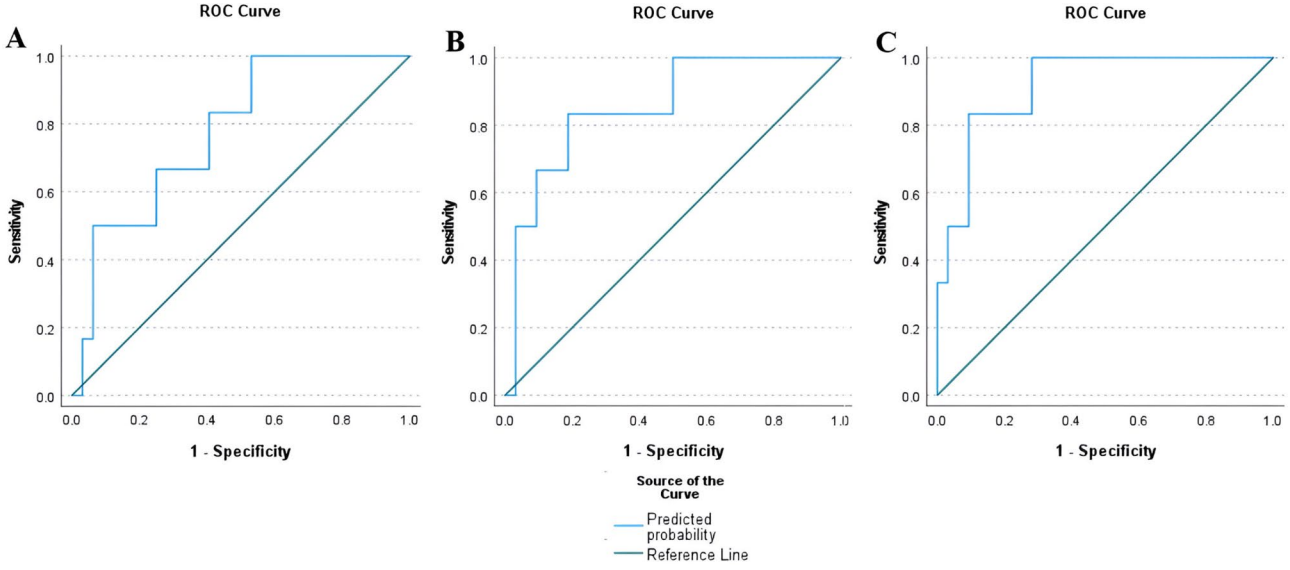

Fig. 3 Area under the ROC of the integrated urinary AKI biomarker models 2 h (**A**), 6 h (**B**), and 24 h (**C**) post-CT

high risk of developing AKI. Also, damage biomarkers offer a potential solution to guide clinicians in their therapeutic decisions to prevent AKI outcomes [14].

Several candidate biomarkers have been tested in search of the most sensitive and specific indicator of drug-induced nephrotoxicity, including NGAL [15], interleukin-18 [15] and KIM-1 [16]. Only a few published studies assessed the applicability of urinary L-FABP as a biomarker for the early diagnosis of AKI in children [17, 18], while there are no published studies on the usefulness of VNN-1 as a specific indicator of drug-induced nephrotoxicity in pediatric population.

In the present study, 15.79% patients developed AKI within 48 h of cytotoxic treatment with CIS and or IFO. Previous studies reported the wide extent of CIS and IFO induced nephrotoxicity, with the incidence ranging 8–40% [19]. Possible explanation for such range could be the difference in sample size, study protocols and AKI definitions.

Severe toxicity was reported in infants and children below 5 years of age, who may be more vulnerable to tubular toxicity. One could speculate that there may be age-related differences in drug metabolism and detoxification [20]. Median age of patients in our study who experienced CIS/IFO-related AKI was 1.5 years, notably younger then patients who did not develope AKI, although, due to a small sample size, statistical significance was not reached.

Low skeletal muscle mass accompanied by lower serum creatinine levels and with higher eGFR along with decreased total body water leading to increased serum drug concentration, may contribute to nephrotoxicity by facilitating excess drug dosing, resulting in reaching higher and potentially toxic serum drug concentrations [21]. Children who developed AKI in this study tended to have lower BSA, compared with those in non-AKI group, which is probably consequence of generally younger age of patients in AKI group. Also, it should be noted that 5/6 patients under the age of 5 developed AKI.

Other risk factors that increase the chance for nephrotoxicity are higher dose of CIS (> 50 mg/m^2) per cycle as well as concomitant administration of IFO [22]. Among 6 patients who developed AKI in our study, two patients were treated with single HD of CIS and one patient received combined CIS + IFO treatment. We presume that larger sample size could confirm this result by reaching statistical significance.

Recently, it was demonstrated that VNN-1 may be a promising biomarker showing potential for the early detection of AKI in numerous kidney diseases and drug-related renal tubular damage [8, 23]. Hosohata and co-authors found that urinary levels of VNN-1 increased prior to the rise in the levels of conventional biomarkers (serum creatinine, urinary NAG, KIM-1 and NGAL) in rodents experiencing renal tubular injury induced by the administration of nephrotoxic agents [8].

In normal conditions, the level of KIM-1 is very low in kidneys, but it was found that renal ischemic or toxic injury increased the level of KIM-1 by the up-regulation of mRNA in proximal tubule cells, and that renal and urinary levels of KIM-1 remain elevated for an extended period following kidney injury [24]. Likewise, NGAL is normally expressed in healthy individuals in very small amounts in various types of cells (kidneys, gastrointestinal tract, respiratory system, and neutrophils) [25]. In kidneys, NGAL is excreted from the epithelial cells of loop of Henle, and in healthy individuals is detectable in very low amount in urine. Immediately after acute kidney injury, increased production of NGAL, by up-regulation of mRNA, appears in the damaged tubules and as a result of that, the increased urinary and plasma NGAL levels could be found [25]. VNN-1 is highly expressed in normal kidney tissues of both humans and rodents [26]. It is proposed that the origin of uVNN-1 in drug-induced AKI models is most likely from the damaged renal parenchyma. According to the Hosohata et al., a significant rise in uVNN-1 occurs 2 to 5 days after cisplatin administration, suggesting that renal VNN-1 began to leak into the urine 2 days after exposure to high dose of cisplatin and continued to leak at least until 5 days after exposure [27].

In our study, all urinary biomarkers were significantly increased during the first 6 h after cytotoxic treatment while serum creatinine concentrations did not change significantly during the study period. The median increases in urinary biomarkers during the first 6 h after treatment were 529.8–2194% in AKI vs. 302.2-429.8% in non-AKI group depending of tested biomarker, but statistically significant differences between the two groups were not found.

The highest levels of uVNN-1 adjusted according to urine creatinine in non-AKI and AKI group of patients were 6 h after chemotherapy, but the difference between those two groups was not statistically significant. In all other time-points, levels of uVNN-1/uCr were insignificantly higher in non-AKI compared to AKI group of patients. There are no data on the role of uVNN-1 in identifying early stages of kidney injury in pediatric patients undergoing chemotherapy.

Data are limited on the role of uL-FABP in identifying early stages of kidney injury in pediatric patients undergoing chemotherapy. Given that in 42 adults, treated with CIS, uL-FABP predicted AKI with AUC over 0.95, we assumed that this biomarker could be useful in detecting AKI in children with cancer during chemotherapy [28]. The highest median increases in uL-FABP/uCr ratio was observed 6 h after chemotherapy in the AKI group of patients. But, the largest AUC of uL-FABP in our patients occured 24 h after chemotherapy, which was

later compared with Yanishi et al. finding. In the abovementioned study, acute renal function deterioration was predicted by increased uL-FABP excretion within 6 h after receiving CIS-CT and, in those with AKI, the increase in uL-FABP excretion preceded the rise in sCr by over 2 days.

Shahbazi et al. compared uNGAL/uCr ratio with serum creatinine in CIS nephrotoxicity prediction [29]. Autors reported that changes in uNGAL/uCr ratio had significant increase at 24 and 48 h compared to baseline after CIS infusion in the AKI group of patients. Shahbazi et al. reported that uNGAL/uCr ratio at 24 h after CIS infusion predicted AKI with measured AUC of 0.80 [29]. Ghadrdan and coauthors found that in patients ($N=35$) with different solid tumors treated with CIS, uKIM-1/uCr 24 h/baseline and uNGAL/uCr 24 h/baseline predicted AKI with measured AUC of 0.78 and 0.77, respectively [30]. The urinary samples were obtained at 0 h, 6 h and 24 h after CIS infusion. In the same study, the uNGAL/uCr and uKIM-1/uCr ratios rapidly increased during the follow-up period, with the peak at 6 h after drug administration [30].

Our findings regarding the uNGAL as a biomarker of kidney function were in line with previously mentioned studies. During the current study, the uNGAL/uCr ratio also rapidlly increased and reached the maximum values at 6 h after chemotherapy and then decreased at 24 h after chemotherapy administration in AKI and non-AKI patients. According to our results, uNGAL/uCr ratio 24 h after chemotherapy infusion demonstrated a moderate predictive value for AKI with measured AUC of 0.719.

In contrast to the Ghadrdan et al. findings [30] regarding KIM-1 as a biomarker of renal function, in our study no statistically significant difference in uKIM-1/uCr ratio in any time point was found between AKI and non-AKI groups.

With the exception of uL-FABP and uNGAL measured 24 h after the treatment, other two tested urinary biomarkers alone did not meet our expectations regarding the ability to predict early onset subclinical kidney damage in patients treated with CIS and IFO.

The difference in our finding compared to previous reports on chemotherapy induced AKI, might be explained by the limited number of sampling, short follow-up period (urine samples were collected only up to 24 h after chemotherapy) as well as that in most of those patients, it was the second course of chemotherapy. In addition to that, the median age of patients in whole study group was 5 years, and median BMI was 15.85 kg/m^2. Taking into account median age and muscle mass of our patients, the use of serum creatinine as a marker of renal function in this group of patients may have led to underestimation of AKI diagnosis. Also, it is possible that increased urinary L-FABP, KIM-1, NGAL and VNN-1 levels in some patients, classified as non-AKI group, may be due to mild renal injury that does not result in increased serum creatinine (subclinical AKI). In addition, hyperhydration may also affect ability to detect serum creatinine rise.

Considering the features of tested urinary biomarkers, we constructed a model of integrated-combined parameters. Our model, which combined all four urinary biomarkers measured at each study time-point, were investigated in more details. This analysis showed moderate (combined urinary biomarkers measured at 2 h) to significant (combined urinary biomarkers measured at 6 and 24 h) diagnostic accuracy of combined urinary biomarkers toward AKI prediction in patients treated with chemotherapy. The results of our study indicate that a combination of urinary biomarkers (including KIM-1, NGAL, L-FABP and VNN-1) may allow for early detection of AKI following chemotherapy, not only better than serum creatinine, but also the individual biomarkers. As far as we know, there are no published prospective studies in the literature that examined the role of combination of those four urinary biomarkers in the prediction of chemotherapy induced AKI in children.

We measured only urinary levels of tested biomarkers in our study. The reason for that was easily accessible and non-invasive nature of sample collection, which enables rapid and serial sampling and is of particular interest in case of special population groups such as children.

During the follow-up period, 14/38 (36.84%) patients died and four (10.53%) patients were lost to follow-up. Three (21.43%) of that passed away where classified as AKI patients according to used criteria. Main cause of death in our group of patients (13 patients) was progression of oncological disease. None of the patients during study period required renal replacement therapy (RRT). Since none of the patients included in the study required RRT and also did not die due to kidney injury, we did not use mortality and RRT as outcomes.

Some limitations of the study should be noted. This is a single center study with a cross-sectional design, limited sample size, and low number of AKI patients. The incidence of all pediatric tumors is small, and even smaller for specific tumors as in the AKI group, thus making it difficult to obtain a bigger sample size and to have comparable groups. Patients with AKI according to KDIGO criteria were younger and with lower muscle mass than patients without AKI, which together with other factors may have influenced serum creatinine levels and its significance as a marker of kidney damage, especially for this group of critically ill patients. Some studies suggest that cystatin C provides a more accurate assessment of kidney function in children on chemotherapy [31, 32]. The explanation for not using cystatin C as a biomarker of kidney function in our study was financial limitations.

These limitations could influence the final results, and this requires a cautious interpretation of our findings. The follow-up period was limited, precluding assessment whether the increase in urinary biomarkers corresponds to a sustained decline in renal function over the long term. Therefore, further studies with larger sample size, comparable groups with propensity score matching, longer follow-up period and greater sampling frequency are suggested.

Conclusion

According to tested urinary biomarkers, early kidney tubular damage occurs as early as 2–6 h after CIS and/or IFO administration. Due to well-known limitations, the use of AKI definition based on changes in serum creatinine and urine output carries the risk of untimely identification of patients receiving CIS and/or IFO who are at risk of developing AKI. The implementation of damage-associated urinary biomarkers specific to tubular injury in AKI definition is essential for this population.

Considering the multifactorial etiology of chemotherapy-induced AKI, and taking into account unique characteristics of different biomarkers, there is a need to explore the combined utilization of VNN-1 with KIM-1, NGAL or L-FABP as a potentially valuable tool for the diagnosis and prediction of renal damage. Further research is needed to validate diagnostic and prognostic utility of urinary KIM-1, NGAL, L-FABP and VNN-1 in prediction of early kidney damage in pediatric patients during chemotherapy.

Abbreviations

AKI	Acute kidney injury
CIS	Cisplatin
IFO	Ifosfamide
KIM	1–kidney injury molecule–1
NGAL	Neutrophil gelatinase–associated lipocalin
L	FABP–liver fatty acid binding protein
VNN	1–Vanin–1
KDIGO	Kidney Disease Improving Global Outcomes
CKD	Chronic kidney disease
HD	High dose
LD	Low dose
BSA	Body surface area
eGFR	Estimated glomerular filtration rate
sCr	Serum creatinine
ELISA	Enzyme–linked immunoassay
ROC	Receiver operating characteristic curve
AUC	Area under receiver operator curve
IQR	Interquartile range
STS	Soft tissue sarcomas
RMS	Rhabdomyosarcoma
ES	Ewing sarcoma
NB	Neuroblastoma
ALL	Acute lymphoblastic leukemia
BL	Burkitt lymphoma
GCT	Germ cell tumors
HB	Hepatoblastoma
HCC	Hepatocellular carcinoma
PPB	Pleuropulmonary blastoma–PPB
NPC	Nasopharyngeal carcinoma

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Author contributions
M-L.G. wrote the manuscript; K-S.J. performed laboratory and statistical analysis, participated in data interpretation, assisted in manuscript preparation and provided important intellectual contribution; P.D. assisted in the preparation of the manuscript. N.S. assisted in statistical analysis; L.J. assisted in data collection and analysis, participated in revising the paper; R.P. and M.G. assisted in data collection and participated in revising the paper; M.J. and V.B. performed laboratory analysis; P.A. assisted in preparing the draft version of the manuscript and preparing files for submission; P.A.A. designed the study and made substantial intellectual contribution. All authors have reviewed and approved the final version of the manuscript for publication.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
All procedures performed were in accordance with the ethical standards of the institutional research committee at which the studies were conducted (approval number 29/IV-10) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from the parents or legal guardian of each participant included in the study.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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