Minireview

Urinary protein biomarkers of kidney injury in patients receiving cisplatin chemotherapy

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Impact statement

There is growing interest in using urinary protein biomarkers to detect acute kidney injury in oncology patients prescribed the nephrotoxic anticancer drug cisplatin. We aim to synthesize and organize the existing literature on biomarkers examined clinically in patients receiving cisplatin-containing chemotherapy regimens. This minireview highlights several proteins (kidney injury molecule-1, beta-2-microglobulin, neutrophil gelatinase-associated lipocalin, calbindin, monocyte chemotactic protein-1, trefoil factor 3) with the greatest promise for detecting cisplatin-induced acute kidney injury in humans. A comprehensive review of the existing literature may aid in the design of larger studies needed to implement the clinical use of these urinary proteins as biomarkers of kidney injury.

Abstract

Despite recent progress in the development of novel approaches to treat cancer, traditional antineoplastic drugs, such as cisplatin, remain a mainstay of regimens targeting solid tumors. Use of cisplatin is limited by acute kidney injury, which occurs in approximately 30% of patients. Current clinical measures, such as serum creatinine and estimated glomerular filtration rate, are inadequate in their ability to detect acute kidney injury, particularly when there is only a moderate degree of injury. Thus, there is an urgent need for improved diagnostic biomarkers to predict nephrotoxicity. There is also interest by the U.S. Food and Drug Administration to validate and implement new biomarkers to identify clinical and subclinical acute kidney injury in patients during the drug approval process. This minireview provides an overview of the current literature regarding the utility of urinary proteins (albumin, beta-2-microglobulin, N-acetyl-D-glucosaminidase, kidney injury molecule-1, neutrophil gelatinase-associated lipocalin, and cystatin C) as biomarkers for cisplatin-induced AKI. Many of the well-studied urinary proteins (KIM-1, NGAL, B2M, albumin) as well as emerging biomarkers (calbindin, monocyte chemotactic protein-1, and trefoil factor 3) display distinct patterns of time-dependent excretion after cisplatin administration. Implementation of these biomarker proteins in the oncology clinic has been hampered by a lack of validation studies. To address these issues, large head-to-head studies are needed to fully characterize time-dependent responses and establish accurate cutoff values and ranges, particularly in cancer patients.

Keywords: Urinary biomarkers, cisplatin, acute kidney injury, kidney injury molecule-1, neutrophil gelatinase-associated lipocalin

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Introduction

Despite the recent development of new immunotherapies and anticancer drugs, cisplatin (*cis*-diamminedichloroplatinum(II)) continues as an important component of chemotherapeutic regimens for the treatment of solid tumors. Use of cisplatin can be limited by acute kidney injury (AKI), which occurs in about one-third of patients.[1] In an attempt to decrease the incidence of AKI, clinicians employ preventive measures including hydration and diuresis to enhance cisplatin excretion and reduce renal exposure. Early histopathological studies in rats revealed the first signs of nephrotoxicity as evidenced by acute proximal tubular necrosis.[2] In humans, cisplatin largely injures the proximal and distal convoluted tubules of the kidneys and to some extent, the collecting ducts.[3,4] The incidence of nephrotoxicity increases with the cumulative dose of

cisplatin in patients and typically occurs at doses above 50 mg/m^2.[1] Clinically, cisplatin nephrotoxicity is detectable through increases in serum creatinine (SCr) and blood urea nitrogen (BUN) concentrations, as well as electrolyte imbalances. Progressive and permanent damage may occur with successive treatments. The mechanisms of cisplatin-induced kidney injury have been extensively studied. Cisplatin actively accumulates in renal tubular cells due to the presence of basolateral uptake transporters such as the copper transporter 1 and organic cation transporter 2.[5–7] Once inside cells, the chloride atoms of cisplatin become labile and are replaced by water molecules to subsequently form hydrated, electrophilic species that target cellular or mitochondrial DNA, RNA and proteins.[8] Studies in rats and mice have also suggested that cisplatin is further biotransformed into highly reactive thiols that injure tubule cells.[9–11] A number of biochemical and cellular processes are perturbed leading to oxidative and nitrative stress, inflammation, lipid peroxidation, and organelle damage ultimately resulting in the activation of apoptotic or necrotic pathways (reviewed in Karasawa and Steyger[12]).

Recently, it was demonstrated that patients prescribed cisplatin have small but permanent declines in renal function.[13] Thus, there is great interest in identifying nephrotoxicity early in patients treated with cisplatin. Current clinical methods, such as SCr and estimated glomerular filtration rate (eGFR), require a substantial decline in kidney function in order to detect clinical AKI.[14] However, there has been a recent surge of research activity aimed at testing the utility of urinary protein biomarkers as a noninvasive and sensitive means of diagnosing drug-induced nephrotoxicity in patients. In 2008, the U.S. Food and Drug Administration (FDA) approved seven urinary protein biomarkers for use in preclinical submissions for regulatory decision-making.[15] These included: kidney injury molecule-1 (KIM-1), clusterin, albumin, total protein, beta 2-microglobulin (B2M), cystatin C, and trefoil factor 3 (TFF3). For clinical detection of AKI, the FDA has approved a point-of-care device, which measures tissue inhibitor of metalloproteinase 2 (TIMP2) and insulin-like growth factor binding protein 7 (IGFBP7), in critically ill patients. There are several clinical studies that have evaluated the utility of urinary proteins as biomarkers of cisplatin-mediated AKI. Moreover, there is interest in using biomarkers to diagnose subclinical AKI in patients with tubular damage in the absence of significant changes in eGFR or SCr.[16] In 2016, the U.S. FDA encouraged the exploratory use of eight protein biomarkers of AKI in early clinical trials.[17]

The ideal biomarker would be noninvasive and obtained from available sources such as urine and should exhibit high sensitivity and specificity to diagnose patients with AKI. In addition, biomarkers should be rapidly quantified, reflect the severity of the injury, and be able to stratify patients according to risk and/or prognosis. Often, the diagnostic performance of a novel biomarker includes the calculation of area under the curve receiver operating characteristic (AUC-ROC) to differentiate patients with AKI from those without AKI. The specificity and sensitivity of the biomarker are compared to a current clinical diagnostic standard. An AUC-ROC value of 0.50 indicates poor diagnostic ability because there is only a 50–50 chance that the biomarker can identify patients with AKI. An ideal biomarker would exhibit an AUC-ROC of 1.00 (100% sensitivity and 100% specificity). However, since an insensitive clinical measure, often SCr, is routinely used for calculating AKI AUC-ROC values, most novel biomarkers appear to exhibit poor performance, particularly in patients with subclinical disease.[18,19]

The purpose of this minireview is to provide an overview of the current literature investigating urinary proteins as potential indicators of cisplatin-induced AKI. The discussion focuses primarily on clinical studies evaluating albumin, B2M, N-acetyl-D-glucosaminidase (NAG), KIM-1, neutrophil gelatinase-associated lipocalin (NGAL), and cystatin C. Notably, these biomarker proteins, with the exception of B2M, were encouraged by the U.S. FDA for exploratory evaluation of AKI in early clinical trials of new drugs in the 2016 Letter of Support document.[17] Brief highlights will also address additional proteins that are emerging as possible biomarkers of cisplatin AKI. Comprehensive tables with key features of each study are included and summarized in each section.

Albumin

Albumin is a high molecular weight protein (66.5 kDa) that is not normally detected in urine due to the absence of significant filtration and the presence of reabsorption by proximal tubules.[20] However, albumin can be found in the urine of patients with glomerular and/or tubular kidney injury.[20] A clinical study comparing patients with one of the two types of injury to healthy individuals suggested that higher levels of urinary albumin were observed with glomerular injury, while moderately elevated levels of albumin excretion (less than 500 mg/24 h) tended to be a sign of tubular injury.[20] Following this work, studies were conducted to assess urinary albumin as a biomarker of cisplatin-induced AKI (Table 1). After cisplatin infusion, urinary albumin concentrations were elevated as early as 4 h,[21] with a peak between 4 and 10 days and decline from the peak value around or before two weeks.[22–24] The magnitude of increases in albumin excretion from baseline varied greatly across clinical studies and populations of patients. In one set of patients ($n = 14$) receiving cisplatin therapy at a dose of 20 mg/m^2 daily for 5 days, urinary albumin concentrations were elevated (2.9-fold) within 5 days after infusion compared to pretreatment levels and concentrations observed in healthy control subjects.[25] In this study, renal damage was not detected by less sensitive, routine laboratory tests such as SCr. The magnitude of changes in urinary albumin concentrations across multiple cycles of cisplatin was measured in the same set of patients and found to be similar from cycle-to-cycle when high doses of cisplatin were prescribed (40 mg/m^2 for five days every three weeks).[26] By comparison, in patients receiving low-dose cisplatin (20 mg/m^2 for five days), baseline excretion of albumin in urine for 24 h was significantly increased by the third cycle of cisplatin compared to the first cycle.[27] Some data have suggested that early time-points (within 24 h) have no predictive utility for

Table 1. Changes in urinary albumin concentrations in patients prescribed cisplatin-containing regimens.

# Patients analyzed	Mean cisplatin dose	Cisplatin naïve (yes/no)	# Patients with clinical AKI	Highest mean/median fold change from baseline during the observation period	Timing of peak change	References
3	38 mg/m^2	Unknown	$n=3$ 23% ↓ eGFR by Day 13	U: ↑ 17-fold	Day 5–8	[29]
8	50–75 mg/m^2 (6 cycles)	Yes	NE	U: ↑ 10-fold	Day 5	[30]
57	63.4 mg/m^2	Mixed	$n=1$	U: ↑ 2-fold	Day 10	[31]
33	75 mg/m^2	Yes	10/33 by Day 4 25% ↓ eGFR	U: ↑ 5.6-fold in AKI group U: ↑ 3.4-fold in no-AKI group	Day 4 AKI Day 3 no-AKI	[21]
10	100 mg/m^2 (3 cycles)	Yes	$n=2$	U: ↑ 2-fold	Day 1 (20 h)	[28]
14	100 mg/m^2 (over 5 days)	No	None	U: ↑ 2.9-fold	Day 5	[25]
16	100 mg/m^2 (over 5 days) (2 cycles)	Unknown	None	U: Cycle 1: Up to ↑ 10-fold U: Cycle 2: Up to ↑ 5.3-fold	Cycle 1: Day 5–6 Cycle 2: Day 4–7	[22]
41	100 mg/m^2 (over 5 days) (3 cycles)	Unknown	NE	U: Cycle 1: ↑ 290-fold U: Cycle 2: ↑ 28-fold U: Cycle 3: ↑ 5-fold ($p > 0.05$)	Cycle 1: Day 9 Cycle 2: Day 9	[27]
23	200 mg/m^2 (over 5 days) (3 cycles)	Unknown	Mean 25% ↓ eGFR Day 9	U: Cycle 1: at least ↑ 30-fold	Cycle 1: Day 9	[26]
5	375 mg/m^2 (over 5 days)	Yes	None	U: ↑ 17.4-fold during U: ↑ 33-fold 1-week after	Day <5	[23]

AKI: acute kidney injury; eGFR: estimated glomerular filtration rate; NE: not evaluated; S: serum; U: urine.
Each publication provides different definitions for the diagnosis of clinical acute kidney injury.

AKI (AUC-ROC value of 0.52).[21] However, by day 4, the AUC-ROC value increased to 0.7 in patients diagnosed with clinical AKI, although time-dependent elevations in urinary albumin were seen in both AKI and no-AKI groups.[21] Since urinary albumin excretion is also an indicator of glomerular injury, very large and/or delayed increases in urinary albumin may reflect more extensive damage to the entire nephron.[23,24,28] Nonetheless, the current data largely support maximal increases in urinary albumin concentrations within 4 to 10 days after cisplatin infusion in patients with clinical and subclinical AKI (Table 1).

Beta-2-microglobulin

Beta-2-microglobulin (B2M) is a low molecular weight protein (~13 kDa) that is typically reabsorbed by renal proximal tubule cells after filtration.[20] Therefore, detection of B2M in urine indicates an impairment of tubular function. B2M has become one of the most commonly utilized urinary proteins for monitoring cisplatin-induced AKI (Table 2). Several studies across solid tumor types and cisplatin dose ranges have observed a rise in urinary B2M levels following cisplatin treatment even in the absence of clinically detectable AKI.[31–36] As a result, B2M may not necessarily have high predictivity to distinguish between patients with clinical and subclinical AKI.[37] In one study, patients that had abnormal increases in urinary B2M did not subsequently develop nephrotoxicity.[38] One reason could be that increased systemic production of B2M may occur in the presence of underlying malignancy. Several studies have shown that baseline urinary B2M concentrations are elevated in cancer patients who have not received chemotherapy, compared to healthy volunteers.[31,32,39] Nonetheless, increases in urinary B2M following cisplatin therapy occur as early as 12 h[32] and generally peak between 3 and 6 days (Table 2).[25,27,31,33,40] Urinary B2M levels decline about 1 to 2 weeks after cisplatin administration[23,25,29,40] and are in the normal range at least three months after cessation of cisplatin-containing chemotherapy ($n=35$).[41] The magnitude of B2M elevation is attenuated with successive cycles of chemotherapy,[27,32,34] although no significant correlations were observed between previous exposure to cisplatin, the dose of cisplatin, and urinary B2M levels.[35] While most of these studies have focused on urinary B2M excretion, it is important to note that serum B2M does not change significantly following cisplatin therapy.[38]

N-acetyl-beta-D glucosaminidase

N-acetyl-beta-D glucosaminidase (NAG) is a lysosomal enzyme identified in the proximal and distal tubules of rat kidneys.[42] Due to its large molecular weight (130 kDa), NAG is not normally filtered and its presence in the urine largely reflects tubular destruction (reviewed in Hong and Chia[43]). Over the past few decades, numerous studies have evaluated urinary NAG concentrations as a surrogate marker of cisplatin AKI in patients (Table 3). Early work revealed that urinary NAG rises in response to cisplatin, but not carboplatin, containing chemotherapy regimens.[36,44] Urinary NAG levels generally peaked within one week after cisplatin treatment, typically between 3 and 5 days, with variability in maximal changes from baseline (3 to 13-fold both in the absence or presence of AKI).[30,36,44] In these patients, there was no clear relationship between maximal NAG changes and a clinical diagnosis of AKI. As a result, there is limited utility in using NAG to predict clinical AKI secondary to cisplatin. For example, in one study there was no difference in urinary NAG levels between groups of patients distinguishable by a 20% decline in eGFR.[45] Similarly, NAG was unable to distinguish between

Table 2. Changes in urinary beta-2-microglobulin concentrations in patients prescribed cisplatin-containing regimens.

# Patients analyzed	Mean cisplatin dose	Cisplatin naïve (yes/no)	# Patients with clinical AKI	Highest mean/median fold change from baseline during the observation period	Timing of peak change	References
35	27–145 mg	No	None	U: ↑ 8.8-fold	Day 0.5	[35]
8	30–90 mg/m^2	Yes	$n=1$	U: ↑ up to 10-fold	Day 3	[40]
20	50 mg/m^2 (6 cycles)	Yes	Unknown	U: ↑ 2-fold	Day 0.5	[32]
57	63.4 mg/m^2	Mixed	$n=1$	U: ↑ 3.3-fold	Day 3	[31]
14	70 mg	Unknown	None	U: ↑ 10-fold	Day 4	[36]
11	<80 mg/m^2	Unknown	Unknown	U: No difference between AKI and non-AKI		[37]
19	80 mg/m^2	Yes	None	U: ↑ 6-fold	Day 1 (1–3 hr)	[38]
14	100 mg/m^2 (over 5 days)	No	None	U: ↑ 4.9-fold	Day <5	[25]
41	100 mg/m^2 (over 5 days) (3 cycles)	Unknown	NE	U: Cycle 1: ↑ 474-fold U: Cycle 2: ↑ 551-fold U: Cycle 3: ↑ 63-fold	Cycle 1: Day 6 Cycle 2: Day 6 Cycle 3: Day 6	[27]
11	100 mg/m^2 (over 5 days) (6 cycles)	Yes	None	U: ↑ 2- to 5-fold	Day 3	[33]
22	100 mg/m^2 (5 cycles)	Yes	None	U: ↓ 1.7-fold	Days 21+	[34]
12	120 mg/m^2	No	NE	U: ↑ 5.7-fold	Day <6	[39]
30	200 mg/m^2 (over 5 days) (3 cycles)	Unknown	Mean 25% ↓ eGFR Day 9	U: Cycle 1: up to 80-fold	Cycle 1: Day 9	[26]
5	375 mg/m^2 (over 5 days)	Yes	None	U: ↑ 13-fold	Day <5	[23]

AKI: acute kidney injury; eGFR: estimated glomerular filtration rate; NE: not evaluated; S: serum; UK: Unknown; U: urine.
Each publication provides different definitions for the diagnosis of clinical acute kidney injury.

Table 3. Changes in urinary N-acetyl-D-glucosaminidase concentrations in patients prescribed cisplatin-containing regimens.

# Patients analyzed	Mean cisplatin dose	Cisplatin naïve (yes/no)	# Patients with clinical AKI	Highest mean/median fold change from baseline during the observation period	Timing of peak change	References
8	30–90 mg/m^2	Unknown	NE	U: Up to ↑ 2.4-fold	Day 3	[40]
3	38 mg/m^2	Unknown	$n=31.3$-fold ↓eGFR by Day 13	U: Up to ↑ 4.4-fold	Day 3–10	[29]
20	50 mg/m^2 (6 cycles)	Yes	NE	U: Up to ↑ 2.6-fold	Day <4	[32]
8	50–75 mg/m^2 (6 cycles)	Yes	NE	U: ↑ 3-fold	Day 4	[30]
24	70 mg/m^2	Unknown	$n=9$ 20% ↓ eGFR ↑ 2.1-fold by Day 6 AUC-ROC: 0.66	U: ↑ 3-fold in 20% ↓ eGFR subjects; U: ↑ 2.1-fold in others	Day 3	[45]
14	70 mg	Unknown	None	U: ↑ 1.9-fold	Day 4	[36]
11	<80 mg/m^2	Unknown	Unknown	U: No difference between AKI and non-AKI		[37]
22	100 mg/m^2 (5 cycles)	Yes	None	U: ↑ 2.2-fold	Day 1.5	[34]
16	100 mg/m^2 (over 5 days) (2 cycles)	Unknown	None	U: Cycle 1: ↑ 3-fold U: Cycle 2: ↑ 2-fold	Day 5-6	[22]
12	120 mg/m^2	No	NE	U: ↑ 11.8-fold	Day 1.5-2	[39]
5	176 mg	Unknown	$n=4$ ↑ 1.5-fold SCr	U: ↑ 2- to 6-fold	Day 5-10	[46]
5	200 mg/m^2 (over 4 days)	Yes	None	U: Up to ↑ 5.7-fold	Day 4	[44]
30	200 mg/m^2 (over 5 days) (3 cycles)	Unknown	Mean 25% ↓ eGFR Day 9	U: Cycle 1: Up to ↑ 7-fold	Cycle 1: Day 6	[26]
5	375 mg/m^2 (over 5 days)	Yes	None	U: ↑ 13.6-fold	Day <5	[23]

AKI: acute kidney injury; AUC-ROC: area under the curve receiver operating characteristic; eGFR: estimated glomerular filtration rate; NE: not evaluated; S: serum; U: urine.
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AKI-positive ($n=30$) and AKI-negative ($n=12$) samples in lung cancer patients treated with cisplatin.[37] Long-term studies have demonstrated that urinary NAG concentrations return to normal levels by at least 3 months after 2 to 7 courses of cisplatin.[41] Furthermore, another report suggested that urinary NAG concentrations may return to baseline as early as 2 weeks after cisplatin treatment.[23] Interestingly, there was no evidence of an increase in

Table 4. Changes in urinary kidney injury molecule-1 concentrations in patients prescribed cisplatin-containing regimens.

# Patients analyzed	Mean cisplatin dose	Cisplatin naïve (yes/no)	# Patients with clinical AKI	Highest mean/median fold change from baseline during the observation period	Timing of peak change	References
57	63.4 mg/m^2	Mixed	$n=1$	U: ↑ 2.8-fold	Day 10	31
24	70 mg/m^2	Unknown	$n=9$ 20% ↓ eGFR by Day 6 AUC-ROC: 0.55	U: ↑ 2-fold	Day 3	45
22	75 mg/m^2	Yes	$n=8$ AUC-ROC: 0.94	U: ↑ 1.6-fold AKI U: ↑ 1.1-fold no-AKI	Day 3	49
11	<80 mg/m^2	Unknown	$n=10$ Day 3 AUC-ROC: 0.858	U: ↑ 6-fold	Day 7	37
27	80 mg/m^2	Yes	$n=2$	U: ↑ 4.6-fold ($n=27$)	Day 7	50
106	Intraoperative	Unknown	$n=42$	U: ↑ <2-fold ($n=106$)	Day 2	51

AKI: acute kidney injury; AUC-ROC: area under the curve receiver operating characteristic; eGFR: estimated glomerular filtration rate; NE: not evaluated; S: serum; U: urine.
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baseline NAG levels after additional courses of cisplatin treatment (up to 6).[22,32,34] NAG, similar to B2M, did not correlate with accumulated cisplatin dose.[40] Taken together, NAG concentrations increase in the urine of patients with subclinical and clinical AKI within 3 to 5 days after cisplatin treatment but do not reflect the degree of injury or the cumulative dose of cisplatin received.

Kidney injury molecule-1

Kidney injury molecule-1 (KIM-1) is a type I cell membrane glycoprotein (104 kDa) that is highly conserved across rodents, dogs, primates, and humans.[47] KIM-1 is also a phosphatidylserine receptor found on renal epithelial cells that recognizes apoptotic cells and enables their clearance by phagocytosis.[48] During proximal tubular injury, KIM-1 mRNA is up-regulated and the ectodomain of KIM-1 protein (90 kDa) is shed from the brush border membrane into the urine. KIM-1 is a prominent and promising biomarker for various etiologies of AKI and there have been several studies utilizing KIM-1 for cisplatin-induced AKI (Table 4). Urinary KIM-1 concentrations were shown to predictably increase in the presence of clinically detectable cisplatin-induced AKI with the peak value occurring with or following a rise in SCr. Among lung cancer patients ($n=11$), 10 patients were diagnosed with cisplatin-induced AKI on day 3 based on an elevation in BUN > 20 mg/dL and/or a rise of 50% SCr level from baseline.[37] Urinary KIM-1 levels also exhibited time-dependent increases in these patients. In a representative patient receiving cisplatin, urinary KIM-1 concentrations peaked on day 7 as reflected in a 6-fold increase from baseline. Analysis of cisplatin AKI-positive ($n=30$) and AKI-negative ($n=12$) samples revealed a high AUC-ROC value of 0.858 suggesting a strong predictive value between urinary KIM-1 concentrations and a clinical diagnosis of AKI. Similarly, in a study of patients receiving their first cycle of cisplatin-containing chemotherapy (N = 22), eight patients developed AKI by day 3, defined by >1.5-fold elevation in SCr.[49] A significant time-dependent elevation in absolute and creatinine-normalized KIM-1 levels was detected in the AKI group and peaked on day 3. The AUC-ROC value for KIM-1 was 0.94 on day 3 after cisplatin treatment. Notably, serum KIM-1 concentrations did not differ between the AKI and non-AKI groups at any of the time points. In another study of cisplatin-naïve patients treated with cisplatin for various solid tumors ($n=27$), two patients developed a 50% increase in SCr.[50] The absolute and creatinine-normalized concentrations of KIM-1 in all patients increased between days 3 and 14, with a peak on day 7.

Interestingly, many studies have also shown that patients with subclinical AKI also display time-dependent increases in urinary KIM-1 levels.[31,49,51] However, the clinical relevance of KIM-1 changes in subclinical AKI is not entirely clear. For example, KIM-1 displayed a diminished ability to predict nephrotoxicity at three days (AUC-ROC value 0.55) when using a modest change in eGFR to diagnose nephrotoxicity (20% decline in eGFR) in urothelial carcinoma patients with AKI ($n=9$) or no-AKI ($n=15$).[45] Nonetheless, even patients with <20% decline in eGFR exhibited time-dependent elevations in urinary KIM-1 concentrations. Another published study in cisplatin-treated patients with subclinical AKI ($n=57$) supports this trend.[31]

Neutrophil gelatinase-associated lipocalin

The NGAL gene encodes for a small protein (25 kDa) bound to gelatinase that is secreted from human neutrophils.[52–54] NGAL sequesters iron and prevents bacterial growth. In a transcriptomic study to identify ischemia-related genes, NGAL was significantly induced within the kidneys of mice.[55] Additional work revealed that NGAL is up-regulated and enriched in proliferating mouse proximal tubule cells after ischemia.[55] Secretion of NGAL protein into the urine has recently gained interest for use as a biomarker of AKI. There is a robust time-dependent increase in urinary NGAL protein concentrations from 0.5 to 3 days after cisplatin therapy that largely precedes elevations in SCr (Table 5).[21,56,57] In one pediatric study, the dose of cisplatin correlated with post-cisplatin

Table 5. Changes in urinary neutrophil gelatinase-associated lipocalin concentrations in patients prescribed cisplatin-containing regimens.

# Patients analyzed	Mean cisplatin dose	Cisplatin naïve (yes/no)	# Patients with clinical AKI	Highest mean/median fold change from baseline during the observation period	Timing of peak change	References
60	50 mg/m^2	Yes	13% ↓ eGFR Day 5	U: ↑ 1.5-fold	Day 0.08	59
24	50–80 mg/m^2	Unknown	$n=2$ AUC-ROC: 0.80	U: ↑ 2.5-fold in AKI subjects U: ↑ 10-fold in no-AKI subjects	Day 2	56
21	56 mg/m^2 Total dose = 129 mg/m^2	No	$n=10$ >50% ↑ SCr AUC-ROC: 0.64 (Day 1) Age-adjusted	U: No change up to Day 4		58
57	63.4 mg/m^2	Mixed	$n=1$	U: No change on Day 3 or 10		31
24	70 mg/m^2	Unknown	$n=9$ 20% ↓ eGFR by Day 6 AUC-ROC: 0.64	U: ↑ 1.3-fold	Day 3	45
33	75 mg/m^2	Yes	10/33 by Day 4 25% ↓ eGFR	U: ↑ 4-fold in AKI No increase in no-AKI	Day 2 AKI	21
11	<80 mg/m^2	Unknown	$n=10$ Day 3 AUC-ROC: 0.608	U: ↓ 6-fold	Day 7	37
46	140 mg	Yes	$n=12$ 25% ↑ SCr	U: ↑ >60-fold in AKI	Day 7	57
14	Unknown	Unknown	None	U: ↑ 2.3-fold cisplatin	Unknown	60

AKI: acute kidney injury; AUC-ROC: area-under-the curve receiver operating characteristic; eGFR: estimated glomerular filtration rate; NE: not evaluated; S: serum; U: urine.
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infusion concentrations of NGAL in urine ($r=0.73$, $n=21$).[58] Moreover, urinary levels of NGAL have been suggested as a means to differentiate patients with clinical AKI versus subclinical or no AKI. In one clinical study, significant elevations in NGAL at day 1 preceded a modest increase in SCr on day 3 (20%) prior to a maximal SCr increase on day 7.[57] In fact, urinary NGAL levels were significantly higher in patients with AKI at days 1, 2, 3, and 15 after cisplatin compared to those not exhibiting clinical AKI. Importantly, an increase in NGAL at day 2 was found to be a significant independent predictor of AKI. A study of patients ($n=33$) revealed an AUC-ROC value for NGAL of 0.87 at 12 and 24 h.[21] Other studies have also revealed similar findings with an AUC-ROC value of 0.80 at 24 h signifying important predictive value for NGAL to reflect AKI.[56] Recent data also suggest that elevations in urinary NGAL may even be able to detect AKI as early as 2 h.[59] Interestingly, patients with a slow recovery from AKI had earlier and higher increases in urinary NGAL levels.[57] The time points selected for NGAL quantification are important. Several studies that assessed urinary NGAL at or past day 3 have a lesser ability to detect nephrotoxicity in patients receiving cisplatin.[31,37,45] Lastly, urinary NGAL may also identify AKI following treatment with other platinum analogs. In one study, urinary NGAL concentrations increased 2.3-fold, 2.7-fold, and 1.5-fold from baseline in response to chemotherapy containing cisplatin, carboplatin, and oxaliplatin, respectively.[60]

Notably, concentrations of NGAL in serum have little ability to detect cisplatin-induced AKI.[57,61] Serum NGAL levels were similar in patients with and without cisplatin-induced AKI.[57]

Cystatin C

Cystatin C is part of a family of cysteine proteinase inhibitors (13 kDa) expressed ubiquitously in all nucleated cells.[62] Cystatin C is primarily eliminated by glomerular filtration, and therefore serum cystatin C concentrations have been used as an endogenous marker of GFR. Since cystatin C is also catabolized by renal tubular epithelia, the presence of cystatin C in the urine indicates tubular injury. Most clinical studies investigating the relationship between cystatin C and cisplatin AKI have largely focused on changes in the serum rather than the urine (Table 6). Serum cystatin C concentrations were elevated 41% by day 3 in patients receiving cisplatin ($n=27$).[50] Smaller changes (1.1-fold) in serum cystatin C have been reported in other studies where no or minimal clinical AKI had occurred.[59,62,63] Caution should be taken when interpreting changes in serum cystatin C as levels can be affected by corticosteroids, hyperthyroidism, and other conditions.[64–66] Because of the modest changes observed in serum and the sensitivity of this protein to be influenced by concomitant diseases, cystatin C may not be an ideal biomarker for cisplatin AKI.

There is little change in urinary cystatin C concentration in the first 8 h of cisplatin treatment[21]; however, 2-fold increases may be evident at later time points (day 3).[31] Additional work is needed to characterize the utility of

Table 6. Changes in serum and urinary cystatin C concentrations in patients prescribed cisplatin-containing regimens.

# Patients analyzed	Mean cisplatin dose	Cisplatin naïve (yes/no)	# Patients with clinical AKI	Highest mean/median fold change from baseline during the observation period	Timing of peak change	References
60	50 mg/m^2	Yes	13% ↓ eGFR Day 5	S: ↑ 1.1-fold	Day 5	59
35	50 mg/m^2	Unknown	No SCr change	S: ↑ 1.1-fold	Day 5	62
34	60 mg/m^2 (3 cycles)	Yes	NE	S: ↑ 1.1-fold After first cycle	Days 14–28	61
57	63.4 mg/m^2	Mixed	N = 1	U: ↑ 1.9-fold	Day 3	31
33	75 mg/m^2	Yes	10/33 by Day 4 25% ↓ eGFR	U: No change at 8h No difference in AKI and no-AKI groups		21
27	80 mg/m^2	Yes	N = 2	S: ↑ 41% $n = 9$ ↑ 50%	Day 3	50
123	120 mg/day	Yes	NE	S: Up to ↑ 1.1-fold	Day 21	67

AKI: acute kidney injury; eGFR: estimated glomerular filtration rate; NE: not evaluated; S: serum; U: urine.
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urinary cystatin C concentrations for identifying subclinical and clinical AKI in oncology patients receiving cisplatin.

Calbindin

Calbindin is a 28 kDa calcium-binding protein found primarily in the distal tubules and collecting ducts of the kidneys.[68] The mechanistic role for calbindin in kidney injury is unknown; however, two contradictory studies in cisplatin-treated rats have observed either an increase or decrease of calbindin in the urine and an elevation in serum calbindin.[69,70] Two studies have quantified urinary calbindin concentrations following cisplatin administration in cancer patients (Table 7). In both studies, calbindin levels were enhanced in the urine in the absence of clinically detectable AKI. There was a 23-fold increase in urinary calbindin and a 7-fold increase in serum calbindin which peaked by day 10 in cisplatin-treated patients ($n = 14$).[36] In this study, patients receiving the analog carboplatin did not exhibit changes in urinary calbindin concentrations. Similarly, another study revealed a 8-fold increase in urinary calbindin by day 10 after cisplatin infusion, in the absence of SCr changes.[31] These data suggest calbindin excretion into urine is elevated in response to cisplatin treatment; however, further studies are required to understand the mechanistic role of calbindin in AKI.

Insulin-like growth factor binding protein-7 and tissue inhibitor of metalloproteinase-2

Insulin-like growth factor binding protein-7 (IGFBP7) (30 kDa) and tissue inhibitor of metalloproteinases-2 (TIMP2) (68 kDa) are cell-cycle arrest proteins released into urine upon kidney injury.[74,75] IGFBP7 is expressed in proximal tubules and up-regulated upon insult, whereas TIMP2 is constitutively expressed in the distal tubules of human kidneys.[76] The U.S. FDA has approved an immunoassay, the NephroCheck® test which detects TIMP2 and IGFBP7 in the urine and calculates their product to generate an AKIRisk® Score within 20 min. The intended use of NephroCheck® is for the clinical risk assessment for moderate to severe AKI within the next 12 h specifically in ICU patients (at least 21 years of age) who have or have had acute cardiovascular and/or respiratory compromise within the past 24 h. There has been interest in assessing the utility of NephroCheck® for other causes of AKI including drug-induced toxicity. In one clinical study, 4 of 32 patients receiving cisplatin developed AKI, and the AUC-ROC value for TIMP2×IGFBP7 was 0.92 within 72 h.[73] However, another study that measured the two biomarkers individually found that there was no change in IGFBP7 concentrations 24 h after cisplatin administration, whereas a 1.1-fold increase in TIMP2 levels could be observed.[71] While 13 patients developed AKI in this study, the AUC-ROC value for the product of TIMP2×IGFBP7 was only 0.46 at 24 h. A third study of 46 patients also showed no significant changes from baseline for either TIMP2 or IGFBP7 as well as for TIMP2×IGFBP7 at days 3 and 10 after cisplatin treatment.[72] However, additional studies are required to further validate this test in cisplatin-treated patients and to find the optimal time-points for clinical use in ambulatory patients at risk of AKI.

Additional urinary proteins

Other promising urinary protein biomarkers have been evaluated in studies of patients receiving cisplatin. Clusterin is a secreted protein (80 kDa) up-regulated during kidney injury[77] that possesses anti-apoptotic properties (reviewed in Rosenberg and Silkensen[78]). In two studies, there was a 2- to 2.5-fold increase in urinary clusterin concentrations between days 7 and 10 following cisplatin treatment.[31,50] Similarly, the urinary protein monocyte chemotactic peptide-1 (MCP-1) (13 kDa) has shown some promise as an indicator of subclinical or clinical AKI.[79] MCP-1 is a potent proinflammatory chemokine that plays a role in monocyte recruitment to sites of injury and was found to be increased in the proximal tubules and urine following cisplatin injury in rats.[80] MCP-1 was elevated 1.7- to 3.8-fold between days 7–10 in patients treated with cisplatin.[31,37] One study also reported an AUC-ROC value of 0.85 for urinary MCP-1 and AKI on day 7.[37]

Table 7. Changes in urinary concentrations of additional proteins in patients prescribed cisplatin-containing regimens.

	# Patients analyzed	Mean cisplatin dose	Cisplatin naïve (yes/no)	# Patients with clinical AKI	Highest mean/median fold change from baseline during the observation period	Timing of peak change	References
Calbindin	14	70 mg	Unknown	None	U: ↑ 23-fold	Day 10	36
	57	63.4 mg/m^2	Mixed	$n = 1$	U: ↑ 8.3-fold	Day 10	31
MCP-1	11	<80 mg/m^2	Unknown	$n = 10$ Day 3 AUC-ROC: 0.85	U: ↑ 3.8-fold	Day 7	37
	57	63.4 mg/m^2	Mixed	$n = 1$	U: ↑ 1.7-fold	Day 10	31
Clusterin	27	80 mg/m^2	Yes	$n = 2$	U: ↑ 2.5-fold	Day 7	50
	57	63.4 mg/m^2	Mixed	$n = 1$	U: ↑ 1.9-fold	Day 10	31
IGFBP7	45	59.9 mg/m^2	No	$n = 13$ No difference between AKI and no AKI	U: No change Day 1		71
IGFBP7	46	64.2 mg/m^2	No	$n = 1$	U: No change Day 3 or 10		72
TIMP2×IGFBP7	45	59.9 mg/m^2	No	$n = 13$ AUC-ROC: 0.46	U: No change Day 1		71
TIMP2×IGFBP7	32	78 mg/m^2	No	$n = 4$ <Day 3 AUC-ROC: 0.92	NE		73
TIMP2×IGFBP7	46	64.2 mg/m^2	No	$n = 1$	U: No change Day 3 or 10		72
TIMP2	45	59.9 mg/m^2	No	$n = 13$ No difference between AKI and no AKI	U: ↑ 1.1-fold	Day 1	71
TIMP2	46	64.2 mg/m^2	No	$n = 1$	U: No change Day 3 or 10		72
GST-pi	57	63.4 mg/m^2	Mixed	$n = 1$	U: ↑ 1.6-fold	Day 10	31
IL-18	57	63.4 mg/m^2	Mixed	$n = 1$	U: No change Day 3 or 10		31
Osteopontin	57	63.4 mg/m^2	Mixed	$n = 1$	U: No change Day 3 or 10		31
TFF3	57	63.4 mg/m^2	Mixed	$n = 1$	U: ↑ 2-fold	Day 10	31

AKI: acute kidney injury; AUC-ROC: area under the curve receiver operating characteristic; eGFR: estimated glomerular filtration rate; GST-pi: Glutathione-*S*-transferase-pi; IGFBP7: Insulin like growth factor binding protein-7; IL-18: Interleukin-18; MCP-1: monocyte chemotactic peptide-1; NE: not evaluated; S: serum; TIMP2: Tissue inhibitor of metalloproteinases-2; TFF3: Trefoil factor 3; U: urine.
Each publication provides different definitions for the diagnosis of clinical acute kidney injury.

Trefoil factor 3 (TFF3) is a small peptide hormone (~9 kDa) secreted by mucus-producing cells and enriched in proximal tubules.[81,82] TFF3 is involved in epithelial surface repair through inhibition of apoptosis and promotion of cell survival and migration.[83] Interestingly, urinary TFF3 concentrations have been found to decline in response to toxicant-induced renal injury in rats (carbapenem, gentamicin, cisplatin).[82,84] Only one study has quantified urinary TFF3 levels in patients receiving cisplatin. In this study, a 2-fold increase from baseline was observed on day 10 in patients that did not exhibit clinical AKI using traditional clinical diagnostic measures.[31] Further studies are required to understand why TFF3 protein concentrations increase in humans, but decrease in rodents, following cisplatin exposure.

The regulation of various isoforms of glutathione-*S*-transferase (GST), an enzyme involved in the detoxification of cisplatin, has been studied in response to cisplatin administration. GST-alpha (51 kDa), which is primarily expressed in proximal tubules following injury[85] (reviewed in McMahon *et al*.[86]), was increased in response to cisplatin injury in rats.[69,87–89] Excretion of GST-pi (47 kDa), a marker for distal tubular damage,[85] was evaluated in the urine of cisplatin-treated patients without clinical AKI and found to be moderately elevated by day 10 (1.6-fold).[31]

There are still other biomarkers that have yet to be evaluated in cisplatin-treated patients including fatty acid-binding protein 1 (FABP1), a 15-kDa protein localized in the cytoplasmic regions of proximal tubules of human kidney.[90,91] Notably, rodents do not express FABP1 in their kidneys. However, mice expressing the human FABP1 protein had increased shedding of FABP1 into urine following cisplatin treatment that correlated with the extent of injury.[92] Quantification of FABP1 in the clinical setting of cisplatin-induced AKI is still lacking. Osteopontin (~40 kDa) has shown great promise as a biomarker in *in vivo* studies of rats treated with cisplatin.[84,93–96] However, only one study evaluated osteopontin in cisplatin-treated patients without clinical AKI and did not detect a significant change on days 3 or 10.[31] Further investigation of these additional proteins is needed to better understand their utility as biomarkers of cisplatin-induced clinical or subclinical AKI.

Conclusion

It is well known that SCr has significant limitations that hinder the sensitive and timely identification of AKI, particularly when there is only a moderate degree of damage to the kidneys. Urinary protein biomarkers play an

important role in our ability to predict drug-induced injuries. In the case of cisplatin, sensitive urinary biomarkers could provide clinicians with greater information to detect kidney injury and could aid in selecting doses for subsequent rounds of chemotherapy. The ideal AKI biomarker should be noninvasive, reflective of the degree of injury, and be unaffected by inter-individual and external variation such as concomitant medications or diseases. The implementation of novel urinary biomarkers in the clinic is lagging due to a lack of sufficient validation and inconsistencies across studies. To address these issues, larger head-to-head studies are needed to identify time-dependent responses and to establish accurate cutoff values and ranges, particularly in cancer patients. Aside from protein biomarkers, the release of microRNAs and exosomes into the urine also shows great promise for recognizing kidney injury in patients. A combination of "omic" strategies, such as protein and microRNA biomarkers, may represent one option for the early and sensitive detection of AKI.

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DECLARATION OF CONFLICTING INTERESTS

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