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# Biomarkers of drug-induced kidney toxicity

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## Abstract

Blood urea nitrogen (BUN) and serum creatinine are imperfect markers of kidney function because they are influenced by many renal and non-renal factors independent of kidney function. A biomarker that is released directly into the blood or urine by the kidney in response to injury may be a better early marker of drug-induced kidney toxicity than BUN and serum creatinine. Urine albumin and urine protein, as well as urinary markers kidney injury molecule-1 (KIM-1), β2-microglobulin (B2M), cystatin C, clusterin, and trefoil factor-3 (TFF-3) have been accepted by the Food and Drug Administration (FDA) and European Medicines Agency as highly sensitive and specific urinary biomarkers to monitor drug-induced kidney injury in preclinical studies and on a case-by-case basis in clinical trials. Other biomarkers of drug-induced kidney toxicity that have been detected in the urine of rodents or patients include IL-18 (interleukin-18), NGAL (neutrophil gelatinase-associated lipocalin), Netrin-1, liver type fatty acid binding protein (L-FABP), urinary exosomes, and TIMP2 (insulin-like growth factor –binding protein 7)/IGFBP7 (insulin-like growth factor binding protein 7), also known as NephroCheck®, the first FDA-approved biomarker testing platform to detect acute kidney injury (AKI) in patients. In the future, a combined use of functional and damage markers may advance the field of biomarkers of drug kidney toxicity. Earlier detection of drug-induced kidney toxicity with a kidney specific biomarker may result in the avoidance of nephrotoxic agents in clinical studies and may allow for earlier intervention to repair damaged kidneys.

## Introduction

The kidney is especially susceptible to drug injury for the following reasons[1]: 1) the kidney receives 20–25% of the resting cardiac output which exposes it to more circulating drug than other organ systems, 2) tubules concentrate the filtrate and thereby are exposed to higher concentrations of drugs, 3) transporters can further increase the intracellular concentrations of drugs, and 4) the tubules have high energy requirements which makes them susceptible to nephrotoxic injury. Drug-induced kidney injury is therefore commonly encountered during drug development. Unfortunately, traditional serum markers of kidney injury such as creatinine or BUN are insensitive markers of AKI. Novel biomarkers play an important role in drug trials by allowing an earlier detection of drug-induced kidney injury. Detecting renal toxicity during preclinical experiments allows for either a reformulation of compounds or complete avoidance of drugs that induce high levels of nephrotoxicity. It is still estimated

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that only 40–60% of animal findings are predictive of toxicities in humans,[2] which suggests that there is room for innovation and improvement in the detection of renal toxicity during drug development.

Serum creatinine and BUN have historically been used to diagnose AKI. AKI is defined as a rise in serum creatinine of at least 0.3 mg/dL or 1.5 times baseline. [3, 4] Creatinine is a small molecule generated in muscle that can serve as a functional marker of AKI, but numerous factors adversely affect its utility to diagnose AKI. For example, the Jaffe reaction creatinine assay is affected by non-creatinine substances including glucose, uric acid, ketones, cephalosporins, furosemide, hemoglobin, paraproteins, paraquat, and diquat which may lead to false elevations in serum creatinine values. [5] Also, serum creatinine may change due to non-renal factors independent of kidney function such as age, gender, race, muscle mass, nutritional status, total parenteral nutrition, infection, protein intake, catabolic states, and volume status. [5–9] Ingestion of creatine supplements or cooked meat can cause increases in serum creatinine, and restriction of dietary protein may result in decreases. Serum creatinine may also change due to the inhibition of renal secretion that is independent of kidney function. For example, medications like trimethoprim, cimetidine, and salicylates alter the tubular secretion of creatinine leading to changes in serum creatinine independent of glomerular filtration rate (GFR). [5, 9] Serum creatinine increases at 2 weeks after moderate to intense resistance training likely due to increased muscle mass. [10] Intense exercise can increase creatinine by increasing muscle breakdown. [11] In addition, serum creatinine is not sensitive to the loss of kidney reserve as evidenced by the very small change in serum creatinine after the loss or donation of one kidney with a normal remaining kidney. [12] Finally, alterations in serum creatinine may lag several days behind actual changes in GFR. [9, 13] BUN is also a marker of kidney function, however, it is also suboptimal for the diagnosis of AKI as it is also altered by non-renal factors such as protein intake, catabolic state, upper gastrointestinal bleeding, volume status, and therapy with high-dose steroids. [6–8]

Because of the inadequacies of creatinine and BUN, there has been a push from investigators and regulatory agencies to consider biomarkers that are released directly into the blood or urine by the kidney to be the more sensitive and specific early markers of drug-induced toxicity. Earlier detection of drug-induced kidney toxicity with a kidney specific biomarker may result in the avoidance of nephrotoxic agents and earlier initiation of specific therapies to repair the damaged kidney. For example, cystatin C has been incorporated into some drug-dosing algorithms for vancomycin because it rises in response to vancomycin toxicity earlier than creatinine. [14]

An ideal biomarker of AKI would allow the early detection of drug-induced kidney toxicity before an increase in serum creatinine and/or BUN, which would differentiate drug-induced kidney toxicity from other causes on AKI and would predict long term kidney outcome and mortality. There may not be one biomarker of kidney toxicity that applies to all drugs and all patient situations as both the characteristics of the drug as well as patient-specific characteristics may affect biomarker performance. A panel of biomarkers will likely be needed to accurately show drug toxicity across an array of conditions.

There are many types of nephrotoxicity (e.g. minimal change glomerulonephritis caused by NSAIDs (Nonsteroidal anti-inflammatory drugs); acute interstitial nephritis caused by NSAIDs antibiotics or proton pump inhibitors; pre-renal failure caused by ACE (Acetylcholinesterase) inhibitors, NSAIDs or diuretics. Because biomarker discovery in drug-induced kidney toxicity has focused on drugs that cause injury to the proximal and distal tubules, this review will focus primarily on drugs that cause injury primarily to areas such as cisplatin, methotrexate, aminoglycosides, tenofovir, radiocontrast dyes, calcineurin inhibitors (cyclosporine and tacrolimus), and amphotericin B.

## Food and Drug Administration (FDA)-accepted urinary biomarkers to monitor drug-induced kidney injury

The first formal recognition of safety biomarkers for regulatory decision-making marks a milestone in the use of biomarkers to help in the development of new drugs. Following the submission of drug toxicity studies and analyses of biomarker performance to the FDA and European Medicines Agency (EMEA) by the Predictive Safety Testing Consortium's (PSTC) Nephrotoxicity Working Group, seven renal safety biomarkers have been qualified for limited use in nonclinical and clinical drug development to help guide safety assessments. [15] The urinary kidney biomarkers kidney injury molecule-1 (KIM-1), albumin, total protein, B2M, clusterin, TFF-3 and cystatin C are accepted by the FDA and EMEA as highly sensitive and specific urinary biomarkers to monitor drug-induced kidney injury in preclinical studies and on a case-by-case basis in clinical trials. [2] These FDA-accepted biomarkers will be discussed first. Promising biomarkers of nephrotoxicity are shown in Table 1.

### KIDNEY INJURY MOLECULE-1 (KIM-1)

KIM-1 is an epithelial cell adhesion molecule found in the proximal tubules that contains a novel immunoglobulin domain. KIM-1 mRNA and protein are expressed at low levels in normal kidneys but are increased dramatically in post-ischemic kidneys. [16–19] There are a large number of animal studies show that KIM-1 production is also increased in proximal tubules after drug-induced injury. [20] Urinary KIM-1 is a noninvasive, rapid, sensitive, and reproducible biomarker when used for the early detection of cisplatin-induced AKI in rats. [19] One day after cisplatin administration, there is a three- to fivefold increase in the urinary KIM-1, but no increase in plasma creatinine, BUN, urinary N-acetyl-beta-glucosaminidase (NAG), glycosuria, or proteinuria. In another study, tissue and urinary expression of KIM-1 were measured following administration of three different nephrotoxins in rats: S-(1,1,2,2-tetrafluoroethyl)-l-cysteine (TFEC), folic acid, and cisplatin, and again marked increases in KIM-1 expression were detected in proximal tubule epithelial cells. [18] Zhou et al. injected rats with gentamicin, mercury or chromium and showed that KIM-1 is more sensitive and specific for early AKI than BUN, serum creatinine or NAG. [21] In models of cisplatin, gentamicin, and cyclosporine toxicity, urinary KIM-1 significantly outperforms serum creatinine and BUN as an early marker of toxicity. [22] The role of KIM-1 was examined in adriamycin toxicity by Kramer et al., and urinary KIM-1 levels correlate with both proteinuria and interstitial damage. [23] Finally, male Sprague-Dawley rats were dosed daily for 1, 3, or 5 days with known nephrotoxicants gentamicin, bacitracin, vancomycin, and

cisplatin, or known hepatotoxicants ketoconazole, 1-naphthyl isothiocyanate and 4,4-diaminodiphenylmethane. [24] Histopathologic evaluation and clinical chemistry revealed renal proximal tubular necrosis in rats treated with the nephrotoxicants, but none from those treated with the hepatotoxicants. A profile of 48 genes was measured in the kidney and the genes displaying the highest expression changes are KIM-1, lipocalin 2 (Lcn2), and osteopontin.

There are many studies showing that urinary KIM-1 is an early biomarker of ischemic AKI in humans. [20] Human studies of urinary KIM-1 as a biomarker of nephrotoxicity are fewer and focus predominantly on cisplatin toxicity. For instance, urinary KIM-1, monocyte chemoattractant protein-1 (MCP-1), neutrophil gelatinase-associated lipocalin (NGAL), NAG, and β2-microglobulin (B2M) were measured in 11 lung cancer patients the day before cisplatin administration and on days 3, 7, and 14 after cisplatin administration. [25] Urinary KIM-1 and MCP-1, but not NGAL, NAG, or B2M, are significantly higher in patients with AKI. ROC curve analyses revealed that urinary KIM-1 and MCP-1, but not NGAL, can detect cisplatin-induced AKI with high accuracy. In another study evaluating a longitudinal cohort of 108 patients with malignant mesothelioma receiving intraoperative cisplatin therapy, urinary KIM-1 as well as microRNA (miR)-21, −200c, and −423 increased significantly over time after cisplatin administration but could not be used to distinguish patients with or without AKI. [26] In a study of 57 patients with solid tumors receiving outpatient cisplatin therapy ( 25 mg/m$^2$), urine was collected at baseline, 3 days and 10 days. Serum creatinine is largely unchanged after cisplatin infusion. Urinary B2M is threefold higher by day 3, and urinary KIM-1 and TFF-3 are elevated twofold by day 10. [27] In another study of 22 patients on cisplatin treatment, urinary KIM-1 was measured before treatment and on days 1, 3 and 5 after treatment. The increase in urinary KIM-1 precedes the increase in creatinine in patients that developed AKI, and KIM-1 predicts cisplatin-induced AKI in early stages with high sensitivity and specificity. [28] However, in a study of 24 patients with urothelial carcinoma who received cisplatin-based chemotherapy, 9 of the 24 patients with more than 20% decline in eGFR did not have significant elevations in urinary KIM-1, NGAL, or NAG on day 3. [29] Less is known about the performance of KIM-1 following administration of other nephrotoxins; but in premature neonates, urinary KIM-1 increased after gentamicin treatment. [30]

In summary, in animal models of cisplatin, gentamicin and cyclosporine toxicity, urinary KIM-1 significantly outperforms serum creatinine and BUN as an early marker of toxicity. In humans that develop cisplatin-induced AKI, urinary KIM-1 consistently increases before serum creatinine.

## ALBUMINURIA AND PROTEINURIA

Albumin is a high molecular weight protein (66.5 kDa) that is not normally detected in urine in quantities greater than 30 mg/g creatinine. Albumin can be found in the urine of patients with glomerular or tubular kidney injury, but higher levels of urinary albumin are usually observed with glomerular injury, with tubular injury generally showing less than 500 mg/24 h of albumin excretion. [31]

The roles of albuminuria and proteinuria as markers of drug-induced nephrotoxicity have been most thoroughly investigated in patients receiving cisplatin. In the study referenced above, among 57 patients that received cisplatin, there is a 2-fold increase in urinary albumin on day 10 after cisplatin independent of AKI. [27] In another study of 33 patients receiving cisplatin, there is a 5.6- fold increase in urinary albumin on day 4 in AKI patients and a 3.4-fold increase on day 3 in no AKI patients. [32] In 41 patients that received a mean dose of cisplatin of 100 mg/m$^2$ over 5 days, there are large increases in urinary albumin after cycles 1 and 2 of cisplatin.[33] The effect of three cycles of high-dose cisplatin (40 mg/m2 day for 5 days) on renal tubular function was determined in 30 patients. [34] Proteinuria, albuminuria, and aminoaciduria, together with an increase of B2M and NAG excretion rates are observed during each treatment cycle. [34] These studies made clear that urinary tubular proteins increased after each dose of cisplatin; however, it is unclear whether increases in urinary protein correlate with either the development of clinically significant AKI as measured by serum creatinine or the need for dialysis.

In addition to cisplatin, the impact of cyclosporine on levels of proteinuria has been investigated in both animals and humans. In the kidneys of pups of pregnant rats treated with cyclosporine, glomeruli number is lower, systolic and diastolic blood pressure are increased, and albuminuria is increased versus the controls. [35] Male Wistar rats administered cyclosporine (20 mg/kg/day) for 21 days alone have increased serum creatinine, and increased spot urine albumin-creatinine ratio. [36] Other studies showed that cyclosporine induces proteinuria in rats, [37] and importantly that proteinuria is a sign of cyclosporine nephrotoxicity.[38]

In summary, much higher levels of urinary albumin are observed with glomerular injury than with tubular injury. In rodents, albuminuria is a marker of cisplatin nephrotoxicity. Cisplatin increases albuminuria, but the clinical relevance is uncertain.

## BETA-2 MICROGLOBULIN (B2M)

B2M is an 11 kDa protein that is produced by all cells expressing major histocompatibility complex (MHC) class I antigen, the main source being activated lymphocytes. [39] Synthesis is increased in disease states in which there is increased proliferation of lymphoid cells such as infection, auto-immune diseases, or certain neoplasms. B2M is freely filtered by the glomerulus and completely reabsorbed by proximal tubular cells. Impaired uptake as a result of tubular injury results in increased B2M urinary excretion, and thus B2M is considered a direct marker of tubular dysfunction. However, increased B2M production or isolated glomerular disease may increase urinary excretion as well. [2]

B2M has become one of the most commonly utilized urinary proteins for monitoring cisplatin-induced AKI in patients. [40] In one study, 20 patients with ovarian cancer treated with cisplatin were followed for 24 weeks to evaluate the nephrotoxicity of cisplatin. [41] After each cisplatin administration, there is a prompt, reversible, and dose-dependent increase in the urinary excretion of B2M. However, associations with B2M increase and the subsequent development of clinical AKI were not studied. In the study of 57 patients treated with cisplatin described above, B2M is threefold higher by day 3 which is an earlier observed increase than that seen in urinary KIM-1, TFF3, or calbindin. [27]

To determine the value of B2M as an indicator of long-term cisplatin-induced nephrotoxicity, chromium-51 labeled ethylenediamine tetraacetic acid ($^{51}$Cr-EDTA) clearance and urinary excretion of B2M were measured in 41 patients receiving cisplatin. [51] Cr-EDTA clearance decreases to nearly half after three cycles of cisplatin and remains at this decreased level during the observation period (24 months). B2M levels dramatically increase in the urine after cycles 1, 2 and 3 of cisplatin chemotherapy. [33] However, in another study looking at $^{51}$Cr-EDTA in 18 patients treated with cisplatin, the degree of GFR reduction correlates neither with peak B2M excretion nor the time to peak levels.[42] The study concluded that it was not possible to predict the long-term nephrotoxicity of cisplatin by measuring urinary B2M.

There have also been trials in which B2M increases does not occur in response to cisplatin administration. For instance, five courses of cisplatin (100 mg/m$^2$) were given to 22 patients with ovarian cancer. [43] Serum creatinine, creatinine clearance, urinary osmolality, and urinary B2M were within the reference ranges and did not change significantly in any patient. [43]

B2M has also been extensively studied as a marker of cyclosporine toxicity. In heart transplant patients, there is a significant positive correlation between serum B2M level and blood cyclosporine concentration suggesting that serum B2M can be used to define cyclosporine tubular toxicity. [44] In 77 bone marrow transplant recipients, the highest levels of B2M was found during renal function impairment due to cyclosporine-induced nephrotoxicity. [45] In 12 patients treated with cyclosporine for rheumatoid arthritis, serum creatinine increased by a mean of 50% in nine of 11 patients followed for 26 weeks, and urinary B2M excretion was significantly increased in these cases. The authors concluded that urinary B2M excretion is a very sensitive parameter for renal tubular damage.[46]

Acute rejection and acute cyclosporine toxicity are common causes of kidney allograft dysfunction in the early post-transplant period. The question is whether B2M can be used to differentiate acute rejection from acute cyclosporine toxicity. B2M was measured daily in serum and urine in 49 patients undergoing renal transplantation. [47] In cases of cyclosporine toxicity, B2M was increased in the urine while serum B2M decreased. In cases of acute rejection, there was an increase in serum B2M with only a moderate elevation in urine. The study concluded that parallel determination of B2M in serum and urine can differentiate between cyclosporine nephrotoxicity and rejection in 91% of the cases. [47] Thirty-seven patients followed for 4 weeks after kidney transplant were divided into 4 groups based on post-transplant course: normal, acute rejection, cyclosporine nephrotoxicity and delayed graft function (defined as the need for dialysis in the first week after kidney transplant). [48] High urinary B2M preceded the increase in serum creatinine in cyclosporine nephrotoxicity and urinary B2M decreased with decrease in cyclosporine dose. However, in 55 renal transplant patients, there was an increase of glomerular proteins like albumin in the urine but no increase in tubular proteins like urine B2M in either acute rejection or acute cyclosporine toxicity. [49] In a study of 30 patients, urinary B2M was increased with biopsy-proven rejection compared to kidney transplant patients without rejection. [50] Thus there is no widely accepted pattern of urinary B2M that differentiates acute rejection from acute cyclosporine toxicity and more studies are needed to precisely determine the role of urinary

B2M as a marker of kidney diseases. [51] A complicating factor is that serum B2M can be a marker of kidney function [51] and B2M can be cleaved in an acid urine.[52]

Toxicity due to gentamicin and other aminoglycosides has been shown to increase urine B2M. In an animal study, 16 urinary biomarkers were measured in rats after gentamicin administration. Only B2M and albuminuria showed an increase that correlated with histopathology. [53] Rises in B2M after gentamicin use have also been demonstrated in neonates [54] as well as in adults. [55] In a study of 52 aminoglycoside-treated patients, serum creatinine and daily 24-hour urinary B2M were measured. [56] An elevation in B2M excretion above the baseline value occurred in 37 out of 52 patients while the serum creatinine concentration rose in only 17 out of 52 patients. An increased B2M excretion greater than 50 mg/day preceded the serum creatinine rise by 2 to 7 days. An abnormal baseline B2M is not a risk factor for a subsequent rise in creatinine concentration or vice versa.

B2M has been investigated less thoroughly in the setting of treatment with other known nephrotoxins, but similar results have been observed. For instance, in children receiving ifosfamide without cisplatin, proximal tubular toxicity was indicated by increased urine B2M excretion (n = 11), and generalized aminoaciduria (n = 10) in 10 out of 11 children. [57] Urinary B2M has also been shown to predict renal dysfunction in HIV-infected patients initiated on tenofovir-containing antiretroviral therapy. [58–59]

In summary, urinary B2M increases after administration of a number of nephrotoxic agents including cisplatin, cyclosporine, and gentamicin. However, the link between an increase in urinary B2M and the development of clinical AKI remains uncertain. Furthermore, the correlation between short-term B2M rise and long-term GFR reduction remains unclear based on current data.

## CLUSTERIN

Clusterin is a glycosylated protein involved in both apoptotic and anti-apoptotic pathways and is found in a number of organ systems including the kidney. [60] Clusterin has two known isoforms in humans, a nuclear form that is pro-apoptotic, and a secretory form that is anti-apoptotic. [61] Both isoforms are involved in a variety of cellular functions including DNA repair, cell cycle regulation, and apoptotic cell death. Clusterin expression has been associated with tumorigenesis and the progression of various malignancies. In the kidney, clusterin is found in the tubules where it has anti-apoptotic effects and mediates cell protection, lipid recycling, cell attachment, and aggregation. [60] After tubular injury, clusterin gene expression is known to be upregulated. Importantly, the clusterin protein is not thought to be able to be filtered through the glomeruli, and thus its detection in urine is considered to be an exclusive marker of damage to tubular cells.[60]

Clusterin in the urine has been studied as a biomarker of nephrotoxicity in animals, but few studies are currently available in humans. In a large series of studies featuring 739 animals designed to assess the value of nephrotoxicity markers (urinary total protein, cystatin C, B2M, and clusterin), urinary clusterin outperforms BUN and serum creatinine in the detection of proximal tubular injury elicited by cisplatin, gentamicin, vancomycin, tacrolimus, puromycin, and doxorubicin. As expected, given that clusterin is not found in

glomeruli or freely filtered, it does not perform as well as other markers in the detection of glomerular injury. [62] Another large series of 22 rat studies evaluated 12 kidney toxicants and 10 compounds with toxicities that induced combinations of both tubular and glomerular disease. A total of 12 urinary biomarkers were evaluated, and KIM-1, clusterin, and albumin were the most sensitive and specific markers of drug- induced tubular injury. [63] Clusterin again performed poorly in the detection of glomerular injury.

To further ascertain the value of clusterin in different kidney diseases, clusterin was measured in urine in animal models of renal ischemia, polycystic kidney disease, and focal segmental glomerulosclerosis (FSGS). In the models of bilateral renal ischemia and polycystic kidney disease (PKD), both diseases that impact tubules, there is a significant increase in proteinuria and in the urinary excretion of clusterin. Rats with FSGS have pronounced proteinuria and albuminuria but do not excrete increased levels of clusterin in urine, further demonstrating that clusterin is useful in differentiating between tubular and glomerular forms of proteinuria. [64] Finally, another important study in male Sprague-Dawley and Han-Wistar rats given cisplatin, gentamicin, or N-phenylanthranilic acid (NPAA) evaluated clusterin along with 3 other urinary markers (α-glutathione-S-transferase (α-GST), μ-GST, and renal papillary antigen-1 (RPA-1)). Clusterin correlates well with injury to multiple areas of the nephron including the proximal tubule, distal tubule, and collecting duct, particularly when tubular regeneration is present. [65] These data were submitted for qualification review by the EMEA and FDA who concluded that these biomarkers can be used in conjunction with traditional clinical chemistry markers and histopathology in good laboratory practice rodent toxicology studies [15].

Clusterin has been specifically evaluated with cisplatin use and was found to increase in the urine after both low and high dose cisplatin. [66] In one study, male Sprague-Dawley rats were treated with cisplatin for 1, 3, 5, 7, or 14 days at 1 mg/kg/day. After one day of treatment there were increases in urinary clusterin; positive KIM-1 immunostaining was also observed in the outer medulla of the kidney in the absence of functional effects. After 3 days of treatment, clusterin immunostaining was positive, and KIM-1 protein levels in urine increased more than 20-fold. Urinary clusterin and tissue KIM-1 were found to be the most sensitive biomarkers for the detection of cisplatin-induced kidney damage, which accurately corresponded with histopathologic damage.[67]

In a study of gentamicin nephrotoxicity, urinary clusterin was compared to NAG in rats given gentamicin daily for 2 months. [68] While the excretion rates of both proteins rose rapidly, peaked, and then declined, only clusterin values stayed significantly above control values throughout the entirety of the study.

In summary, clusterin performs well in the detection of proximal tubule injury, including nephrotoxic injury induced by cisplatin or gentamicin. Evidence suggests that clusterin along with KIM-1 are two of the earliest markers of proximal tubular injury. However, clusterin has the added benefit of reflecting injury to multiple territories of the nephron including the distal tubule and collecting duct, but not glomerulus-which is advantageous for biomarker use and interpretation. It is important to note, though, that nearly all data on clusterin are derived from animal models, and that human clinical data on the correlations

between urinary clusterin and short and long-term outcomes have not been adequately studied. [2]

## TREFOIL FACTOR 3 (TFF3)

TFF3 is a small peptide hormone secreted by mucus-producing epithelial cells especially within the gastrointestinal tract and it is involved primarily in the protection and restoration of epithelial surfaces. In the kidney, it is produced by cells of the collecting ducts, but its physiological function within the kidney is not known. [2]

TFF3 levels are increased significantly in the setting of chronic kidney disease (CKD) in the serum [69] as well as urine. [70] Certain patient populations (African descent, diabetes and antihypertensive medication use) have higher baseline urinary TFF3 concentrations, which might indicate ongoing repair of chronic damage in the kidneys, and might predict progression of disease.[71] TFF3, along with 14 other biomarkers, was evaluated in 2,948 Framingham Heart Study participants. Urinary TFF3 elevations predicted all-cause mortality, and were associated with coexistent kidney disease at the time of death. [72] More human studies are needed, but TFF3 rises in CKD may act similarly to albuminuria in predicting progression and mortality.

In studies of drug-toxicity conducted primarily in rodents, urinary TTF3 levels actually *decreased* rather than increased. One rat study evaluated renal toxicants affecting multiple territories of the nephron. These included six proximal tubule toxicants (cisplatin, gentamicin, carbapenem A, thioacetamide, hexachlorobutadiene (HCB) and D-serine), two papillary toxicants (phenylanthranilic acid (NPAA) and propyleneimine), one glomerular toxicant (doxorubicin), one renal vascular toxicant (cyclosporine A), and one kidney tubulo-interstitial toxicant (allopurinol). Whereas albuminuria increased in the setting of tubule damage, there was a marked reduction in the levels of TFF3. [73] In a study of cisplatin toxicity in rats, there was an increase in the kidney expression of KIM-1 and clusterin, a decrease in TFF3, and no change in B2M five days after 1.0 mg/kg or 2.5 mg/kg cisplatin treatment. [74]

Only one study so far has evaluated urinary TFF3 as a marker of nephrotoxicity in humans, and curiously an increase in TFF3 was observed. In the same study of 57 patients with solid tumors receiving outpatient cisplatin therapy noted previously, both urinary KIM-1 and TFF3 showed a 2-fold elevation by day 10. [27] Why TFF3 increased in the human study but decreased in the rat studies is uncertain.

In summary, TFF3 shows promise as a prognostic marker in clinical and subclinical CKD, but very little is known about the performance of TFF3 with regards to nephrotoxicity. In cisplatin nephrotoxicity studies, TFF3 fall in rat models but rise in human patients treated for solid organ tumors. The role of TFF3 in nephrotoxicity needs further investigation, especially in human subjects.

## CYSTATIN C

Butler et al. in 1961 studied the urine proteins of 223 individuals by starch gel electrophoresis and found a new urine protein fraction in the post gamma globulin fraction

that was named cystatin C. [75] Cystatin C is approximately 13 KdA and is produced by all nucleated cells; It is freely filtered by the glomerulus, and is completely reabsorbed by the proximal tubules.[76] While serum cystatin C is a functional marker of GFR similar to serum creatinine, it is not affected by muscle mass, diet, gender, or tubular secretion. However, the accuracy of serum cystatin C is adversely impacted by abnormalities of thyroid function [77] and glucocorticoid therapy. [78–79]

In studies using $^{51}$Cr-EDTA clearance as the reference, serum cystatin C was identified as a more accurate measure of GFR than creatinine. [80] Serum cystatin C and cystatin C based formulae are as good in estimating GFR as the Modification of Diet in Renal Disease (MDRD) formula. [81] Serum technetium- 99m diethylenetriamine pentaacetic acid (Tc 99m DTPA) clearance best correlates with creatinine clearance (r = 0.957) and cystatin C (r = 0.828), compared to B2M (r = 0.767) and creatinine (r = 0.682). [82] In another study, serum creatinine, serum cystatin C and the clearance of the iodinated contrast dye iopromide (reference standard) were compared in 127 patients undergoing cardiac catheterization. Serum cystatin C showed a higher non-parametric correlation (r = 0.805) to the iopromide clearance that either serum creatinine (r = 0.652) or Cockcroft-Gault estimation (r = 0.690). [83]At a multinational meeting held in 2002 in Germany,[84] it was agreed that 1) serum cystatin C is at least equal if not superior to serum creatinine as a marker of GFR; 2) serum cystatin C is independent of height, gender, age, and muscle mass; 3) certain patients such as children, the elderly, and those with reduced muscle mass may benefit from its use as a marker of GFR.

Urinary cystatin C is an early biomarker of ischemic AKI and nephrotoxicity in humans.[85] Because cystatin C is fully reabsorbed in the proximal tubules under normal conditions, its presence in the urine indicates tubular dysfunction.

Cystatin C has been used in numerous studies of nephrotoxicity. Cisplatin is the agent with the most cystatin C nephrotoxicity data to date, but results have been mixed. In some studies of patients receiving cisplatin, serum cystatin C increased as expected. [86–87] For example, in 41 patients that received cisplatin, there was a 21% increase in serum cystatin C levels that correlate with a 23% loss of inulin clearance. There were no significant changes in serum creatinine levels.[88] A cystatin C based equation was also shown to accurately estimate GFR in children with solid and CNS tumors receiving nephrotoxic chemotherapy. [89] However, other studies looking at GFR calculated by serum cystatin C were not found to be reliable compared to renal scintigraphy [90] or even compared to serum creatinine. [91] Urinary cystatin C, similar to serum cystatin C, has also had mixed results as a marker of cisplatin-induced nephrotoxicity. In 33 patients that received 75 mg/m$^2$ of cisplatin, there was no increase in urinary cystatin C at 8 hours after cisplatin and no difference in urinary cystatin C between AKI and non-AKI groups. [32] However, in another study, there was a 2-fold increase in urinary cystatin C at 3 days after cisplatin administration. [27] In a meta-analysis of 6 studies, cystatin C also had better diagnostic accuracy than creatinine as a test for glomerular dysfunction in 206 children undergoing treatment for cancer.[92]

Serum cystatin C has been investigated as an early marker of nephrotoxicity with agents besides cisplatin, often with better results. In one study, 54 patients received amphotericin B

for 1 week, and serum cystatin C and creatinine were measured at days 0, 7, and 14 after treatment. The Cystatin C-based GFR equation correlated significantly with the Cockcroft-Gault estimate at the early time period of treatment with amphotericin B. [93] In patients that received radiocontrast dye, the increase in serum cystatin C paralleled the increase in serum creatinine [94] or, in another study, increased earlier than serum creatinine. [95] Serum cystatin C concentration alone was shown to be a sensitive marker to monitor renal function during and after high dose methotrexate infusion in pediatric leukemia patients. [96] Serum cystatin C was a better marker of GFR than serum creatinine concentration or creatinine clearance in cystic fibrosis patients receiving amikacin, and serum cystatin C levels and cystatin C clearance showed greater ability to predict amikacin clearance during therapy than creatinine clearance. [97] In a rat model of colistin nephrotoxicity, serum cystatin C correlated better with tubular injury on histology than does serum creatinine. [98] In 81 children receiving aminoglycosides, serum cystatin C was found to be an early AKI biomarker and predictive of persistent AKI. [99] Finally, serum cystatin C has been shown to better predict vancomycin-induced nephrotoxicity than serum creatinine, and has been used to improve medication safety as part of vancomycin drug-dosing algorithms.[100]

In summary, serum and urinary cystatin C show mixed results in the detection of nephrotoxicity due to cisplatin, but serum cystatin C is able to effectively predict AKI due to aminoglycosides, amphotericin B, radiocontrast dye, high dose methotrexate, and vancomycin. Due to its effectiveness to diagnose AKI induced by a variety of agents, serum cystatin C has become an important guide for regulatory decision making in drug development. [62]

## Other biomarkers of drug-induced kidney toxicity

Besides the FDA-accepted biomarker panel discussed above, there are other important biomarkers of drug-induced kidney toxicity reported in the literature. These biomarkers include IL-18, NGAL, Netrin-1, FABP, urinary exosomes, TIMP2 and IGFBP7 (also known as Nephrocheck®).

### INTERLEUKIN-18 (IL-18)

IL-18 is a pro-inflammatory cytokine that is important in both innate and acquired immunity. [101, 102] Activated macrophages expressing high levels of IL-18, and IL-18 is thought to play an important role in host defenses against infections and neoplasms. Other cells that express IL-18 are mononuclear cells, keratinocytes, osteoblasts, intestinal and renal epithelial cells, and dendritic cells.

IL-18 has been widely investigated within the context of ischemic AKI and has consistently been shown in animals to rise quickly following an ischemic insult. Mice injected with IL-18 neutralizing antiserum prior to an ischemic insult had less functional and histological evidence of ischemic AKI. [103, 104] IL-18 binding protein transgenic mice or IL-18 deficient mice were protected against ischemic AKI, and administration of IL-18 binding protein protected against ischemic AKI. [105, 106] In humans, numerous studies from the Translational Research involving Biomarkers of Early Acute Kidney Injury (TRIBE-AKI) network have shown that urine IL-18 increased within 6 hours of an ischemic insult and at least a day

before the increase in serum creatinine. These studies have also shown that urinary IL-18 is a marker of both short- and long-term prognosis in ischemic AKI. [85]

The use of IL-18 as a marker of nephrotoxicity has not been fully investigated, but early studies showed some promise. High dose cisplatin in mice resulted in an increase in IL-18 in kidney and urine. Cisplatin-induced AKI was associated with an increase in urinary interleukin (IL)-1 beta, IL-18, and IL-6. [107] In another study, serum tumor necrosis factor (TNF), serum and urinary IL-18, and urinary KIM-1 were increased on day 3 after high dose cisplatin in mice. [108] Furthermore, while IL-18 increased in the kidney and urine following cisplatin administration, neutralization of IL-18 did not protect against cisplatin-induced AKI in mice demonstrating that IL-18 is a marker rather than a mediator of cisplatin-induced AKI.[107]

Urinary IL-18 is a marker of nephrotoxic AKI in humans, although the number of studies and the sample sizes within those studies are limited. In 23 patients undergoing cisplatin-based chemotherapy, urinary excretion of leucine aminopeptidase, NGAL, cystatin C, liver fatty acid-binding protein (L-FABP), and IL-18 were increased 3 hours after chemotherapy and 72 hours before the increase in serum creatinine. [109] Urine NGAL and IL-18 showed promise as early AKI diagnostic tests in children treated with ifosfamide and may have a potential role in drug toxicity monitoring. [110] Urinary IL-18 or NGAL are early biomarkers of contrast-induced nephropathy (CIN) and urinary IL-18 was associated with cardiac outcomes in patients after coronary angiography. [111, 112] Urinary IL-18 has also been shown to be a marker of chronic nephropathy in children after chemotherapy with ifosfamide, cisplatin, and/or carboplatin.[79]

In summary, IL-18 is a well-known marker of ischemic AKI. Limited data in animals and humans suggests that urinary IL-18 also functions as a marker of nephrotoxin-induced AKI, but these studies are small (see above) and need to be confirmed with larger cohorts of patients.

## NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN (NGAL)

NGAL is a 21-kD protein of the lipocalin superfamily which acts to bind iron during times of infection or inflammation. NGAL is a critical component of innate immunity to bacterial infection and is expressed by immune cells, hepatocytes and renal tubular cells in various disease states. [113–115] NGAL is a small secreted polypeptide that is protease resistant and thus may be easily detected in the urine. NGAL gene products are massively upregulated in the kidney and urine after ischemic AKI in rats and mice. [114] Furthermore, NGAL appears in the urine very early after ischemic AKI and precedes the appearance of other urinary markers such as NAG or B2M. Both urine NGAL and urine IL-18 were increased in the urine 6 hours post cardiac surgery in adults and children that develop AKI. [116, 117] NGAL is arguably the best-studied novel markers and has been tested in a wide range of clinical settings and patient groups.

NGAL has been frequently investigated as a marker of cisplatin nephrotoxicity. NGAL has been detected in the urine of mice in the early stage of cisplatin-induced nephrotoxicity, [33] and has also been shown to be an early biomarker of cisplatin-induced AKI in humans. Nine

studies that looked at urinary NGAL in patients receiving cisplatin were recently summarized, [40] and in 6 of the 9 studies there was an increase in urinary NGAL after cisplatin chemotherapy in patients with and without clinical AKI. [40] The area under the ROC curve for AKI prediction in these studies varied from 0.6 to 0.8.[40]

NGAL has been shown to be an early marker of toxicity in animal studies using aminoglycosides. Repeated administration of gentamicin to male Sprague-Dawley rats for 1, 3, or 7 days resulted in a dose- and time-dependent increase in the expression of KIM-1 and NGAL in the kidney. [118] There was also an early increase in KIM-1 and NGAL in the urine. Changes in gene and protein expressions correlated with worsening histology and preceded the increase in serum creatinine. NGAL was also shown to be a sensitive urinary biomarker of AKI in dogs or rats receiving gentamicin.[119–121] These animal studies demonstrated that NGAL may represent an early, sensitive, noninvasive urinary biomarker for ischemic and nephrotoxic kidney injury.

The usefulness of urine NGAL has been investigated with a variety of other nephrotoxic agents as well. For example, in patients receiving amphotericin B, AKI could be detected 3.2 days earlier through urinary NGAL compared to serum creatinine. [122]

In summary, urinary NGAL is a promising biomarker of cisplatin-induced AKI in humans. Animal studies suggest that NGAL may be valuable in the detection of nephrotoxicity with a number of other agents as well, but larger human studies are required.

## NETRIN

Netrins are laminin-like molecules with a distinctive domain organization [123] that belong to the laminin-related family of axon-guidance molecules.[124] Netrin-1, −3, and −4 are encoded by distinct genes. Mouse netrin-1 shares 52% amino acid identity with mouse netrin-3. Netrins act via two receptors, "deleted in colon cancer" (DCC) and uncoordinated 5 (UNC5). Netrins play a role in axonal guidance including development of mammary gland, lung, pancreas, and blood vessels, inhibition of leukocyte migration and chemoattraction of endothelial cells. [125] Netrin-1 is a potent inhibitor of leukocyte chemoattraction. The kidney has high levels of netrin expression.

Netrin-1 expression is increased early in the tubules during ischemic AKI. In mice, urinary netrin-1 levels increased markedly within 3 hours of ischemia-reperfusion, reached a peak level at 6 hours, and returned to near baseline by 72 hours. Serum creatinine significantly increased only after 24 hours of reperfusion. [127] In humans, netrin-1 was analyzed in urine samples from 10 healthy controls, 22 recipients of a renal allograft, 11 patients with ischemic AKI, 13 with AKI associated with sepsis, 9 with radiocontrast dye-induced AKI, and 8 with drug-induced AKI. Urinary netrin-1 levels normalized for urinary creatinine were significantly higher in patients with AKI compared to healthy controls. [126]

In summary, the value of netrin-1 as a biomarker of early drug-induced kidney injury has not been well examined and requires further study.

## LIVER-TYPE FATTY ACID BINDING PROTEIN (L-FABP)

FABPs are a family of carrierproteins for fattyacids and other lipophilic substances such as eicosanoids and retinoids. FABPs facilitate the transfer of fatty acids between extra- and intracellular membranes. L-FABP binds fatty acids and transports fatty acids to the mitochondria or peroxisomes, where the fatty acids are metabolized via β-oxidation and provide energy for tubular epithelial cells. Human L-FABP is a relatively recent biomarker that is elevated in the urine in a number of renal disease states including ischemia-reperfusion injury, contrast-induced nephropathy, diabetic nephropathy, and, notably, chronic kidney disease (CKD).[127]

L-FABP studies of nephrotoxicity are limited to a handful of animal studies. In one study, urinary L-FABP was measured in mice with ischemic AKI and cisplatin-induced AKI. [50] In both ischemic AKI and cisplatin-induced AKI, urinary L-FABP was increased before BUN. In both conditions, urinary L-FABP showed a better correlation with histology injury scores and GFR, as measured by inulin clearance, than BUN or NAG. In a mouse model of folic acid-induced nephropathy, urinary L-FABP was correlated with the degree of tubulointerstitial damage, and the protein expression levels of human L-FABP in both the kidney and urine significantly correlated with the degree of tubulointerstitial damage, the infiltration of macrophages, and the deposition of type I collagen. [128] Finally, HIV/HCV co-infected patients with lower body weight treated with tenofovir had increased urinary L-FABP.[129]

In summary, L-FABP is a promising and relatively new biomarker, but its role as a marker of early drug-induced kidney injury requires further study.

**Exosomes—**Exosomes are 50–90 nm vesicles that are created inside the cell when a segment of the cell membrane invaginates and is endocytosed. Urinary exosomes can be released from every segment of the nephron as well as from podocytes and can contain molecules not detected otherwise in urine. To illustrate, in one study, exosomes were isolated by differential centrifugation in rats and humans with AKI. [130] The exosomes were found to contain activating transcription factor 3 (ATF3) detected by Western blot. ATF3 was found in the concentrated exosomal fraction, but not in whole urine. ATF3 was present in urine exosomes in rat models of AKI before the increase in serum creatinine. ATF3 was found in exosomes isolated from patients with AKI but not from patients with CKD or the controls.

An exciting element of urinary exosomes is that they may offer insight into cellular regulatory pathways. [130] Exosomes have been found to contain transcription factors which can give information about both the upstream gene activation in AKI and downstream protein production. In the study above, urine was collected from two AKI rodent models (cisplatin or ischemia-reperfusion). ATF3 and Wilms Tumor 1 (WT-1) were found in the concentrated exosomal fraction, but not in whole urine. ATF3 was continuously present in the urine exosomes of rat models following cisplatin and ischemia-reperfusion injury, at times earlier than the increase in serum creatinine, and WT-1 was found to detect early podocyte injury.

Proteomic analysis of urinary exosomes can identify candidate biomarkers for the diagnosis of AKI. For instance, exosomal fetuin-A was identified as an exosomal marker that was increased in rats with cisplatin-induced AKI compared to control rats. [131]

In summary, the discovery of urinary exosomal vesicles has opened a new field of biomarker research [132] which shows significant promise. At present, however, exosomal biomarkers are not ready for use in large-scale drug trials.

## TIMP2 AND IGFBP7

TIMP2 is the product of a gene belonging to a human gene family and TIMP2 proteins are natural inhibitors of the matrix metalloproteinases. TIMP2 can also suppress proliferation of endothelial cells in response to angiogenic factors. IGFBP7 regulates the availability of insulin-like growth factors in tissues and modulates IGF binding to its receptors. IGFBP7 stimulates cell adhesion and cancer growth. TIMP2 and IGFBP7 are also markers of cell cycle arrest. Renal tubular cells enter a period of G1 cell cycle arrest after ischemia or sepsis. Both TIMP2 and IGFBP7 are involved in the $G_1$ cell-cycle arrest phase that occurs during the very early phases of cellular stress. Detection of cell cycle arrest may serve as a biomarker of impending tubular damage in AKI. It is believed that TIMP2 and IGFBP7 are expressed in the tubular cells in response to DNA damage and other forms of injury. [133] TIMP2 and IGFBP7 block the effect of the cyclin-dependent protein kinase complexes on cell-cycle promotion which results in G1 cell-cycle arrest for short periods of time to prevent damaged cells from dividing.

A combination of urinary TIMP2 and IGFBP7, known as NephroCheck®, is the first platform approved by the FDA; it is marketed as a biomarker of AKI and its approved use is currently limited to critically ill patients. An AKI risk score (which includes urinary [TIMP2] x [IGFBP7] in the calculation) of greater than 0.3 has been shown to be an accurate predictor of renal outcomes and mortality in critically ill patients in the intensive care unit (ICU) with a variety of AKI etiologies. [134–136] Urinary [TIMP2] x [IGFBP7] has also been studied in adults and children cardiac surgery. For instance, in 40 adults undergoing transcatheter aortic valve implantation (TAVI), early elevation of urinary [TIMP2] x [IGFBP7] after TAVI was associated with the development of postoperative AKI with a diagnostic accuracy that was superior to that of serum creatinine. [137] In a study of 94 infants, urine [TIMP2]x[IGFBP-7] was significantly higher in patients with AKI at 12 hours after cardiopulmonary bypass initiation; notably, the acute injury score that effectively predicted AKI was greater than that utilized in adults (i.e., 0.78, 95% confidence interval 1.47–6.11). [138] Urinary [TIMP2] x [IGFBP7] was recently the primary biomarker in a successful interventional trial to reduce AKI following cardiac surgery. The effect of a management strategy consisting of optimization of volume status and hemodynamics, avoidance of nephrotoxic drugs, and prevention of hyperglycemia was evaluated in high risk cardiac surgery patients using a cutoff of urinary [TIMP2] x [IGFBP7] > 0.3. [139] AKI was significantly reduced with the intervention compared to the controls, and rates of moderate to severe AKI were also significantly reduced.

Despite these broad successes, [TIMP2] x [IGFBP7] has not been shown definitively to be of benefit in the detection of nephrotoxicity in humans. [140] In one study, urinary [TIMP2] x

[IGFBP7] and serum creatinine levels were measured before and 24 hours after cisplatin administration in lung cancer patients. Cisplatin-associated AKI was detected in 13 patients (28%) out of 45 enrolled. [141] There was no difference between creatinine, IGFBP7 and (IGFBP7 × TIMP2)/1000 (the at risk score) levels before and after treatment with cisplatin. However, in another study, urinary [TIMP2] x [IGFBP7] was found to be a useful tool for early identification of patients who are at risk for cisplatin-induced AKI. [142] In this study, urine samples were collected before cisplatin injection and 12 hours after the end of chemotherapy. Four patients out of 58 developed AKI within 72 hours. [TIMP2] x [IGFBP7] predicted the development of AKI with an impressive area under the receiver operating characteristic curve (AUC; 95% confidence interval) of 0.92 (0.80–1.00).

In summary, [TIMP2] x [IGFBP7] is an FDA approved biomarker that is especially useful in critically ill patients but has not been conclusively shown to predict nephrotoxicity in stable patients. However, its ability to identify early AKI in critically ill patients may allow for reduced dosing of known nephrotoxic medications in the intensive care unit (ICU), which is certainly of use in patient care, if not in drug trials per se.

## Biomarkers reflecting individual response to immunosuppressants

At the recent Barcelona Consensus on biomarker-based immunosuppressive drugs management in solid organ transplantation, biomarkers that help in assessing the risk of rejection and personal response to the immunosuppressant drug were discussed in an effort to help tailor immunosuppression to the needs of the individual patient. [143] In contrast to biomarkers of nephrotoxicity, biomarkers reflecting the individual response to immunosuppressants were discussed. Direct determination of drug targets may help to better assess the individual response to the immunosuppressant. For example, measuring inhibition of calcineurin activity by cyclosporine or tacrolimus, measuring inhibition of inosine-monophosphate-dehydrogenase activity (IMPDH) by mycophenolate, or measuring inhibition of the mTOR complex by everolimus or sirolimus. At the Consensus Conference, recommendations were made for the most appropriate analysis of a proposed panel of preliminary biomarkers, most of which are currently under clinical evaluation in ongoing multi-center clinical trials. [143]

## Conclusion

Drug-induced kidney injury is a problem that frequently hampers the drug development process. Unfortunately, BUN and creatinine are not sensitive markers for AKI, and significant nephrotoxicities can be missed until late in the testing process or even after a drug is approved. For this reason, drug developers and regulatory bodies are turning to novel biomarkers that can directly detect glomerular or tubular damage. In fact, the FDA and EMEA have approved a panel of 7 biomarkers that can be used to detect nephrotoxicity in drug trials.

In this review, we focused on the urinary biomarkers approved by the FDA and EMEA, which are KIM-1, albumin, total protein, B2M, clusterin, TFF3, and cystatin C. We also discussed a number of other markers that may someday play major roles in nephrotoxicity

studies including IL-18, NGAL, netrins, L-FABP, urinary exosomes, and [TIMP2] x [IGFBP7]. Many of these markers have been shown to rise (or fall, in the case of TFF3) early after the administration of known nephrotoxins such cisplatin or gentamicin. However, the correlation between rises in these biomarkers and the development of clinically significant AKI is unclear. Even less is known about the correlation between a change in these biomarkers and long-term impacts on kidney function. While these biomarkers show tremendous promise and may greatly improve the utility of preclinical trials and the safety of clinical trials, there is still a great deal to learn about these novel markers of drug-induced kidney toxicity.

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**Table 1:**

Most promising biomarkers of nephrotoxicity

| Biomarker | Drug |
|---|---|
| Urinary KIM-1 | cisplatin, gentamicin, cyclosporine |
| Albuminuria | cisplatin |
| Beta-2 microglobulin | cisplatin, cyclosporin, gentamicin |
| Clusterin | cisplaitin, gentamicin |
| Serum cystatin C | aminoglycosides, amphoterin B, radiocontrast dye, high dose methotrexate, vancomycin |
| Urine NGAL | cisplatin |

Ngal=Neutrophil gelatinase-associated lipocalin

**Table 2:**

Human studies of biomarkers of nephrotoxicity

| Biomarker | Drug | Study population | End point | Sensitivity/specificity | Ref |
|---|---|---|---|---|---|
| Urine KIM-1 | days 3, 7 and 14 after cisplatin | N=11. Lung cancer. | AKI defined as increase in BUN or SCr | AUC = 0.858 to detect AKI | 25 |
| Urine KIM-1 | cisplatin | N=111. malignant mesothelioma | Stage 1,2 or 3 AKI (AKIN Classification) | KIM-1 increased after cisplatin but could not distinguish AKI vs. no AKI | 26 |
| Urine KIM-1, B2M, TFF3, albuminuria | cisplatin ( 25 mg/m$^2$) | N=57. Solid tumor | no patients developed AKI (Subclinical AKI) | B2M, 3-fold higher day 3 (P < 0.0001 vs. baseline) urinary KIM-1, TFF3 2-fold higher day 10 (P = 0.002) albuminuria 1.5-fold higher at day 3 (P=0.008) | 27 |
| Urine KIM-1 | days 1, 3 and 5 after cisplatin 75 mg/m$^2$/day | N=22 | stage 1,2 or 3 AKI (AKIN classification) | AUC=0.94 to detect AKI at day 1 | 28 |
| Urinary vanin-1, KIM-1, NGAL and NAG | cisplatin | N=24. urothelial cancer | AKI: > 20% decline in eGFR in first 6 days | vanin-1 elevated early on day 3 before increase in SCr. Sensitivity 66.7%, specificity 83.3% KIM-1, NGAL not increased | 29 |
| Urine NGAL, urine cystatin C, albuminuria | days 1, 3 and 5 after cisplatin 75 mg/m$^2$/day | N=33 | AKI: eGFR decrease >25% within 12 hrs | NGAL AUC=0.865 to detect AKI cystatin C AUC=0.53 to detect AKI albuminuria AUC=0.52 to detect AKI | 32 |
| Urine NGAL | amphotericin B | N=24 | stage 1,2 or 3 AKI (AKIN classification) | AUC=0.68 to 0.89 to detect AKI urine NGAL increased 3 days before SCr | 122 |
| Urine B2M, NAG | cisplatin 50 mg/m$^2$/day | N=20 | no change in GFR | B2M, NAG increases after dose of cisplatin despite no change in GFR | 41 |
| Urine B2M | cisplatin | N=18 | decrease in GFR | 2 to 5-fold increase in B2M timing and peak of B2M not correlate with GFR | 42 |
| Urine B2M, NAG, AAP | cisplatin 100 mg/m$^2$ | N=22 ovarian cancer | no change in GFR | B2M not increased NAG, AAP increase 2 days after cisplatin | 43 |
| Serum B2M | CYA | N=33 heart transplant | decline in kidney function. CYA blood levels | correlation between B2M and decline in kidney function and CYA blood levels | 44 |
| Serum B2M | CYA | N=77 bone marrow transplant | increase in SCr | correlation between B2M and increase in SCr (P<0.001) | 45 |
| Urine B2M, albuminuria | CYA | N=12 rheumatoid arthritis | 50% increase in SCr | 10- fold increase in B2M 2- fold increase in albuminuria | 46 |

| Biomarker | Drug | Study population | End point | Sensitivity/specificity | Ref |
|---|---|---|---|---|---|
| Urine and serum B2M | CYA | N=49 kidney transplant | renal dysfunction due to CYA or rejection | increased urine B2M with CYA toxicity; Increased serum B2M with rejection | 47 |
| Urine B2M | CYA | N=37 kidney transplant | increase in SCr | increase in B2M before increase in SCr | 48 |
| Urine B2M | aminoglycosides | N=52 | increase in SCr | increased B2M preceded the increase in SCr by 2 to 7 days | 56 |
| Serum cystatin C | cisplatin | N=41 | inulin-based GFR | cystatin C better marker of decreased GFR than SCr. | 88 |
| Serum cystatin C | chemotherapy not specified | N=28 children | isotope-based GFR | cystatin C based GFR equation more accurate than SCr based GFR equation | 89 |
| Serum cystatin C | cisplatin | N=36 lung cancer | isotope –based GFR | no correlation between cystatin C based GFR equation and isotope based GFR | 90 |
| Serum cystatin C | chemotherapy not specified | N=206 Children 6 studies meta-analysis | creatinine clearance, inulin based GFR, isotope based GFR | AUC=0.890 to detect decreased GFR. serum cystatin C performed better than SCr. | 92 |
| Serum cystatin C | days 0, 7, 14 after amphotericin B | N=54 | creatinine based GFR equation | cystatin C-based GFR equation correlated with creatinine- based GFR equation | 93 |
| Serum cystatin C | radiocontrast dye | N=87 CKD | SCr | cystatin C correlated with increase in SCr | 94 |
| Serum cystatin C | 5, 24 and 48 hours after radiocontrast dye | N=41 | SCr | cystatin C increased earlier than SCr | 95 |
| Serum cystatin C, serum NGAL | 24, 36, 48, 72 hours after high dose methotrexate 100 mg/m$^2$ | N=20 children | SCr | cystatin C increased at 36 hours SCr, NGAL not increased | 96 |
| Serum cystatin C | 3, 5, 7, 10 and 12 days after amikacin | N=71 children cystic fibrosis | SCr amikacin concentrations | cystatin C better marker of GFR than SCr. cystatin C greater ability to predict amikacin clearance than SCr | 97 |

KIM-1 kidney injury molecule 1, BUN=blood urea nitrogen,SCr=serum creatinine, B2M-beta- macroglobulin, TFF=trefoil factor 3, AAP=alanine aminopeptidase, NGAL=Neutrophil gelatinase-associated lipocalin, NAG=N-acetyl-B-glucosaminidase, AUC=area under the Receiver Operating Characteristic curve [AUC], GFR=glomerular filtration rate, eGFR=estimated glomerular filtration rate, . CYA=cyclosporine A, AKI=acute kidney injury, AKIN=acute kidney injury network, CKD=chronic kidney disease.