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Emergence of biomarkers in nephropharmacology

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Abstract

Blood-urea nitrogen, serum creatinine and urine output have long been used as markers of kidney function despite their known limitations. In the past few years, a number of novel biomarkers have been identified in the urine and blood that can detect kidney injury early. Although, to date, none of these biomarkers are in clinical use, many have been validated as reliable and sensitive, allowing detection of kidney injury before serum creatinine levels rise and urine output drops. These markers have been evaluated in great detail in animal models and to a lesser extent in humans in postcardiopulmonary bypass and sepsis. There is relatively scarce data on the use of these biomarkers in the detection of kidney injury associated with the use of pharmacologic agents. The purpose of this article is to summarize these data and highlight the potential utility of these biomarkers in nephropharmacology.

Keywords

biomarker; drug-induced renal failure; kidney injury; nephrotoxins

As the kidneys receive approximately 25% of cardiac output, approximately 1.5 l/min blood flow, almost any drug has the potential to have a range of effects on the kidney. Various classes of pharmacological agents have long been known to affect kidney function through their hemodynamic effects and some to cause acute kidney injury (AKI). While some drugs cause injury mediated by allergic interstitial nephritis, many are known to cause direct tubular or glomerular injury. These classes include, but are not limited to, nonsteroidal anti-inflammatory agents, chemotherapeutic agents, calcineurin inhibitors and antimicrobials, in particular, aminoglycosides. This article will focus on drugs that have been associated with kidney injury, and the use of emerging biomarkers to accurately predict nephrotoxicity before significant and potentially irreversible renal injury occurs.

Early recognition of nephrotoxicity, a major focus of nephropharmacology, can be a daunting challenge. Current clinical practice in monitoring drug effects on the kidney relies on the measurement of serum creatinine and estimated glomerular filtration rates (GFR),

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determined from serum creatinine concentration, which is based on various formulae despite well-recognized limitations [1,2]. Conceptually, the limitations of these biomarkers are in reliance on significant reduction in the filtration function of the kidney and the absence of specific markers of cellular injury. Reduction in the kidney's filtration function can be recognized only after a threshold is reached, owing to limitations in the measurement of serum creatinine and the nonlinear relation between the serum creatinine concentration and the GFR. Therefore, any significant cellular injury is missed until it reaches a level that causes a significant reduction in GFR, usually approximately 50%. Secondly, even clinically significant reductions in GFR can be missed if they do not reach the threshold.

The limitations in the reliance on creatinine measurements led to the realization that novel biomarkers are needed for early diagnosis, assessment of the site and the severity of kidney injury. During the last decade, there has been intense research to identify more sensitive and specific biomarkers to aid in the recognition of renal injury, using novel methodologies, including proteomics, metabolomics, gene arrays and reverse transcription PCR, as well as measuring suspect markers by use of conventional methods, such as sandwich ELISA and enzyme assays [3–9]. Numerous candidates, including kidney injury molecule (Kim)-1, *N*-acetyl- β -D-glucosaminidase (NAG), neutrophil gelatinase-associated lipocalin (NGAL), lactate dehydrogenase (LDH), albumin, total protein, low-molecular-weight proteins, such as β_2 -microglobulin (β_2 M), immune globulin light chains, retinol-binding protein (RBP), cystatin C, cytokines, trefoil factor 3, fatty acid-binding protein and renal papillary antigen, have emerged as potential markers (Table 1). These biomarkers have mainly been investigated in the setting of AKI associated with cardiopulmonary bypass, sepsis, contrast-induced nephropathy and trauma-associated acute renal failure, and there are relatively sparse data on their usefulness in the setting of kidney injury associated with pharmacological agents [10–15]. However, the pathophysiologic mechanisms that lead to AKI in these diverse conditions share common pathways, and the biomarkers that appear of potential value would be expected to be useful in drug-associated renal injury as well. Characterization of the pattern of these biomarkers in response to pharmacological agents will not only aid in early recognition, but also in assessing the severity and, potentially, identify the site of injury. Although, at present, none of these candidate markers have yet gained acceptance into clinical practice, several have emerged as potentially useful and most likely to be adopted into clinical use. In a recent review of 'high-quality' publications, serum cystatin C, urine IL-18 and urine Kim-1 were reported to be best for the identification of established AKI. For early diagnosis of AKI, serum cystatin C, urine NGAL, IL-18, glutathione-S-transferase (GST)- π and γ -GST performed best. Urine NAG, Kim-1 and IL-18 performed the best for prediction of mortality risk after AKI [16]. There are a large number of candidate markers for early detection of AKI, especially in the setting of ischemia/reperfusion and sepsis in the intensive care unit setting. The pharmacology community has a special interest in early biomarkers owing to the obvious implications in drug discovery and development in the preclinical phases [17,18]. This article will focus on some of the major candidate renal biomarkers that have been explored in the setting of kidney injury associated with various nephrotoxic drugs [13].

Cystatin C

Cystatin C, a potent inhibitor of cysteine proteases, is a 13-kDa protein encoded by the *CST3* gene and is present in all nucleated human cells. It is a low-molecular-weight protein that is positively charged at physiologic pH and, therefore, relatively freely filtered by the glomerulus [19]. It is completely reabsorbed by normal proximal tubules and catabolized almost completely such that there is no appreciable quantity excreted in the urine. Unlike creatinine, nonrenal variables do not seem to have much effect on cystatin C levels and many investigations have demonstrated that serum levels reflect GFR better than serum

creatinine, and could also be viewed as a glomerular biomarker [20]. Furthermore, increased cystatin C levels in the urine, usually expressed as the cystatin C:creatinine ratio, has the advantage of indicating tubular injury. Cystatin C can be measured accurately by nephelometry. The utility of cystatin C measurements has been evaluated in populations at high risk of AKI, usually in the critical care setting, and the results are mixed [20]. In one prospective study of patients on cisplatin, cystatin C had a better predictive value than serum creatinine as a marker of early renal injury [21]. In an experimental study in rats monitoring nephrotoxic effects of gentamicin, carbapenem and doxorubicin, serum cystatin C level was reported to be superior to blood-urea nitrogen and creatinine, especially after gentamicin treatment. Benohr and colleagues correlated their measurements with careful histomorphologic evaluations, and proposed cystatin C as the best biomarker for predicting both renal injury and recovery from renal failure [21]. The authors also pointed out that, for more sensitive prediction of AKI, a panel of serum and urine biomarkers, including one or more additional biomarkers, such as Kim-1, osteopontin, NGAL, clusterin and trefoil factor 3, along with cystatin C measurements, may be necessary. Cystatin C was also demonstrated to be a sensitive and useful biomarker for early detection of renal insufficiency in transplant patients on calcineurin inhibitors (both cyclosporine and tacrolimus) despite the observations that corticosteroids can slightly elevate cystatin C levels [22–24].

Taken together, the animal data and clinical experience point to serum and urine cystatin C measurements as superior to conventional biomarkers, such as urine output, urine protein, urine albumin, and serum blood-urea nitrogen and creatinine. Therefore, cystatin C has the theoretical advantage of simultaneously being a marker of both glomerular and tubular dysfunction. Adoption of cystatin C measurements for routine assessment of kidney function may improve the nephropharmacologist's ability to better manage potentially nephrotoxic drugs, including aminoglycoside antibiotics, chemotherapeutic agents, such as cisplatin and doxorubicin, and calcineurin inhibitors in transplant patients.

Kidney injury molecule-1

Kidney injury molecule-1 is a type I cell membrane glycoprotein, which is a phosphatidylserine receptor on renal epithelial cells that recognizes apoptotic cells, directing them to lysosomes and, thereby, converting the normal proximal tubule cell into a phagocyte. Its elevation in the context of tubular injury has also led many to believe that it is involved in tubular dedifferentiation and regeneration after ischemic and nephrotoxin-mediated injury [25–27]. Kim-1 mRNA levels are known to be highly elevated in response to injury [25–27]. The expression of Kim-1 is especially pronounced in the S3 segment of the proximal tubule [25–28] and has been used to detect tubular injury in the past. By *in situ* staining, Kim-1 expression has been used to diagnose acute tubular necrosis. It is also known that its ectodomain is shed into urine, as a result of metallo-peptidase cleavage, and is then released into the urine [28–31]. Its expression in the urine has been utilized as an early and sensitive marker for AKI in association with gentamicin and other nephrotoxicants [29–32].

Among the most frequently used chemotherapeutic agents, cisplatin and adriamycin are known nephrotoxins. Cisplatin is known to cause injury to the proximal tubule in the S3 segment near the corticomedullary junction – the major site of its toxicity [31,33,34]. It is also known to affect the loops of Henle and the distal tubules [35]. The potential usefulness of Kim-1, as well as other molecules, was evaluated in some detail in experimental models of AKI with cisplatin. Tonumura *et al.* recently investigated the nephrotoxicity of intravenous cisplatin in rats [36]. Data were also collected from a group of controls and from a set of rats treated with puromycin, a known glomerular toxin. Cisplatin was infused at previously established nephrotoxic concentrations that had been confirmed by

histopathological changes [27]. Urine samples were collected between -4 and 0 h before, and 20–24 h, 44–48 h and 68–72 h after administration. Rats were then euthanized and their kidneys removed for histologic examinations. The levels of each biomarker were plotted separately on a receiver operating characteristic curve analysis. The urinary concentration or activity of several biomarkers, urine total protein, urine albumin, Kim-1, clusterin, GST- α and - μ , LDH and aspartate aminotransferase were elevated in a time-dependent manner in the group treated with cisplatin. Among these biomarkers, urine albumin, Kim-1, GST- α , LDH, GST- μ and urine total protein were elevated significantly within 24 h of administration. A bimodal increase in NAG at 24 and 72 h after administration was also seen. The biopsy specimens from rats exposed to cisplatin showed characteristic proximal tubular necrosis and inflammatory cell infiltration at the corticomedullary junction. In the receiver operating characteristic curve analysis, candidate biomarkers Kim-1 and GST- μ demonstrated perfect sensitivity, while GST- α , LDH and urine albumin also demonstrated very high accuracy and sensitivity. Since Kim-1 is expressed exclusively in the proximal tubule in response to injury, the authors interpreted it to represent the injury to the proximal tubule. Several studies have demonstrated expression of Kim-1 in the S3 segment of proximal tubules by immunohistochemistry [4,37]. Elevation in GST- μ , a biomarker expressed in the distal tubules and the loop of Henle [38], was taken as evidence of injury to those sites. In the same study, the set of rats exposed to puromycin, a known glomerular toxin, revealed a very different profile of biomarker expression, with NGAL being the predominant biomarker and Kim-1 expression having a weak association with kidney injury. Therefore, Kim-1 appears to be a promising biomarker in the detection of early kidney injury during treatment with potentially nephrotoxic drugs, such as cisplatin, aminoglycoside antibiotics and nephrotoxic heavy metals [26,27,30,32,36,39–41]. A rapid dipstick test that is now being evaluated to detect Kim-1 may accelerate the introduction of this test to routine practice [42].

NGAL & IL-18

Neutrophil gelatinase-associated lipocalin is a 25-kDa ubiquitous protein that belongs to the lipocalin family, originally extracted from neutrophils, but also found in many tissues in the body, including the kidney, and GI and respiratory tracts [43–45]. Although NGAL is present at very low concentrations in both the kidney tissue and the serum of normal individuals, its levels in both serum and urine increase in AKI of diverse etiologies, including contrast-induced nephropathy, and cisplatin, aminoglycoside, vancomycin or cyclosporine nephrotoxicity [36,43–47]. There is evidence that both ischemic and nephrotoxic injury increases production of NGAL in kidney tubules, which contributes to the increased urinary concentrations seen in AKI, and, as the kidney clearance declines, serum levels rise. NGAL was identified as one of several genes upregulated within the first few hours after ischemic renal injury in a mouse model [44]. NGAL is upregulated and can be detected in the urine of mice 3 h after cisplatin administration, and has been proposed as an early biomarker for diagnosing AKI [44]. NGAL may have renoprotective properties, and it may protect kidney cells from apoptosis and oxidative stress by binding to ferric complexes [48]. Many studies have demonstrated that the serum levels of NGAL may increase up to tenfold, urinary concentrations up to 100-fold in nephrotoxic and ischemic AKI, and the NGAL elevations precede the rise in serum creatinine and predict AKI before measurable decline in GFR can be noted [10,14,19,36,44,45,47–51]. Although NGAL levels have been noted to increase in inflammatory states, this elevation is less marked than that seen in kidney injury, and increased serum and urine concentrations of NGAL appear to be relatively specific to kidney tissue [48]. The level of NGAL in serum and urine can be measured, conveniently, by ELISA, and it deserves consideration among biomarkers of early nephrotoxicity.

Urine IL-18 is an inflammatory cytokine and, similar to NGAL, it is also a marker of inflammation. In many studies of biomarker evaluations the sensitivity and specificity of IL-18 was similar to that of NGAL in predicting early renal injury as well as the severity of renal injury [16]. Compared with other biomarkers, such as Kim-1 and cystatin C, IL-18 appears to be relatively less sensitive, but it continues to attract interest because its measurement in urine is easy and convenient [16,48]. However, to date, most studies that have evaluated IL-18 are in the setting of ischemic kidney injury, delayed allograft kidney function or in critically ill patients [14,16,48,52]. Although Faubel *et al.* reported IL-18 to be predictive of renal injury in cisplatin nephrotoxicity [53], its potential value in the setting of nephrotoxic renal injury has not been widely explored.

N-acetyl- β -D-glucosaminidase

N-acetyl- β -D-glucosaminidase is a proximal tubule lysosomal enzyme, along with other kidney-derived enzymes, including LDH, alkaline phosphatase and γ -glutamyl transferase, and has been widely explored as a potential biomarker for early detection of renal injury [34]. Increased urinary levels of NAG have been taken as evidence for proximal tubule injury because its high molecular weight – over 150 kDa – precludes glomerular filtration and, thus, urinary excretion can reliably be attributed to renal tubules [48]. Increased urinary excretion of NAG has been reported with nephrotoxic drugs, including gentamicin [41], calcineurin inhibitors [54], cisplatin, nedaplatin [34] and IFN- α , as well as following cardiopulmonary bypass procedures [14,55,56]. In patients diagnosed with AKI, elevations in NAG levels have correlated with the need for dialysis, as well as with death [57]. Urinary NAG appears to be a sensitive biomarker, and even subtle injury in proximal tubule epithelial cells results in shedding of NAG into the urine. Furthermore, the amount of shed enzyme seems to correlate with the severity of tubule injury [52]. NAG appears to be an attractive candidate biomarker for early detection of renal injury, because, in addition to its sensitivity, it can be measured easily and inexpensively by colorimetric spectroscopy. However, increased urinary NAG levels have been observed in conditions without kidney injury and some heavy metals have been observed to suppress its production [52]. Furthermore, in a comparative analysis of potential biomarkers in the setting of nephrotoxicants, including gentamicin, mercury and chromium, Zhou *et al.* reported NAG elevations are variable, dependent on the nature of the nephrotoxicant and inferior to Kim-1 [41]. Therefore, the nonspecificity of NAG appears to be a major limitation for it to be a reliable biomarker for predicting renal injury during drug discovery and development.

Netrin-1

Netrin-1 is perhaps the newest candidate biomarker of renal injury and is an interesting molecule. Netrin-1 is a 50–75-kDa laminin-like protein that belongs to a family of proteins involved in axon guidance, has chemotrope properties and functions as a cell survival factor in the nervous system [58,59]. Netrin-1 may have possible roles in neovascularization, cell adhesion and tumorigenesis. Netrin-1 is not present or minimally expressed in normal kidneys, but is dramatically increased in the kidney and urine after renal injury. In a recent study using four types of mouse kidney injury model, including ischemia–reperfusion and three nephrotoxic insults – cisplatin, endotoxin and folic acid – netrin-1 excretion in the urine was detected as early as 1 h after kidney injury, reached a 30–40-fold increase by 3 h and peaked at 6 h after the insult. Urinary excretion of netrin-1 preceded any increase in blood-urea nitrogen and serum creatinine by many hours, underscoring its potential value as an early biomarker [58–60]. Interestingly, netrin-1 appears to be renoprotective with the potential to ameliorate kidney injury, and protected the kidney in an ischemic model of AKI by countering apoptosis, and promoting cell proliferation and regeneration [61]. More recently, Ramesh *et al.* determined netrin-1 levels by ELISA in urine samples from healthy

controls, recipients of a renal allograft, and patients with AKI caused by ischemia, sepsis, radio-contrast dye and nephrotoxic drugs, including gentamicin, cisplatin, bactrim, aztreonam and cefazolin [60]. Urinary netrin-1 levels, normalized for urinary creatinine, were significantly higher in all subject groups compared with healthy controls. Netrin-1 levels preceded the rise in serum creatinine and urea nitrogen, and started to decline as kidney function improved. Therefore, netrin-1 emerged as a potentially universal biomarker that could be used in early recognition of a wide range of kidney injury types and also appeared to be a prognostic indicator of renal recovery [59,60].

Low-molecular-weight proteins

Increased urinary excretion of filtered low-molecular-weight proteins, including β_2 M, α_1 -microglobulin (α_1 M), RBP and polyclonal immunoglobulin light chains, has long been recognized as a potential biomarker of toxic kidney injury, especially with aminoglycosides and cadmium nephropathy [62,63]. In a recent study comparing the renal effects, efficacy and safety with once-daily abacavir/lamivudine versus tenofovir/emtricitabine, increased urinary excretion of both β_2 M and RBP were noted in the tenofovir/emtricitabine arm without any change in estimated GFR between the two arms [64]. Although cystatin C is also a low-molecular-weight protein (13 kDa), owing to the detailed studies evaluating its utility as a biomarker it is discussed separately. Low-molecular-weight proteins are smaller than albumin and most are relatively positively charged; therefore, they are filtered by the glomerulus and almost completely reabsorbed by proximal tubule cells, such that there is minimal or no excretion in the urine. Since proximal tubule injury may reduce the kidney's ability to endocytose these proteins, their presence in the urine in increased quantities can be taken as evidence of renal injury.

β_2 -microglobulin

β_2 -microglobulin, an 11.8-kDa protein, is the light chain of the MHC I antigen expressed on the cell surface of all nucleated cells. β_2 M attracted attention nearly 40 years ago and, similar to cystatin C, was also proposed as a more accurate biomarker to estimate the early and subtle changes in the GFR [62]. Increased urinary β_2 M excretion has been reported to be an early marker of tubular injury in aminoglycoside nephrotoxicity, preceding elevations in serum creatinine by 4–5 days [63]. However, the utility of β_2 M as a biomarker has been limited by its instability in the urine, with rapid degradation at room temperature and in urine with a pH of less than 6.0.

α_1 -microglobulin

α_1 -microglobulin is a 31-kDa protein synthesized by the liver, which is mostly bound to IgA. Its free form is filtered by the glomerulus and reabsorbed by proximal tubule cells [48]. Unlike β_2 M, urinary α_1 M is stable over the range of pH found in routine clinical practice. It has been reported to be a sensitive biomarker for proximal tubular dysfunction, especially during IFN- α -2b treatment, and its appearance in urine precedes the rise in serum creatinine by several days [56]. Conditions such as liver disease and HIV infection alter α_1 M production and, thus, its utility in these conditions may be suboptimal.

Retinol-binding protein

Retinol-binding protein is a 21-kDa protein that is synthesized in the liver and is responsible for transporting vitamin A from the liver to other tissues. Similar to other low-molecular-weight proteins, it is relatively freely filtered by the glomerulus and reabsorbed by the proximal tubule to be catabolized to its amino acid constituents. Increased plasma levels and urinary excretion of RBP correlated with renal injury associated with aminoglycoside and cephalosporin [48,62]. Other low-molecular-weight proteins, including lysozyme and

polyclonal immunoglobulin light chains, have been explored as potential biomarkers in various kidney injury models, but evaluations in the setting of nephrotoxics are limited. Furthermore, many of these proteins' synthesis are altered in various inflammatory conditions and, therefore, more studies are needed to validate them as useful biomarkers in nephrotoxicology.

There are numerous other molecules that hold promise as potential biomarkers of renal injury associated with ischemic and nephrotoxic injury, such as trefoil 3 [65], renal papillary antigens-1 and -2 [31], sodium–hydrogen exchanger isoform-3, fetuin A, clusterin and osteopontin [14,18,33,36,39,65–67]. Although, among these candidate molecules, urinary fatty acid binding protein appears promising based on studies in cisplatin-induced AKI [33], validation of this marker awaits additional studies.

Conclusion

There is a need for easy to measure, reproducible and noninvasive biomarkers to detect early and subtle kidney injury during preclinical studies with new drugs, and during treatment with known nephrotoxic drugs. It has long been recognized that reliance on serum creatinine results in delayed diagnosis of renal injury. Numerous molecules have been evaluated as potential biomarkers of early renal injury with reasonable sensitivity and specificity. Most of these biomarkers were identified in the setting of AKI, caused by either ischemia, or sepsis, and their utility in the setting of drug nephrotoxicity has not been examined in great detail. However, many of these molecules were demonstrated highly promising as biomarkers of drug nephrotoxicity. This article demonstrates that, among these novel biomarkers, cystatin C, Kim-1 and urinary low-molecular-weight proteins are most likely to enter clinical use in the foreseeable future. The development of a dipstick test for Kim-1 would no doubt be a big step in this direction [42]. Cystatin C also appears likely to find its way into routine clinical practice soon as it is one of the most exhaustively studied biomarkers and demonstrated sufficient sensitivity and specificity in both ischemic and toxic kidney injury [16,18,21,68]. Furthermore, cystatin C is easy to measure accurately and most studies demonstrated that it could detect kidney injury 1–2 days before creatinine [21,68]. Among the recently identified candidate molecules, netrin-1 appears to be most intriguing. Its urinary excretion and kidney levels, similar to Kim-1, increased in AKI of diverse etiologies with very high sensitivity and specificity, pointing to its potential to be a universal biomarker for kidney injury [59,60]. Preliminary data also suggest that netrin-1 can be useful in protecting kidneys from toxic and ischemic injury, and can even be of therapeutic value. Furthermore, levels of netrin-1 seem to correlate with renal injury and decline during recovery, indicating that it can be of prognostic value [59,60].

The pathophysiology of kidney injury involves diverse and complex mechanisms, and different insults affect different components of the kidney. Depending on the type and site of the insult kidney impairment may range from subtle functional abnormalities, such as transport and acidification defects, leading, for example, to Fanconi syndrome, or cell death in the form of apoptosis or necrosis in different segments of the kidney tubules. Therefore, different segments may give rise to different biomarkers. The proximal tubule cells are the most abundant cell type in the kidney and most likely to be affected by toxic or ischemic injury. Therefore, it is not surprising that most promising markers, including Kim-1, and netrin-1, derive from the proximal tubule cells. Similarly, low-molecular-weight proteins, including increased urinary excretion of cystatin C, reflect the renal tubules impaired ability to reabsorb them.

Future perspective

Despite vigorous efforts, it is clear that there is no single marker that can satisfactorily replace creatinine. The pathophysiology of kidney injury involves diverse and complex mechanisms, and different insults affect different components of the kidney. Depending on the type, site and intensity of the insult, the functional impairment, as well as the pattern of biomarker production, may vary. Therefore, it is quite likely that, rather than a single agent, a panel of markers will come into clinical use and, rather than an absolute value, the pattern in which they rise will give clues towards the site and severity of injury. This information will be vital for physicians treating patients with medications that are nephrotoxic and will also aid in the process of development of future medications by determining their nephrotoxic potential. With a convenient dipstick already available for Kim-1, we hope that, in the future, measuring for other biomarkers is also going to become simple and affordable.

Executive summary

- With a large functional reserve in the kidney, a significant proportion of renal injury can occur before serum creatinine rises and estimated glomerular filtration rate declines. There is ongoing research to identify and validate more sensitive biomarkers that can detect acute kidney injury (AKI) prior to a detectable rise in serum creatinine and blood-urea nitrogen.
- An impressive array of potential biomarkers have emerged, which include, but are not limited to, kidney injury molecule-1, *N*-acetyl- β -D-glucosaminidase, neutrophil gelatinase-associated lipocalin, netrin-1, lactate dehydrogenase, albumin, total protein, low-molecular-weight proteins, such as β -2 microglobulin, immune globulin light chains, retinol binding protein, cystatin C, cytokines, trefoil factor 3, fatty acid binding protein and renal papillary antigen.
- These biomarkers have been investigated in-depth in the context of AKI, secondary to ischemia reperfusion, contrast exposure and sepsis, but there are relatively scarce data on their use in the setting of kidney injury associated with pharmacologic agents.
- This article highlights the studies that involve these biomarkers in the context of drug-induced AKI.
- Cystatin C is a positively charged low-molecular-weight protein that is freely filtered at the glomerulus, completely reabsorbed by normal proximal tubules and catabolized almost completely so there is no appreciable quantity excreted in the urine, and, therefore, increased cystatin C levels in the urine, usually expressed as cystatin C:creatinine ratio, indicate tubular injury, while serum levels can be used to estimate changes in glomerular filtration rate more sensitively than creatinine.
- There is evidence that cystatin C level is superior to conventional markers of kidney function, especially in the context of aminoglycosides and calcineurin inhibitors.
- Kidney injury molecule-1 is a type I cell membrane glycoprotein that sheds its ectodomain into the urine in AKI. This marker is easy to measure and has been found to be useful as an early biomarker in drug-induced nephrotoxicity, secondary to cisplatin and aminoglycosides.
- Neutrophil gelatinase-associated lipocalin, a lipocalin originally extracted from neutrophils, but found in many tissues in the body, including the kidney, and GI

and respiratory tracts. Its levels in both serum and urine increase in AKI of diverse etiologies. It has been validated as a marker for early detection of AKI in drug-induced AKI, secondary to cisplatin, contrast dye, aminoglycosides, vancomycin and cyclosporine.

- Urine IL-18, an inflammatory cytokine, is similar to neutrophil gelatinase-associated lipocalin in its sensitivity and specificity in the setting of AKI, although studies investigating drug-induced AKI are lacking.
- *N*-acetyl- β -D-glucosaminidase is a proximal tubule lysosomal enzyme that has been widely studied as a biomarker for AKI, with its early rise associated with the need for dialysis and death. Although sensitive, *N*-acetyl- β -D-glucosaminidase is not as specific as some of the other markers, but has been found useful in the detection of AKI secondary to gentamicin, calcineurin inhibitors, cisplatin, nedaplatin and IFN- α .
- Netrin-1 is a protein belonging to the laminin family, known for its role in neuronal development and tumor growth, usually not present in the urine but is dramatically increased within hours of renal injury. Recently, it has been validated in many different forms of AKI and proposed as a universal marker; drug-induced renal effects were studied with cisplatin, and calcineurin inhibitors gentamicin, bactrim, aztreonam and cefazolin.
- Low-molecular-weight proteins (β_2 -microglobulin, α_1 -microglobulin, retinol-binding protein and polyclonal immunoglobulin light chains) are usually filtered at the level of the glomerulus, but are reabsorbed and metabolized by tubular cells, and, thus, their levels are low or undetectable in the urine. They have been known to be elevated in the urine after AKI caused by variety of insults, including drugs such as gentamicin, tobramycin, cisplatin, cadmium, tenofovir and emtricitabine.
- To date, the most useful biomarkers identified originate from the proximal tubules cells, which are the most abundant type of cells in the kidney.
- Biomarker elevation is representative of injury at the level of the tissue rather than the conventional markers that are representative of level of function of the entire urinary system.
- Markers of injury are likely to be of great use in the process of development of new drugs as toxic effects to the kidney are a known limiting factor in the utility of many potential pharmacological agents.

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Table 1

Selected biomarkers associated with drug-induced renal injury.

Biomarker	Source	Methodology	Drugs	Ref.
Cystatin	Urine and blood	Nephelometry	Gentamicin, cisplatin, carbapenem, doxorubicin and calcineurin inhibitors	[20–24,36]
Kim-1	Urine and <i>in situ</i>	ELISA, genomics, proteomics and immunohistochemistry	Cisplatin, gentamicin, folic acid, vancomycin, cadmium, mercury and cyclosporine	[18,27,28,30–32,36,39–41,69–71]
NGAL	Urine	ELISA and genomics	Gentamicin, cyclosporine, cisplatin and contrast dye	[36,44,47,51]
IL-18	Urine	ELISA	Ischemic AKI and intensive care unit patients	[14,16,48,52]
RBP, β_2 M and α_1 M	Urine	ELISA	Gentamicin, tobramycin, cisplatin, cadmium, tenofovir and emtricitabine	[18,62–64]
Netrin-1	Urine, blood and <i>in situ</i>	ELISA and immunohistochemistry	Gentamicin, cisplatin, bactrim, aztreonam and cefazolin	[58–60,72]
NAG	Urine	Enzyme assay	Cyclosporine, tacrolimus, gentamicin, cisplatin and IFN- α	[34,41,52,54,56]
RPA-1 and -2	<i>In situ</i>	Immunohistochemistry and ELISA	Gentamicin, cisplatin and valproic acid	[31]

α_1 M: α_1 -microglobulin; β_2 M: β_2 -microglobulin; AKI: Acute kidney injury; Kim: Kidney injury molecule; NAG: *N*-acetyl- β -D-glucosaminidase; NGAL: Neutrophil gelatinase-associated lipocalin; RBP: Retinol-binding protein; RPA: Renal papillary antigen.