Review Article

Biomarkers of Acute Kidney Injury

Chen, H.,* Avital, Y. and Segev, G.

Koret School of Veterinary Medicine, The Hebrew University Veterinary Teaching Hospital,
The Hebrew University of Jerusalem.

* **Corresponding author:** Dr. Hilla Chen, DVM, Department of Small Animal Internal Medicine, Hebrew University Veterinary Teaching hospital, Koret School of Veterinary Medicine, The Hebrew University of Jerusalem, P.O. Box 12, Rehovot 76100, Israel. Tel: 972-3-9688588, Fax: 972-3-9604079. Email: hillach@gmail.com

ABSTRACT

Acute kidney injury (AKI) is common in dogs and cats and is associated with substantial morbidity and mortality. Serum creatinine is the most commonly utilized marker for renal function, but is neither sensitive nor specific for early detection of renal injury. Therefore, there is a need for sensitive renal biomarkers that will facilitate the diagnosis of early kidney injury. Early detection of AKI will enable timely intervention and thus is expected to improve the outcome. Renal biomarkers may also aid in assessing the severity of the disease, indicate repair processes and predict the outcome. It is unlikely though, that a single biomarker will provide all this information, but rather an array of biomarkers will have to be used. This paper reviews the main biomarkers currently under investigation in veterinary medicine, including their advantages and limitations.

Keywords: Biomarkers; Dog; Cat; SDMA; AKI.

INTRODUCTION

Acute kidney injury (AKI) is characterized by an abrupt and sustained decrease in the glomerular filtration rate (GFR) (1). It is a common disorder in companion animals and humans, and is associated with high treatment costs as well as high morbidity and mortality. Four phases are currently recognized in the pathogenesis of AKI: initiation, progression, maintenance, and recovery (1). Using the common clinicopathologic markers (e.g., serum creatinine [sCr]), the disease is characteristically recognized only in the maintenance phase, when clinical signs are overt.

Despite advances in the management of AKI, including the introduction of renal replacement therapies, the mortality rate among human and animal patients remains unacceptably high. Over the past 50 years, mortality rates of human patients with AKI in intensive care units have remained as high as 70% (2). One of the speculated reasons for the high mortality is the late recognition of the disease and consequently the narrow window of opportunity for therapy. Therefore, early recognition of the disease before overt renal failure is evident, is crucial to allow timely and thus potentially more effective therapy. The need for early diagnosis is further emphasized in veterinary medicine, because renal replacement therapies are not readily available.

Limitations of serum creatinine concentration

Despite the diagnostic advancements made in other medical fields (e.g., the use of biomarkers in cardiology), sCr is still being used as the marker for kidney function despite its multiple shortcomings, including: 1) High variability among dog breeds and therefore, including all dogs under one reference range results in a very wide reference range and decreased sensitivity and specificity. Consequently, sCr is not expected to rise above the reference range in most dog breeds until ~75% of nephrons become non-functional. 2) sCr is affected by extra-renal factors, particularly muscle

mass, therefore lacks specificity. 3) sCr is a functional marker thus it is blinded to kidney injury that is not accompanied by decreased kidney function. 4) sCr does not represent the severity of the dysfunction until a steady-state has been reached. Consequently, substantial changes in GFR at the early stages of kidney injury are associated with relatively small changes in sCr.

The above limitations of sCr are reflected by the findings of several studies indicating that small, and even transient, increase in sCr in human patients is detrimental. In one study, as little as 0.5 mg/dL increase in sCr was associated with increased in-hospital mortality (3). In another study, a transient increase in sCr (for 1–3 days) was also associated with increased odds ratio for in-hospital mortality (4). Finally, even a small and transient increase in sCr in patients that were discharged from the hospital, was associated with the need for chronic dialysis over the ensuing three years (5). The aforementioned limitations and studies imply that relying on sCr as the only marker of kidney function does not provide all the information needed to accurately assess kidney function, to promote timely manner intervention, and to determine the prognosis.

Renal biomarkers – characteristics

In the recent years, research in nephrology has been growing in an attempt to identify sensitive and specific biomarkers. A biomarker should meet several requirements to be considered an ideal marker: 1) be detectable in urine and/or plasma; 2) assays should be readily available and cost effective; 3) the biomarker would have high prediction of kidney injury (namely high sensitivity and specificity); 4) the biomarker would provide information regarding the etiology; 5) provide information regarding the location of the injury (i.e., glomerulus, tubules); 6) would be associated with the severity of the injury; 7) would indicate both kidney injury and repair processes and 8) would predict the likelihood of recovery and the outcome. It is yet to be determined when and if biomarkers would fulfill all of these requirements; however, it is unlikely that a single biomarker will provide all this information. More likely, an array of biomarkers will be needed, each of which will provide one piece of the puzzle, and all together will provide a comprehensive picture. A panel of biomarkers might be cost prohibitive; therefore, only a subset of biomarkers that are complimentary to each other will have to be selected for such a panel, each will provide specific and unique information.

POTENTIAL UTILITIES OF BIOMARKERS

Early diagnosis of kidney injury

Renal biomarkers have several potential advantages, of which early diagnosis is considered the major one (6). It has been shown that kidney injury can be identified using sensitive biomarkers days before any increase in sCr is documented (7), thus measurement of biomarkers may direct the clinician's attention to ongoing kidney damage, before there is a measurable reduction in kidney function. For example if a nephrotoxic drug is being administered, it would be more rational to monitor the patient using biomarkers with the capability to indicate kidney injury, rather than using markers that can only indicate presence of kidney failure. Once kidney injury is identified, the drug can be discontinued before kidney failure occurs. Such practice is expected to be associated with better outcome, since once kidney function decreases, recovery is expected to be prolonged and the patient might die before the kidney had the opportunity to recover (even if the latter is possible).

Screening patients at risk for AKI

Biomarkers indicating active kidney injury are likely to be more sensitive compared with markers indicating decreased function (i.e. surrogates for GFR); therefore the former should be utilized to screen patients with high risk for kidney injury. It has been shown that the prevalence of AKI in hospitalized patients is relatively high when using human criteria to diagnose AKI (8). Nevertheless, human criteria as well as the International Renal Interest Society (IRIS) guidelines rely on changes in sCr (or urine production), and therefore probably represent an underestimation of the true prevalence of AKI in hospitalized patients in general, and specifically in the ICU setting. Early recognition of kidney injury in hospitalized patients will allow identification, and potentially elimination of the cause and might facilitate therapy initiation before the injury progresses to failure, therefore a better outcome is to be expected. The current limitation of this approach is the low availability of commercial assays for assessment of candidate biomarkers; yet, if proven useful, availability of such assays is expected to increase in future years.

Differentiating upper and lower urinary tract infection

A recent study of neutrophil gelatinase-associated lipocalin (NGAL) demonstrated increased urinary NGAL concentration in a subset of dogs with apparent lower urinary tract disease, potentially indicating upper urinary tract involvement (9). Increase in urinary NGAL concentration might be the result of local inflammation within the lower urinary system, and not necessarily due to kidney damage, since NGAL originates also from neutrophils that are being recruited as part of the local inflammatory process (10). Nonetheless, recent unpublished data suggest that other biomarkers, which are kidney specific, are also increased in dogs with apparently lower UTI, indicating that some of these dogs have subclinical pyelonephritis and ongoing kidney injury, which would go unnoticed based on the current conception. It is thus possible that in the absence of sensitive and specific tools to aid differentiating upper and lower urinary tract infection, some of the patients with apparent cystitis, in fact suffer from pyelonephritis, and are consequently being undertreated. With the growing availability of kidney-specific biomarkers, differentiation between upper and lower urinary tract infection may become simpler when UTI is documented, and sequential changes in the biomarker concentrations can then guide treatment.

Markers of chronic kidney disease progression

The traditional conception is that AKI and chronic kidney disease (CKD) represent two distinct processes of kidney damage. AKI represents rapidly progressing active damage due to various etiologies, whereas CKD represent slowly progressive damage. Lately, it has been suggested that AKI and CKD might not be separate entities, as they are influenced by various similar conditions, share common risk factors and ultimately impact each other (AKI is a risk factor for CKD and vice versa) (11). In both dogs and cats with CKD, the etiology is often unidentified, but regardless of the etiology, there is gradual replacement of the normal kidney parenchyma with inflammation and scar tissue. Results of recent studies indicate that active kidney damage is present in a subset of dogs with apparently stable (based on sCr) CKD. This finding might account, at least in part, for the wide variation in progression rate of CKD that occurs among dogs and cats (12, 13). Presence of active kidney injury, demonstrated by increased concentrations of biomarkers, likely predicts a risk for rapid progression of the disease, whereas absence of active kidney injury, predicts a more slowly progressive kidney disease. The documentation of ongoing active kidney damage in patients with CKD might suggest that the pathophysiology of AKI and CKD share more characteristics than currently recognized, and the main difference between these two types of kidney damage is the rate of disease progression (11).

Identification of markers of kidney damage in dogs and cats with CKD might also facilitate the diagnosis of IRIS Stage I CKD, which is currently challenging with the available tools. Early identification of the disease will allow early intervention, aimed to preserve kidney function. Moreover, assessment of biomarkers indicating active kidney damage in animals with CKD will facilitate the investigation of novel therapeutic interventions. Active injury biomarkers will be monitored sequentially after the application of these therapies, at the same manner as alanine aminotransferase is being used to assess the efficacy of different interventions during the management of liver diseases. The currently available markers of kidney damage preclude such an assessment, as short-term benefits are unlikely to alter kidney function, despite either benefit or harmful effects.

CANDIDATE BIOMARKERS

Symmetric dimethylated arginine

Symmetric dimethylated arginine (SDMA) is a methylated form of the amino acid arginine. There are 3 main species of methylated arginine: monomethylarginine, asymmetric dimethylarginine, and SDMA. All are derived from intranuclear methylation of L-arginine by protein-arginine methyltransferase and released into the circulation after proteolysis (14, 15). SDMA is primarily eliminated through the kidneys by renal filtration and excretion (16), therefore it is a potential endogenous marker of GFR (17, 18). SDMA was shown to be closely associated with measured GFR in people (17, 18), and was found as an independent predictor of all-cause and cardiovascular mortality (19). In human patients after ischemic stroke, SDMA was strongly associated with renal function and clinical outcome (20). Additional studies in people have established SDMA as a more sensitive and specific marker of renal function compared with sCr (20-22).

In veterinary medicine, there is growing evidence regarding the utility of SDMA as an early marker of kidney dysfunction. As creatinine, SDMA is a filtration marker,

but might have several potential advantages. The most important one is that SDMA is not influenced by muscle mass and therefore, its reference range is more uniform among dog breeds. Creatinine is a major metabolite of muscle breakdown and thus its serum concentration largely depends on muscle mass. Consequently, kidney function may be overestimated in cachectic or geriatric animals with low muscle mass, or underestimated in animals with high muscle mass (23-25). In a prospective study of cats, the correlation between muscle mass, creatinine and SDMA concentrations was examined; GFR was measured and body mass composition was evaluated using dual-energy X-ray absorptiometry (23). This study demonstrated that muscle mass and GFR, both decreased with age, but so did creatinine, implicating that sCr is affected by muscle mass, and likely does not reflect renal function accurately in this specific population. On the other hand, SDMA concentration increased with age, demonstrating that this marker is not affected by alterations in muscle mass and thus might serve as a better marker for renal function. In a parallel prospective study of dogs, correlations between muscle mass, creatinine and SDMA concentrations were studied over 6 months (25). Age and muscle mass, significantly correlated with creatinine, while SDMA did not, indicating that sCr concentration is influenced by muscle mass, whereas SDMA concentration is not.

A recent study reported earlier detection of CKD in cats when using SDMA as a marker of kidney function compared with sCr (6). In this study, increased SDMA concentration was associated with an average of 40% decrease in GFR, preceding the diagnosis of CKD using sCr by an average of 17 months. In dogs with X-linked hereditary nephropathy (XLHN), SDMA was superior to sCr for early identification of kidney function (26).

Despite several experimental and clinical studies indicating that SDMA is a promising marker of kidney function, the absence of a practical analytical method precluded its use in the clinical setting. Lately, a chemistry assay had become available for routine veterinary use and the IRIS has recently recognized SDMA as a biomarker for kidney function in dogs and cats.

Gamma-glutamyl transpeptidase

Gamma-glutamyl transpeptidase (GGT) is a proximal tubular brush border enzyme. Due to its large molecular weight (126 kDa), GGT does not undergo glomerular filtration, therefore its presence in the urine is indicative of tubular damage (27). In dogs, increased urine GGT activity was documented as early as one day following AKI initiation, whereas sCr increased only seven days later (28), suggesting that urine GGT activity is an early marker of AKI. In another study, urinary GGT-to-creatinine ratio was associated with the severity of proximal tubular lesions in dogs with pyometra (29). GGT-to-creatinine ratio was also found as an early marker in gentamicin-induced nephrotoxicosis in dogs (30). Early detection of AKI based on urine GGT activity was also demonstrated in a study including dogs with naturally occurring renal disease (31). In the latter study urine GGT activity of dogs with CKD overlapped with the activity of healthy dogs suggesting that GGT is not a sensitive diagnostic marker for CKD (31). Other studies found that urinary GGT-to-creatinine ratio is not as highly sensitive and specific as was previously suggested. Urinary GGT-to-creatinine ratio was increased in dogs poisoned by a non-nephrotoxic agent (*Nerium oleander*) (32) and was an insensitive marker of cisplatin induced AKI (33). One of the shortcomings of GGT is that it is not stable, even if frozen (34), limiting its clinical and research utility.

N-acetyl-β-glucosaminidase

N-acetyl-β-glucosaminidase (NAG) is a high molecular weight (150 kDa) lysosomal enzyme found in many mammalian tissues, serum and urine and is abundantly present in cells of the renal proximal tubules (35). Urinary NAG concentrations are increased in human patients with nephrotoxicity (36), in diabetic nephropathy (37) and following cardiopulmonary bypass procedure (38). Increased NAG activity has also been shown to precede increases in sCr by 12 hours to 4 days (39), allowing early therapeutic intervention. NAG activity is also associated with the severity of injury, as its activity was associated with the need for renal replacement therapy as well as with mortality (40).

In dogs, urinary NAG to creatinine ratio was significantly increased in CKD compared with healthy controls (41), and was also higher in dogs with CKD or pyelonephritis compared to healthy dogs or dogs with UTI (42). Its decreased specificity though is due to the fact that it is influenced by proteinuria (43). In hyperthyroid cats, NAG index was unable to identify geriatric cats at risk of developing azotemia (44), nor differentiate non-azotemic

from azotemic hyperthyroid cats before treatment with methimazole (45).

Cystatin C

Cystatin C is a small, non-glycosylated, 13-kDa protein belonging to the super family of cysteine proteinase inhibitors (46). Cystatin C is constantly produced by nucleated cells and released into the circulation. Due to its low molecular weight, cystatin C is freely filtered by the glomerulus, it is then reabsorbed and catabolized in the proximal renal tubules without re-entering the bloodstream or being excreted in the urine (47). Due to the aforementioned characteristics, cystatin C can be utilized as a filtration marker and marker of tubular function. It is considered superior to sCr in detecting changes in renal function in humans (48).

Serum and plasma cystatin C were investigated as alternative markers for renal function in dogs (49, 50). Unlike sCr, cystatin C was unaffected by sex, age, muscle mass and other non-renal disease such as neoplasia or infection (46, 49, 50); therefore it might serve as a better surrogate for GFR. Preliminary studies in dogs suggest that serum cystatin C is an early marker of kidney dysfunction in critically ill dogs (51), in dogs with visceral Leishmaniasis (52), and in dogs with CKD and diabetes mellitus (53). Though preliminary results are encouraging, further investigation is warranted, particularly due to the reported biological fluctuations of plasma cystatin C concentrations associated with eating (50).

Since cystatin C is reabsorbed by the proximal renal tubules, its urine concentration is an indicator of renal tubular function (54). Urinary cystatin C to creatinine ratio was found to be a specific marker of tubular dysfunction in dogs (55). In a model of gentamycin induced AKI in beagles, urinary cystatin C was the most sensitive of 14 markers evaluated (56). Conversely, in dogs with CKD due to Leishmaniasis, cystatin C was not an early marker, increasing only at the late stages of the disease when dogs are already azotemic (57). A human assay for cystatin C was validated in cats in a study demonstrating that urinary cystatin C is higher in cats with CKD compared with healthy controls (58). However this finding was not in agreement with a recent study suggesting that increased urinary cystatin C concentration is not a consistent finding in cats with CKD and does not accurately differentiate CKD cats from healthy controls (59); thus, cystatin C is neither sensitive nor specific marker for kidney function in cats.

Kidney Injury Molecule-1

Kidney Injury Molecule-1 (KIM-1) is a transmembrane glycoprotein, which is markedly up-regulated during AKI, and its urine concentration is a sensitive marker of AKI in human patients (60). In critically ill or septic human patients, urinary KIM-1 was found as an early marker of AKI, but was not associated with the progression rate of the disease or with the need for renal replacement therapy (61).

Information regarding the utility of KIM-1 in veterinary medicine is scarce. KIM-1 was undetectable in healthy dogs and was increased in dogs with gentamicin induced AKI, and preceded detectable elevations in sCr and BUN concentrations (56). KIM-1 was also recently evaluated in cats with kidney disease (62). KIM-1 was expressed in specific segments of the nephron and detected in urine of cats at risk of AKI.

Alpha 1 microglobulin and Beta 2 Microglobulin

Low molecular weight proteins such as Alpha 1 microglobulin (α1M) and Beta 2 microglobulin (β2M) are freely filtered by the glomerulus and reabsorbed by the tubules (63). Increased urinary concentration of these proteins, indicate tubular uptake impairment and therefore may be used as markers of proximal tubular injury.

β2M is an 11.8 kDa protein, which makes part of the major histocompatibility class I, and is expressed on all nucleated cells. After dissociating from the complex, it is freely filtered by the glomerulus, and almost entirely reabsorbed and catabolized by the proximal tubular cells (64). During kidney injury, its reabsorption rate is reduced and consequently urinary concentration increases, as was demonstrated in humans after nephrotoxic exposure, cardiac surgery and renal transplantation (65, 66). It is considered an early marker of tubular injury as its urinary concentration rises 4-5 days before sCr exceed the upper limit of the reference range (67). One of the major limitation of this marker is its instability in urine (especially when urine pH drops below 6.0), limiting the utility of this marker (68).

α1M is a small protein (27-33 kDa), synthesized by the liver and bound to IgG in the circulation. It is normally filtered by the glomerulus and reabsorbed by the proximal tubule, therefore might serve as a sensitive marker of tubular integrity. It was found to be increased even before histological damage is evident (69), and unlike β1M, it is stable in a range of urinary pH levels (70).

Retinol Binding Protein

Retinol Binding Protein (RBP) is a low molecular weight protein (21 kDa), synthesized by the liver and functions as a carrier protein for retinol (Vitamin A1). When unbound, it is freely filtered and then metabolized and reabsorbed by the proximal renal tubules, thus has been suggested as a marker of proximal tubular dysfunction (71). In one study, high urinary RBP (uRBP) concentration was predictive of the need for hemodialysis in ICU human patients (72). Increased uRBP concentration was also found to be a highly sensitive indicator of renal tubular dysfunction in humans, preceding increases in urinary NAG activity (73). Additionally, increased uRBP concentration was documented as an early marker of AKI in infants, following birth asphyxia (74).

Several veterinary studies have evaluated the utility of RBP as an early marker of AKI. In a study of XLHN, increased uRBP to creatinine ratio correlated with sCr and GFR. Moreover, RBP continued to increase as the disease progressed, while other biomarkers peaked and reached a plateau early in the course of disease (43). URBPs were assessed in dogs with pyometra as markers of proximal tubular function. URBP concentrations were significantly increased compared with healthy controls. Furthermore, 68% of 17 non-azotemic dogs had increased urinary biomarker concentrations. The results of this study suggest that the occurrence of AKI in dogs with pyometra is higher than previously perceived when using routine markers (75). In a study characterizing kidney damage during naturally occurring canine heatstroke, uRBP was also increased, often before any increase in sCr was documented (76).

Neutrophil gelatinase-associated lipocalin

Neutrophil gelatinase-associated lipocalin (NGAL) is the most studied biomarker thus far in veterinary medicine (77, 78). It is a 25 kDa protein, originally discovered in the granules of neutrophils, and is released in response to bacterial infection (79). Its antibacterial function is not yet clear, but it seems to play a bacteriostatic role, due to its ability to bind siderophores and sequester iron (6). NGAL is also present in various organs such as trachea, lung, stomach, colon and kidneys (80). In an experimentally induced ischemic AKI in rodents, NGAL was the first protein to increase. Its expression in the proximal tubules increased by 4-fold after 3 hours of unilateral ischemic reperfusion injury (81).

In a prospective study evaluating NGAL as a marker of kidney injury in dogs, urinary NGAL to creatinine ratio (UNCR) was higher in dogs with AKI compared with other urinary tract diseases (CKD, UTI). Receiver operator characteristics analysis, performed to assess UNCR as a predictor of AKI had an area under the curve of 0.94 (9). Interestingly, the median UNCR of the non-azotemic, IRIS Grade 1-AKI dogs, was significantly higher compared with other groups, but did not differ from that of dogs in the azotemic AKI group, indicating that UNCR increases before sCr, and thus may be used as an early marker of the disease.

In experimentally induced AKI, gentamicin was administered to five dogs until an AKI was documented, as defined by 50% increase in sCr compared to baseline. During the first 16 days, sCr remained unchanged but UNCR increased as from day 7 and preceded the increase in sCr by approximately 7 days (7). Increased NGAL was also documented during experimentally induced hemorrhage leading to hypotension and reperfusion injury in seven anesthetized Greyhound dogs, indicating that it is a sensitive marker of ischemic injury (82).

Despite being a very sensitive and an early marker of kidney injury, NGAL's specificity is questionable, as it originates from multiple tissues as well as from neutrophils and therefore may increase during inflammation and other disease processes accompanied by neutrophil stimulation (e.g., sepsis, lower urinary tract diseases). In humans, serum NGAL concentrations were up to 80% higher in septic AKI patient compared to non-septic AKI patients (79). A study of 15 septic dogs without evidence of AKI, undergoing emergency laparotomy, demonstrated increased urinary and serum NGAL concentrations compared with a control group undergoing intervertebral disk surgery, raising questions regarding the specificity of NGAL (83). Finally, NGAL was found highly variable among different age groups and genders in people and its serum concentration is influenced by co-existing CKD, systemic inflammation, anemia hypoxia and other inflammatory conditions such as eczema, colitis, and several solid tumor malignancies (6).

Interleukin 18

Interleukin 18 (IL-18) is a proinflammatory cytokine involved in activation and production of helper T cells, IgG and interferon-γ. It is produced and stored in the intercalated cells of the distal convoluted tubules, as well as at the proximal convoluted tubules and the collecting duct (84).

A study of induced nephrotoxic AKI in mice, found IL-18 to be an indicator of tubular injury (85). Urinary IL-18 concentrations were higher in AKI patients compared with those with CKD, UTI, and prerenal azotemia (86). In another study, human patients who underwent cardiopulmonary bypass and developed AKI, had increased IL-18 levels 4-6 hours following surgery, peaking at 12 hours post surgery, while sCr concentrations peaked only after 48-72 hours (87).

In a study of the predictive ability for survival of pediatric AKI patients, IL-18 concentration was higher in patients with AKI, compared with the control group, and was positively correlated with the stage of AKI, but was not an accurate outcome indicator (81). In another study of human patients with ARDS, urinary IL-18 was found a predictive marker of AKI, preceding the elevation in sCr concentrations by 1 to 2 days (88). Nonetheless, IL-18 may increase in non-renal inflammatory diseases, such as arthritis, inflammatory bowel disease, pulmonary diseases and hepatitis, therefore its specificity to kidney diseases is limited (84).

Future biomarkers

Serum inosine, urinary cystatin B and urinary clusterin, are some of the promising biomarkers currently under investigation. These markers are alluring, as kidney specific assays are being developed to assess their concentration in dogs and cats. Therefore, they are considered more specific and encompass the potential to predict accurately active and ongoing injury processes. In addition to early detection of AKI, preliminary studies suggests their usefulness in documenting subclinical active kidney injury associated with concurrent urinary diseases or other systemic pathologies that may influence the kidney.

SUMMARY

Kidney biomarkers might change in the next years some of the common paradigms in veterinary nephrology. At this point, data regarding the utilization of such biomarkers in veterinary medicine is still scarce, and further research is warranted. Each of the investigated biomarkers exhibits advantages and weaknesses and it is thus most likely only an array of biomarkers will provide all the necessary information.

DECLEARED CONFLICT OF INTEREST

There was no conflict of interest or any financial conflict in this study.

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