REVIEW

Biomarkers in the assessment of acute and chronic kidney diseases in the dog and cat

A. R. COBRIN*, S. L. BLOIS*, S. A. KRUTH*, A. C. G. ABRAMS-OGG* AND C. DEWEY†

*Ontario Veterinary College Department of Clinical Studies, University of Guelph, Guelph, Ontario, Canada N1G 2W1
†Ontario Veterinary College Department of Population Medicine, University of Guelph, Guelph, Ontario, Canada N1G 2W1

In both human and veterinary medicine, diagnosing and staging renal disease can be difficult.
Measurement of glomerular filtration rate is considered the gold standard for assessing renal function but methods for its assessment can be technically challenging and impractical. The main parameters used to diagnose acute and chronic kidney disease include circulating creatinine and urea concentrations, and urine-specific gravity. However, these parameters can be insensitive. Therefore, there is a need for better methods to diagnose and monitor patients with renal disease. The use of renal biomarkers is increasing in human and veterinary medicine for the diagnosis and monitoring of acute and chronic kidney diseases. An ideal biomarker would identify site and severity of injury, and correlate with renal function, among other qualities. This article will review the advantages and limitations of renal biomarkers that have been used in dogs and cats, as well as some markers used in humans that may be adapted for veterinary use. In the future, measuring a combination of biomarkers will likely be a useful approach in the diagnosis of kidney disorders.

INTRODUCTION

Kidney diseases commonly affect dogs and cats, and are often associated with guarded to poor outcomes in their later stages (Polzin 2011). The overall prevalence of chronic kidney disease (CKD) is 0-5 to 7% in dogs and 1-6 to 20% in cats (Lund et al. 1999, Polzin 2010). The prevalence increases to 15% in dogs over 10 years of age and up to 31% in cats over 15 years of age (Lulich et al. 1992, Polzin 2010, O’Neill et al. 2013). Depending on International Renal Interest Society (IRIS) stage and progression of disease, the reported median survival times (MSTs) with CKD varies widely, as those cats and dogs with a lower IRIS stage live longer than those with more advanced stages (Jacob et al. 2002, Boyd et al. 2008, Parker & Freeman 2011). Cats with CKD have a reported MST of 1512 days for IRIS Stage II, with 778 and 103 days for Stages III and IV, respectively (Boyd et al. 2008). Dogs with CKD are reported to have an MST of 226 days when all IRIS stages are considered (O’Neill et al. 2013). Proteinuria contributes to a decreased survival time in both dogs and cats, whereas hypertension is associated with decreased survival time only in dogs (Jacob et al. 2003, 2005, Syme et al. 2006, Jepson et al. 2007, King et al. 2007).

Acute kidney injury (AKI) has a poor prognosis: mortality rates associated with AKI are 50 to 60% in companion animals, with many deaths occurring shortly after diagnosis (Vaden et al. 1997, Worwag & Langston 2008, Thoen & Kerl 2011). Key factors in the high mortality rate associated with AKI are the delayed detection of this condition due to insensitive diagnostic tests, the subtlety of early signs delaying presentation to a veterinarian, and the rapid progression of kidney injury associated with nephrotoxins such as ethylene glycol or lilies. AKI is associated with a high mortality rate of approximately 45 to 64% in humans, especially critically ill patients (Bagshaw 2005, Bellamo 2008). Given a lack of effective therapies for AKI, early identification and supportive management may improve survival (Slocum et al. 2012).

Despite the clinical importance of kidney diseases, their early diagnosis can be challenging. Measurement of glomerular filtration rate (GFR) is thought to be the best method for assessing renal function as it is directly proportional to functional renal mass (Kerl & Cook 2005). However, techniques for measurement of GFR can be challenging for many practices because of the need for specialised equipment and the rigorous sampling procedures required by some methods (Gaspari et al. 1997, Kerl...
& Cook 2005). Serum creatinine concentration is often used to estimate GFR and stage CKD, but is a relatively insensitive marker of renal function in humans and dogs (Narayanan & Appleton 1980, Finco et al. 1999, Braun et al. 2003, Peake & Whiting 2006).

Novel serum and urine biomarkers are being evaluated in human and veterinary patients in an attempt to improve the ability to accurately diagnose both acute and chronic kidney diseases including the potential for early disease detection. Furthermore, these biomarkers help to identify the various processes that cause kidney disease such as glomerular damage and tubular stress or dysfunction. An ideal biomarker to detect kidney disease would be specific, able to detect both AKI and CKD, sensitive for detecting early disease, capable of documenting extent or severity of disease and of monitoring disease progression, adept at injury location, predictive of clinical outcome, non-invasive, of low cost and rapidly available from a reference laboratory or point-of-care assay (Oberbauer 2008, Frangogiannis 2012, Slocum et al. 2012, Urbach et al. 2011). There are several renal biomarkers, with various advantages and disadvantages, which are being investigated in veterinary medicine (Table 1). The purpose of this review article is to evaluate the current evidence on renal biomarkers in

| Table 1. The clinical indications, advantages, disadvantages, and common assay methodology of selected serum and urinary biomarkers of renal disease reported in veterinary medicine |
|---|---|---|---|---|---|
| **Biomarker** | **Sample(s) needed** | **Condition(s) leading to marker elevation** | **Advantages** | **Disadvantages** | **Common method(s) of measurement** |
| **Surrogate markers of GFR** |  |
| Creatinine | Serum | Declining GFR Various non-renal causes | Widely available Inexpensive Familiar assay Most accurate in steady state GFR | Minor assay interference from non-creatinine chromogens (e.g. proteins, glucose, ketoacids) Non-linear relationship with GFR Proportional to patient muscle mass Influenced by pre and post-renal azotaemia and hydration status Higher creatinine levels in breeds with increased muscle mass (e.g. Boxers, Greyhounds, sled dogs, Birmans) | Jaffe (alkaline picrate) reaction Enzymatic reactions |
| **Markers of tubular dysfunction** |  |
| Cystatin C | Serum, urine | Proximal tubular damage causing decreased reabsorption | Good marker of GFR in early stages of renal disease | Questionable effects of age and weight in dogs Not consistently shown to be superior to creatinine as a marker of GFR | Particle-enhanced turbidimetric immunoassay (PETIA) |
| RBP | Urine | Proximal tubular damage causing decreased reabsorption | Stable in acidic urine and frozen samples Useful for monitoring chronic disease due to progressively increases in later disease stages | Wide intra-individual variation in feline CKD and hyperthyroidism | ELISA Western blot |
| α1-microglobulin | Urine | Proximal tubular damage causing decreased reabsorption | Stable in acidic urine | Decreased by hepatic disease | Western blot |
| β2-microglobulin | Plasma, urine | Proximal tubular damage causing decreased reabsorption | Good predictor of GFR in dogs | Unstable in acidic urine Decreased sensitivity for monitoring disease progression Affected by proteinuria, hyperthyroidism, diabetes mellitus, alkaline urine pyuria, long-term storage, ±sex | ELISA Western blot |
| NAG | Urine | Proximal tubular damage causing increased release | Can measure activity from spot urine sample Marker of AKI secondary to pyometra, leishmaniasis and nephrotoxins | Lack specificity as NAG B Isozyme associated with protein processing and lysosomal activity | Enzymatic colorimetric assay |
| GGT | Urine | Proximal tubular damage causing increased release | Can measure activity from spot urine sample Used to identify nephrotoxicity secondary to gentamicin | Unstable in acidic urine, Hematuria and pyuria cause assay interference | Spectrophotometric assay |
| NGAL | Urine, plasma, or serum | Tubular damage causing increased release | Samples stable with freeze-thaw cycles | Hematuria and pyuria may cause assay interference Malnourished, inflammation and infection may decrease specificity | ELISA (several species-specific assays, including dogs) |

GFR glomerular filtration rate, CKD chronic kidney disease, AKI acute kidney injury, ELISA enzyme-linked immunooabsorbant assay, RBP retinol-binding protein, NAG N-acetyl β-D-glucosaminidase, GGT γ-glutamyl transpeptidase, NGAL neutrophil gelatinase-associated lipocalin
Renal biomarkers in veterinary medicine

... veterinary medicine for the dog and cat and to examine some biomarkers currently used in human medicine and the current evidence of their potential utility in veterinary species.

**SURROGATE MARKERS OF GFR**

**Creatinine**

Creatinine is a by-product of endogenous muscle metabolism (Peake & Whiting 2006, Linnetz & Graves 2010). Creatinine is distributed in the body water, is almost entirely freely filtered at the glomerulus, is not absorbed by the renal tubules and undergoes minimal tubular secretion. As such, it inversely correlates with GFR and is an indicator of renal function (Jacobs et al. 1991, Guyton & Hall 2006, Lefebvre 2011).

**Staging kidney disease using creatinine**

Circulating creatinine concentration is the most widely used marker of renal function in human and veterinary medicine. Creatinine is a primary variable in kidney disease staging schemes including the RIFLE classification (Risk of renal dysfunction, Injury to the kidney, Failure of renal function, Loss of renal function and End-stage renal disease) and the Acute Kidney Injury Network (AKIN) criteria (Bellomo et al. 2004, Bagshaw et al. 2008, Lee et al. 2011). Modified RIFLE and AKIN scores have been applied to dogs with AKI and have been shown to predict outcome (Lee et al. 2011, Thoen & Kerl 2011). The IRIS system uses creatinine concentration to stage dogs and cats with CKD (IRIS 2007), and IRIS stage has been strongly associated with survival time (Boyd et al. 2008). A modified IRIS classification to define AKI grades is based on serum creatinine concentration, urine output and the need for renal replacement therapy (Cowgill 2012). Even when circulating creatinine concentration is within the reference interval, increases from baseline are considered significant in human medicine and this concept is the key to the RIFLE staging system and AKIN criteria (Bellomo et al. 2004, Bagshaw et al. 2008). Similar trends are also being recognised in veterinary medicine. For example, the definition for IRIS AKI Grade I includes non-azotaemic patients demonstrating a progressive increase in baseline circulating creatinine concentration ≥26.4 µmol/L within 48 hours (Cowgill 2012).

**Limitations of creatinine**

Using circulating creatinine concentration to diagnose kidney disease has significant limitations. Creatinine measurement is insensitive for early renal insufficiency; at least 75% loss of functional nephrons occurs before creatinine increases above the reference interval (Lefebvre 2011). In addition, the relationship between GFR and serum creatinine is nonlinear: in early kidney disease, large reductions in GFR result in relatively small increases in circulating creatinine concentration, whereas the reverse is true in more advanced kidney disease (Braun et al. 2003, Peake & Whiting 2006). Therefore, serial creatinine measurement may not accurately detect deterioration in kidney function, especially in rapidly progressive conditions such as AKI (Finco et al. 1995, Thomas et al. 2011). Non-renal factors that can influence creatinine concentration include muscle mass, renal tubular secretion of creatinine in male dogs and various causes of pre and postrenal azotaemia such as hydration status (Finco & Duncan 1976, Finco et al. 1995). Despite these limitations, many of the studies assessing the utility of new renal biomarkers compare their performance to that of circulating creatinine concentration in detecting kidney disease. In human medicine, some of the limitations of creatinine as a surrogate marker for GFR are circumvented by using an estimation of GFR incorporating other factors besides serum creatinine; the estimated GFR (eGFR) is calculated using various formulas that incorporate factors such as creatinine, age, sex, ethnicity and body mass (Levey et al. 2009). Similar equations for calculating eGFR have not been described for dogs and cats, however, factors such as age, sex, bodyweight and breed can all affect measured GFR values (Lefebvre et al. 2004, Bexfield et al. 2008, Lefebvre 2010).

**Cystatin C**

Cystatin C is a cysteine proteinase inhibitor produced constitutively by all nucleated cells (Abrahamson et al. 1986, Grubb 2001, Monti et al. 2012). Cystatin C is freely filtered at the glomerulus and is absorbed and catabolised in the cells of the proximal tubule, with minimal tubular secretion (Grubb 2001). While there is minimal urinary excretion of cystatin C in healthy patients, its reabsorption is reduced when renal tubular damage occurs resulting in increased concentrations of cystatin C in the urine (Uchida & Gotoh 2002).

**Cystatin C in humans**

Circulating cystatin C is primarily used as a GFR marker in assessment of CKD in humans. Various human studies have shown that cystatin C is more sensitive for detecting reduced GFR than creatinine and better able to detect small changes in GFR in the same patient (Kyhse-Andersen et al. 1994, Herget-Rosenthal et al. 2004a). However, sensitivity of circulating cystatin C concentration to detect AKI in humans has been variable (Herget-Rosenthal et al. 2004a, Royakkers et al. 2011). Cystatin C can have high intra-individual variability in humans, requiring at least a 37% difference from the mean reference value to indicate a significant difference and potentially complicating interpretation of serial results (Keevil et al. 1998).

**Cystatin C in dogs**

Measurement of circulating cystatin C concentration has been evaluated as a GFR marker in dogs using commercially available particle-enhanced turbidimetric immunoassay (PETIA), particle enhanced nephelometric immunoassay (PENIA) and enzyme-linked immunosorbent assay (ELISA) methods (Miyagawa et al. 2009). Western Blot analysis demonstrated cross-reactivity of anti-human cystatin C antibody to canine cystatin C, validating its use in canine studies (Almy et al. 2002). While one study showed that age, bodyweight and sex did not affect cystatin C in healthy dogs (Wehner et al. 2008), another showed that cystatin C was higher in very young or old healthy dogs and in those more than 15 kg (Braun et al. 2002).
Cystatin C can be measured in canine urine or serum. Concentrations of urinary or serum cystatin C were significantly elevated in dogs with renal disease of various causes compared to those without, and correlated strongly with GFR measured by creatinine or iothexol clearance in both healthy dogs and those with renal disease (Wehner et al. 2008, Miyagawa et al. 2009). Cystatin C correlated more strongly with GFR measured by creatinine clearance than serum cystatin concentration (Wehner et al. 2008). However, another canine study found that serum cystatin C was not superior to creatinine concentration as a marker of GFR (Almy et al. 2002). Previous studies describing cystatin C in renal disease have been performed in populations with variable types of renal injury, which might have major influences on renal function and therefore account for the variation in results obtained. Overall, further studies are required to determine if cystatin C is a better marker for kidney disease than creatinine concentration, however, cystatin C may be superior especially in early stages of renal damage (Grubb 2001, Laterza et al. 2002). Currently, no studies have explored the utility of cystatin C in detecting AKI in dogs and cats.

**MARKERS OF TUBULAR DYSFUNCTION**

**Low molecular weight proteins: Retinol-binding protein**

Retinol-binding protein (RBP) is a 21-kDa low molecular weight protein (LMW) protein synthesised in the liver (Roberts et al. 1990, Barbosa de Deus & de Paulo Castro Teixeira 2008). Unbound RBP is filtered at the glomerulus and is almost completely reabsorbed and catabolised in the proximal tubular cells (Raila et al. 2010, Smets et al. 2010a). Like other LMW proteins, RBP can be detected in the urine when there is tubulointerstitial damage impairing reabsorption (D’amico & Bazzi 2003).

**Retinol-binding protein in humans**

Urinary RBP is usually measured in humans using an ELISA (Topping et al. 1986) and is a sensitive indicator of renal tubular damage in humans (Kirsztajn et al. 2002). In addition, urinary RBP has been shown to predict disease progression and prognosis in humans with glomerulopathies (Kirsztajn et al. 2002, Barbosa de Deus & de Paulo Castro Teixeira 2008). While RBP is often used in the detection of CKD in humans, it has also been used to predict AKI in infants following birth asphyxia (Roberts et al. 1990).

**Retinol-binding protein in dogs**

RBP has been identified in canine and feline urine using Western blot, and ELISA assays have been validated in dogs and cats (van Hoek et al. 2008, Maddens et al. 2010, Smets et al. 2010a). Urinary RBP has been used as a marker of decreased tubular function in dogs with CKD. In a study comparing urinary biomarkers in young and older healthy dogs to CKD dogs, the urinary RBP to creatinine ratio (uRBP/c) was significantly elevated in CKD dogs and strongly correlated with urea and creatinine concentrations (Smets et al. 2010a). In addition, age did not appear to affect uRBP/c (Smets et al. 2010a). In dogs with X-linked hereditary nephropathy, a model of progressive proteinuric nephropathy, uRBP/c correlated most strongly with serum creatinine, GFR, and histological lesions, and demonstrated progressive increases in values compared to other markers of renal function, even in late stage kidney disease (Vinge et al. 2010, Nabity et al. 2012). Urinary RBP/c was significantly elevated in azotaemic dogs in another study, but was not sensitive enough to detect decreasing GFR in non-azotaemic dogs (Raila et al. 2010). Further study is needed to determine if RBP is a sensitive marker of proximal tubular dysfunction, as well as GFR, in dogs.

**Retinol-binding protein in cats**

Urinary RBP is elevated in cats with CKD or hyperthyroidism, compared to healthy cats, and normalises in cats without pre-existing CKD after establishment of euthyroidism. However, there is large inter-individual variation in RBP concentrations in CKD and hyperthyroid cats (van Hoek et al. 2008, Van Hoek et al. 2009a,b).

In summary, studies in dogs suggest that RBP is a promising marker of tubular dysfunction in CKD. However, assessment of tubular function using RBP in hyperthyroid cats is problematic secondary to large inter-individual variation, and may indicate that this is not a suitable marker of feline renal tubular injury.

**Low molecular weight proteins (LMW): α1 and β2-microglobulins**

α1-Microglobulin, a 27-kDa anti-inflammatory protein, and β2-microglobulin, an 11.8-kDa protein expressed on all nucleated cells, are also LMW markers of proximal tubular dysfunction (Penders & Delanghe 2004). Unlike RBP and α1-microglobulin, a major limitation in the measurement of β2-microglobulin is its instability in acidic urine (Bernard et al. 1987, Penders & Delanghe 2004).

**Low molecular weight proteins in humans**

Urinary excretion of α1- and β2-microglobulin are used to detect AKI and CKD in humans, and can be measured by ELISA and radioimmunoassay (Reichert et al. 1995, Bazzi et al. 2001, Branten et al. 2005). Urinary α1-microglobulin was significantly associated with the severity of histological lesions in humans with membranous nephropathy, and was able to predict progression to CKD (Bazzi et al. 2001). In humans with acute tubular necrosis, elevation in urinary α1-microglobulin concentration predicts the need for renal replacement therapy and may indicate a poor prognosis (Herget-Rosenthal et al. 2004b). Because α1-microglobulin is produced in the liver, hepatic disease can decrease concentrations, interfering with the ability to detect renal disease in patients with concurrent hepatic disease (Penders & Delanghe 2004).

Urinary β2-microglobulin excretion was more sensitive than serum creatinine concentration for the detection of AKI in humans, preceding elevation of serum creatinine by several days (Tölkoff-Rubin et al. 1988). Urinary excretion of β2-microglobulin was shown to be a strong predictor for progression of renal insufficiency and can predict survival in humans with membranous nephropathy (Branten et al. 2005).
Low molecular weight proteins in dogs

Urinary α1-microglobulin concentrations have been measured in dogs with Western blot (Vinge et al. 2010). An ELISA has been validated for measuring canine β2-microglobulin, and this protein can also be measured using Western blot (Nabity et al. 2012). Several canine studies have examined the utility of α1- and β2-microglobulin for the detection and monitoring of CKD. Urinary α1-microglobulin concentrations progressively increased over time in dogs with Alport syndrome, a model of progressive glomerular disease, and were significantly increased compared to normal dogs (Vinge et al. 2010). When compared to other markers of tubular injury, urinary β2-microglobulin concentration was a significant and independent predictor of GFR in dogs with X-linked hereditary nephropathy (Nabity et al. 2012). However, in the later stages of canine CKD, urinary β2-microglobulin concentrations were fairly constant, potentially decreasing its utility for monitoring disease progression (Nabity et al. 2012). α1- and β2-Microglobulin appear to be promising markers of proximal tubular damage in dogs, and may be sensitive to progressive changes in renal injury.

Enzymuria: N-acetyl-β-D-glucosaminidase and γ-glutamyl transpeptidase

N-acetyl-β-D-glucosaminidase (NAG) and γ-glutamyl transpeptidase (GGT) are proximal tubular enzymes involved in protein processing.

N-acetyl-β-D-glucosaminidase and γ-glutamyl transpeptidase in humans

Detection of NAG and GGT may be best suited for detection of AKI rather than CKD as enzymuria may reflect acute tubular dysfunction instead of more chronic ongoing damage. (Greco et al. 1985, Skalova 2005, Brunker et al. 2009). Total NAG activity can be subdivided by isoenzyme: NAG A is associated with tubular protein processing and lysosomal activity, NAG B may be more indicative of renal tubal damage. (Marchewka et al. 2001, Sato et al. 2002a, Jepson et al. 2009). While small amounts of NAG and GGT are excreted normally in urine, tubular dysfunction greatly increases their excretion (Braun & Lefebvre 2008). Urinary NAG and GGT measurements are expressed as a ratio to urinary creatinine concentration: NAG index and GGT index, respectively (Grauer et al. 1995, Skalova 2005, Brunker et al. 2009). Non-renal factors including urine pH and long-term sample storage may affect urinary NAG and GGT activity (Uechi et al. 1998, Jepson et al. 2009). Urinary NAG activity is a widely used measure of tubular function, and elevations have been detected in humans with kidney disease secondary to various conditions (Skalova 2005). Urinary GGT is not as extensively studied in humans but appears to be elevated in acute proximal tubular damage (Westhuyzen et al. 2003).

N-acetyl-β-D-glucosaminidase and γ-glutamyl transpeptidase in dogs

Several studies have examined the utility of urinary NAG to detect canine CKD. Urinary NAG was significantly elevated in dogs with X-linked hereditary nephropathy compared to control dogs, and was detectable before a creatinine elevation (Nabity et al. 2012). In addition, NAG was the only marker that significantly increased before a detectable elevation in urine protein:creatinine ratio, indicating that it may be a useful marker of early kidney disease (Nabity et al. 2012). However, NAG did not perform as well as RBP in the detection of progressive changes and was not useful in monitoring later stages of renal disease in one study, while another study revealed a large overlap of NAG index values in healthy and CKD dogs (Smets et al. 2010a, Nabity et al. 2012). Increased NAG activity has also been used to detect the onset of AKI associated with pyometra and leishmaniasis (Palacio et al. 1997). While some studies found a relatively increased NAG index in male dogs compared to females, this is not a consistent finding (Uechi et al. 1994, Sato et al. 2002b). In addition, one study found increased NAG in dogs with poorly controlled diabetes mellitus although it is possible that these dogs had nephropathy secondary to diabetes (Sato et al. 2002b).

Urinary GGT has been used primarily to detect AKI in dogs. In dogs with gentamicin-induced nephrotoxicity, GGT index increased before creatinine elevation (Greco et al. 1985, Rivers et al. 1996). Urinary GGT values were significantly elevated in AKI versus healthy dogs, but not significantly different between healthy and CKD dogs. However, there was considerable overlap in GGT values between the groups, and enzyme activity did not correlate with histological lesions in this study (Heiene et al. 2001).

N-acetyl-β-D-glucosaminidase and γ-glutamyl transpeptidase in cats

An enzymatic colourimetric NAG assay has been validated in dogs and cats, whereas GGT is measured via a spectrophotometric assay (Jepson et al. 2010, Smets et al. 2010b). Urinary NAG and GGT activities increased earlier than detectable changes in serum creatinine concentration in induced feline glomerulonephritis, and correlated with presence of histological lesions (Bishop et al. 1991). However, this study did not specifically correlate urinary NAG and GGT elevations to clinical signs of disease (Bishop et al. 1991). Another study found a moderate correlation between NAG index and proteinuria in azotaemic and non-azotaemic cats (Jepson et al. 2010). However, the measured NAG may be increased secondary to lysosomal protein processing in states of proteinuria, rather than tubular damage, potentially confounding results of these studies. Other studies have shown poor correlation between creatinine concentration and the NAG index in healthy cats and cats with urinary tract disease, and NAG index was not predictive of development of azotaemia in geriatric cats or those with treated hyperthyroidism (Sato et al. 2002a, Jepson et al. 2009, Jepson et al. 2010). The inconsistent results of the above studies, as well as high inter-assay variability detected in one study, suggest that NAG may not be a good marker of CKD in cats (Jepson et al. 2010).

To date, veterinary research shows that NAG and GGT may be well suited for detection of early tubular injury. In ongoing kidney injury, depletion of tubules decreases enzyme excretion.
Neutrophil gelatinase-associated lipocalin

Neutrophil gelatinase-associated lipocalin (NGAL) is a 25-kDa protein expressed in neutrophils as well as many epithelial cells including the renal proximal tubule, loop of Henle and collecting ducts (Schmidt-Ott et al. 2006, Soni et al. 2010). A commercially available monoclonal antibody ELISA is commonly used in the research setting to measure plasma, serum and urine NGAL concentration (Devarajan 2008, Soni et al. 2010). Newer assays have been developed for clinical use in humans, including a point-of-care plasma assay and a standardised urine immunoassay, both of which correlate well with the ELISA (Devarajan 2008, Soni et al. 2010).

Neutrophil gelatinase-associated lipocalin in humans

In human medicine, NGAL has been primarily investigated as a marker of AKI. Elevated NGAL concentrations have been used to detect AKI secondary to cardiac surgery, contrast induced nephropathy, critical illnesses and other disease processes (Hirsch et al. 2007, Nickolas et al. 2008). Urinary NGAL was highly sensitive and specific for predicting AKI and clinical outcomes (such as need for renal replacement therapy and in-hospital mortality), and was superior to other markers of renal disease including NAG, α1-microglobulin and creatinine (Nickolas et al. 2008).

Urine and serum NGAL concentrations correlate well with GFR studies in humans with CKD secondary to various diseases, and are superior to some other markers such as cystatin C (Brummer et al. 2006, Bolognano et al. 2007, Ding et al. 2007, Mitsnefes et al. 2007).

Neutrophil gelatinase-associated lipocalin in dogs

A canine-specific NGAL ELISA has been validated in dogs to quantify NGAL activity in urine (Nabity et al. 2012). The urine NGAL to creatinine concentration ratio (uNGAL/c) was elevated in the early stages of X-linked hereditary nephropathy in dogs, and correlated well with serum creatinine concentration, GFR, other urine biomarkers (RBP, β2-microglobulin and NAG) and renal fibrosis (Nabity et al. 2012). However, similar to β2-microglobulin and uNAG/c, uNGAL/c plateaued during later stages of nephropathy, potentially limiting the ability of these biomarkers to detect progressive late-stage changes. In addition, uNGAL/c was elevated in healthy puppies possibly due to urinary contamination with prepubertal leukocytes (Nabity et al. 2012). In dogs with AKI, urinary NGAL concentrations increased earlier than a detectable elevation of creatinine outside the reference interval (Palm et al. 2012). Other preliminary investigations have shown elevated serum and urinary NGAL in dogs with kidney diseases compared to controls (Le Roy et al. 2011, Cobrin et al. 2012). NGAL is elevated secondary to tubular damage, but is not kidney-specific (Devarajan 2008). NGAL shows promise in the assessment of kidney disease, but further studies are needed especially to determine its utility in detecting progression of disease as well as the effect of concurrent conditions.

FUTURE PERSPECTIVES

Kidney injury molecule-1

Kidney injury molecule-1 (KIM-1) is a type 1 cell membrane glycoprotein that is expressed at low to undetectable levels in the normal kidney (Ichimura et al. 1998). KIM-1 is considered to be highly specific for the detection of renal proximal tubular injury, and is primarily used to detect AKI. Expression of KIM-1 is markedly up-regulated 24 to 48 hours after ischaemic or toxic injury to the renal proximal tubules, and is also expressed in renal fibrosis and inflammation (Ichimura et al. 1998, Han et al. 2002, 2007, Vaidya et al. 2006, Bonventre 2009). The ectodomain of KIM-1 is shed into urine and is stable in urine for extended periods (Ichimura et al. 1998, Han et al. 2002, 2007, Vaidya et al. 2006, Bonventre 2009). Urinary KIM-1 can be measured by ELISA or rapid immunochromatographic assay dipstick; these tests correlate with tissue expression (Han et al. 2002, Vaidya et al. 2009).

Kidney injury molecule-1 in humans

There have been several human studies evaluating KIM-1, especially in AKI, and the FDA has approved urinary KIM-1 as a biomarker of kidney injury when assessing renal safety of drugs (Bonventre 2009, Vaidya et al. 2009, 2010). Expression of KIM-1 was markedly increased in renal tissue of humans with acute tubular necrosis, and was more sensitive than routine histopathology in identifying proximal tubular injury (Zhang et al. 2007). Increasing concentrations of urinary KIM-1 predicted adverse outcomes including requirement for renal replacement therapy and in-hospital mortality (Liangos et al. 2007). KIM-1 concentrations are also elevated in CKD, but to a lesser extent (Han et al. 2002). Currently, prospective clinical trials investigating KIM-1 in cats with kidney disease are ongoing at the authors’ institution. KIM-1 appears to be a promising marker of AKI, but currently studies are lacking in companion animals.

Interleukin-18

Interleukin-18 (IL-18) is a pro-inflammatory cytokine constitutively produced and stored in the intercalated cells of the late distal convoluted tubule, the connecting tubule and the collecting duct (Gauer et al. 2007). During ischaemic AKI and ischaemic reperfusion injury, stored IL-18 is cleaved, activated and released from tubular cells leading to neutrophil infiltration in the kidney and eventual excretion of IL-18 into urine (Melnikov et al. 2001, Parikh et al. 2004, Leslie & Meldrum 2008). IL-18 also promotes inflammation via activation of T cells and natural killer cells in addition to macrophages (Wu et al. 2008). IL-18 measurement is used to detect AKI, although IL-18’s pro-inflammatory role is not unique to the kidneys: it is also a mediator in non-renal inflammatory diseases such as arthritis, lung injury, inflammatory bowel disease and myocardial ischaemia as well as
Interleukin-18 in humans

Urinary IL-18 is measured using an ELISA (Parikh et al. 2004). In a human AKI study, elevated urinary IL-18 preceded a detectable increase in serum creatinine concentration by 24 to 48 hours and was a predictor for mortality after development of AKI (Parikh et al. 2005). Elevated urinary IL-18 was also a predictor of delayed graft rejection in renal transplant patients (Parikh et al. 2005). While there are currently no published studies evaluating urinary IL-18 concentrations in dogs and cats with renal disease, studies of IL-18 in rodents and humans have demonstrated that IL-18 plays a role in tubular injury and inflammatory diseases of the kidney with its main utility as a marker of AKI (Leslie & Meldrum 2008).

Conclusion

The development of clinically useful renal biomarkers is currently an active field of research. Many human studies have demonstrated that novel biomarkers are superior to circulating creatinine concentration in the assessment of GFR (cystatin C), in diagnosing AKI (NGAL, KIM-1, IL-18 and NAG) or for assessing CKD (cystatin C, RBP). Furthermore, the value of these markers extends beyond the general assessment of AKI, CKD and GFR; more specifically, RBP, the microglobulins and NAG A detect tubular dysfunction secondary to proximal tubular damage, GGT detects nephrotoxicity secondary to gentamicin use, NGAL detects ischaemic renal injury throughout the tubules, and KIM-1 along with IL-18 detect ischaemic AKI and acute tubular necrosis. However, most markers require further, larger clinical trials before widespread clinical implementation. In addition, while many assays have been validated and investigated in dogs and cats, some markers lack published clinical studies, and as such, the utility of many of these markers in dogs and cats has yet to be determined. Many current studies compare the performance of renal biomarkers to circulating creatinine concentration, an insensitive marker of renal function. Future areas of research should include comparing the performance of renal biomarkers to studies that more accurately estimate GFR such as cystatin C concentration, iohexol clearance or scintigraphy, as well as direct comparisons to other biomarkers.

There are multiple renal markers being studied and it can be tempting to ask which one is the most promising. Biomarkers typically have differing accuracy depending on disease timeframe or stage, may be specific to lesion location, and may differ depending on method of measurement. Therefore, it is unlikely that any single marker will be ideal and comprehensive, fulfilling all the desired qualities of a renal biomarker. While creatinine has its limitations (e.g. decreased sensitivity during early disease, influence of muscle mass), it is still a useful and practical marker for kidney disease; better utilisation of creatinine by recognising small changes early in the disease process could improve diagnosis. As such, it is likely that in the future a panel of biomarkers along with creatinine, rather than a single test, will have the most clinical utility for assessing AKI and CKD of various causes and types of injury in humans as well as dogs and cats.

Conflict of interest

None of the authors of this article has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

References

Abrahamson, M., Barrett, A., Salvesen, G., et al. (1986) Isolation of six cysteine proteinase inhibitors from human urine. Their physicochemical and enzyme kinetic properties and concentrations in biological fluids. Journal of Biological Chemistry 261, 11282-11289


Journal of Small Animal Practice  • Vol 54  • December 2013  • © 2013 British Small Animal Veterinary Association 653

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hepatitis and multiple sclerosis; thus it is not specific to renal injury (Parikh et al. 2004, Urbach et al. 2011).


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