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Urinary biomarkers of renal function in dogs and cats: a review

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ABSTRACT: Kidney diseases commonly affect dogs and cats. Early diagnosis of renal impairment may be challenging even when urinalysis is used to provide additional information. Serum creatinine concentration is often used in the diagnosis, but it is a relatively insensitive marker of renal function. Particular attention is aimed at the investigation of certain molecules that may occur in urine at elevated levels as a result of glomerular or tubular dysfunction. These changes may be found before the increase of serum creatinine levels. This review article summarises reports of urine biomarkers and their utility in detecting early kidney disease in dogs and cats. Detection of multiple urinary biomarkers in diagnosis of acute kidney injury and chronic kidney disease may increase specificity and sensitivity. Early diagnosis of reduced renal functional mass allows early therapeutic interventions which may decrease morbidity and mortality.

Keywords: microalbuminuria; neutrophil gelatinase-associated lipocalin; *N*-acetyl-β-D-glucosaminidase; gamma-glutamyl transpeptidase; immunoglobulin G

Abbreviations

AKI = acute kidney injury; **ARF** = acute renal failure; **CKD** = chronic kidney disease; **CRP** = C-reactive protein; **GFR** = glomerular filtration rate; **GGT** = gamma-glutamyl transpeptidase; **HMW** = high molecular weight; **ICU** = intensive care unit; **IgG** = immunoglobulin G; **NAG** = *N*-acetyl-β-D-glucosaminidase; **NGAL** = neutrophil gelatinase-associated lipocalin; **PTH** = parathormone; **RBP** = retinol binding protein; **UAC** = urinary albumin to creatinine ratio; **uGGT** = urinary gamma-glutamyl transpeptidase; **uNAG** = urinary *N*-acetyl-β-D-glucosaminidase; **UNCR** = urinary neutrophil gelatinase-associated lipocalin to creatinine ratio; **UPC** = urinary protein to creatinine ratio; **uRBP** = urinary retinol binding protein; **UTI** = urinary tract infection

Contents

1. Introduction

Kidney diseases are common in dogs and cats, and are often associated with poor prognosis in later stages (Polzin 2011). Acute kidney injury (AKI) is associated with a sudden onset of renal parenchymal damage with subsequent impairment of renal function. Severe injury can lead to acute renal

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failure (ARF), which is characterised by the highest morbidity and mortality rates. The high mortality associated with ARF is caused by delayed detection of this condition due to insensitive diagnostic tests (Cowgill and Langston 2011). Chronic kidney disease is a commonly diagnosed condition in the geriatric population. The prevalence increases to up to 15% in dogs over 10 years of age and to up to 31% in cats over 15 years of age (Lulich et al. 1992; O'Neill et al. 2013). Chronic kidney disease is typically progressive and irreversible, regardless of the initiating cause.

Traditionally, measurement of serum creatinine concentration is used for the diagnosis of kidney disease. Nevertheless, it is insensitive, because increases in serum creatinine are mild and often remain within the reference range, until approximately 75% of all nephrons are no longer functional (Polzin 2011). Measurement of glomerular filtration rate (GFR) provides the most accurate assessment of renal function and is the most sensitive method for early detection of kidney dysfunction. However, many clearance tests are costly and time-consuming and not suitable for widespread use as a screening test (Kerl and Cook 2005). Thus, the diagnosis of renal dysfunction at an early stage represents a challenge for practitioners.

Evaluation of certain urinary proteins has shown promise in determining the localisation and severity of renal damage in people with renal disease. Similar studies were performed in dogs and cats and revealed some markers that can be used in the detection of glomerular or tubular dysfunction earlier than conventional methods. An ideal biomarker for detection of kidney disease should be specific, non-invasive, and sensitive enough to allow early detection of the disease. Further, it should allow specification of the extent or severity of disease, be suitable for monitoring progression, as well as informative with respect to the localisation of the injury and the clinical outcome. Measurement of biomarkers should be inexpensive and readily available from a reference laboratory or point-of-care assay (Cobrin et al. 2013). Almost all urinary biomarkers are expressed as a ratio to urine creatinine concentration. Because the total amount of creatinine excreted daily in urine changes little, dividing by the urine creatinine concentration effectively adjusts the marker concentration for variations in urine volume and concentration. This method allows the measurement of the urinary marker in spot urine samples without the necessity of 24-hour urine collection (Pressler 2011). The purpose of this article is to review the current knowledge of urinary biomarkers described for dogs and cats.

2. Potential markers of glomerular dysfunction

2.1. Albumin

Albumin is the main serum protein. It is produced by the liver and acts as a carrier protein, which is essential for maintaining oncotic pressure. Albumin is not normally present in large quantities in glomerular filtrate because of its size (69 kDa) and glomerular selective permeability. The glomerular filtrate of healthy dogs and cats contains only 2–3 mg/dl of albumin compared with about 4 g/dl found in plasma. This small amount of albumin is almost completely reabsorbed by tubular epithelial cells and degraded by lysosomes. This reabsorption occurs primarily in proximal convoluted tubules and reduces the concentration of albumin in normal urine to < 1 mg/dl (Russo et al. 2002; Grauer 2011). Therefore, albuminuria usually reflects kidney dysfunction, either by glomerular damage, which allows increased leakage of albumin or by tubular damage, which decreases the ability of the nephron to degrade the even small amount of albumin in the glomerular filtrate. Albuminuria of glomerular origin is usually of greater magnitude (Newman et al. 2007).

Conventional urine dipsticks are the standard initial screening test for detection of proteinuria; urine protein concentrations must be approximately 30 mg/dl or greater to be detected by this method. This is called overt proteinuria. Microalbuminuria is defined as an albumin concentration of 1–30 mg/dl in urine normalised to a specific gravity of 1010 (Lees 2004). Microalbuminuria may be detected using E.R.D.-HealthScreen (Heska, Colorado, USA), a semi-quantitative species-specific point-of-care test (Mardell and Sparkes 2006; Whittemore et al. 2006). The most accurate method to measure albumin in canine urine is an automated immunoturbidimetric method, calibrated and validated for the measurement of canine albumin (Murgier et al. 2009). Urinary albumin is similar in voided and cystocentesis samples. It is stable for four months at –20 °C, and for 12 months at –80 °C (Smets et al. 2010a) Albuminuria is not affected by micro-

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scopic haematuria. Albuminuria may be more likely in dogs with pyuria and concurrent haematuria or bacteriuria (Vaden et al. 2004). Urine albumin/creatinine ratios from spot urine samples accurately reflect the quantity of albumin excreted in the urine over a 24-hour period (Grauer et al. 1985). The prevalence of microalbuminuria in healthy dogs may range between 19–24.7% (Jensen et al. 2001; Pressler et al. 2001; Radecki et al. 2003). Mild exercise has a negligible effect on albumin concentration (Gary et al. 2004).

Albuminuria that is persistent or that is of increasing magnitude may be the earliest clinical indicator of glomerular disease. Reported causes of increased urinary albumin are summarised in Table 1. In critically ill dogs, microalbuminuria is associated with shorter survival (Vaden et al. 2010; Whittemore et al. 2011). In cats, microalbuminuria was associated with the presence of underlying disease (e.g. neoplasia, infections, inflammatory or immune-mediated diseases and endocrine disorders) (Whittemore et al. 2007; Atkins et al. 2011).

Microalbuminuria was not found in healthy obese dogs (Tefft et al. 2014). Nevertheless, Tvarijonaviciute et al. (2013) reported a significant decrease of the urinary albumin/creatinine ratio in healthy dogs after weight loss. Microalbuminuria was not observed in dogs with hyperadrenocorticism (Lien et al. 2010), cisplatin-induced azotemia (Autio et al. 2007) or in Labradors and Golden Retrievers exposed to *Borrelia burgdorferi* (Goldstein et al. 2007).

Nevertheless, the role of microalbuminuria in the early diagnosis of kidney dysfunction is debatable (Raila et al. 2010). The detection of microalbuminuria in non-renal disorders raises the question about the specificity of this condition for diagnosis of renal disease. In addition, the presence of urinary albumin is not site-specific. It may be caused by both glomerular and tubular impairment, although marked albuminuria is typically associated with glomerular disorders.

2.2. Immunoglobulin G

Immunoglobulin G (IgG) is a high molecular weight protein (160 kDa) that plays an important role in the humoral response. It is unable to pass through an intact glomerular barrier. Detection of higher amounts of IgG in urine reflect glomerular damage (D'Amico and Bazzi 2003; Maddens et al. 2010a).

In urine, IgG may be measured by sandwich ELISA (Maddens et al. 2010b; Nabity et al. 2012). An increased amount of urinary IgG in urine was found in dogs with X-linked hereditary nephropathy, where a strong correlation with UPC was revealed. Increased urinary IgG was described to occur before an increase in UPC, although this finding was not statistically significant in comparison with unaffected animals (Nabity et al. 2012). Other causes of increased levels of urinary IgG are shown in Table 1.

2.3. C-reactive protein

C-reactive protein (CRP) is an acute phase protein with an increased serum concentration in many inflammatory diseases (Nakamura et al. 2008). Due to its size (115 kDa), CRP is not able to pass through the intact glomerular barrier. The presence of CRP in urine is the result of glomerular dysfunction (Maddens et al. 2010b). It has been proposed as a renal marker to detect glomerular dysfunction in different disorders (Table 1). Nevertheless, in one study of dogs with chronic kidney disease (CKD), urinary CRP was not increased (Smets et al. 2010b). For CRP to appear in urine, its plasma concentration must be increased and the glomerular barrier must be sufficiently damaged to allow HMW protein filtration. Patients evaluated in the mentioned study presumably had normal plasma levels of CRP or their glomerular barriers were not severely affected. Thus, further studies are needed to evaluate the diagnostic value of CRP in urine.

3. Potential markers of tubular injury

3.1. Enzymuria

Several urinary enzymes are used for the evaluation of impaired renal function. Under normal conditions, enzymatic activities of urine may originate from serum (as glomerular filtrate), renal tubular cells, and the urogenital tract (epithelial cells, glandular secretion, and semen). The contribution of serum enzymes to urine is negligible for most urinary enzymes because they are relatively

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Table 1. Reported causes of increased levels of urinary markers of impaired renal function in dogs and cats

Urinary marker	Causes of urinary marker elevation
Markers of glomerular impairment	
Albumin	X-linked hereditary nephropathy (before the onset of overt proteinuria) (Lees 2002) Glomerular disease in Soft Coated Wheaten Terriers (Vaden et al. 2001) Chronic kidney disease, CKD with hypertension (Bacic et al. 2010) Hypertension without CKD (Surman et al. 2012) Systemic disease which may secondarily affect the kidneys (Whittemore et al. 2006) Diabetic nephropathy in dogs and cats (Struble et al. 1998; Al-Ghazlat et al. 2011) Severe inflammatory response syndrome (Schaefer et al. 2011) Lymphoma and osteosarcoma in dogs (Pressler et al. 2003) Hypercortisolism (Smets et al. 2012)
Immunoglobulin G	X-linked hereditary nephropathy (Nabity et al. 2012) Snake envenomation (Hrovat et al. 2013) Pyometra (Maddens et al. 2011) Leishmaniasis (Solano-Gallego et al. 2003; Zaragoza et al. 2003a) Leptospirosis (Zaragoza et al. 2003b) Hypercortisolism (Smets et al. 2012)
C-reactive protein	Pyometra (Maddens et al. 2010b) Babesiosis (Defauw et al. 2012) Leishmaniasis (Martinez-Subiela et al. 2013)
Markers of tubular impairment	
N-acetyl-β-D-glucosaminidase	Leishmaniasis (Palacio et al. 1997) X-linked hereditary nephropathy (Nabity et al. 2012) Heartworm disease with cardiac impairment (Uechi et al. 1994b) Pyometra (Maddens et al. 2010b) Experimental immune complex glomerulonephritis (Bishop et al. 1991) Acute renal failure experimentally induced in cats (NAG-B isoenzyme) (Sato et al. 2002a) CKD in cats (Jepson et al. 2010a) Hypercortisolism (Smets et al. 2012)
Gamma-glutamyl transpeptidase	Aminoglycoside-induced nephrotoxicity (Greco et al. 1985; Grauer et al. 1995; Rivers et al. 1996) Renal insufficiency associated with pyometra (De Schepper et al. 1989) Heartworm disease with cardiac insufficiency (Uechi et al. 1994b) Leishmaniasis (Palacio et al. 1997) Envenomation by the common European adder (Palviainen et al. 2013) Experimental immune complex glomerulonephritis in cats (Bishop et al. 1991)
Neutrophil gelatinase-associated lipocalin	Acute kidney injury (Hsu et al. 2014a; Segev et al. 2013; Zhou et al. 2014) Chronic kidney disease (Hsu et al. 2014a; Steinbach et al. 2014)
Retinol binding protein	Chronic kidney disease (Smets et al. 2010b) Pyometra related renal impairment (Maddens et al. 2010b) Babesiosis (Defauw et al. 2012) Severe inflammatory response syndrome (Schaefer et al. 2011) Envenomation by cytotoxic or neurotoxic snakes (Hrovat et al. 2013) X-linked hereditary nephropathy before the onset of azotemia (Nabity et al. 2012) Chronic renal failure and hyperthyroidism in cats (Van Hoek et al. 2008) Hypercortisolism (Smets et al. 2012)
Beta2-microglobulin	Early stages of X-linked hereditary nephropathy (Nabity et al. 2012)
Cystatin C	Severe CKD in dogs with leishmaniasis (Garcia-Martinez et al. 2015)
Cauxin	Tubular impairment (Myiazaki 2007)
Clusterin	Drug-induced acute kidney injury (Zhou et al. 2014)

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large (> 80 kDa) and unable to pass through normal glomerulus.

Tubular enzymes are typically located within proximal tubular cells. This portion of the nephron is a metabolically active segment that can be easily damaged. Enzymes are subsequently released into the urine (Clemo 1998).

One problem with using urinary enzymes to assess renal damage is that they are sometimes too sensitive; elevations may be present in the absence of any other measurable renal abnormalities, and it may be present for only a short period of time after renal damage. Evaluation of more than one enzyme at multiple time intervals is therefore recommended (Clemo 1998).

3.1.1. *N*-acetyl-β-D-glucosaminidase

N-acetyl-β-D-glucosaminidase (NAG) is a high molecular weight (150 kDa) lysosomal enzyme that may be detected in many mammalian tissues, serum and urine. NAG is abundantly present in cells of the renal proximal tubule (Bourbouze et al. 1984). It consists of several isoenzymes (Skalova 2005). Two main types of isoenzymes (NAG-A and NAG-B) are present in the renal proximal tubular cells. NAG-A is located in the soluble intralysosomal compartment and NAG-B is bound to the lysosomal membrane. In healthy humans and animals, urinary NAG activity is low. NAG-A is mainly detected in urine as a result of exocytosis. In cases of renal damage, an increase in the proportion of NAG-B has been reported. NAG-B is associated with the lysosomal membrane, so it would not be lost into the urine unless the tubular cells were damaged. Thus NAG-B is considered to be a useful and more sensitive indicator of renal tubular impairment (Sanchez-Bernal et al. 1991; Sato et al. 2002a).

In dogs and cats, an enzymatic colorimetric NAG assay has been validated (Jepson et al. 2010a; Smets et al. 2010a). No circadian variations in urinary NAG (uNAG) excretion in dogs and cats were detected (Uechi et al. 1994a; Uechi et al. 1998). Thus, enzyme activity and its ratio to urine creatinine in non-periodically collected samples is sufficient for the detection of renal function impairment. No significant difference was found between the uNAG in young and older healthy dogs (Smets et al. 2010b). Some studies revealed significant differences in NAG activity between male and female dogs with a higher uNAG to creatinine ratio in males (Nakamura et al. 1983; Reusch et al. 1991; Brunker et al. 2009). It is considered that the increased NAG activity in the urine of male dogs is due to contamination with semen, which is an important source of NAG (Higashiyama et al. 1983; Yoshida et al. 1989). However other studies did not reveal differences between males and females (Uechi et al. 1994a; Sato et al. 2002b). In cats, no differences were found between males and females (Sato et al. 2002a; Jepson et al. 2010a). Changes in urine pH do not affect the urinary NAG in dogs (Brunker et al. 2009). Older human reports showed a negative influence of alkaline urine (pH > 8) on total NAG activity, nevertheless individual isoenzyme activities were stable in a wide range of pH values (Mandic et al. 2005). Urinary NAG concentrations are similar in samples obtained by normal voiding and cystocentesis. Urinary NAG activity is less stable after storage at –20 °C as well as –80 °C (for 12 months) (Smets et al. 2010a).

Urinary NAG activity is commonly used for detection of early renal damage. In humans, evaluation of NAG activity in urine is used to monitor the nephrotoxic effects of aminoglycosides, heavy metals or in the diagnosis of diabetic nephropathy. Higher urinary NAG was found in patients with nephrotic syndrome or developmental kidney abnormalities (Skalova 2005).

Increased NAG activity was found in different diseases before the increase in serum creatinine level or increase of UPC (Table 1). Excretion of NAG into urine during mid to late-stage renal disease was relatively constant (Nabity et al. 2012). The increase in uNAG was significantly higher in CKD dogs (Smets et al. 2010b) and dogs with pyelonephritis, whereas in dogs with uncomplicated urinary tract infection (UTI) uNAG was normal (Sato et al 2002b). Activity of uNAG may be used for evaluation of the renal function of dogs submitted to therapy with non-steroidal anti-inflammatory drugs (Borges et al. 2013).

In cats with CKD, an increased amount of NAG was found and in cats with feline lower urinary tract disease, normal values were observed. The NAG index in cats with CKD may be indicative of ongoing lysosomal activity due to proteinuria rather than active proximal tubular cell damage (Jepson et al. 2010a).

Urinary NAG alone is not sufficient for the prediction of the development of azotemia in geriatric cats (Jepson et al. 2009). Further, it cannot be used

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to differentiate non-azotemic from azotemic cats in cats with hyperthyroidism before treatment with methimazole. Nevertheless, uNAG could be useful for the monitoring of progressive renal damage during medical therapy for hyperthyroidism (Lapointe et al. 2008)

3.1.2. Gamma-glutamyl transpeptidase

Gamma-glutamyl transpeptidase is located in the proximal tubular brush border. Urinary GGT may be measured using a commercial testing kit (Uechi et al. 1998; Brunker et al. 2009). No circadian variations and no differences between the sexes in urinary GGT were observed (Uechi et al. 1998; Brunker et al. 2009). The urinary GGT index is influenced by the pH of the urine sample; in alkaline urine, the values are higher (Brunker et al. 2009).

Urine activity of g-glutamyl transpeptidase is commonly used for the detection of acute kidney injury. Reported disorders associated with increased values of uGGT are shown in Table 1. Urinary GGT activity has great potential in safety assessment studies (Narita et al. 2007; Borges et al. 2013) Nevertheless, Crivellenti et al. (2014) reported false positive results of urinary GGT measurement in dogs poisoned by a non-nephrotoxic agent (*Nerium oleander*).

3.1.3. Alkaline phosphatase

Alkaline phosphatase is a brush-border enzyme. Its increased activity in urine has been associated with proximal tubular damage in dogs (Heiene et al. 1991). Excretion of urinary alkaline phosphatase is used to evaluate renal function in different conditions (Lobetti and Joubert 2000; Lobetti and Lambrechts 2000; Heiene et al. 2001; Raekallio et al. 2006).

3.2. Neutrophil gelatinase-associated lipocalin

Neutrophil gelatinase-associated lipocalin (NGAL), also called lipocalin 2, is a glycoprotein with a molecular weight of 25 kDa and a member of the lipocalin family (Xu et al. 1994). It was initially purified from neutrophils during infection and inflammation (Kjeldsen et al. 1993). In neutrophils, NGAL is found in granules and is considered an important component of the innate immune response to bacterial infections. It has the ability to bind and inhibit bacterial iron siderophores that are synthesised to scavenge iron and promote bacterial growth (Flo et al. 2004). However, NGAL is also expressed in the uterus, prostate gland, salivary glands, bone marrow, stomach, colon, trachea, lungs, liver, and kidneys (Cowland and Borregaard 1997) NGAL is upregulated in response to inflammatory signals, including epithelial injury associated with acute kidney injury (Kjeldsen et al. 2000) In animal models, NGAL is highly expressed during ischaemic renal injury (Mishra et al. 2004a; Supavekin et al. 2003). In a rat model of toxic AKI, NGAL was induced predominantly in proximal tubule cells (Mishra et al. 2004b). Because of its early and substantial increase after kidney injury, it is considered one of the early and robust biomarkers of AKI. In human medicine, NGAL is typically determined in patients after cardiac surgery, as a predictor for AKI (Mishra et al. 2005; Wagener et al. 2006).

In veterinary medicine, NGAL is a novel and promising biomarker that has been shown to increase in dogs with kidney disease. A canine-specific NGAL ELISA has been validated for dogs to quantify NGAL activity in urine (Nabity et al. 2012). Lee et al. (2012) showed an association between an increase in urinary NGAL and the development of AKI in dogs after surgery of different types. The significant increase of NGAL in urine was detected as early as 12 h after surgery, much earlier than the increase in serum creatinine. Urinary NGAL was recommended as a sensitive and specific biomarker for the detection of AKI, for prediction of nephrotoxicity, and to screen patients at risk for AKI (Table 1) (Lee et al. 2012; Kai et al. 2013; Hsu et al. 2014a; Zhou et al. 2014). NGAL was found to be a more sensitive biomarker for the detection of gentamicin-induced renal proximal tubular toxicity than NAG (Zhou et al. 2014). Urine NGAL concentrations were highly correlated with serum creatinine concentrations. Among CKD dogs, death was associated with significantly higher uNGAL concentrations compared with survivors (Hsu et al. 2014a). Recent studies in rats and children found that urinary NGAL increases significantly in the presence of urinary tract infection (Ichino et al.

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2009; Decavele et al. 2011). In comparison between healthy non-azotemic dogs, dogs with lower urinary tract disorder and negative urine culture and dogs with UTI, the highest level of urinary NGAL and UNCR was found in dogs with UTI. The recommendation is to perform urine culture and to consider the results when interpreting urinary NGAL values in dogs with suspected kidney injury to avoid misdiagnosis (Daure et al. 2013). UNCR was increased significantly in dogs with lower urinary tract diseases compared with healthy dogs. The presence of neutrophils attributable to inflammation in lower urinary tract diseases could be the origin of the increased NGAL concentrations in urine. Nevertheless, an increase in uNCR in lower urinary tract diseases slightly decreases the specificity of this marker for AKI (Segev et al. 2013). The specificity may be increased by determining the molecular forms of uNGAL which allows more precise detection of different urinary tract diseases (Hsu et al. 2014b).

3.3. Retinol binding protein

Retinol binding protein (RBP) is 21 kDa protein synthesised in the liver. It circulates in plasma, where 90% of it is bound to the 55 kDa protein transthyretin. The unbound fraction of RBP is freely filtered through the glomeruli and is catabolised after reabsorption in the proximal tubules. Only small amounts of RBP should be excreted in the urine of healthy dogs. Increased levels of uRBP are expected in dogs with proximal tubule disorders. Thus, uRBP has been suggested as a sensitive marker of proximal tubule dysfunction in dogs (Raila et al. 2000; Forterre et al. 2004).

Urinary RBP may be measured using validated ELISA assays. It is not affected by the sampling method (cystocentesis vs. normal voiding). RBP in urine is stable after storage at –20 °C and –80 °C. Urinary RBP showed a very mild increase in samples with 3+ haematuria (Smets et al. 2010a).

Increased levels of urinary RBP were reported in different disorders (Table 1) In dogs with X-linked hereditary nephropathy, an increase in uRBP was correlated with renal progression. Thus, measurement of uRBP might be clinically useful for both early detection and monitoring of CKD in dogs (Nabity et al. 2011).

In cats, the only study performed to date with respect to RBP revealed an increase of RBP in the urine of cats with chronic renal failure and hyperthyroidism (Van Hoek et al. 2008).

3.4. Microglobulins

Alpha1-microglobulin is a 27 kDa immunomodulatory glycoprotein produced by the liver. It belongs to the lipocalin superfamily. Free α1-microglobulin passes through the glomerulus and is reabsorbed by the proximal tubules. Normal urine contains very small amounts of α1-microglobulin. In conditions with disturbed tubular function, reabsorption of α1-microglobulin is reduced and increased amounts are found in urine (DeMars et al. 1989; Penders and Delanghe 2004). Alpha1-microglobulin is stable at different urine pH values, and at room temperature for seven days (Donaldson et al. 1989). In humans, α1-microglobulin is a marker for proximal tubular damage (Penders and Delanghe 2004).

Beta2-microglobulin is a low molecular weight protein (11.8 kDa) that is present on the surface of all nucleated cells (Nakajima et al. 2001). It is unstable in acidic urine (Donaldson et al. 1989). Increased levels of urinary β2-microglobulin were observed in dogs with X-linked hereditary nephropathy, where it increased during the early stage of the disease (Nabity et al. 2012).

3.5. Cystatin C

Cystatin C is a 13 kDa non-glycosylated protein that is produced at a constant rate by all nucleated cells. It is a member of the cystatin superfamily of cystein proteinase inhibitors (Randers et al. 1998). As a consequence of its low molecular weight and stable production rate, the serum concentration of cystatin C is mainly determined by GFR. Thus, it is used for the evaluation of GFR and is considered superior to serum creatinine in detecting renal dysfunction in humans (Newman et al. 1995; Dharnidharka et al. 2002). In dogs, serum cystatin C may be an alternative to serum creatinine for screening dogs with decreased GFR due to chronic renal failure (Almy et al. 2002). Nevertheless, Pagitz et al. (2007) showed that the biological variance of cystatin C and creatinine in the serum of dogs is similar.

Due to its size, cystatin C is freely filtered through the glomerulus and is reabsorbed in the proximal

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tubules by megalin-mediated endocytosis where it is completely catabolised (Tenstad et al. 1996; Kaseda et al. 2007). Under normal conditions, only small quantities of cystatin C can be found in definitive urine. With proximal tubular damage, urinary cystatin C increases (Conti et al. 2006). Urinary cystatin C was suggested to be more sensitive than other low molecular weight proteins. However, the measurement of total proteinuria is required, because massive proteinuria has been shown to inhibit tubular reabsorption of cystatin C causing higher urinary cystatin C concentrations (Thielemans et al. 1994; Tkaczyk et al. 2004; Ghys et al. 2014a).

In veterinary medicine, a primary report described an assay for measuring canine urinary cystatin C and suggested that the urinary cystatin C to urinary creatinine ratio was a promising marker for evaluating renal tubular function (Monti et al. 2012). This ratio could be used to distinguish dogs with renal disease from dogs without renal disease. Sasaki et al. (2014) found urinary cystatin C to be the most sensitive index of kidney injury in dogs with gentamicin-induced acute kidney injury. In dogs with leishmaniasis, urine cystatin C concentrations were increased only in severe, azotemic stages of CKD suggesting that urine cysC will not allow identification of dogs with early kidney disease (Garcia-Martinez et al. 2015). In cats, a human assay for urinary cystatin C measurement was validated. Cats with CKD exhibited a significantly higher urinary cystatin C to urinary creatinine ratio when compared with healthy cats (Ghys 2014b).

The first results seem promising but further studies are needed to evaluate urinary cystatin C as an early marker of renal damage in dogs and cats.

3.6. Cauxin

Cauxin is a 70 kDa carboxylesterase which is secreted from the proximal straight tubular cells into the urine (Miyazaki et al. 2003). In normal cats, it is a major urinary protein. Excretion of cauxin appears to be age-dependent, with no cauxin evident in the urine of cats younger than three months. Higher urinary cauxin levels are found in intact male cats when compared with neutered males and females (Miyazaki et al. 2006a). Cauxin regulates the production of felinine, a putative pheromone precursor. It is therefore suggested that cauxin may play an important role in behavioural territorial marking in cats (Miyazaki et al. 2006b). In cats with tubulointerstitial nephritis, lower urinary levels and renal expression of cauxin was found. It was suggested that urinary cauxin may be a novel clinical indicator of cats with tubular damage (Miyazaki 2007). In geriatric cats, the urine cauxin-to-creatinine ratio was significantly higher in the group that later developed azotemia. Nevertheless, additional research is needed to determine whether cauxin may be suitable as a marker of renal proximal tubular cell damage (Jepson et al. 2010b).

3.7. Clusterin

Clusterin is a glycoprotein (70–80 kDa) synthesised in many tissues and found in various physiologic fluids (Rosenberg and Silkensen 1995). In human kidney, clusterin is expressed during various kidney diseases (Rosenberg and Paller 1991). In dogs, clusterin was recently measured as a potential marker of drug-induced acute kidney injury. Together with NGAL, clusterin was found to be the most sensitive biomarker for detection of gentamicin-induced renal proximal tubular toxicity (Zhou et al. 2014).

4. Conclusions

Renal disorders are common in dogs and cats. Due to the insensitivity of routine blood analysis, early diagnosis may be challenging. Evaluation of specific biomarkers in urine may allow early detection of reduced renal functional mass and differentiation of various renal and non-renal disorders and assist with localisation of damage. In cases of acute kidney injury, early detection of renal function impairment, before overt clinical abnormalities are present and development of azotemia, may allow initiation of medical management when it may be more effective. In CKD, early diagnosis facilitates renoprotective and therapeutic interventions that may slow down the progression of kidney disease. In addition, it allows more effective monitoring of the rate of decline in renal function over time and may help to determine the efficacy of the treatment.

Some of the biomarkers described above are well established for routine use in early diagnosis of

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kidney disorders. For other markers, further studies are needed to evaluate their suitability for determining the localisation and severity of renal damage and to assess their predictive value.

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