Abstract: Current conventional tests of kidney damage and function in blood (serum creatinine and urea nitrogen) and urine (urine protein creatinine ratio and urine specific gravity) are widely used for diagnosis and monitoring of kidney disease. However, they all have important limitations, and additional markers of glomerular filtration rate and glomerular and tubular damage are desirable, particularly for earlier detection of renal disease when therapy is most effective. Additionally, urinary markers of kidney damage and function may help localize damage to the affected portion of the kidney. In general, the presence of high- and intermediate-molecular weight proteins in the urine are indicative of glomerular damage, while low-molecular weight proteins and enzymes in the urine suggest tubular damage due to decreased reabsorption of proteins, direct tubular damage, or both. This review aims to discuss many of these new blood and urinary biomarkers in domestic veterinary species, focusing primarily on dogs and cats, how they may be used for diagnosis of renal disease, and their limitations. Additionally, a brief discussion of serum creatinine is presented, highlighting its limitations and important considerations for its improved interpretation in domestic species based on past literature and recent studies.
Introduction

Urinalysis has contributed to medical diagnoses for thousands of years. However, it was not until the routine use of clinical chemistry approximately 50–60 years ago that measurement of renal biomarkers became commonplace in human and veterinary medicine. This allowed for both an improved understanding of the renal system and ability to diagnose renal disease. Historically, renal biomarkers have focused on kidney function testing, and this is the basis for current conventional tests in blood (serum creatinine [sCr], urea or urea nitrogen [UN]) as endogenous indicators of glomerular filtration rate (GFR). We are increasingly recognizing the need to identify renal disease at an early stage, when therapeutic options are most effective. While sCr and UN play an important role in the diagnosis of kidney disease, their limitations create poor confidence for their use as early indicators of disease. New markers of renal function aim to overcome these limitations. In addition, there are now many urinary markers that can detect kidney damage and help localize that damage to the compartment of the kidney that is affected.

Many excellent and comprehensive reviews of renal biomarkers have been published focusing on the human literature, with several recent reviews available in veterinary medicine. This review aims to comprehensively summarize the field in veterinary medicine, particularly focusing on recent advances in renal biomarker research.

Renal Biomarkers: Blood

Endogenous markers of GFR

Creatinine

Serum creatinine is the most widely used endogenous marker for estimating GFR, and its metabolism, measurement, and diagnostic significance in dogs have previously been reviewed. While the present review focuses primarily on new tests of renal function, it is important to consider factors that can either enhance or limit the clinical use of sCr in order to optimally help clinicians and clinical pathologists interpret the value of this conventional test. In particular, accurate interpretation of the literature, population-specific reference intervals, trending of sCr, and consideration of muscle mass influence and analytic variability are all needed to best interpret sCr in dogs and cats. Of note, although creatinine is referred to as sCr throughout this manuscript, creatinine is also commonly measured in plasma.

Nephron mass vs nephron function. It is widely accepted that at least 75% of nephron mass must be lost before sCr increases above the reference limit. The original source for this statement likely originates from partial nephrectomy studies in dogs. However, the statement is often misinterpreted as 75% loss of renal function vs mass. In partial nephrectomy studies, 3/4th loss of renal mass corresponded to an approximately 50–60% or 35–45% decrease in renal function based on inulin clearance one month or 13 months post surgery, respectively. The much lower decrease in function as compared with the percentage of nephron loss is due to compensatory changes in remaining nephrons (ie, compensatory renal hypertrophy). Furthermore, using an age- and breed-specific reference limit (sCr ≥ 106 mmol/L or 1.2 mg/dL) along with frequent monitoring, adolescent dogs with rapidly progressive kidney disease due to X-linked hereditary nephropathy (XLHN) demonstrated increased sCr after GFR had decreased an average of 48% (range 39–68%). Based on these studies, sCr can be more sensitive for detecting decreased renal function than has been historically assumed.

Value of population-specific reference intervals. While sCr is not as poorly sensitive as generally believed, its inability to consistently detect < 50% decline in kidney function at least partly stems from reference intervals that are overly wide for patients with low baseline
sCr. Since current methodologies are highly specific for creatinine, the wide reference intervals largely stem from biologic differences in sCr among individuals. Serum creatinine has relatively high individuality in dogs and cats, meaning that variability between individuals is much higher than the variability observed within a single animal. Serum creatinine is influenced by age and particularly by breed in dogs and cats and, to a lesser extent, in cats. It might also be influenced by sex and the veterinary clinic evaluating the patient. Therefore, sCr would benefit from age- and breed-specific reference intervals, ideally (although not practically) for every individual instrument and laboratory.

Trending of serum creatinine. Small increases in sCr even within the reference interval can reflect significant decreases in GFR in an individual patient, particularly since variation in sCr within an individual healthy dog or cat is minimal over weeks to months and even years. In fact, the critical difference or reference change value for detecting a significant increase or decrease in sCr is only 23–27 μM/L (0.3 mg/dL) in clinically healthy dogs, and 17% (corresponding to similar absolute values as in dogs) in clinically healthy cats. Thus, the sensitivity of sCr for detecting early kidney disease can be improved by evaluating serial fasted sCr measurements in an individual animal (trending) to look for increases that likely reflect worsening renal function. This concept of detecting small but clinically relevant increases in sCr is actively being adopted in cases of acute kidney injury (AKI), illustrated by the International Renal Interest Society (IRIS) Grading of AKI. In this grading scheme, an increase in sCr ≥ 26 μmol/L (0.3 mg/dL) within a 48-hour period is a criterion for identifying Grade I and Grade II AKI (www.IRIS-Kidney.com). Furthermore, in adolescent dogs with XLHN, trending of sCr detected an average of 27% (range 5–49%) decrease in GFR. Despite heightened awareness of small, but clinically relevant increases in sCr over a short time frame, more recognition is needed with slowly progressive CKD, in which small increases might occur over many months or years.

Nonrenal influences. It is well known that an inherent limitation of sCr is its dependence on muscle mass. Serum creatinine can overestimate renal function in cachectic, geriatric, and very young patients as well as in small-breed dogs. Additionally, sCr increases might be offset by ongoing muscle wasting in animals with CKD and in elderly animals, as supported by a recent study of geriatric cats, where both sCr and GFR were lower in cats > 15 years old as compared with cats < 15 years old. Therefore, even careful monitoring of sCr over time in an individual patient is an unreliable indicator of declining GFR if concurrent muscle wasting is present. Additionally, as might be expected with any endogenous marker of GFR, hydration and blood volume status as well as urinary tract obstruction or rupture can influence sCr. Particularly early on, these changes do not necessarily reflect inherent kidney function, further hampering the sensitivity and specificity of sCr for kidney disease.

Analytic challenges. Finally, sCr is plagued by inconsistencies in its measurement between instruments and laboratories, which can result in markedly different results. While most reference laboratory instruments have excellent precision and provide results of similar magnitude among instruments, recent studies illustrate the high imprecision and bias possible with some instruments and among different laboratories. In normal to mildly azotemic samples, one study using reference laboratories found differences of up to 40 μmol/L (0.45 mg/dL) in the same sample measured by the same laboratory and up to 50 μmol/L (0.57 mg/dL) among different laboratories, even when excluding 2 laboratories (out of 10) with extreme outliers. Larger differences were noted in moderately to markedly azotemic samples: excluding outlier results from 3 laboratories, up to a 68 μmol/L (0.77 mg/dL) difference was observed within the same laboratory, and 117 μmol/L (1.32 mg/dL) difference between laboratories. A still larger variation in sCr measurement was observed among 99 veterinary practices, where results ranged from 80 to 200 μmol/L (0.9–2.26 mg/dL) for a single sample spiked with 112 μmol/L (1.25 mg/dL) creatinine.

These results highlight that many instruments/laboratories are performing below the total allowable error (TEa) guideline for sCr (TEa ≤ 20%) set by the Quality Assurance and Laboratory Standards Committee of the American Society for Veterinary Clinical Pathology (ASVCP). Even with TEa ≤ 20%, analytic variability alone (instrument precision and bias) could account for an increase or decrease in sCr ≥ 18 mmol/L (0.2 mg/dL) in normal to mildly azotemic samples, which is approaching the critical difference for detecting a significant change in sCr in clinically healthy dogs and cats. To minimize this analytic variability, it is particularly important that serial determinations of sCr are measured on a single instrument that is subjected to a strict quality assurance program.
In summary, sCr can be a sensitive marker of declining renal function if careful monitoring and/or appropriate reference intervals are used in animals with relatively stable muscle mass. However, because of both inherent biologic and current analytic limitations of sCr, additional endogenous markers of GFR would aid in the early diagnosis of renal disease. Two recently studied markers are cystatin C and symmetric dimethylarginine (SDMA). An excellent recent review of cystatin C is available. Therefore, only SDMA will be further discussed below.

**Symmetric dimethylarginine (SDMA)**

Symmetric dimethylarginine is a methylated amino acid (arginine) of similar size to creatinine (SDMA: 202 daltons [Da]; creatinine: 113 Da). Symmetric dimethylarginine is formed by posttranslational methylation of arginine by type 2 protein arginine methyltransferases, and it is released into circulation following proteolysis. Symmetric dimethylarginine was originally discovered 45 years ago in human urine. Concentrations of Symmetric dimethylarginine in protein fractions from various organs in rats demonstrated SDMA to be highest in the brain and relatively high in the liver, lung, kidney, spleen, and small intestine as compared with heart, muscle, skin, and blood. The kidneys are the major source of SDMA excretion, and SDMA does not appear to be reabsorbed by the tubules for reutilization.

Symmetric dimethylarginine was first shown to be increased in human patients with CKD over 20 years ago; however, SDMA has only recently gained prominence as an endogenous marker of GFR in people, correlating strongly with renal function based on inulin clearance. Symmetric dimethylarginine has largely been believed to be inert, reducing nitric oxide synthesis indirectly by competing with L-arginine uptake in cells. However, a recent study demonstrated a direct effect of SDMA, in which uncoupling of endothelial nitric oxide synthase resulted in superoxide anion production in glomerular endothelial cells. In addition, a recent review highlights several studies that support a proinflammatory role of SDMA. However, chronic infusion of SDMA in healthy mice had no evident effect on renal structure or function as well as cardiac function.

**Measurement and stability.** Mass spectrometry is the gold standard for SDMA measurement, as it uniquely and accurately detects the molecule. However, recent studies in people have used a commercially available ELISA with at least one study demonstrating good precision, although accuracy was not assessed. An accurate and precise liquid chromatography–mass spectrometry (LC–MS/MS) assay has recently been developed for SDMA measurement in dogs and cats. In addition, a high-throughput immunoassay with similar performance to the LC–MS/MS method has been developed and validated, allowing SDMA to become a standard analyte on chemistry panels offered by IDEXX Laboratories, Inc. Symmetric dimethylarginine is stable in canine and feline serum and plasma for at least 7 days at room temperature, and 14 days at 4°C and with up to 3 freeze–thaw cycles (between −80°C and room temperature). While no published studies are available regarding the long-term stability of SDMA in frozen samples, anecdotal evidence supports that it is stable for at least 5 years when frozen at −20°C or −80°C (IDEXX, Laboratories, Inc., Personal Communication).

**Reference limit.** In clinically healthy animals, SDMA concentrations are similar across studies and species, despite differences in methodology and animal populations. Two studies in healthy human subjects determined the reference interval using LC–MS/MS as 0.32–0.65 μmol/L (6.5–13.1 μg/dL) and 0.225–0.533 μmol/L (4.5–10.8 μg/dL). Using serum from 120 healthy adult dogs of varying ages and breeds, a 95% reference interval for SDMA was calculated as 6–13 μg/dL, and in both dogs and cats, the upper reference limit using the LC–MS/MS methodology was set at 14 μg/dL. Furthermore, a range of 7.3–12.4 μg/dL (0.36–0.61 μmol/L) was observed in 21 healthy geriatric cats in a recent study. However, in juvenile dogs, SDMA might occasionally reach or exceed the 14 μg/dL reference limit. In older studies using high-performance LC, SDMA in healthy dogs ranged from 0.22 to 0.61 μmol/L (4.4–12.3 μg/dL), although values up to 14.1 μg/dL (0.7 μmol/L) were observed in a group consisting of both healthy dogs and dogs with mitral regurgitation.

**Nonrenal influences.** Currently, SDMA’s rate of production is thought to be relatively constant, although in theory, altered arginine methylation and/or protein breakdown could contribute to increased or decreased serum concentrations. Furthermore, SDMA does not appear to be highly protein bound, and its clearance is similar to that of creatinine, supporting that it is freely filtered through the glomerulus. It is largely renally excreted; however, hepatic clearance of SDMA was observed in people, and a small degree of uptake by the gut was observed in rats. Unlike asymmetric
dimethylarginine (ADMA), which is largely degraded by dimethylarginine dimethylaminohydrolase (DDAH)\textsuperscript{57}, enzymatic degradation does not seem to be a major factor in SDMA elimination. However, some degree of enzymatic degradation of SDMA is possible\textsuperscript{55,58}, and in particular, alanine-glyoxylate aminotransferase 2 (Agxt2), which is largely present in the liver but also kidney, can metabolize SDMA.\textsuperscript{59} Symmetric dimethylarginine does not appear to be significantly metabolized by renal tubules.\textsuperscript{30} No studies were identified that determined whether or not SDMA can be secreted from the tubules or reabsorbed for recirculation, although these processes seem unlikely.\textsuperscript{28}

Various studies, typically measuring SDMA as an afterthought to ADMA, have found a variety of external factors to show a lack of statistically significant and/or clinically relevant influence on SDMA concentrations in people or rats, including weight loss\textsuperscript{60}, inflammation\textsuperscript{61}, diabetes\textsuperscript{62}, and estrogen therapy.\textsuperscript{63,64} Diet also does not appear to influence SDMA in people, even with lysine and arginine loading.\textsuperscript{28,65,66} Furthermore, SDMA in people is minimally influenced by obesity\textsuperscript{67}, gender, age\textsuperscript{68}, and polycystic ovary syndrome.\textsuperscript{69}

In cats and dogs, several nonrenal influences have been investigated. Notably, SDMA is not influenced by muscle mass, the major nonrenal influence on sCr. In healthy geriatric cats and adult dogs, SDMA did not correlate with lean body mass.\textsuperscript{43,70} Furthermore, in healthy growing dogs from 2 months to approximately one year of age, SDMA did not correlate with age, weight, or body condition score, whereas sCr strongly correlated with these variables.\textsuperscript{8} Intriguingly, SDMA did not increase as GFR (normalized to body weight) decreased in these growing dogs.\textsuperscript{8} While this could suggest a nonrenal influence on SDMA in adolescent dogs, the normalization method for clearance techniques needs further investigation in young, growing dogs before ascribing clinical significance to stable SDMA concentrations during growth. In addition, in healthy dogs participating in a dietary trial (comparing a renal diet with or without supplementation of L-carnitine and fish oil), SDMA significantly decreased over 6 months (from an average of 13.3 to 6.5 µg/dL).\textsuperscript{70} While the authors speculate that this decrease could be due to improved renal function secondary to a dietary effect, no clearance techniques were performed in these dogs, and nonrenal causes for this decrease cannot be ruled out.

In studies evaluating additional nonrenal variables in dogs, SDMA does not appear to be influenced by breed, body size, sex, age (in adults), diurnal variation, white-coat effect, or short-term administration of angiotensin-converting-enzyme inhibitors.\textsuperscript{49,50,52} In one study, SDMA demonstrated a mild but statistically significant correlation with adjusted body weight only when excluding creatinine as an explanatory variable, and the authors speculated that this was due to lower GFR in the larger dogs.\textsuperscript{52}

**Renal disease in veterinary medicine.** Several recently published studies of SDMA in dogs and cats have shown strong evidence for SDMA as an endogenous surrogate marker for GFR, particularly in animals with renal disease. In dogs, SDMA correlated strongly with inulin clearance in a partial nephrectomy model of CKD \((r = −.851)\textsuperscript{51}\) and with iohexol clearance using serial measurements in adolescent dogs with XLHN \((r = −.95)\).\textsuperscript{8} In studies combining both healthy cats and cats with CKD, correlations of SDMA or 1/SDMA with iohexol clearance were \(r = −.79\)\textsuperscript{43} and \(r = .82\)\textsuperscript{71}, respectively. These were similar to correlations of sCr with clearance. Furthermore, in healthy geriatric cats, the correlation of SDMA with iohexol clearance was higher \((r = −.72)\) than that observed for sCr \((r = −.50)\).\textsuperscript{22} Symmetric dimethylarginine correlation with sCr was also strong in both dogs and cats when including those with kidney disease \((r = .72–.95)\textsuperscript{8,43,71,72}\), whereas in clinically healthy dogs and cats, the correlation between SDMA and sCr was much lower \((r = .32–.46)\).\textsuperscript{43,52,70}

Importantly, SDMA appears to detect a decrease in GFR prior to sCr when based on reference limits in cats and dogs. In 21 geriatric laboratory cats with naturally occurring CKD, SDMA increased by the time azotemia (sCr > 2.1 mg/dL) developed in all cats, and SDMA increased above its reference limit an average of 17 months earlier (up to 48 months earlier) than sCr.\textsuperscript{43} Symmetric dimethylarginine also shows promise as a sensitive screening test for CKD in cats. Using iohexol clearance to estimate GFR and > 30% decrease from the median of clinically healthy controls as the gold standard for decreased GFR, SDMA had perfect sensitivity and negative predictive value (100%), indicating that cats with SDMA < 14 µg/dL did not have decreased GFR.\textsuperscript{43} Specificity and positive predictive value (PPV) were slightly lower (91% and 86%, respectively), due to 2 cases where SDMA was mildly increased with a GFR only 25% lower than the median.\textsuperscript{43} However, this could indicate that SDMA can detect < 30% decrease in GFR in cats. In comparison, the upper reference limit of sCr provided perfect specificity and PPV (100%), indicating that cats with sCr above the reference interval (sCr > 2.1 mg/dL) all had > 30% decline in
GFR. However, sensitivity was quite poor (17%), indicating that many cats with decreased GFR are undetected when using this reference limit. Since specificity and sensitivity depend on the reference limit used, certainly use of a lower reference limit for sCr would have improved its sensitivity in this study, particularly since sCr in the clinically healthy cats was ≤ 1.6 mg/dL.

In dogs with rapidly progressive CKD due to XLHN, SDMA increased an average of 4–5 weeks prior to an increase in sCr and decrease in GFR, based on their respective reference limits. However, when trending both sCr and SDMA in individual dogs, SDMA increased an average of only 2 weeks prior to an increase in sCr. Notably, SDMA identified ≤ 34% (range, −6–34%) decrease in GFR when compared with the GFR of unaffected, age-matched dogs in this colony, regardless of whether the increase in SDMA was based on the reference limit, trending, or comparison with healthy controls. In some dogs, SDMA increased even before iohexol clearance was below that observed in unaffected, age-matched dogs. This was in contrast to sCr, which detected a 5–68% decrease in GFR.

In summary, SDMA appears to be a useful endogenous marker of GFR and is particularly promising as a screening test for early detection of decreased renal function. Furthermore, because it is not influenced by lean body mass and is less variable among different dog breeds, SDMA could prove especially useful in those patients with poor muscle mass or ongoing muscle loss, where sCr would provide an unreliable estimate of GFR. Further studies are needed in veterinary medicine to determine possible extra-renal influences on SDMA concentration.

**Markers of altered metabolism**

**Fibroblast growth factor-23 (FGF-23)**

Fibroblast growth factor-23 (FGF-23), recently reviewed in dogs and cats with CKD, is a phosphaturic hormone that is secreted by osteoblasts and osteocytes into the blood in response to increased serum phosphorus. Fibroblast growth factor-23 results in increased urinary phosphorus excretion by inhibiting sodium-dependent phosphorus reabsorption in the proximal tubule, and it decreases intestinal reabsorption of phosphorus via diminished calcitriol. Blood concentration of FGF-23 has also been shown to increase as GFR decreases in both people and cats. While studies in human medicine have evaluated the pathogenesis of plasma FGF-23 in CKD and secondary renal hyperparathyroidism, few studies are available in domestic animals, and these have focused on feline CKD in geriatric (> 9 years) cats.

**Measurement, stability, and reference range.** A human FGF-23 ELISA has been validated for the detection of plasma FGF-23 concentrations in cats, with acceptable precision, reproducibility, and dilutional linearity. Plasma FGF-23 was stable for up to 7 days at 22°C and 14 days at −20°C as well as up to 4 freeze–thaw cycles. A reference range determined for geriatric cats (56–700 pg/mL) was higher and broader than that used in adult people, which could be due to subclinical CKD in the cats used to establish the reference range, diet, or species-specific factors.

**Renal disease in veterinary medicine.** Plasma FGF-23 concentration was significantly increased at baseline in geriatric cats that developed azotemia within the following 12 months compared with those that remained nonazotemic, and plasma FGF-23 increased significantly with increasing IRIS stage of CKD. Additionally, the presence of hyperphosphatemia was associated with greater plasma FGF-23 compared with normophosphatemic cats within the same IRIS stage. Plasma phosphate concentration was shown to be an independent predictor of FGF-23 concentration. Furthermore, plasma FGF-23 concentrations decreased significantly from baseline in geriatric cats with stable azotemic CKD fed a reduced phosphate renal diet for 4–8 weeks. This FGF-23 decrease was observed even in cats that were normophosphatemic (plasma phosphate ≤ 4.5 mg/dL), although phosphate and PTH concentrations remained unchanged. Hyperthyroid cats with azotemic CKD or that developed azotemia after becoming euthyroid had significantly higher baseline plasma FGF-23 than hyperthyroid cats that remained nonazotemic after treatment. However, after treatment for hyperthyroidism, plasma FGF-23 increased both in cats that remained non-azotemic and those that became azotemic, and overall baseline plasma FGF-23 concentration was not a predictor for the development of azotemia post treatment in hyperthyroid cats.

Other renal biomarkers that have been evaluated in the serum and plasma of domestic species include cystatin C in dogs and cats and neutrophil gelatinase-associated lipocalin (NGAL) and homocysteine, as markers of inflammation in dogs (Table 1).
<table>
<thead>
<tr>
<th>Renal Biomarker</th>
<th>Location of Production</th>
<th>Type of Protein/Molecule</th>
<th>Type of Biomarker</th>
<th>Validation/Species</th>
<th>Values in Healthy Animals</th>
<th>Affected in AKI, CKD, or Both</th>
<th>Nonrenal Influences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine</td>
<td>Muscle</td>
<td>Cyclic derivative of creatine</td>
<td>Endogenous indirect GFR marker</td>
<td>Dogs/Cats</td>
<td>Dogs: 0.5–1.5 mg/dL(^{226}) Cats: 0.8–1.8 mg/dL(^{226}) (but depends on breed/species/instrument)</td>
<td>Both</td>
<td>Muscle mass; meat diet; hydration status</td>
</tr>
<tr>
<td>Cystatin C</td>
<td>All nucleated cells</td>
<td>LMW protein and proteinase inhibitor</td>
<td>Endogenous indirect GFR marker</td>
<td>Dogs/Cats</td>
<td>Dogs: &lt; 2.28 mg/L(^{83,86-89,227-229}) Cats: &lt; 1.95 mg/L(^{82,84,85,90})</td>
<td>CKD: Dogs(^{85,86-89,227-229}), cats(^{82,85,90}) (Presumably also AKI)</td>
<td>Obesity and weight loss in dogs(^{96})</td>
</tr>
<tr>
<td>SDMA</td>
<td>All nucleated cells</td>
<td>Methylated amino acid (arginine)</td>
<td>Endogenous indirect GFR marker</td>
<td>Dogs/Cats</td>
<td>Dogs and cats: &lt; 14 µg/dL(^{5,41,48-52})</td>
<td>CKD: Dogs(^{43,71,72}), cats(^{43}) (Presumably also AKI)</td>
<td>Dietary phosphorus; hyperthyroidism(^{79})</td>
</tr>
<tr>
<td>FGF-23</td>
<td>Osteocytes and osteoblasts</td>
<td>Phosphaturic hormone</td>
<td>Marker of altered phosphorus metabolism</td>
<td>Cats</td>
<td>Cats: 56–700 pg/mL(^{80})</td>
<td>CKD: Cats(^{78-81})</td>
<td>Inflammation(^{230})</td>
</tr>
<tr>
<td>NGAL</td>
<td>Neutrophils, kidney, bronchus, stomach, small intestine, pancreas, prostate gland, thymus</td>
<td>LMW glycoprotein</td>
<td>Endogenous indirect GFR marker</td>
<td>Dogs</td>
<td>Dogs: &lt; 21.2 ng/mL(^{91-93})</td>
<td>AKI: Dogs(^{92-93}) CKD: Dogs(^{92-93})</td>
<td></td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>Hepatocytes</td>
<td>Positive acute phase protein</td>
<td>Inflammatory marker</td>
<td>Dogs</td>
<td>Dogs: 3.21 mg/L (range: 2.09–8.60 mg/L(^{97})</td>
<td>Renal diseases in dogs (AKI vs CKD not specified)(^{97})</td>
<td></td>
</tr>
<tr>
<td>Homocysteine</td>
<td>All nucleated cells</td>
<td>Amino acid; Intermediate product of methionine metabolism</td>
<td>Endogenous indirect GFR marker</td>
<td>Dogs</td>
<td>Dogs: 4.35 ± 2.69 µmol/L(^{94})</td>
<td>AKI: Dogs(^{95}) CKD: Dogs(^{95})</td>
<td>Obesity and weight loss in dogs(^{96}); icterus, severe hemolyisis and lipemia(^{95}); cardiac disease(^{95})</td>
</tr>
<tr>
<td>Big-Endothelin-1</td>
<td>Blood vessels, lung, other tissues including kidney medulla</td>
<td>Precursor to endothelin-1, a vasoconstrictor peptide</td>
<td>Inflammation</td>
<td>Dogs</td>
<td>Dogs: 6.51 ± 1.86 pg/mL(^{94})</td>
<td>CKD: Dogs(^{94})</td>
<td>Hypertension and systemic inflammation(^{94})</td>
</tr>
</tbody>
</table>

AKI indicates acute kidney injury; CKD, chronic kidney disease; FGF-23, fibroblast growth factor-23; GFR, glomerular filtration rate; LMW, low molecular weight; NGAL, neutrophil gelatinase-associated lipocalin; SDMA, symmetric dimethylarginine.
Renal Biomarkers: Urine

Renal protein handling

Several publications are available that review the mechanisms of protein handling by the kidney in health and disease. In brief, proteins with a molecular weight < 40 kilodaltons (kDa) (low-molecular weight [LMW] proteins) are able to freely pass through the glomerular filtration barrier, while intermediate-molecular weight (IMW) proteins, those approximate the size of albumin, face increased charge and size restrictions, and high-molecular weight (HMW) proteins (> 100 kDa) are generally completely restricted due to their large size. Healthy tubules reabsorb proteins that are filtered into the tubular space via receptor-mediated endocytosis. Glomerular damage increases the permeability of the glomerular filtration barrier, typically resulting in marked proteinuria, while tubular damage results in mild proteinuria due to decreased reabsorption of proteins, leakage of proteins from the damaged tubular epithelial cells, and upregulation of proteins involved in injury and repair.

When protein biomarkers are quantified in urine, their concentration is often indexed to urine creatinine (eg, urinary biomarker divided by urine creatinine) or urine specific gravity. Indexing urinary biomarkers to urine creatinine assumes that the excretion of urine creatinine is constant between and within individuals, and that both urinary biomarkers and creatinine are inversely proportional to urinary flow rate. When these assumptions are met, an increased or decreased biomarker/creatinine ratio will reflect increased or decreased biomarker excretion. However, when an animal is not in steady state (ie, when renal function is rapidly changing), the assumption of constant urine creatinine excretion may not be correct, as demonstrated in a study of human patients with AKI and post kidney transplantation. Because of this, the practice of indexing to urine creatinine in cases of AKI is questionable. Therefore, some authors have reported concentrations of biomarkers without indexing to creatinine. For purposes of this review, biomarkers that are indexed to urine creatinine are denoted as uBiomarker/c, and those that are unindexed concentrations in the urine are denoted as uBiomarker.

Markers of glomerular damage/dysfunction

Immunoglobulins

Immunoglobulins are large glycoproteins made by plasma cells in the spleen, lymph nodes, and bone marrow, and are involved in antibody-mediated defense. The molecular weight of immunoglobulins G (IgG) and M (IgM) are 150 kDa and 900 kDa, respectively. Serum immunoglobulin A (IgA) is present in monomeric, dimeric, and polymeric forms, with the monomeric form having a molecular weight of 160 kDa. Thus, IgG, IgM, and IgA are HMW proteins that cannot pass through the glomerular filtration barrier in the healthy kidney. However, with glomerular damage, they may pass into the urinary filtrate; thus, they are considered markers of glomerular damage.

Measurement and stability. Species-specific ELISAs are the most common and preferred method for detection of urinary immunoglobulins; however, thus far, detection of IgA in canine urine has only been reported using Western blot. Canine-specific ELISA assays for IgG, IgM, and IgA are available (Bethyl Laboratories, Montgomery, TX, USA). Serum immunoglobulin A ELISA assays have been validated showing acceptable mean intra- and inter-assay variabilities, spiking recovery, and dilutional linearity. True stability testing of IgG in urine has not been evaluated; however, uIgG/c in canine urine samples stored for 8 years at −80°C were similar to those stored for 2 years at −80°C.

Values in healthy animals. Mean urinary IgG/creatinine (uIgG/c) is generally < 3 mg/g, with maximum values < 10 mg/g observed in all but one study (Table 2). Published studies currently are not available describing the urine concentration of IgM in healthy animals; however, in the authors’ personal experience, urinary IgM concentration is low in clinically healthy dogs. IgA was undetectable in the urine of healthy dogs using Western blot.

Nonrenal influences. A few studies found that urinary IgG concentration is not significantly altered by hematuria/hemoglobinuria or pyuria/urinary tract infection. However, in the authors’ experience in dogs with primarily proteinuric CKD, uIgG/c was significantly higher in dogs with hematuria, and fractional excretion of IgG (IgG_FE) was higher in dogs with pyuria/bacteriuria compared with dogs with inactive urine sediment. Similarly, urinary IgM/
creatinine (uIgM/c) and fractional excretion of IgM (IgM_FE) were both significantly increased with hematuria and pyuria/bacteriuria in these dogs (J.A.H., M.B.N., unpublished data).

Renal disease in veterinary medicine. Acute kidney injury (AKI): Urinary IgG is the main immunoglobulin evaluated in diseases known to cause AKI, with fewer studies evaluating urinary IgA. Dogs with AKI due to a variety of causes, including *Babesia rossi*, leishmaniasis, and leptospirosis, have demonstrated increases in uIgG/c or IgG and IgA on Western blot, supporting glomerular damage. However, as expected, canine leptospirosis resulted primarily in increased LMW proteins on sodium dodecyl sulfate polyacrylamide gel electrophoresis, consistent with interstitial nephritis. Interstitial nephritis. Interestingly, in dogs with snake envenomation, UPC was not increased despite significantly increased uIgG/c vs control dogs at baseline and 24 hours later.

Pyometra can cause significant increases in uIgG/c and UPC, with a positive correlation between UPC and uIgG/c. This increase is typically transient, decreasing significantly after ovariohysterectomy, and in some cases, uIgG/c returns to values that are not significantly different from healthy dogs. A low proportion of bitches with pyometra also had detectable urinary IgA. These studies support that altered glomerular permselectivity can be present in dogs with pyometra, and indeed, histopathology demonstrated glomerulosclerosis as the most common glomerular lesion in these dogs. However, tubular atrophy, interstitial inflammation, and fibrosis were often present as well, and the role of pyometra vs previously underlying renal disease in these older dogs is uncertain.

### Table 2. Summary of urinary biomarkers of glomerular damage/dysfunction in small animals.

<table>
<thead>
<tr>
<th>Renal Biomarker</th>
<th>Location of Production</th>
<th>Type of Protein/Biomarker</th>
<th>Validation/Species</th>
<th>Values in Healthy Animals</th>
<th>Affected in AKI, CKD, or Both</th>
<th>Nonrenal Influences</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA</td>
<td>Plasma cells in spleen, lymph nodes, bone marrow</td>
<td>Antibody; HMW protein</td>
<td>Dogs[107]</td>
<td>Dogs: uIgA/c: &lt; 10 mg/g[105,112-115]</td>
<td>AKI: Dogs[107-109,112-115]</td>
<td>Hematuria*; Pyuria/bacteriuria*</td>
</tr>
<tr>
<td>IgM</td>
<td>Plasma cells in spleen, lymph nodes, bone marrow</td>
<td>Antibody; HMW protein</td>
<td>Dogs[106]</td>
<td>Dogs: uIgM/c: &lt; 10 mg/g[105,110,112-115]</td>
<td>AKI: Dogs[106]</td>
<td>Hematuria*; Pyuria/bacteriuria*</td>
</tr>
<tr>
<td>Transferrin</td>
<td>Primarily liver; other tissues as well</td>
<td>Iron transport protein</td>
<td>Cats: uTf: 0.09 ± 0.42 mg/dL[127]</td>
<td>CKD: Dogs[117,118]</td>
<td>Cats[127,134]</td>
<td></td>
</tr>
</tbody>
</table>

AKI indicates acute kidney injury; Alb_FE, fractional excretion of albumin; CKD, chronic kidney disease; HMW, high molecular weight; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; TXB2, thromboxane B2; uALB, urinary albumin concentration; uALB/c, urinary albumin/urinary creatinine; uCRP/c, urinary C-reactive protein/urinary creatinine; uIgG/c, urinary immunoglobulin G/urinary creatinine; uTF, urinary transferrin concentration; uTXB2/c, urinary thromboxane B2/urinary creatinine. *Personal observations.
Currently there are no studies evaluating urinary IgM in companion animals with AKI.

**Chronic kidney disease (CKD):** Urinary IgG and IgM have been shown to increase in dogs with CKD. In dogs with CKD due to X-linked hereditary nephropathy (XLHN), uIgG/c increased in early stages of renal disease while uIgG/c remained low in healthy age-matched littermates. Urinary IgG/c often increased before UPC and continued to increase in mid to late stages of disease progression. Furthermore, uIgG/c was moderately to highly positively correlated with most glomerular and tubulointerstitial (TI) lesions based on histopathology. Urinary biomarker concentrations and their fractional excretions were measured in dogs with naturally occurring CKD to correlate biomarkers with types of renal damage (glomerular vs TI) and their association with survival. Immunoglobulin G (uIgG/c and IgG_FE) and IgM (uIgM/c and IgM_FE) demonstrated moderate, positive correlations with glomerular damage based on light and electron microscopy (r = .44–.58 and r = .41–.58, respectively), which were similar to that observed for UPC (r = .45–.57). Immunoglobulin M_FE also correlated moderately well with TI damage (r = .49). Markedly increased uIgM/c and uIgG/c were associated with immune complex-mediated glomerulonephritis (ICGN), while lower uIgM/c was observed in juvenile nephropathies, nonimmune complex-mediated glomerulonephropathies, and primary tubular disease. Both IgM_FE and IgG_FE were significantly associated with faster time to death due to renal disease in these dogs.

Urinary IgG has been evaluated in several studies of dogs with increased cortisol due to either exogenous or endogenous sources, since excess cortisol causes proteinuria, likely by altering the glomerular filtration barrier. In aged Beagles treated over 24 weeks with hydrocortisone, uIgG/c and UPC progressively increased, while tapering and cessation of hydrocortisone treatment resulted in decreased uIgG/c and UPC. In dogs with hyperadrenocorticism, uIgG/c was significantly higher than in clinically healthy controls, supporting glomerular dysfunction. Finally, in dogs treated with trilostane or hypophysectomy for ACTH-dependent hyperadrenocorticism, uIgG/c decreased up to 15-fold posttreatment. However, uIgG/c did not completely return to levels comparable to healthy dogs in all cases, consistent with persistence of proteinuria in 38% of dogs 12 months posttreatment.

Additional urinary proteins indicating glomerular damage/dysfunction

**Albumin.** The use of urinary albumin (Alb) in dogs as a renal biomarker has recently been reviewed. Albumin is a negative acute phase, IMW (approximately 65 kDa) serum protein synthesized primarily by hepatocytes. It is considered primarily to be a marker of glomerular damage, but uAlb can also be present with tubular or vascular damage. Measurement of uAlb has been validated in dogs, and uAlb/c is elevated in both AKI and CKD, including renal damage due to nephrotoxic drugs (methyl tharadimide tablets and gentamicin), snake envenomation, pyometra, and hypercortisolism. Pyometra caused transient albuminuria that significantly decreased or returned to values not significantly different from healthy dogs after ovariohysterectomy. Cats with stage 1 CKD had significantly higher concentrations of uAlb than normal cats, and uAlb concentration had higher sensitivity and specificity for detection of CKD compared with plasma creatinine.

**C-reactive protein.** C-reactive protein (CRP) is a positive acute phase protein in dogs, made primarily by hepatocytes. The MW of CRP ranges from 110 to 144 kDa, therefore, its presence in the urine supports glomerular damage. In dogs with various causes of both AKI and CKD, including renal damage due to leishmaniasis, babesiosis, snake envenomation (24 hours post envenomation), pyometra (24 hours post envenomation), and pyometra treated by ovariohysterectomy had uCRP/c values that decreased to values not significantly different from healthy dogs. In contrast, a significant difference was not found in uCRP/c between healthy dogs and those with CKD in one study, although uCRP was undetectable in the healthy dogs while increased in 3 of 10 dogs with CKD. Age does not seem to have an effect on uCRP/c in healthy dogs.

Thus far, only one study evaluated the concentration of serum CRP in dogs with naturally occurring renal disease. In this study, serum CRP was significantly increased in dogs with reduced GFR, and the authors suggested that stimulation of the inflammatory acute phase response may be implicated in the pathogenesis of renal disease in dogs.

Other urinary protein markers of glomerular damage not listed in Table 2 that have been shown to increase in CKD include transthyretin, adiponec-
Markers of tubular damage/dysfunction

The proteins discussed in the next section are abnormally present in the urine due to decreased tubular reabsorption (retinol-binding protein [RBP], neutrophil gelatinase-associated lipocalin [NGAL], cystatin C), upregulation of proteins involved in injury and repair (NGAL), and decreased production by damaged tubules (Tamm–Horsfall protein [THP]). Proteins present due to release from damaged tubular epithelial cells are discussed in the urinary enzyme section. Additional examples of urinary biomarkers present due to these 4 mechanisms are presented in Table 3.

Retinol-binding protein (RBP)

Retinol-binding protein is a 21-kDa lipocalin that acts as the transport protein for retinol in plasma. Retinol-binding protein is primarily produced in the liver but also in the kidney, lungs, spleen, brain, stomach, heart, and skeletal muscle. Retinol-binding protein circulates in a complex with transthyretin (TTR), which has a molecular weight of 54 kDa. Retinol-binding protein by itself is a LMW protein that can freely pass through the glomerular filtration barrier; however, the TTR-RBP complex is too large to pass through the glomerular filtration barrier in the healthy kidney. Retinol-binding protein in the renal filtrate is reabsorbed by tubular epithelial cells. Tubular damage and/or competition for reabsorption by the presence of abnormally large amounts of protein (ie, with glomerular damage) results in decreased reabsorption of RBP with subsequent loss of RBP into the urine. Glomerular damage could also contribute to urinary RBP due to loss of the TTR-RBP complex.

Measurement and stability. Human RBP immunoassays have been validated for dogs and cats, with adequate intra-assay and inter-assay variabilities (canine urine and plasma and feline urine), spiking recovery (canine urine), and dilutional linearity along a specific range of the standard curve (canine urine). A canine-specific RBP ELISA was recently marketed (ICL); however, validation and use of this kit has not yet been published. Retinol-binding protein concentration is similar in cystocentesis vs voided urine samples from clinically healthy dogs. Retinol-binding protein appears to be relatively stable in canine urine samples when frozen, ideally at –80°C. All but one study normalized urinary RBP concentration to urinary creatinine (uRBP/c).

Values in healthy animals. In healthy dogs and cats, RBP is generally undetectable or minimally detectable in the urine by Western blot. Using immunoassays to quantify urinary RBP in healthy dogs, the highest reported uRBP/c was 0.9 mg/g, with most studies reporting means and medians < 0.15 mg/g regardless of assay used (Table 3). In healthy cats, urinary RBP was below the limit of assay detection, and it was not detectable in healthy sheep by 2-dimensional gel electrophoresis and mass spectrometry.

Nonrenal influences. In dogs, age does not appear to have a major influence on uRBP/c, as no significant differences were found for uRBP/c between healthy young and older dogs. However, a mild but statistically significant negative correlation with age was found in young adolescent dogs, likely due to low creatinine excretion in very young dogs. Pyuria, bacteriuria or positive urine culture, and at least mild to moderate hematuria and hemoglobinuria do not seem to significantly affect uRBP or uRBP/c; however, in one study, a mild increase in uRBP was seen in markedly hematuric samples. In the authors’ experience, fractional excretion of RBP (RBP_FE) was significantly higher in pyuric/bacteriuric samples vs inactive sediments from dogs with proteinuric CKD (J.A.H., M.B.N., unpublished data). To the authors’ knowledge, nonrenal influences on urinary RBP have not been published in cats.

Renal disease in veterinary medicine. AKI: Naturally occurring AKI (eg, pyometra, babesiosis due to Babesia rossi, and envenomation by cytotoxic and neurotoxic snakes) transiently increases uRBP and uRBP/c in dogs, presumptively indicating tubular dysfunction. Histologic confirmation of tubular damage in dogs with pyometra found that dogs with severe TI lesions on histopathology had higher uRBP/c (in the 75th percentile) compared with dogs demonstrating milder lesions. Typically, uRBP/c decreased significantly after ovariohysterectomy in these dogs, often to values comparable to healthy dogs. Urinary RBP has also been detected with 2-dimensional gel electrophoresis in the urine of sheep with AKI due to ketoprofen overdose.

tin and ferritin in dogs, and apolipoprotein-H in cats.
Table 3. Summary of urinary protein and enzyme biomarkers of tubular damage/dysfunction in small and large animals.

<table>
<thead>
<tr>
<th>Renal Biomarker</th>
<th>Location of Production</th>
<th>Type of Protein/Biomarker</th>
<th>Mechanism for Altered Excretion</th>
<th>Validation / species</th>
<th>Values in Healthy Animals</th>
<th>Affects in AKI, CKD, or Both</th>
<th>Nonrenal Influences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary proteins</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Clusterin</td>
<td>Renal tubules</td>
<td>Glycoprotein</td>
<td>Increased production</td>
<td>Dogs(^{231})</td>
<td>Dogs: uClu/c: 0.27 units/g; uClu: 38.5 ng/mL(^{231})</td>
<td>AKI: Dogs(^{125,281})</td>
<td></td>
</tr>
<tr>
<td>Cystatin C</td>
<td>All nucleated cells</td>
<td>LMW protein and proteinase inhibitor</td>
<td>Decreased reabsorption</td>
<td>Dogs(^{232})</td>
<td>Cats: below limit of quantification of assay(^{233})</td>
<td>AKI: Dogs(^{126}); CKD: Dogs(^{80,232}, cats^{26,233})</td>
<td>Diabetes in cats(^{233})</td>
</tr>
<tr>
<td>KIM-1</td>
<td>Renal tubules</td>
<td>Glycoprotein</td>
<td>Increased production</td>
<td>Undetectable in healthy cats(^{234})</td>
<td></td>
<td>Both, but mostly with acute processes AKI: Dogs(^{125}), cats(^{234}); a lamb(^{176})</td>
<td></td>
</tr>
<tr>
<td>NGAL</td>
<td>Neutrophils, kidney, bronchus, stomach, small intestine, pancreas, prostate gland, thymus</td>
<td>LMW glycoprotein</td>
<td>Decreased reabsorption and increased production</td>
<td>Dogs(^{90,106,110,164})</td>
<td>Dogs: uNGAL/c: &lt; 6 µg/g; uNGAL: &lt; 21.2 ng/mL(^{91,93,161,164})</td>
<td>AKI: Dogs(^{92,93,125,126,161,165}); CKD: Dogs(^{91-93,106,110,161,165})</td>
<td>Inflammation/urinary tract infection/pyuria (dogs); uNGAL/c decreased with body mass (dogs); Age (dogs) (^{110,164})</td>
</tr>
<tr>
<td>RBP</td>
<td>Primarily liver; also other organs (kidney, lungs, spleen, brain, stomach, heart, skeletal muscle)</td>
<td>Vitamin A carrier protein</td>
<td>Decreased reabsorption</td>
<td>Dogs(^{105,110,144})</td>
<td>Cats: uRBP/c: &lt; 0.15 mg/g; Cats: Undetectable(^{145}); Sheep: Undetectable(^{152})</td>
<td>AKI: Dogs(^{105,133,115,151}); CKD: Dogs(^{105,106,110,116-119,123,124,147})</td>
<td>Marked hematuria;(^{146}) Pyuria/bacteriuria (RBP_FEP(^*)); Negative correlation with age in young adolescent dogs; Treatment with hydrocortisone (dogs)(^{110}); Urolithiasis (cats)(^{235})</td>
</tr>
<tr>
<td>THP</td>
<td>Epithelial cells of thick ascending limb of loop of Henle and distal convoluted tubule</td>
<td>Glycoprotein</td>
<td>Decreased production</td>
<td>Cats(^{235})</td>
<td>Dogs: uTHP/c: 10–65 mg/g; Cats: uTHP: 49.2 ± 35.5 µg/mL(^{235})</td>
<td>CKD: Dogs(^{117,144,151,174,175}); cats(^{134})</td>
<td></td>
</tr>
<tr>
<td>Urinary enzymes</td>
<td>Renal tubular brush border enzyme</td>
<td>Enzyme</td>
<td>Released from brush border</td>
<td>Dogs(^{91})</td>
<td>Dogs: uAAP/c: 0.7–9.0 U/g(^{191})</td>
<td>AKI: Dogs(^{125})</td>
<td></td>
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(continued)
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<thead>
<tr>
<th>Table 3 (continued)</th>
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<tr>
<td><strong>ALP</strong></td>
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<tr>
<td><strong>Enzyme</strong></td>
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<tr>
<td><strong>Dogs</strong></td>
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<tr>
<td><strong>Horses</strong></td>
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<tr>
<td><strong>Cats</strong></td>
</tr>
<tr>
<td><strong>Enzyme Released from brush border</strong></td>
</tr>
<tr>
<td><strong>Dogs</strong></td>
</tr>
<tr>
<td><strong>Horses</strong></td>
</tr>
<tr>
<td><strong>Cats</strong></td>
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<tr>
<td><strong>Cauxin</strong></td>
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<tr>
<td><strong>Carboxylesterase</strong></td>
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<tr>
<td><strong>Dogs</strong></td>
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<tr>
<td><strong>Horses</strong></td>
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<tr>
<td><strong>Cats</strong></td>
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<tr>
<td><strong>Enzyme Released from brush border</strong></td>
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<tr>
<td><strong>Dogs</strong></td>
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<tr>
<td><strong>Horses</strong></td>
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<td><strong>Cats</strong></td>
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<tr>
<td><strong>GGT</strong></td>
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<tr>
<td><strong>Enzyme Released from brush border</strong></td>
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<tr>
<td><strong>Dogs</strong></td>
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<td><strong>Horses</strong></td>
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<td><strong>Cats</strong></td>
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<tr>
<td><strong>Enzyme Released from brush border</strong></td>
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<tr>
<td><strong>Dogs</strong></td>
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<td><strong>Horses</strong></td>
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<td><strong>Cats</strong></td>
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<tr>
<td><strong>NAG</strong></td>
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<tr>
<td><strong>Enzyme Released from brush border</strong></td>
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<tr>
<td><strong>Dogs</strong></td>
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<tr>
<td><strong>Cats</strong></td>
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<tr>
<td><strong>Enzyme Leakage</strong></td>
</tr>
<tr>
<td><strong>Dogs</strong></td>
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<tr>
<td><strong>Cats</strong></td>
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<tr>
<td><strong>Enzyme Released from brush border</strong></td>
</tr>
<tr>
<td><strong>Dogs</strong></td>
</tr>
<tr>
<td><strong>Cats</strong></td>
</tr>
</tbody>
</table>

AAP, alanine aminopeptidase; AKI, acute kidney injury; ALP, alkaline phosphatase; CKD, chronic kidney disease; GGT, gamma glutamyl-transpeptidase; KIM-1, kidney injury molecule-1; LMW, low molecular weight; NAG, N-acetyl-β-D-glucosaminidase; NGAL, neutrophil gelatinase-associated lipocalin; RBP, retinol-binding protein; RBP_FE, fractional excretion of retinol-binding protein; THP, Tamm–Horsfall protein; uAAP/c, urinary alanine aminopeptidase/urinary creatinine; uALP, urinary alkaline phosphatase concentration; uALP/c, urinary alkaline phosphatase/urinary creatinine; uClu, urinary clusterin concentration; uClu/c, urinary clusterin/urinary creatinine; uGGT, urinary gamma glutamyl-transpeptidase; uGGT/c, urinary gamma glutamyl-transpeptidase/urinary creatinine; uNGAL, urinary neutrophil gelatinase-associated lipocalin concentration; uNGAL/c, urinary neutrophil gelatinase-associated lipocalin/urinary creatinine; uRBP, urinary retinol-binding protein/urinary creatinine; uTHP, urinary Tamm–Horsfall protein concentration; uTHP/c, urinary Tamm–Horsfall protein/urinary creatinine

*Personal observations
CKD: More studies have evaluated uRBP for detection of tubular dysfunction in dogs and cats with CKD than with AKI, and dogs with CKD have significantly increased uRBP\textsuperscript{151}. uRBP/c\textsuperscript{105,110,117,123,124,144,150} and RBP\_FE\textsuperscript{124,144} compared with healthy dogs. However, whether this increase is primarily present due to tubular damage as opposed to presence of proteinuria is controversial, as discussed below.

In dogs with CKD due to XLHN, uRBP/c was increased prior to the onset of azotemia but after onset of proteinuria, and urinary RBP increased with disease progression throughout all disease stages (based on sCr), with the most pronounced increase in mid to late stages of renal disease.\textsuperscript{110,118,150} Furthermore, uRBP/c had the strongest correlation with both glomerular and TI lesions compared with other biomarkers of renal function, and it correlated most strongly with conventional measures of disease severity (sCr, GFR, and interstitial fibrosis).\textsuperscript{110} Despite this, uRBP/c was not a significant independent predictor of GFR based on multivariate analysis. It was concluded that uRBP/c might be useful for detecting early tubular damage before an obvious increase in sCr.\textsuperscript{110}

When biomarkers were correlated with histologically proven renal damage in dogs with naturally occurring CKD due to a variety of causes, uRBP/c and RBP\_FE were moderately correlated with glomerular and TI damage, with RBP\_FE having the second strongest correlation with TI damage following sCr.\textsuperscript{106} RBP\_FE also increased significantly with each increase in IRIS stage, while uRBP/c only increased significantly in IRIS stages 3 and 4. Both uRBP/c and RBP\_FE were significantly associated with time to death due to renal disease when evaluated individually; however, in a multivariate analysis with other biomarkers, neither uRBP/c nor RBP\_FE was significantly associated with survival.\textsuperscript{106}

Despite the promise of uRBP for early detection of CKD and monitoring of progression, another study concluded that proteinuria influenced uRBP/c more than decreased renal function based on sCr and plasma creatinine clearance. This conclusion was based on finding uRBP/c to be significantly greater in dogs with proteinuria and borderline proteinuria compared with azotemic, nonproteinuric dogs, and the inability of uRBP/c to detect reduced GFR.\textsuperscript{124} Dogs with hyperadrenocorticism had higher uRBP/c compared with control dogs; however, UPC was also significantly higher in these dogs compared to controls.\textsuperscript{116} Additionally, in dogs with ACTH-dependent hyperadrenocorticism, uRBP/c decreased significantly after treatment with hypophysectomy\textsuperscript{119}, such that posttreatment median uRBP/c values were within the range reported for healthy dogs. Urinary RBP/c also decreased after treatment with trilostane; however, the decrease was not significant, and several dogs had persistent proteinuria.\textsuperscript{119} These cumulative results suggest that ACTH-dependent hyperadrenocorticism results in decreased tubular protein reabsorption; however, the degree of reversibility and posttreatment values for uRBP/c depend on the type of treatment (hypophysectomy vs trilostane) and persistence of proteinuria.\textsuperscript{119} Finally, in aged dogs treated with hydrocortisone, uRBP/c increased with treatment and decreased posttreatment.\textsuperscript{111}

In cats, urinary RBP evaluation has focused on those with CKD, hyperthyroidism, or both. These studies have shown that uRBP/c is significantly increased in cats with either CKD\textsuperscript{134,145} or untreated hyperthyroidism\textsuperscript{145,149} compared with healthy cats. These findings suggest that hyperthyroidism causes tubular dysfunction in cats, and this tubular dysfunction may be at least partly reversible, as uRBP/c often decreased significantly (although was still detectable, unlike in healthy cats) in cats treated with radioiodine.\textsuperscript{148,149} However, because hyperthyroidism can mask CKD in cats, and CKD may be revealed after treatment for hyperthyroidism, increased uRBP/c in hyperthyroid cats could reflect CKD in some animals. Supporting this theory, uRBP/c decreased significantly in cats that did not develop azotemia post treatment for hyperthyroidism, but it remained elevated in cats that did develop azotemia posttreatment. Thus, in hyperthyroid cats, it was suggested that uRBP/c might be a marker of reversible tubular dysfunction in healthy kidneys, but also a marker of irreversible damage in cats with preexisting CKD.\textsuperscript{148}

Neutrophil gelatinase-associated lipocalin (NGAL)

Neutrophil gelatinase-associated lipocalin is a member of the family of lipocalin-binding proteins originally isolated from the specific granules of human neutrophils\textsuperscript{153,154}, but it is also present in many normal tissues including the kidney.\textsuperscript{155} Neutrophil gelatinase-associated lipocalin is upregulated in epithelial cells in neoplastic\textsuperscript{155,156} and inflammatory\textsuperscript{156} processes. The original proposed function of NGAL was as a bacteriostatic agent that binds bacterial siderophores to prevent iron acquisition.\textsuperscript{157} Neutrophil gelatinase-associated lipocalin has since been found to be involved in many cellular mechanisms including renoprotection\textsuperscript{158}, and NGAL’s many functions have been reviewed.\textsuperscript{159} Neutrophil gelatinase-associated lipocalin is a LMW protein that freely passes...
through the glomerular filtration barrier and is reabsorbed almost completely by the proximal tubules in the healthy kidney.\textsuperscript{158} With renal damage and protein overload, reabsorption of NGAL in the proximal tubules is impaired. In addition, there is increased synthesis of NGAL by damaged tubular epithelial cells.\textsuperscript{160} Three different molecular weight forms of NGAL are present in canine urine\textsuperscript{161}, including a 25 kDa monomeric protein that appears to originate from renal tubular epithelial cells and is associated with renal damage, a 45–50 kDa dimeric protein that is the predominant form released by neutrophils and appears most often with pyuria, and an NGAL/matrix metalloproteinase (MMP) 9 heterodimer complex that occurs with both renal injury and pyuria and hematuria.\textsuperscript{153,161,162} Although ELISAs that distinguish between different molecular forms of human NGAL are available, canine NGAL ELISAs are currently unable to make this discrimination.\textsuperscript{163}

\textbf{Measurement and stability.} In domestic species, NGAL has been evaluated in urine, serum, and plasma in dogs, and it has been partially validated using canine-specific ELISAs with acceptable intra-assay and inter-assay variabilities, dilutional linearity, and spiking recovery in canine urine and plasma.\textsuperscript{93,106,110,164} Neutrophil gelatinase-associated lipocalin has also been validated in canine serum, but results were not published.\textsuperscript{91} Neutrophil gelatinase-associated lipocalin appears to be relatively stable in canine urine, as there were minimal effects on uNGAL after 4 freeze–thaw cycles, and no significant differences in uNGAL/c were observed in samples collected up to 8 years apart, stored at −80°C.\textsuperscript{110} No difference in uNGAL/c was apparent between cystocentesis and voided samples\textsuperscript{165} or between 2-hour spot urine samples and 15-hour collection samples.\textsuperscript{166} The 3 different molecular weight forms of NGAL can be differentiated in canine urine samples using Western blot.\textsuperscript{161} Values in healthy dogs are reported in Table 3.

\textbf{Nonrenal influences.} In one study, healthy dogs < 4 months of age often had much higher uNGAL/c than dogs > 4 months of age, but it was suggested that this was likely due to preputial neutrophil contamination from voided urine samples from the youngest dogs.\textsuperscript{110} In another study, there was no correlation of uNGAL/c with age in adult dogs, although uNGAL (not normalized) was weakly but positively correlated with age. Furthermore, this same study found that both uNGAL and uNGAL/c decreased with body mass.\textsuperscript{164} Interestingly, peripheral WBC count does not correlate with overall serum NGAL (sNGAL)\textsuperscript{92}; however, dogs with monomeric uNGAL had significantly higher peripheral WBC and neutrophil counts than dogs without the uNGAL monomer.\textsuperscript{161}

Pyuria and urinary tract infections (UTI) can markedly influence urinary NGAL concentrations. Several studies showed that both uNGAL and uNGAL/c were significantly higher in dogs with pyuria, UTI, and other lower urinary tract diseases (such as transitional cell carcinoma and calcium oxalate urolithiasis), both with and without azotemia, compared with healthy controls.\textsuperscript{161,164,165} In the authors’ experience in dogs with proteinuric CKD, uNGAL/c was higher in dogs with active vs inactive sediments (J.A.H., M.B.N., unpublished data). Although dogs with lower urinary tract diseases had increased uNGAL/c compared with control dogs, values were still lower than dogs with renal disease.\textsuperscript{161,165} A uNGAL/c cutoff of > 2.57 μg/g and a uNGAL cutoff of > 3.38 ng/ml had sensitivities and specificities in the 70–80% range for dogs with vs without a UTI.\textsuperscript{164} Furthermore, the 50 kDa dimer form of NGAL was shown to be more common in urine from dogs with pyuria, while the NGAL/MMP-9 complex was found in dogs with pyuria and hematuria.\textsuperscript{161}

Other nonrenal diseases (gastritis, protein losing enteropathy, hepatic disease, enteritis, portal shunt, bone fracture, intervertebral disk disease) do not appear to affect uNGAL.\textsuperscript{161}

\textbf{Renal disease in veterinary medicine. AKI:} While studies of drug-induced AKI consistently demonstrate increases in urinary NGAL, mixed results are reported with regard to timing of this increase, which could at least partly be due to differing protocols, particularly with varying concentrations of gentamicin used. Two separate studies in dogs given gentamicin found that uNGAL/c increased early (as early as day 1 post administration) compared with other markers of nephrotoxicity (such as sCr and UN) and was correlated with the severity of tubular damage, including tubular cell necrosis, degeneration and regeneration, tubular cell hyaline droplet formation, and hyaline casts.\textsuperscript{166,167} It was concluded that uNGAL/c was a sensitive and predictive marker of gentamicin-induced nephrotoxicity.\textsuperscript{166} However, a third study found that although uNGAL/c was significantly correlated with GFR and increased at the first time point evaluated (4 days after gentamicin administration), the increase was not statistically significant until the second time point, at 8 days post administration. Thus, in this study, uNGAL/c was not superior to more traditional markers
of renal function. In AKI induced by administration of intravenous polymyxin B for 7 days, a dose-dependent increase in uNGAL/c was observed, with significantly increased uNGAL/c in the mid- and high-dose groups on day 2. Finally, Beagle dogs administered methyl cantharidimide tablets over 30 days demonstrated significant increases in uNGAL in the high-dose group, while middle- and low-dose groups did not have significant changes in uNGAL.

Repeatedly, studies have shown promise for NGAL as a marker of naturally occurring AKI in dogs. Urinary NGAL, uNGAL/c, and plasma NGAL (pNGAL) were all significantly greater in dogs with azotemia from natural causes (whether due to AKI or CKD) vs healthy control dogs, and extremely high pNGAL differentiated AKI from CKD in one study while extremely elevated uNGAL/c was seen in dogs with AKI compared to both CKD and lower urinary tract diseases in another study. Furthermore, uNGAL/c was increased in nonazotemic (IRIS Grade I) AKI, indicating the presence of kidney injury before sCr increased outside of the reference interval. Similarly, in dogs that developed AKI postoperatively, median uNGAL was significantly greater 12 hours post surgery and remained elevated for at least 3 days, while sCr did not show a significant increase until 24 hours post surgery. Urinary NGAL and uNGAL/c were also shown to be significantly increased (average of 24-fold and 41-fold, respectively) from baseline within 2 hours of reperfusion using a hemorrhage and colloid fluid resuscitation model of renal injury in Greyhounds. Thus far, uNGAL/c, uNGAL, and sNGAL have not been shown to be predictors of survival in dogs with AKI.

**CKD:** Dogs with CKD have demonstrated increased uNGAL/c, uNGAL, sNGAL, and pNGAL compared with healthy dogs and dogs with UTI or other lower urinary tract diseases. Similar to findings in AKI studies, increases in uNGAL/c occurred early in the development of CKD in dogs with XLIH. Serum NGAL and fractional excretion of NGAL (NGAL_FE) also increased with increasing IRIS stages in dogs with proteinuric CKD. Furthermore, uNGAL/c and NGAL_FE correlated moderately to strongly with both glomerular and TI lesions in dogs with CKD. Finally, higher sNGAL and NGAL_FE are associated with shorter survival compared with lower values in dogs with CKD. Serum NGAL concentration was actually concluded in one study to be a better predictor of clinical outcomes than sCr for dogs with CKD that were already azotemic; however, this was not repeatable in a second study of dogs with CKD that were predominantly proteinuric but included both azotemic and nonazotemic dogs.

**Additional urinary proteins indicating tubular damage/dysfunction**

Although typically much more limited in their evaluation, numerous other urinary proteins have been studied as tubular biomarkers in domestic animals. Several of these are presented briefly below, while others with minimal references are listed in Table 3.

**Tamm–Horsfall Protein.** Tamm–Horsfall protein (THP, otherwise called uromodulin) is a 100-kDa protein present in the thick ascending limb of the loop of Henle and the distal convoluted tubule. Tamm–Horsfall protein is one of the major urinary proteins present in healthy dogs. The biologic function of the protein is still not fully understood, but it is believed to have roles in water and electrolyte balance in the thick ascending limb of Henle’s loop, defense against UTI, prevention against the formation of kidney stones, and in innate immunity of the kidney. Normal urine has high concentrations of THP, and significantly reduced urinary THP concentrations and uTHP/c are seen in dogs and cats with renal disease, including CKD. Furthermore, uTHP/c correlates negatively with plasma creatinine concentration and UPC. Therefore, reduced urinary THP might be a marker of distal tubular damage in dogs and cats.

**Cystatin C.** Cystatin C is a LMW protein (13 kDa), and its presence in urine is a marker of proximal tubular damage. A recent review includes studies of urinary cystatin C; therefore, only key information is provided in Table 3.

Other urinary protein markers of tubular damage and dysfunction not listed in Table 3 that have been shown to increase in AKI include calbindin-D28K, CD1d, and heat shock protein 1B in sheep, and interleukins 2, 7, and 8, monocyte chemoattractant protein-1, granulocyte macrophage colony-stimulating factor, and keratinocyte-derived chemokine in dogs. Urinary proteins that were increased in dogs with CKD include vitamin D-binding protein, α1-microglobulin, β2-microglobulin, apolipoprotein A1, megalin, and cubulin. In cats with CKD, urinary cystatin M, transforming growth factor-β, and interleukin-8 were increased while
vascular endothelial growth factor was decreased.\textsuperscript{179}

**Urinary enzymes**

Renal tubular epithelial cells contain enzymes which have been explored as biomarkers of tubular damage (Table 1). The most commonly studied enzymes in domestic animals include N-acetyl-\(\beta\)-D-glucosaminidase (NAG), a lysosomal enzyme\textsuperscript{180}, and gamma-glutamyl transpeptidase (GGT) and alkaline phosphatase (ALP), which are brush border enzymes.\textsuperscript{181} Cauxin has also been studied in cats, and a brief discussion of this marker will be included at the end of the enzyme section. Two isoenzymes of NAG occur in the kidney: NAG-A and NAG-B. In people, only NAG-A can be detected in urine from patients without renal disease, whereas renal damage and disease results primarily in increased excretion of NAG-B.\textsuperscript{182–184} Of note, both NAG-A and B are detectable in healthy cats.\textsuperscript{185} Although present in the serum and other tissues and cells, urinary NAG, GGT, and ALP originate from the renal tubules in the absence of glomerular damage.\textsuperscript{186–189}

**Measurement and stability.** Activity of urinary enzymes is expressed as units per liter, and an activity index is typically calculated by normalizing to urinary creatinine concentration. Validation of assays for urinary NAG activity (uNAG) has been performed in dogs and cats; however, to the authors’ knowledge, validation of assays for urinary GGT (uGGT) and ALP (uALP) activity has not been performed in domestic animals.\textsuperscript{110,190,191} In canine urine, overall intra-assay and inter-assay variabilities, dilutional linearity, and spiking recovery for NAG were acceptable.\textsuperscript{110,123,191} However, canine and feline urine samples with lower concentrations of NAG tended to show higher coefficients of variation.\textsuperscript{110,190} NAG activity is relatively stable in urine at -80°C for at least one year; however enzyme degradation, which is not prevented by addition of a protease inhibitor, is still possible.\textsuperscript{110,146,190,191} Studies assessing stability at room temperature, 4°C, and -20°C have shown contrasting findings, although NAG appears to be stable for at least one month at -20°C in both feline and canine urine.\textsuperscript{110,146,190,191} Up to 4–5 freeze–thaw cycles have been shown to significantly affect urinary NAG activity in dogs and cats.\textsuperscript{110,190} Thus, it is recommended to keep urine samples frozen at -80°C and to limit the number of freeze–thaw cycles. No significant difference in uNAG activity was seen between voided and cystocentesis samples from dogs.\textsuperscript{146} While uGGT activity was relatively stable at 4°C for at least 4 days in dogs\textsuperscript{192}, in equine urine, uGGT was not stable after storage at -20°C, 4°C, and 25°C by 72 hours, with the greatest decrease in enzyme activity occurring after freezing (-20°C).\textsuperscript{193}

**Values in healthy animals.** Urinary NAG, GGT, and ALP activities are generally present at low levels in healthy domestic animals (Table 3). In the urine of healthy cats, the NAG-A isoenzyme activity was 2–3 times higher than NAG-B.\textsuperscript{185}

**Nonrenal Influences.** Age does not appear to affect uNAG/c in dogs.\textsuperscript{146,194} However, 3 of 4 studies found uNAG/c was significantly higher in males than females.\textsuperscript{191,195–197} Additionally, uNAG/c decreased after castration of male dogs; therefore, the increase in NAG activity in males may be due to the enzyme content of sperm.\textsuperscript{191,196} In cats, no significant difference in uNAG/c or NAG isoenzymes was found between sexes.\textsuperscript{185,190}

Spot measurements of uGGT/c and uNAG/c were significantly correlated with 24-hours urine GGT and uNAG excretion, respectively.\textsuperscript{192,198} However, large intra- and inter-individual variability was noted for uNAG/c.\textsuperscript{191} In cats, there was no apparent circadian variation in NAG or GGT excretion, and while short (4 hours) urine collection periods estimated 24-hour uNAG/c and uGGT/c, there was greater variability in enzyme indices over the shorter collection time period.\textsuperscript{199}

In both dogs and cats, hematuria, pyuria, and bacteruria/UTI without concomitant pyelonephritis does not appear to affect uNAG or uNAG/c.\textsuperscript{146,185,197} However, dogs with lower UTI accompanied by pyelonephritis had markedly increased uNAG/c values, likely indicating the presence of tubular damage.\textsuperscript{197} Further studies are needed to determine if uNAG/c can accurately detect pyelonephritis. While changes in urine pH did not seem to affect uNAG/c in dogs\textsuperscript{195}, in cats, NAG activity was decreased with alkaline urine pH\textsuperscript{199} or demonstrated a weak negative association with urine pH.\textsuperscript{190} Furthermore, induction of muscular inflammation with injection of Freund’s complete adjuvant resulted in decreased uGGT/c in horses, and it was suggested that the presence of inflammation may affect the reliability of uGGT/c as an index of renal damage.\textsuperscript{200} One study reported that uGGT/c was increased after the administration of a nonnephrotoxic agent in dogs and concluded that this reflected false positive results.\textsuperscript{201} However, mild tubular damage might still have been missed on ultrastructural examination given the tendency for such lesions to be focally distributed.
Renal disease in veterinary medicine. AKI: Urinary enzymes, particularly NAG and GGT, have been most extensively studied as early markers of AKI by chemical (drug) induction in domestic species. Gentamicin, especially high doses, significantly increased uNAG/c and uGGT/c in dogs, and uNAG/c increased as early as 24 hours post administration, and continued to increase throughout the study period.\textsuperscript{126,167,198,202} Even therapeutic doses of gentamicin in dogs resulted in a 2-fold increase in uGGT/c, although without statistically significant changes in sCr, UPC, or USG.\textsuperscript{203} Similarly, horses treated with a therapeutic dose of gentamicin had significantly increased uGGT/c without increased sCr during the treatment period.\textsuperscript{204} Another study found that uNAG/c correlated with the severity of renal lesions and had high accuracy and sensitivity for detection of renal injury compared with UN.\textsuperscript{167} These and other studies support that urinary enzymes are more sensitive and reliable for detecting acute renal tubular damage induced by gentamicin than markers of GFR.\textsuperscript{202}

Other nephrotoxic drugs evaluated in the context of tubular enzymes include polymyxin B (dogs)\textsuperscript{168}, methyl cantharidimide (dogs)\textsuperscript{125}, sulfonamide (a cat)\textsuperscript{185}, neomycin (horses)\textsuperscript{205}, ketoprofen (sheep)\textsuperscript{206}, and mercuric chloride and potassium dichromate (ponies)\textsuperscript{207}, all of which resulted in early increases in uNAG/c, uGGT/c, or both. One study reported early increases in uNAG/c and uGGT/c in dogs administered a therapeutic dose of ketoprofen long-term, but noted that these enzyme indices returned to clinically normal values in both dogs despite continued drug administration.\textsuperscript{208} Overall, administration of nonsteroidal anti-inflammatory drugs (NSAID) at a therapeutic dose did not appear to significantly increase uGGT/c, uNAG/c, or uALP/c in dogs.\textsuperscript{208-212} In horses treated with neomycin, no histologic evidence of renal damage was observed despite increases in uGGT/c.\textsuperscript{205} However, the study does not indicate what types of histologic studies were performed, and therefore, it is possible that renal lesions may have been missed. Additionally, dogs given intravenous polymyxin B for 7 days demonstrated dose-dependent increases in uNAG/c, with significantly increased uNAG/c occurring in the mid- and high-dose groups on day 2.\textsuperscript{168} In one cat given sulfonamide to induce acute renal failure, uNAG/c increased within 24 hours and continued to increase to very high values several days after administration. N-acetyl-β-D-glucosaminidase-A and NAG-B also both increased, with a greater increase in NAG-B.\textsuperscript{185}

Few studies are available evaluating urinary enzyme activity in cases of naturally occurring AKI in domestic animals. Dogs with pyometra have demonstrated increased uNAG/c, uGGT, uGGT/c, uALP, and uALP/c that, in several studies, decreased back into the range of healthy dogs after ovariohysterectomy, supporting transient AKI in these dogs.\textsuperscript{114,115,194,197,213,214} Furthermore, markedly increased uNAG/c was associated with severe TI lesions and reduced GFR in dogs with pyometra.\textsuperscript{115,194} In client-owned dogs with renal disease, uALP/c was a significant indicator of AKI compared to dogs with CKD or healthy dogs (all confirmed histologically), while uGGT/c was not shown to be as useful for distinction.\textsuperscript{215} Significant correlations were not detected between urinary enzyme levels and extent of morphological kidney damage.\textsuperscript{215} Transient increases in uGGT and/or uGGT/c were seen with anesthesia and routine surgery in healthy dogs\textsuperscript{216} and diarrheic calves\textsuperscript{217}, and uGGT/c and uALP/c were increased in dogs with European Adder envenomation.\textsuperscript{147}

The studies in both drug-induced and naturally occurring AKI support that tubular enzymes can be sensitive markers of tubular damage. However, inconsistent results are present regarding the magnitude of enzymuria, whether histologic changes are observed, and whether or not azotemia develops following the insult. Therefore, the usefulness of these markers in predicting clinically significant tubular damage is unknown.

CKD: Of the tubular enzymes, only NAG has been evaluated in dogs and cats with CKD. Urinary NAG/c appears to be increased in most dogs and cats with CKD as compared to healthy controls.\textsuperscript{110,123,197} In dogs with XLHN, mild increases were observed as one of the earliest findings, even before increased UPC; however, it did not continue to increase with disease progression beyond mid-stage disease.\textsuperscript{110} Furthermore, uNAG/c was inconsistently associated with IRIS staging in dogs with proteinuric CKD.\textsuperscript{106,123} Interestingly, in dogs with XLHN, uNAG/c correlated moderately to strongly with both glomerular and TI damage lesions.\textsuperscript{110} In contrast, uNAG/c at the time of biopsy in dogs with naturally occurring CKD (mostly proteinuric and variably azotemic) correlated moderately well with glomerular damage alone and did not correlate with TI damage.\textsuperscript{106} Additionally, uNAG/c correlated moderately to strongly with UPC and uGluG/c in both studies.\textsuperscript{106,110} These findings support that uNAG/c might be a better indicator of glomerular rather than tubular damage in canine proteinuric CKD.

Several studies in cats similarly suggest that uNAG/c trends better with proteinuria than with tubular damage. One study found that when geriatric cats
were grouped according to plasma creatinine concentration, there was no significant difference in uNAG/c between groups, and uNAG/c was not correlated with creatinine. However, when cats were grouped based on proteinuria, cats with borderline proteinuria and overt proteinuria had significantly higher uNAG/c than nonproteinuric cats, and uNAG/c was moderately correlated with UPC.\(^{190}\) uNAG/c was also unable to distinguish azotemic from nonazotemic euthyroid cats.\(^{218}\) Furthermore, uNAG/c was not significantly associated with development of azotemia when evaluated in a multivariate model, as uNAG/c did not provide any benefit over UPC for predicting development of azotemia.\(^{219}\) In contrast, one study found that, although uNAG/c increased in cats with CKD compared to healthy cats, there was no correlation between uNAG/c and severity of proteinuria, although this was based on unindexed, semi-quantitative measurement of proteinuria using a dipstick.\(^{185}\)

Endocrine diseases may also influence urinary NAG activity, presumably due to renal damage. Dogs with hyperadrenocorticism had significantly higher uNAG/c compared with control dogs.\(^{116}\) Furthermore, while several other biomarkers (uALB/c, uIgG/c, uRBP/c, and UPC) demonstrated significant decreases after treatment for hyperadrenocorticism, uNAG/c did not decrease significantly even after treatment with trilostane and hypophysectomy.\(^{119}\) However, several dogs that were treated for hyperadrenocorticism had persistent proteinuria.\(^{119}\) Despite the increases in uNAG/c in dogs with naturally increased cortisol due to hyperadrenocorticism, the same effect was not seen in dogs treated with hydrocortisone, both in comparison to the control group and within the treatment group itself.\(^{111}\) In dogs with diabetes mellitus, increased uNAG/c was observed when the disease was not controlled (ie, hyperglycemia, glucosuria, and ketonuria were present), whereas uNAG/c values were comparable to healthy dogs when blood glucose was controlled.\(^{197}\) Finally, azotemic and nonazotemic cats with hyperthyroidism had higher uNAG/c than healthy cats, and uNAG/c decreased with treatment in both groups.\(^{218}\) In this study, urine microalbumin concentration in hyperthyroid cats was statistically similar among healthy, azotemic, and nonazotemic cats.\(^{218}\)

The majority of studies show that urinary NAG largely trends with proteinuria. For both dogs and cats with CKD, a possible reason behind the stronger correlation of urinary NAG with glomerular damage/proteinuria as compared with TI damage/azotemia could be increased lysosomal activity as opposed to active proximal tubular cell damage. However, when glomerular proteinuria is present, it is reasonable that increased uNAG/c at least partially represents loss of NAG from the blood due to altered glomerular permeability.

**Cauxin.** Cauxin, a 70 kDa enzyme\(^{220}\), is constitutively secreted from the proximal straight\(^{221}\) and distal\(^{220}\) tubular epithelial cells in the urine of domestic (particularly intact males)\(^{220,221}\) and big\(^{222}\) cats. Progressive tubular damage due to CKD caused decreased tissue expression\(^{223}\) and low urine excretion of cauxin\(^{134}\) in cats, suggesting a decreased number of functional tubular epithelial cells. However, when normalized to urinary creatinine concentration, decreased cauxin was not observed in cats with CKD, and in fact, increased urinary cauxin/creatinine values were predictive of development of azotemia in geriatric cats.\(^{224}\)

Other urinary enzymes noted to be increased in AKI but not included in Table 3 include: glutathione-S-transferase\(^{176}\), lactate dehydrogenase\(^{206}\), acid phosphatase\(^{206}\), MMP/pro-MMP\(^{220}\) in sheep, and MMP/pro-MMP 9 in horses.\(^{225}\)

**Conclusions**

The number of widely available tests of kidney function and damage is currently limited, and our interpretation of these tests, particularly creatinine, needs improvement. However, there has recently been significant progress in the evaluation of many new biomarkers in dogs and cats with kidney disease. Most of these biomarkers require further investigation as well as test availability using a reliable, high-throughput, and commercially available platform that has been validated in the sample of interest before their widespread use can be recommended and achieved. Still, studies to date show promise that several of these markers can and will aid in early detection, monitoring, and assessment of prognosis in patients with kidney disease. They can also help localize damage to the kidney.

The evidence for the role of urinary biomarkers in the early detection of tubular damage in AKI is particularly compelling, whereas in animals with proteinuric kidney disease, the influence of filtered proteins (originating from the blood) must be considered. There is also the potential for nonrenal influences, such as hematuria and pyuria, to alter biomarker concentrations. For plasma-derived proteins serving as indicators of tubular reabsorptive function (eg, RBP, cystatin C)
these limitations are not likely to be overcome. However, for indicators of tubular damage, we should strive to use biomarkers that are either exclusively produced by the renal tubules or have renal tubular-specific forms that can be distinguished from circulating forms of the protein. Without such specificity, early, noninvasive detection of tubular damage will remain challenging. Finally, no single biomarker is likely to be sufficient for even one component of altered kidney function or damage, and a panel of tests will be needed in the future for comprehensive evaluation to best determine renal status in an individual animal.

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