Clinical Approach to Advanced Renal Function Testing in Dogs and Cats

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KEYWORDS

• Biomarkers • Fractional excretion • Glomerular filtration rate
• γ-Glutamyl transpeptidase • Microalbuminuria • Urine

KEY POINTS

- Advanced renal function tests may allow earlier detection of reduced renal functional mass and localization of damage to a particular nephron segment, and are required for diagnosis or exclusion of some causes of kidney injury.
- Measurement of glomerular filtration rate (GFR) allows for precise quantitative assessment of remaining filtration and excretion ability by the kidneys.
- Spot samples of simultaneously collected urine and plasma provide clinically reasonable approximations of total daily urine electrolyte excretion.
- The majority of plasma albumin is size and charge excluded from the ultrafiltrate; glomerular damage results in increased filtration of albumin and excretion into the urine. Microalbuminuria may be detected prior to positive reactions on standard urine protein dipstick pads, and before the urine protein:creatinine (UPC) ratio increases above reference range.
- Urinary *N*-acetyl-β-D-glucosaminidase (NAG):creatinine ratio is increased in dogs with chronic kidney disease, pyelonephritis, uncontrolled diabetes mellitus, pyometra, or X-linked hereditary nephropathy but does not differ before versus after control of hyperadrenocorticism with trilostane or transphenoidal hypophysectomy.

Serum biochemical analysis and urinalysis are the mainstay diagnostic tests for initial detection and estimation of severity of kidney disease in dogs and cats. Increased serum creatinine concentration and impaired urine concentrating ability, however, are relatively insensitive for detecting early kidney injury and do not assist in differentiation between glomerular versus proximal or distal tubular damage. Advanced renal

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function tests, including GFR testing, determining fractional excretion (FE) of electrolytes, and assay of urine biomarkers, may allow earlier detection of reduced renal functional mass and differentiation of various renal and nonrenal differential diagnoses and assist with localization of damage. This article reviews the principles, indications, and limitations of these tests and describes their use in sample clinical scenarios.

GLOMERULAR FILTRATION RATE

Serum creatinine concentration is insensitive for detecting kidney injury. Increases in serum creatinine concentration are mild and often remain within reference range, until approximately 60% to 75% of all nephrons are no longer functional. In contrast, measurement of GFR allows for precise quantitative assessment of remaining filtration and excretion ability by the kidneys. Example situations when GFR measurement may provide critical information regarding remaining kidney function beyond serum creatinine concentration alone include diagnostic evaluation of dogs and cats with unexplained polyuria and polydipsia, to avoid overdosing of medications that are excreted by the kidneys or that have potential nephrotoxic effects, and to predict risk of overt renal failure after nephrectomy in dogs or cats with unilateral kidney disease, such as tumors or pyonephrosis.

Several methods for determination of GFR have been validated in dogs and cats, all of which report the volume of plasma, which has been cleared over a given interval of time, per kilogram of patient body weight. After injecting a substance (the marker) that is eliminated solely via filtration through the glomeruli and which then passes into the urine without being reabsorbed or further secreted by the tubules, the rate at which the concentration of marker decreases in successive blood samples allows calculation of the plasma clearance and GFR.[1] Assays that measure the rate of marker appearance in urine are more accurate than those that assay marker disappearance in plasma (because few markers are solely excreted via glomerular filtration without any tubular reuptake or secretion); however, urine assays that allow calculation of renal clearance (vs plasma clearance) are more cumbersome to perform because they require collection of all urine produced in a 24-hour period. Fortunately, plasma clearance assays using blood sampling techniques are sufficiently close to renal clearance, such that in the clinical setting urine collection is not required.[1–4]

Several markers have been validated for measurement of GFR in dogs and cats, including creatinine, cystatin C (CysC), iohexol, and radiolabeled molecules. In people, GFR is most commonly estimated (rather than measured) using serum creatinine concentration, body weight, and correction factors based on a patient's gender and race. Unfortunately, formulae for estimating GFR from serum creatinine have not proved accurate in dogs and cats due to greater individual, gender, and breed variation than occurs in people.[5,6] Intravenous administration of a sterile creatinine bolus is safe and cost effective; however, comparison of various markers suggests that exogenous creatinine GFR assays underestimate true GFR, likely due to some excretion into the gastrointestinal tract and perhaps tubular reuptake.[2,7,8] CysC is an endogenous protein produced by all nucleated cells at a constant rate that undergoes glomerular filtration without tubular secretion; however, commercial assays are limited, and comparative studies in dogs have suggested lower specificity for detection of reduced kidney function than exogenous creatinine GFR.[9–11]

Iohexol GFR measurement uses a marker that can be easily obtained by veterinarians and has been well validated for use in dogs and cats, and a commercial assay is available at a reasonable cost to owners. After intravenous bolus injection of the same iodinated contrast agent used in diagnostic imaging studies, plasma samples are

collected at predetermined times (usually 2, 3, and 4 hours after injection); the volume of injection is based on concentration of elemental iodine within the iohexol.[12] Iodinated compounds are stable for long periods, and plasma samples can be frozen for extended periods of time and assayed later if indicated.[13] Intravenous iohexol can induce acute kidney injury and renal failure in people, particularly in patients with preexisting kidney damage; however, this idiosyncratic drug reaction is rare in dogs and cats.[14,15] Iohexol GFR assays have been safely used to study renal function in healthy dogs and cats,[16–21] in dogs with gentamicin-induced acute kidney injury,[22] in dogs and cats administered various nonsteroidal anti-inflammatory drugs (NSAIDs) after anesthesia,[20,23] and in cats with untreated or post-treated naturally occurring hyperthyroidism.[24–26] The most commonly used commercial assay for iohexol concentrations is offered by the Michigan State University Diagnostic Center for Population and Animal Health (http://www.animalhealth.msu.edu/); this diagnostic laboratory reports the calculated GFR after assaying serial plasma iohexol concentrations.

Radiolabeled markers validated for measurement of GFR currently used in clinical patients include chromium-51 ethylenediaminetetraacetic acid (EDTA) and technetium-99m diethylenetriamine pentaacetic acid (DTPA). These radionucleotides undergo glomerular filtration without tubular reabsorption or excretion and are stable in dog and cat blood samples but have short half-lives in vivo; this permits storage and shipping of samples to outside laboratories for assay of plasma clearance while patients are cleared of radioactivity and able to be released to owners within 24 to 48 hours.[27] Use of radiolabeled markers is limited, however, to specialty practices that are appropriately licensed to perform nuclear medicine–based testing. Radionucleotide GFR assays have been safely used to study renal function in anesthetized dogs,[28] in cats with solid tumors administered nephrotoxic chemotherapeutic agents,[29] and in dogs and cats with naturally occurring (cats with polycystic kidney disease[30] or azotemic chronic kidney disease[31]) or induced (cats with rejection of transplanted kidneys[32] or dogs that had previously undergone renal biopsy[33]) kidney disease (**Box 1**).

Although serial measurement of plasma iohexol or radionucleotides can be used to determine total, or global, GFR, these assays cannot determine the relative contributions of the right versus left kidney (ie, per-kidney GFR) to total renal excretion. Clinical use of per-kidney GFR is most commonly recommended in dogs or cats requiring unilateral nephrectomy (for example, due to presence of a renal tumor) but which have confirmed or suspected bilateral renal dysfunction. In these animals, determining both global and per-kidney GFR allows clinicians to predict the whether removal of the right or left kidney will result in renal failure and worsened quality of life.

Global and per-kidney GFR measurement can be determined using either iohexol or radionucleotide markers, when performed in tandem with advanced diagnostic imaging studies. CT of the abdomen in conjunction with iohexol allows per-kidney uptake of marker to be compared.[17] The ratio of uptake in the right versus left kidneys can then be used to calculate the per-kidney GFR. Gamma camera imaging of the abdomen after bolus administration of radionucleotide allows a similar comparison of marker uptake by each kidney over time and estimation of per-kidney GFR (**Box 2**).[8,34,35]

URINARY FRACTIONAL EXCRETION OF ELECTROLYTES

The kidneys are the primary organs responsible for excretion of electrolytes at times of excess and conservation at times of deficiency. Nonprotein-bound electrolytes are freely filtered through the glomeruli and then reabsorbed by electrolyte-specific exchange receptors throughout the proximal convoluted tubule, loop of Henle, and distal convoluted tubule. The rate of electrolyte reabsorption depends on multiple factors,

Box 1
Clinical scenarios: measurement of glomerular filtration rate

Case 1

An 8-year-old spayed female Shetland sheepdog was evaluated for polyuria and polydipsia of 3 months' duration. The owners reported that the dog had been urinating large volumes of clear urine every 2 to 3 hours and emptying the water bowl multiple times per day; however, the urine stream appeared normal without any associated straining, and the dog was otherwise acting normal with unchanged appetite and activity level. Physical examination of the dog was unremarkable, but the bladder was distended with a large volume of urine. All values on complete blood cell count and serum biochemistry panel were within reference range, although serum creatinine concentration were at the upper end of the reference range (1.2 mg/dL; reference range, 0.3–1.4 mg/dL). Urine specific gravity was 1.009 on 2 different occasions and the remainder of the urinalysis unremarkable. Abdominal radiography revealed that the kidneys were bilaterally smaller than expected and slightly misshapen.

Differential diagnoses for a dog with polyuria and polydipsia and unremarkable physical examination and minimum database may include atypical hypoadrenocorticism, central or primary nephrogenic diabetes insipidus, hyperadrenocorticism infections with lipopolysaccharide-producing bacteria, liver dysfunction, psychogenic polydipsia, and renal insufficiency. Loss of urine concentrating ability occurs in dogs with approximately 66% loss of total nephron function. Azotemia may not be noted, however, until 75% of nephrons are lost; during the intervening period (ie, damage to approximately 66%–75% of total nephrons) dogs may be polyuric and polydipsic due to kidney injury, but serum creatinine and blood urea nitrogen concentrations remain within reference ranges. This scenario, termed *renal insufficiency,* rather than *renal failure*, is of particular concern in this dog given the repeatable isosthenuria and appearance of the kidneys on abdominal radiographs.

Plasma iohexol clearance determination of GFR was performed in this dog using 1 mL/kg of iohexol containing 300 mg/mL elemental iodine/mL (Omnipaque 300 (iohexol) injection, GE Healthcare, Princeton, New Jersey); blood samples were collected 2, 3, and 4 hours after administration. Calculated GFR result was 0.9 mL/min/kg (reference range, 2.89–8.07 mL/min/kg), supporting a presumptive diagnosis of renal insufficiency. Ultrasonographic examination of the abdomen was recommended to better characterize the dog's kidney disease; bilateral dilatation of the renal pelves was noted with echogenic debris. Aerobic bacterial culture of urine resulted in greater than 100,000 colony-forming units/mL of *Staphylococcus aureus* and a 6-week course of amoxicillin-clavulanic acid was prescribed for a presumptive diagnosis of bilateral pyelonephritis. The owners reported that after 2-weeks of therapy, the dog's polyuria and polydipsia had resolved, and 2 weeks after the end of antibiotic therapy, repeat aerobic culture of urine did not result in any growth, and repeat GFR measurement revealed plasma iohexol clearance had increased to 3.27 mL/min/kg.

Case 2

An 11-year-old castrated male Labrador retriever was evaluated for a gradual decrease in activity level over the previous year and left hind limb lameness after playing with the other dogs in the household. Orthopedic examination and radiographs of both hind limbs were consistent with moderate osteoarthritis of both stifles and hocks. Two years before the current evaluation, this dog had been diagnosed with leptospirosis-induced acute renal failure (serum creatinine concentration 5.7 mg/dL); aggressive treatment with intravenous fluids and antibiotics were successful in resolving the azotemia (current serum creatinine concentration is 1.3 mg/dL).

NSAIDs are the mainstay treatment of osteoarthritis in dogs but may induce or exacerbate kidney injury. Unfortunately, these owners declined treatment with alternative analgesics due to cost concerns. Although serum creatinine concentration is within the laboratory reference range, there is a concern that sufficient residual chronic kidney injury may be present from the previous leptospirosis infection, such that there is an increased likelihood of drug nephrotoxicity but not enough to currently result in azotemia. Samples were submitted for determination of plasma iohexol clearance, and calculated GFR was 1.4 mL/min/kg, or approximately 50% of the lower end of the laboratory reference range (2.89–8.07 mL/min/kg). The prescribed NSAID was, therefore, reduced by 50% of the recommended milligram/kilogram dosage; 2 years later, the owners reported that the dog's lameness and activity level were much improved, and serum creatinine concentration remained within reference range.

Box 2
Clinical scenario: measurement of global and per-kidney glomerular filtration rate

An 11-year-old, spayed female domestic shorthaired cat was evaluated for gross hematuria of 2 weeks' duration. On physical examination, a midabdominal mass was palpated in the region of the left kidney, and the right kidney was slightly smaller than expected. Ultrasonographic examination of the abdomen revealed a large mass completely effacing the normal left kidney parenchyma, and several hyperechoic, wedge-shaped lesions in the right kidney extending from the renal medulla up to the cortical surface, consistent with chronic infarction. Serum creatinine concentration was 1.9 mg/dL (reference range, 0.2–1.6 mg/dL).

Based on the serum creatinine concentration and ultrasonographic abnormalities, this cat likely had International Renal Interest Society stage 2 chronic kidney disease. Unilateral nephrectomy was recommended after further diagnostic evaluation failed to reveal any metastases; however, there was concern that further reduction in functional nephrons after nephrectomy would result in worsening of azotemia, uremia, and unacceptable quality of life. Global and per-kidney GFR were, therefore, measured via technetium-99m DTPA and gamma camera imaging to determine both the current total renal function and the percent contribution of the mass-containing kidney.

Global GFR in this cat was 0.6 mL/min/kg (reference range, 1.15–2.73 mL/min/kg). The ratio of nucleotide uptake in the left, mass-containing kidney versus the right kidney was 1:9 (ie, 10% of total GFR vs 90% of total GFR). Based on the low contribution of the left kidney to total renal function, nephrectomy was performed. One month after surgery, the cat's serum creatinine was 2.7 mg/dL, and the owners report no abnormal clinical signs.

including dietary concentrations, renal function, various hormones (including parathyroid hormone and aldosterone), rate of ultrafiltrate flow through the nephron, and need to conserve or excrete water based on intravascular volume status.[36] Calculating the percent excretion of an electrolyte in relation to that electrolyte's serum concentration and correcting for filtration rate based excretion of creatinine is FE.

Although urine collection over a 24-hour period is most accurate for determining FE of electrolytes, spot samples of simultaneously collected urine and plasma provide clinically reasonable approximations of total daily excretion despite some variability.[37] The formula for calculating FE of a given electrolyte, *E*, is

$$\%FE = \frac{(\text{Urine concentration of E}) \times (\text{Plasma concentration of creatinine})}{(\text{Urine concentration of creatinine}) \times (\text{Plasma concentration of E})} \times 100$$

Because of the many factors that may influence FE, there are no definable reference ranges for dogs or cats with plasma electrolyte concentrations within reference range; however, as a general rule, FE of sodium should be low (<1%) whereas FE of potassium is high (up to 25%).[38,39]

Clinical use of electrolyte FE is limited due to the variety of influencing endogenous and exogenous factors. In select cases of increased or decreased serum electrolyte concentrations, however, FE may allow clinicians to prioritize differential diagnoses. Most laboratories that perform serum biochemical analyses are able to assay urine concentrations of creatinine and electrolytes. Dogs and cats in which FE of 1 or more electrolytes is considered should be fed a consistent diet before testing (the author suggests at least 1 week before submission of samples) to minimize food-associated fluctuations in urine electrolyte excretion. Dehydration should also be corrected and normal hydration should persist for several days, because healthy kidneys reabsorb excess sodium in an effort to restore water balance (under the influence of aldosterone). Interpretation of results requires forehand consideration of (1) serum electrolyte concentrations: animals with a given electrolyte concentration greater

than reference range should have greater-than-expected FE of that electrolyte and vice versa; (2) possible endocrine diseases: several endocrine diseases alter renal excretion and reabsorption of electrolytes—for example, excess parathyroid hormone (ie, in animals with primary hyperparathyroidism) promotes calcium reabsorption from the urine and phosphorus excretion, whereas insufficient aldosterone (ie, in animals with hypoadrenocorticism) results in decreased sodium reabsorption and excessive potassium reuptake in the distal convoluted tubule; and (3) renal function: in most dogs and cats with either acute or chronic kidney failure, serum electrolyte concentrations remain within reference range despite widespread nephron injury; this likely occurs because remaining nephrons and undamaged segments of diseased nephrons are able to compensate for the increased per-nephron excretion or reabsorption requirements—a potassium-wasting nephropathy, however, has been historically reported in cats (although the prevalence of this disease is now low for unknown reasons), renal secondary hyperparathyroidism unpredictably alters FE of calcium and phosphorus, and animals with severe polyuria may be unable to appropriately reabsorb electrolytes due to high rate of ultrafiltrate flow through the nephron. Because of these many interrelated factors and the difficulty predicting total expected effect on electrolyte excretion in a given patient, the clinical use of FE is limited; nevertheless, on occasion these tests may be cost-effective when prioritizing a limited number of differential diagnoses (**Box 3**).

URINARY BIOMARKERS OF KIDNEY INJURY

Biomarkers are physiologic molecules (usually proteins) that increase or decrease in association with normal or pathologic processes.[40] As discussed previously, serum creatinine concentration is relatively insensitive for early detection of renal injury; therefore, serum and urinary biomarkers that increase with early kidney damage have been the focus of many studies in people with naturally occurring disease and laboratory animal models of nephrotoxicity. Serum and plasma biomarkers seem less sensitive and have poorer correlation with presence or severity of kidney injury than urinary biomarkers. Clinical use of these biomarker proteins requires normalization to urine creatinine (ie, urine biomarker:creatinine ratio) to correct for changes rate and volume of urine production: with changes in urine volume, biomarker and creatinine concentrations are expected to proportionally increase or decrease.[41] A few urinary biomarker assays are offered by diagnostic laboratories, although clinical validation in dogs and cats is still limited.

Commercially Available Urinary Biomarkers of Renal Injury

Urine albumin/microalbuminuria

The majority of plasma albumin is size excluded and charge excluded from the ultrafiltrate; glomerular damage results in increased passage of albumin into the urine.[42] Although conventional urine dipsticks are the standard initial screening test for detection of proteinuria, urine albumin concentration must be approximately 30 mg/dL or greater to be detected by this method. Normal urine albumin concentration in dogs and cats, however, is significantly lower than this limit of detection: although there are slight differences between these species, the upper end of the reference range is approximately 1 mg/dL.[43] The range between these numbers (1–30 mg/dL) is referred to as mALB, whereas proteinuria greater than 30 mg/dL is termed *overt proteinuria*. Detection of mALB may allow earlier diagnosis of pathologically increased urine protein excretion, which can occur with primary glomerular diseases or extrarenal inflammatory diseases that secondarily damage the kidneys. Just as with overt

Box 3
Clinical scenario: fractional excretion of electrolytes

A 12-year-old spayed female Persian cat was evaluated for routine dental cleaning. The owner reported that the cat was healthy and active and the appetite normal and on physical examination the body condition was appropriate, despite the owner reporting that for the previous 6 months she had been feeding a home-prepared organic, vegetarian mixture of rice, tofu, and blenderized carrots and green beans. She had been previously instructed to supplement the diet with an adult human-strength multivitamin once per day but admits that she has difficulty administering medications by mouth to the cat and has not followed this recommendation for at least 2 months. Complete blood cell count, serum biochemical analysis, and urinalysis were performed before anesthesia for the dental cleaning; the only abnormality noted was moderate hypokalemia (2.1 mEq/L, reference range, 3.2–5.3 mEq/L). Serum concentration of creatinine was within the reported reference range (0.9 mg/dL; reference range, 0.2–1.6 mg/dL), and indirect systolic blood pressure was 175 mm Hg.

Differential diagnoses for hypokalemia in cats included hyperaldosteronism (due to an aldosterone-secreting adrenal tumor), potassium wasting renal disease, diet-related causes (ie, insufficient dietary concentration), and gastric or proximal duodenal obstruction with protracted vomiting and secondary metabolic alkalosis. In a patient with diet-related hypokalemia (as was suspected in this cat) or protracted vomiting (which was unlikely given the reported history), the kidneys would be expected to maximize potassium reabsorption, resulting in a very low to negligible FE of this electrolyte. In contrast, cats with hyperaldosteronism or potassium wasting renal disease would be expected to have an inappropriate FE of potassium. Ultrasonographic examination of the abdomen was recommended to determine whether or not an adrenal mass was present, because hyperaldosteronism in cats often results in hypertension (which was noted during the initial examination). The owner was reluctant to consent to this diagnostic test, however, due to expense.

Given the primary differentials for this cat's hypokalemia (insufficient dietary concentration of potassium or primary hyperaldosteronism), FE of potassium was determined. Urine concentration of creatinine was 250 mg/dL, and urine potassium of creatinine was 15.2 mEq/L. Substituting these values into the FE formula,

$$\%FE = \frac{(\text{Urine concentration of } K^+) \times (\text{Plasma concentration of creatinine})}{(\text{Urine concentration of creatinine}) \times (\text{Plasma concentration of } K^+)} \times 100$$

$$\%FE = \frac{(15.2\ \text{mEq/L}) \times (0.9\ \text{mg/dL})}{(250\ \text{mg/dL}) \times (2.1\ \text{mEq/L})} \times 100 = 2.6\%$$

The low FE of potassium was more consistent with increased reabsorption of electrolytes by the kidneys in response to hypokalemia, and therefore, a presumptive diagnosis of insufficient dietary potassium, resulting in hypokalemia was made. Conversely, hyperaldosteronism would have been expected to result in an inappropriately high potassium FE, because this hormone induces potassium excretion in the distal convoluted tubule. The owner was advised to supplement the cat with a potassium gluconate paste (she declined to feed a more balanced diet). Reevaluation of serum potassium concentration after 3 weeks of paste administration confirmed that all electrolytes were now within the laboratory reference range.

proteinuria, however, mALB may be due to preglomerular, glomerular, or postglomerular causes.[43]

There are strong associations between presence and magnitude of mALB and poor outcome in people. mALB is a strong prognostic indicator for later development of renal failure in diabetic patients; presence of mALB is also correlated with cardiovascular disease and death in patients with type 1 or type 2 diabetes mellitus.[44–46] Successful therapy with angiotensin-converting ednzyme inhibitors and better glycemic control slow the progression of mALB to overt proteinuria and decreases the likelihood of eventual azotemia and end-stage renal disease.[45] Other inflammatory diseases

associated with mALB in people include some neoplasms, inflammatory bowel disease, and acute inflammatory conditions, such as pancreatitis and myocardial infarction; in many of these diseases, the magnitude of mALB correlates with severity.[46] This correlation is particularly evident in people with lung or breast cancer or lymphoma, where presence and magnitude of mALB are associated with histologic subtype, tumor burden, presence or absence of metastatic disease, and median survival time.

The E.R.D.-HealthScreen (Heska, Loveland, Colorado) is a point-of-care test for detection of mALB. These assays are species specific, and separate kits must be purchased for detection of mALB in dogs versus cats. Urine samples are diluted to a standard concentration, thus correcting for urine specific gravity. Unfortunately, the E.R.D.-HealthScreen is only semiquantitative rather than providing a precise measurement of mALB: results are reported as negative, low positive, medium positive, or high positive. Additionally, these tests were developed for use in all dogs and cats, but mALB reference ranges for mALB may vary based on breed and age.[47]

mALB occurs in approximately one-third of dogs and cats presenting to veterinary teaching hospitals for a variety of conditions, and greater than 50% of critically ill dogs have urine protein in the mALB range but do not have increased urine protein:creatinine ratios.[48–50] mALB has been demonstrated to occur before overt proteinuria in dogs with hereditary X-linked nephritis[51] and in soft-coated wheaten terriers with protein-losing nephropathy.[52] Dogs with lymphoma or osteosarcoma have normal urine protein:creatinine ratios but often have mALB; whether urine protein correlates with tumor burden or remission status is unknown.[53] Dogs with heartworm disease develop mALB before overt proteinuria, and histologic evidence of glomerular disease is evident at the time of mALB development.[54] Other inflammatory conditions found in dogs with mALB include renal failure, pancreatitis, and cardiovascular disease, although it is unknown whether presence of mALB correlates with prognosis or glomerular injury.[48,55] Unlike in people, strenuous exercise does not cause transient mALB in dogs.[56] mALB is more common in cats with chronic kidney disease, hypertension, or hyperthyroidism, and presence and greater magnitude of mALB are associated with decreased survival time.[49,50,57–60]

It is still unclear when or how much further diagnostic investigation or therapeutic intervention is indicated in mALB-positive animals. Dogs and cats in which mALB has been detected should first have urine protein:creatinine ratios determined to quantitate the severity of proteinuria. Breeds known to develop hereditary glomerular diseases should likely be monitored regularly, and if magnitude of mALB increases, then further steps should be considered. In dogs or cats with unexpected, persistently positive E.R.D.-HealthScreen results, it may be advised to screen for glomerular diseases and/or extrarenal inflammatory diseases. No doubt, long-term longitudinal studies evaluating the benefit of these recommendations are still needed. It is unknown whether antiproteinuria therapies in animals with mALB are of any benefit.

Urine γ-glutamyl transpeptidase:creatinine ratio

Serum concentrations of the transmembrane amino acid transporter γ-glutamyl transpeptidase (GGT) are commonly used in the diagnostic evaluation of dogs and cats with hepatic or biliary tract disease. GGT is expressed in several other tissues, however, including the apical surface of proximal convoluted tubular epithelial cells, which release small amounts of GGT into the urine. Several studies in dogs with naturally occurring or experimentally induced kidney disease have demonstrated that the urine GGT:creatinine ratio is a sensitive early marker of tubular injury, oftentimes increasing before rises in serum creatinine concentration, decreases in GFR or urine specific gravity, or appearance of casts in urine sediment. Dogs with gentamicin-induced renal

failure have increased urine GGT activity 24 hours after initial dosing, whereas serum creatinine concentration did not increase above reference range until 7 days of drug administration[61–63]; 50% of intact female dogs with pyometra have moderate to high increases in urine GGT:creatinine ratios before ovariohysterectomy, which then gradually decrease over the 10 days after surgery.[64]

Urine GGT is labile, and samples should be assayed within 24 hours or frozen. Reference range in normal dogs for urine GGT:creatinine ratio is wide (1.93–28.57 IU/g), likely due to interindividual variation rather than circadian changes in excretion.[65,66] Anecdotally, and in the personal experience of the author, baseline assay of urine GGT:creatinine in patients of interest followed by serial measurement is of greater clinical utility than comparison to the suggested reference range (**Box 4**).

Selected Investigational Urinary Biomarkers of Renal Injury

Cystatin C

CysC is an inhibitor of endogenous extracellular proteinases that is produced by all nucleated cells, freely filtered across the glomerulus, and reabsorbed by renal tubular cells. Serum concentrations of CysC increase as GFR decreases, and urine concentrations increase after tubular injury. Utility of absolute urine CysC concentrations and CysC:creatinine ratio in the prediction of presence, severity, and outcome of acute kidney injury in people is unclear, because prospective studies have yielded conflicting results. A single study in dogs with either acute kidney injury or chronic kidney disease evaluated urine CysC:creatinine ratio and confirmed greater excretion than in healthy dogs.[67] Several CysC assays are available for research studies using canine plasma or urine but not clinical patients.

Interleukin 18

Interleukin 18 (IL-18) is a proinflammatory cytokine that polarizes helper T cells toward a T_H1 phenotype, induces interferon-γ production and release, and enhances

Box 4
Clinical scenario: urine GGT:creatinine ratio

A 6-year-old castrated male Rottweiler was evaluated for tachypnea associated with aspiration pneumonia due to idiopathic megaesophagus. The dog had been appropriately treated for multiple episodes of bacterial pneumonia over the preceding 2 years, with antibiotic therapy guided by sensitivity testing of aerobic bacterial cultures of transtracheal washings. The most recent culture indicated infection with *Escherichia coli*, resistant to all tested antibiotics other than aminoglycosides and carbapenems. Because of cost concerns, the owners elected home subcutaneous administration of amikacin for treatment; they also voiced monetary concerns when told that repeat thoracic radiographs would be required to guide duration of antibiotic therapy.

Before the first dose of amikacin, urine GGT:creatinine ratio was 3.72 IU/g, urine specific gravity was 1.042 without any casts noted, and serum creatinine concentration was 0.9 mg/dL. Two days after the first injection, the owners reported by tachypnea had resolved; urine GGT:creatinine ratio was 4.55 IU/g with a specific gravity of 1.045 and no casts were noted. Five days after beginning treatment, urine GGT:creatinine ratio had increased further to 7.21 IU/g, urine specific gravity was 1.035, and urine sediment remained benign. Eight days after initiating antibiotic therapy, urine specific gravity was 1.039, but GGT:creatinine ratio had tripled from baseline to 10.48, so amikacin administration was discontinued. Two weeks after discontinuing treatment, a lateral thoracic radiograph revealed resolution of previously noted interstitial and alveolar radio-opacities.

production of complement-activating IgG subclasses. Serum and urinary IL-18 concentrations increase in people with acute kidney injury, chronic kidney disease, and glomerular disease.[68–72] Increased IL-18 mRNA or serum concentrations have been reported in dogs with various inflammatory diseases, including autoimmune thyroiditis,[73] immune-mediated hemolytic anemia,[74] and sinonasal aspergillosis[75]; increased serum concentrations of this cytokine are associated with greater likelihood of death in dogs with immune-mediated hemolytic anemia.[74] Although IL-18 expression has been demonstrated in Madin-Darby canine kidney cells (a commonly used laboratory cell line),[76] in vivo investigations have not been performed in dogs with experimentally induced or naturally occurring kidney disease as yet.

Kidney injury molecule-1

Kidney injury molecule-1 (KIM-1) is a transmembrane protein normally found in healthy proximal convoluted tubular cells and shed into the urine at low concentrations. KIM-1 expression is rapidly up-regulated, however, by tubular epithelium after kidney injury, and KIM-1:creatinine concentration increases. Urinary KIM-1 increases in people with ischemic, nephrotoxic, or septic renal damage, polycystic kidney disease, and renal neoplasia.[77–79] Although in vitro expression of KIM-1 has been confirmed in Madin-Darby canine kidney cells, there are as yet no published in vivo studies of KIM-1 in dogs or cats.[79] A KIM-1 assay is available for canine urine (MILLIPLEX MAP Canine Kidney Toxicity Magnet Bead Panel 1, EMD Millipore Corporation, Billerica, Massachusetts) in conjunction with CysC and clusterin (a proposed biomarker not discussed in this article) but is only marketed for research purposes.

N-acetyl-β-D-glucosaminidase

N-acetyl-β-D-glucosaminidase (NAG) is an intracellular protein that participates in glycosaminoglycan catabolism; glycoproteins reabsorbed by proximal convoluted tubular cells are degraded by NAG and other lysosomal enzymes.[80] Increased urine excretion occurs in people with proximal tubular epithelial cell damage, particularly with acute kidney injury. Urinary NAG:creatinine ratio is increased in dogs with chronic kidney disease,[81,82] pyelonephritis,[81] uncontrolled diabetes mellitus,[81] pyometra,[81] or X-linked hereditary nephropathy[83] but does not differ before versus after control of hyperadrenocorticism with trilostane or transphenoidal hypophysectomy.[84] In cats, urinary NAG:creatinine ratio increases with chronic kidney disease.[85,86] Assays for urine retinol binding protein (RBP) are marketed for research use only.

Neutrophil gelatinase–associated lipocalin

Neutrophil gelatinase–associated lipocalin (NGAL) is an intracellular protein of hepatocytes, neutrophil granules, and epithelial cells, including the tubular epithelium of the thick ascending loop of Henle and collecting ducts.[87] Expression of NGAL is low in healthy tissues but in response to inflammation is up-regulated and plays a role in stabilizing and potentiating matrix metalloproteinases and sequestering iron, thereby inhibiting bacterial growth. Urinary NGAL:creatinine ratio is more sensitive than serum creatinine concentration for detection of acute kidney injury in people, and increases with progression of chronic kidney disease.[88,89] A few studies in dogs have investigated the utility of NGAL as a biomarker of renal damage; increases before serum creatinine concentration have been reported in laboratory dogs with gentamicin nephrotoxicity[90] or X-linked hereditary nephropathy[83] and in privately owned dogs with naturally occurring acute kidney injury or chronic kidney disease.[91] A canine-specific urine NGAL assay is available for research use only (BioPorto Diagnostics, Gentofte, Denmark).

Retinol binding protein

RBPs complex with and transport retinol (vitamin A). The plasma isoenzyme–retinol complex binds to transthyretin, which prevents passage of the low-molecular-weight (21-kDa) RBP protein across the glomerular filtration barrier.[92] In the absence of retinol, RBP undergoes a conformational change that prevents binding to transthyretin, passes into the glomerular ultrafiltrate, and is reabsorbed by proximal convoluted tubule cells. Tubular epithelial injury of any cause in people impairs RBP reabsorption and increases the urine RBP:creatinine ratio. Similarly, increased urinary excretion of RBP has been demonstrated in dogs with chronic kidney disease,[82] untreated hyperadrenocorticism,[84] and X-linked hereditary nephropathy[83] and in cats with untreated hyperthyroidism.[26,93] Assays for RBP can be purchased for laboratory use but are not offered by commercial laboratories for clinical patients.

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