Evaluation of urinary γ-glutamyl transferase and serum creatinine in non-azotaemic hospitalised dogs

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ABSTRACT

Urinary and blood biomarkers for diagnosis of acute kidney injury (AKI) in hospitalised dogs were evalueted. This prospective study included 97 dogs, classified according to the International Renal Interest Society classification into no AKI and AKI grade 1 (48-hour increase in serum creatinine≥0.3 mg/dl and/or urinary production <1 ml/ kg/hour for at least six hours). A total of 62 of 97 dogs (64 per cent) were classified as AKI 1. A statistically significant difference was found between no AKI and AKI 1 in urine protein to creatinine ratio, urinary γ-glutamyl transferase (uGGT) and uGGT/cu (P<0.0001). Thirteen of 97 dogs (13.4 per cent) that developed increased creatinine and change in AKI grade showed high mortality (n=9/13; 69.2 per cent). The receiver operating characteristic (ROC) curve analysis of uGGT/cu index as a marker for AKI grade 1 had an area under the ROC curve of 0.78; optimal cut-off point was 57.50 u/g, with sensitivity and specificity of 75.4 per cent and 75.6 per cent, respectively. Overall intensive care unit mortality was 23.7 per cent (23/97), 13.4 per cent (13/97) of which died during hospitalisation and 10.3 per cent (10/97) within 28 days after discharge. uGGT is an acceptable marker for distinguishing between AKI 1 and no AKI.

Introduction

Acute kidney disease represents a spectrum of diseases associated with a sudden onset of renal parenchymal injury, most typically characterised by generalised failure of the kidneys to meet the excretory, metabolic and endocrine demands of the body.1 2 The term acute kidney injury (AKI) was coined to highlight the need for early recognition of AKI and to make clinicians more aware of the presence of kidney injury early in its course, when therapeutic interventions may be more effective and outcomes more positive.2 3 To better emphasise the concept of AKI, few staging schemes RIFLE (Risk, Injury, Failure, Loss of kidney function, End-stage kidney disease) and AKIN (Acute Kidney Injury Network) have been proposed for human patients to stratify the extent and duration of renal injury and predict clinical outcome.4–6 In human medicine, AKI is most commonly defined as an abrupt reduction in kidney function, generally recognised in hospitalised

patients by an increased serum creatinine concentration or reduced urine output.7 8 In animals, by contrast, AKI most commonly develops outside the hospital setting, in which the abruptness of the disease and the magnitude of changes in glomerular filtration rate (GFR), azotaemia and/or urine production are rarely known or quantified out of the hospital setting.2 9 There have only been two retrospective studies concerning hospital- acquired AKI (HA-AKI) in veterinary medicine and they used the human classification of AKI.10 11 Recently, the International Renal Interest Society (IRIS) adapted this schematic approach to classify and grade the severity of AKI in dogs and cats.2 The IRIS AKI grading scheme is based on serum creatinine, urine production and the requirements for renal replacement therapy.2 IRIS AKI grade 1 includes animals with progressive (hourly or daily) increases in serum creatinine of at least 0.3 mg/ dl within the non-azotaemic range during a 48-hour interval.2 AKI grade 1 also includes animals whose decreased urine production is readily fluid-responsive. A diagnosis of kidney injury at this stage using routine clinical parameters can be difficult.2 3 Biomarkers that predict the presence of kidney injury before clinical abnormalities are present and before the development of azotaemia would greatly facilitate the detection of kidney injury, in order to facilitate medical management and to reduce the progression of AKI.3 In both human and veterinary medicine, there is a recognised need for sensitive and specific markers for early identification of AKI, and potential biomarkers for early recognition of AKI are being investigated.3 12 13 AKI biomarkers could facilitate early diagnosis and provide specific preventive and therapeutic strategies, with an overall improvement in outcome.13 Increased activities of some urinary enzymes suggest injury to renal tubular cells or indicate increased lysosomal activity; thus, specific enzymes (γ-glutamyl transferase or GGT, N-acetyl-β- glucosaminidase or NAG, and alkaline phosphatase) tend to be associated with renal injury.14 15 Several studies15–18 have revealed that the measurement of renal tubular enzyme activities (NAG, GGT and others) is more sensitive in the detection of acute renal damage than the current standard veterinary diagnostic tests.19 GGT is one of the numerous renal tubular enzymes that are leaked in the urine of many mammal species, after tubular injury.20 GGT is not considered a stable enzyme, and its activity does decrease over time but can be measured using the same equipment as for serum GGT.21 In dogs, GGT is a brush border enzyme of the proximal convoluted tubule.19 GGT urinary excretion has thus long been used in nephrotoxicity studies in human beings and also in dogs.17 21–23 It has also been used in dogs with pyometra and with gentamicin-induced and aminoglycoside-induced nephrotoxicity.15 18 22

The aims of the present study were to evaluate the sensitivity and specificity of urinary GGT (uGGT) for the diagnosis of early stages of AKI compared with serum creatinine, and to assess the prevalence of HA-AKI in a population of hospitalised dogs using the IRIS AKI grading system.

MATERIALS AND METHODS

Animals (study population)

One thousand five hundred and ninety-two dogs admitted to the intensive care unit (ICU) at the University of Pisa, Veterinary Medical Teaching Hospital between January 2015 and December 2016 were screened. Of these, 897 (56.3 per cent) were surgery patients and 695 (43.7 per cent) were medical patients. Surgery patients were classified into general surgery (n=673; 75 per cent) and orthopaedic surgery (n=224; 25 per cent). Medical patients were distributed among nephrology and urology (n=299; 43 per cent), cardiorespiratory (n=95; 13.6 per cent), endocrinology (n=98; 14.1 per cent), gastroenterology (n=131; 18.8 per cent), haematology (n=30; 4.3 per cent), non-surgical trauma (n=20; 2.8 per cent) and neurology (n=22; 3.1 per cent).

Study protocol

ICU-admitted dogs (n=1592) were considered (T0) in the study if they met the following criteria (figure 1):

- Non-azotaemic dogs (serum creatinine <1.6 mg/dl).
- ICU hospitalisation of at least 48 hours.

Azotaemia (serum creatinine >1.6mg/dl) and ICU hospitalisation less than 48hours were the exclusion criteria.

For dehydrated patients, T0 was considered only when dehydration was corrected.

For dogs which were considered dehydrated on ICU admission, dehydration percentage was determined on the basis of a specific table,24 and hydration requirements were calculated as the sum of maintenance (60 ml/kg/ day) and dehydration (expressed as percentage) needs, according to the following formula: BW (kg) x per cent dehydration=volume (litre) to correct based on previous guidelines. In and outs were calculated for each patient on ICU admission. During the first 30 minutes from the start of intravenous hydration, patients were monitored for at least every 15minutes (especially for severely dehydrated patients) to evaluate their response in terms of changes in clinical parameters of hydration (skin turgor, mucous membrane moisture and eye position). After the first 30 minutes, patients were reassessed every one to twohours according to

individual needs. The typology of fluids to infuse was dependent on the results of blood gas analysis of each patient. For example, in case of metabolic acidosis, an alkalinised fluid such as Ringer lactate or acetate was preferred to restore pH and electrolyte balance. If needed, once again based on blood gas examination, glucose or potassium or bicarbonate was supplemented in a 'home made', fluid- type 'ad hoc' for every patient. At least three blood gas analyses were assessed for patients per day. The rate of infusion was set on the basis of rehydration goal of the patient, and adjusted on the basis of clinical response (such as refilling time, blood pressure, mucous membrane moisture and colour, and heart rate).

At inclusion (T0) age, sex, breed, leading causes of hospitalisation, diagnosis, mortality (during hospitalisation and within 28 days of discharge) and length of stay (days) were recorded. All dogs were classified according to the IRIS grading staging system for AKI into two groups: (1) no AKI, non-azotaemic dogs that did not develop AKI during hospitalisation; and (2) AKI 1, dogs with normal serum creatinine at inclusion (T0) which developed AKI during hospitalisation (AKI grade 1). In particular, according to the IRIS guidelines, AKI grade 1 was defined as non-azotaemic dogs with documented AKI (historical, clinical, laboratory or imaging evidence of AKI, clinical oliguria/ anuria, volume responsiveness) and/or progressive non-azotaemic increase in serum creatinine (≥0.3mg/dl within 48hours), and/or the presence of oliguria (<1ml/kg/hour) or anuria over sixhours (www.iris- kidney.com).

Sample collection

Serum creatinine, complete urinalysis with urine protein to creatinine ratio (UPC) and uGGT were performed at inclusion (T0) and at 24 (T1) and 48 (T2) hours later. Urine was collected by cystocentesis or catheterisation. For all dogs, serum creatinine, urine production and GGT were assessed at T0, T1 and T2. Urine production was estimated over a six-hour period of time. Calculation of urine production was initiated only after dehydration has been corrected (<24 hours).

uGGT evaluation

Urine samples were centrifuged at 3000 revolutions per minute (rpm) for threeminutes, and the supernatant was separated. The determination of uGGT, generally intended for the determination of GGT in human serum or plasma, was used for quantitative in vitro determination of GGT. A Liasys AMS Assel spectrophotometer (for enzymatic chemical-type immunoturbidimetric and colorimetric analysis) was used on refrigerated samples (+4°C) within 24hours of collection.20 Enzymatic activity was expressed in units per

litre (u/l). Creatinine was measured on the same sample using Jaffe test run on an automated clinical chemistry analyser (SLIM Seac). The uGGT activity (iu/l) was then divided by the urinary creatinine concentration (mg/dl) to obtain the uGGT index (u/g).

Statistical analysis

Distribution of continuous parameters (normal vs non-normal) was assessed using the D'Agostino- Pearson omnibus normality test. The chi-squared test was used to compare the proportion of patients with surgical or medical reasons for hospitalisation between no AKI and AKI grade 1. The same test was used to differentiate the number of survivors and non-survivors between no AKI and AKI 1, and between dogs with uGGT/uc of at least 57.50u/g and less than 57.50u/g. The Mann-Whitney test or unpaired *t* test was used to compare body weight, serum creatinine, urine-specific gravity (USG), UPC, uGGT and uGGT/uc between no AKI and AKI grade 1 on presentation. The same test was used to compare UPC between dogs with uGGT/ uc of at least 57.50u/g and less than 57.50u/g. The correlation between uGGT/uc and serum creatinine and UPC in the no AKI group and AKI grade 1 was assessed using the Pearson or Spearman rank correlation test. The Friedman test was used to compare uGGT/uc and creatinine among different time points (T0, T1 and T2) in AKI 1 dogs. The receiver operating characteristic (ROC) curve analysis was used to select cut-off points and to calculate the corresponding sensitivities and specificities of GGT in the prediction of AKI. Results were considered statistically significant at $P<0.05$.

Results

The study population included 97 dogs non-azotaemic (creatinine ≤1.6mg/dl) at inclusion (T0). For all dogs (n=97), the median values of body weight (BW), serum creatinine, USG, UPC, uGGT, uGGT/uc are reported in table 1.

The median age of all dogs was eight years (range: fivemonths to 15 years). A total of 67 (69 per cent) of 97 dogs underwent general anaesthesia. The median length of stay was three days (range: two days to 20 days). The median baseline creatinine was 1mg/dl (range: 0.5–1.6). According to the IRIS grading system, 35 (36 per cent) of 97 dogs were classified in the no AKI group and 62 (64 per cent) in the AKI grade 1 group. Of the 62 dogs which were classified as AKI 1, 10 dogs were classified on the basis of urine production, 17 on the basis of serum creatinine, and 35 on the basis of both urine production and serum creatinine. For 48 of 62 dogs with AKI 1, urine production was assessed through an indwelling urinary catheter, while in 14 of 62

dogs urine production was estimated by serial daily assessment of bodyweight (median 19.5kg; min 6 kg, max 35kg). All 10 dogs which were classified on the basis of urine production alone had an indwelling urinary catheter. The 14 dogs for which urine production was estimated on the basis of serial daily assessment of bodyweight were distributed between the 17 dogs classified by serum creatinine (n=9) and the 35 dogs classified by a combination of serum creatinine and urinary production (n=5).

On the basis of urine production, 45 of 62 dogs were classified as oliguric (urine output <1ml/kg/hour). On hospital admission, dehydration was estimated to be 5–6 per cent in 20 of 62 dogs with AKI 1, 6–8 per cent in 12 dogs, and 10–12 per cent in 10 dogs. Overhydration was estimated in five of 62 dogs, and normal hydration in 15 dogs.

The number of patients that required surgery was significantly higher (P=0.0191) compared with the number of non-surgery patients in both AKI 1 and no AKI groups. General surgery was the major reason for hospitalisation in both groups (57.1 per cent for no AKI and 43.6 per cent for AKI 1) (figure 2). UPC, uGGT and the uGGT indexed for urinary creatinine (cu) (uGGT/cu) on presentation were significantly higher (P=0.0001) in the AKI 1 than in the no AKI group (table 1, figure 3). Four patients in the AKI 1 group developed multiple episodes of significant hypotension (mean arterial pressure; MAP <60 mmHg) during anaesthesia for up to 15minutes. None of the surgery patients in the no AKI group developed significant hypotension during surgery. A significantly positive correlation was also found between the uGGT/uc index and UPC (P<0.0001, r=0.59) (figure 4). No significant correlation (P=0.8271, r=−0.02) was found between uGGT/uc and serum creatinine. A significant difference (P=0.01) was found between the median values of uGGT/uc at different times (T0, T1 and T2) in AKI 1 dogs (figure 5). Dunn's multiple comparison test showed a significant difference between T0 versus T1 (P=0.02), and T0 versus T2 (P=0.02).

No statistically significant difference (P=0.45) in serum creatinine was found at different times (T0, T1 and T2). The ROC curve analysis of the uGGT/cu index as a predictor of AKI grade 1 compared with no AKI in non-azotaemic patients (P<0.0001) had an area under the ROC curve of 0.78 (95 per cent confidence interval, 0.69–0.88). The optimal cut-off point was 57.50u/g, which corresponded to a sensitivity and specificity of 75.4 per cent and 75.6 per cent, respectively (figure 6). A significant difference in UPC (P<0.0001) was found between dogs with an elevated (>57.50u/g) and normal uGGT/cu index. Three patients in the no AKI group progressed to AKI 1 after the initial 48hours of the study, while the other 32

dogs remained in the no AKI group. In the AKI 1 group, 10 dogs progressed to more advanced stages, while 48 patients came back to baseline creatinine between 48hours and 10 days. Four patients did not come back to baseline creatinine within the hospitalisation.

The overall mortality in the study population was 23.7 per cent (23/97). Of the 23 which did not survive, five patients were euthanased, while 18 died; five (5.1 per cent) of 97 were in the no AKI group and 18 (18.6 per cent) were in the AKI group. In-hospital mortality was 13.4 per cent (13/97), and an additional 10.3 per cent (10/97) died within 28 days after discharge. The mortality rate of dogs which showed a progression in the AKI grade during hospitalisation (13/97) was 69.2 per cent (9/13) (table 2). No statistical difference in mortality rate was found between dogs with or without AKI. There was no significant difference in mortality between dogs with increased versus normal uGGT/uc index (17, 17.5 per cent v 6, 6.2 per cent).

Discussion

In the present study, uGGT and uGGT/uc index seemed to be a promising urinary biomarker in detecting early stages of AKI in hospitalised dogs. uGGT and uGGT/ uc index increased significantly in the AKI 1 group compared with the no AKI group (table 1). The area under the ROC curve (figure 6) shows that uGGT is a moderately sensitive and specific marker which can be used to predict kidney injury earlier than serum creatinine in critically ill dogs. Previous studies demonstrated that uGGT/cu increases significantly in dogs with pyometra and with gentamicin-induced nephrotoxicity.[15 22] Elevated uGGT has been found to be associated with the severity of lesions in proximal tubular cells.[17] According to a recent review and study,[12 25 26] uGGT could also be used to detect AKI in dogs with naturally occurring renal disease. uGGT has also been evaluated in other animal species[20] and in healthy dogs[19 26]; however, to the best of the authors' knowledge, uGGT has never been evaluated as a potential marker of AKI in hospitalised dogs. In clinically healthy dogs, Brunker *et al*[19] reported a normal uGGT/uc cut-off of 1.93–28.57u/g, while in a recent review[25] uGGT/uc of less than 42u/g was considered a normal value. In the present study, the mean value of uGGT/cu in the no AKI group (classified as 'healthy' according to the IRIS staging system) was 39.6 u/g (table 1). This value seems to be very close to the value reported by Hokamp and Nabity[25] for healthy animals. The area under the ROC curve together with the sensitivity and specificity also support these

findings (figure 6). A value of 57.50 u/g was considered the best combination of sensitivity and specificity (75.4 per cent and 75.6 per cent, respectively).

Serum creatinine is conventionally recognised as a primary diagnostic marker for AKI and chronic kidney disease (CKD).[2 27] The sensitivity of creatinine in detecting early kidney disease would be improved by serial evaluation of serum creatinine (trend), which better reflects deterioration in renal function. This concept of detecting small but clinically significant increases in serum creatinine is actively being adopted in cases of AKI, illustrated by the IRIS AKI grading. In this grading scheme, an increase in serum creatinine of at least 0.3 mg/dl within a 48-hour period is a criterion for identifying AKI grades 1 and 2.[2] In the present study, creatinine was not significantly correlated with uGGT/cu (P=0.8271) and did not change significantly during hospitalisation (P=0.45), unlike the uGGT index (P=0.01) (figure 5). Differently from uGGT, single determination of serum creatinine may not be able to diagnose early stages of AKI (table 1).

UPC value also correlated significantly with uGGT/cu (figure 4). UPC increased in the AKI 1 group compared with the no AKI group (table 1), and was significantly increased in dogs with uGGT/cu greater than 57.50 u/g (P=0.0001). UPC is traditionally considered a marker of prognosis and stage of CKD.[27] The IRIS group recommends the analysis of UPC values in guiding clinical decision-making and for monitoring trends in dogs with CKD.[12 27] Proteinuria is also considered an important marker in dogs with pyometra-associated nephropathy.[28] Maddens *et al*[28] found that kidney biopsies indicated tubulointerstitial nephritis in many of the dogs with pyometra and UPC greater than 0.5, and a frequent association with glomerulosclerosis when UPC is greater than 1.00. In a recent study by Segev *et al*[29], the median UPC on presentation was high in dogs with heat stroke compared with healthy control dogs. In this case, UPC was used in association with other markers of kidney function for the characterisation of renal injury and its severity.[29]

In the present cohort of dogs, HA-AKI showed a relatively high incidence. AKI grade 1 was diagnosed in 62 (63.9 per cent) of 97 dogs. The authors hypothesise that the discrepancy between their results and the previous findings may be due to different factors.

First of all is the use of different classification criteria.[10 11 30] Thoen and Kerl[10] found an incidence of AKI of 14.6 per cent in hospitalised dogs using the AKIN criteria. In another retrospective study[11] which

included a larger number of dogs (n=400) classified according to changes in serum creatinine (>0.3 mg/dl), the majority of patients (85.3 per cent) showed no evidence of AKI. A third retrospective study[30] reported only prognostic data based on the modified RIFLE criteria (from human medicine) using a decrease of 25 per cent, 50 per cent and 75 per cent from baseline GFR. The IRIS grading system for AKI is based on an increase in serum creatinine (>0.3 mg/dl) and on the presence of anuria/ oliguria (<1ml/kg/hour) over a six-hour period and/ or readily fluid volume responsiveness.[2] However, it is to be pointed out that the majority of dogs in the present study were diagnosed on the basis of elevation in serum creatinine, rather than on reduction in urine production. As a consequence, the use of different classification criteria might contribute marginally on the higher prevalence of AKI 1 patients in the present study compared with the previous findings. The incidence of AKI in hospitalised dogs, based on an elevation in serum creatinine alone, has been estimated at around 15 per cent.[10] It is therefore possible that HA-AKI is underdiagnosed in veterinary medicine. According to the results of the present study, the majority of dogs with AKI grade 1 (45/62; 72.6 per cent) developed oliguria or anuria (<1 ml/kg/hour) during hospitalisation. Only one retrospective veterinary study evaluated urinary output (UO) in hospitalised dogs with AKI.[31] In Vaden *et al*'s[31] study, 99 dogs with AKI were considered and UO was measured in 44 of 99 dogs. Anuria and oliguria were present in the majority of dogs (18 per cent and 43 per cent, respectively).[31] Although these results seem to agree with the present study, they cannot be directly compared, as Vaden *et al*[31] used a different staging criteria and definition of AKI. On the other hand, it is
possible that some patients have been misclassified due to technical or subjective errors, especially for dogs that did not have urinary catheter placement.

The elevated incidence of AKI in the present study may also be secondary to the high number of dogs with surgical reason for hospitalisation. However, the incidence of significant hypotension during surgery in the patients seemed to be too low to justify the elevated incidence of AKI in this group of dogs. Therefore, other potential causes of AKI should be investigated in patients submitted to recent surgery. Particular attention should be given to changes in renal haemodynamics during the recovery phase from anaesthesia and surgery. With the high prevalence of surgery patients developing AKI during hospitalisation, the authors may also

speculate a redistribution of the glomerular blood flow during anaesthesia. In anaesthetised rats, renal blood flow and GFR were markedly depressed during the recovery from surgery.[32]

When dogs which showed a progression in the AKI grade during hospitalisation (n=13) were considered, the mortality rate was 69.2 per cent (n=9/13) (table 2). In this case, the mortality rate is in agreement with other veterinary studies.[10 11]

On the other hand, no statistically significant difference in mortality rate was found between the number of dogs with AKI grade 1 and no AKI. In this case the mortality was not higher in dogs affected by AKI grade 1. This highlights important data in critically ill dogs. The mortality rate seems to increase with the worsening of AKI grade, which is important in terms of a critically ill population.

In the present study, elevated uGGT and UPC seemed to be early indicators of AKI in non-azotaemic hospitalised dogs, although no association between increased GGT and elevated mortality risk was found. The results of the present study show a potential role of uGGT as an early marker of AKI in hospitalised dogs. Although serum creatinine was recognised as the most widely used marker for AKI diagnosis, uGGT may be used as a complementary tool to increase the diagnostic ability in discovering early stages of AKI. Particularly, GGT may be helpful in identifying AKI 1 stage in hospitalised patients at risk of AKI. With the rapid time of assessment, low cost and good correlation to UPC, uGGT may be part of a bedside panel of markers to use for the diagnosis of AKI in dogs, especially in dogs undergoing general anaesthesia or those exposed to nephrotoxins. The authors conclude that GGT is a moderately sensitive and specific marker of AKI in hospitalised dogs which could be used to screen hospitalised patients at risk of AKI to improve the ability in diagnosing IRIS AKI grade 1 patients. Additional studies are needed to evaluate GGT and UPC in combination with other biomarkers to establish the actual incidence of HA-AKI.

References

1 Cowgill LD, Langston CE. Acute kidney insufficiency. In: Bargtes J, Polzin DJ, eds. Nephrology and urology of small animals: Whiley Blackwell, 2011:472–514.

2. Cowgill LD. Iris grading of acute kidney injury: International Renal Interest Society (IRIS), 2012. http://www.iris-kidney.com/guidelines/grading.html.

3. Segev G, Palm C, LeRoy B, *et al*. Evaluation of neutrophil gelatinase-associated lipocalin as a marker of kidney injury in dogs. *J Vet Intern Med* 2013;27:1362–7.
4. Bellomo R, Ronco C, Kellum JA, *et al*. Acute renal failure - definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. *Crit Care* 2004;8:R204–12.
5. Uchino S, Bellomo R, Goldsmith D, *et al*. An assessment of the RIFLE criteria for acute renal failure in hospitalized patients. *Crit Care Med* 2006;34:1913–7.
6. Srisawat N, Hoste EE, Kellum JA. Modern classification of acute kidney injury. *Blood Purif* 2010;29:300–7.
7. Uchino S, Kellum JA, Bellomo R, *et al*. Acute renal failure in critically ill patients: a multinational, multicenter study. *JAMA* 2005;294:813–8.
8. Thakar CV. Perioperative acute kidney injury. *Adv Chronic Kidney Dis* 2013;20:67–75.
9. Kerl ME, Langston CE. chapter 186: Recognition and Prevention of Hospital-Acquired Acute Kidney Injury. In: Bonagura JD, Twedt DC, eds. Kirk's XV. : Whiley Elsevier, 2014:186: 247–75.
10. Thoen ME, Kerl ME. Characterization of acute kidney injury in hospitalized dogs and evaluation of a veterinary acute kidney injury staging system. *J Vet Emerg Crit Care* 2011;21:648–57.
11. Harison E, Langston C, Palma D, *et al*. Acute azotemia as a predictor of mortality in dogs and cats. *J Vet Intern Med* 2012;26:1093–8.
12. De Loor J, Daminet S, Smets P, *et al*. Urinary biomarkers for acute kidney injury in dogs. *J Vet Intern Med* 2013;27:998–1010.
13. Zheng C-M, Liao M-T, Lin M-Y, *et al*. Biomarkers in Acute Kidney Injury. *Open J Nephrol* 2013;03:51–60.
14. Crivellenti LZ, Mesa JS, Meirelles AÉ, *et al*. False positivity of gamma-glutamyl transpeptidase measurement in urine. *Ren Fail* 2014;36:581–4.
15. Heiene R, Moe L, Mølmen G. Calculation of urinary enzyme excretion, with renal

303 structure and function in dogs with pyometra. *Res Vet Sci* 2001;70:129–37.
304 16. GrecoDS,TurnwaldGH,AdamsR,*etal*.Urinarygamma-glutamyltranspeptidaseactivity
305 in dogs with gentamicin-induced nephrotoxicity. *Am J Vet Res* 1985;46:2332–5.
306 17 Heiene R, Biewenga WJ, Koeman JP. Urinary alkaline phosphatase and 7-glutamyl transferase as
307 indicators of acute renal damage in dogs. *J Small Anim Pract* 1991;32:521–4.
308 18 Rivers BJ, Walter PA, O'Brien TD, *et al*. Evaluation of urine gamma-glutamyl transpeptidase-to-
309 creatinine ratio as a diagnostic tool in an experimental model of aminoglycoside-induced acute renal failure
310 in the dog. *J Am Anim Hosp Assoc* 1996;32:323–36.
311 19 Brunker JD, Ponzio NM, Payton ME. Indices of urine N-acetyl-beta-D-glucosaminidase and gamma-
312 glutamyl transpeptidase activities in clinically normal adult dogs. *Am J Vet Res* 2009;70:297–301.
313 20 Mancinelli E, Shaw DJ, Meredith AL. γ-Glutamyl-transferase (GGT) activity in the urine of clinically
314 healthy domestic rabbits (*Oryctolagus cuniculus*). *Vet Rec* 2012;171:475. 21 Dierickx PJ. Urinary gamma-
315 glutamyl transferase as an indicator of acute nephrotoxicity
316 in rats. *Arch Toxicol* 1981;47:209–15.
317 22 Grauer GF, Greco DS, Behrend EN, *et al*. Estimation of quantitative enzymuria in dogs
318 with gentamicin-induced nephrotoxicosis using urine enzyme/creatinine ratios from
319 spot urine samples. *J Vet Intern Med* 1995;9:324–7.
320 23 Clemo FA. Urinary enzyme evaluation of nephrotoxicity in the dog. *Toxicol Pathol*
321 1998;26:29–32.
322 24 DiBARTOLA S, Bateman S. Introduction to Fluid therapy. DiBartola S, ed. Fluid,
323 Electrolyte, and Acid-Base Disorders in small animal practice. 4th edn: Elsevier,
324 2012:331–50.
325 25 Hokamp JA, Nabity MB. Renal biomarkers in domestic species. *Vet Clin Pathol*
326 2016;45:28–56.
327 26 Nivy R, Avital Y, Aroch I, *et al*. Utility of urinary alkaline phosphatase and γ-glutamyl
328 transpeptidase in diagnosing acute kidney injury in dogs. *Vet J* 2017;220:43–7.
329 27 . http://www.iris-kidney
330 28 Maddens B, Heiene R, Smets P, *et al*. Evaluation of kidney injury in dogs with pyometra

based on proteinuria, renal histomorphology, and urinary biomarkers. *J Vet Intern Med* 2011;25:1075–83.

29 Segev G, Daminet S, Meyer E, *et al*. Characterization of kidney damage using several renal biomarkers in dogs with naturally occurring heatstroke. *Vet J* 2015;206:231–5. 30 Lee YJ, Chang CC, Chan JP, *et al*. Prognosis of acute kidney injury in dogs using *RIFLE* (Risk, Injury, Failure, Loss and End-stage renal failure)-like criteria. *Vet Rec* 2011;168:264.

31 Vaden SL, Levine J, Breitschwerdt EB. A retrospective case-control of acute renal failure in 99 dogs. *J Vet Intern Med* 1997;11:58–64.

32 Walker LA, Buscemi-Bergin M, Gellai M. Renal hemodynamics in conscious rats: effects of anesthesia, surgery, and recovery. *Am J Physiol* 1983;245:F67–74.