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Biomarkers in canine renal disorders

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Abstract
Renal problems in canines are of worrying concern to both pet owners and practitioners throughout the globe as death becomes inevitable if appropriate diagnostic and therapeutic intervention is not carried out at the earliest. Creatinine has been used as a gold standard for screening renal disorders for years in canines. Much research has been carried out to develop and standardise novel renal biomarkers which can effectively diagnose renal issues well in advance than the existing renal biomarkers. In this review, we will discuss the various attributes of existing, timetested renal biomarkers and the prospects of various newly developed and emerging renal biomarkers.

Keywords: Acute kidney injury-chronic kidney disease-creatinine–renal biomarker- urinary enzymes

Introduction
In emergency and critical care unit, dogs are presented with signs and symptoms of moderate to severe damage to the kidneys. Kovarikova (2015) stated that Acute Kidney Injury (AKI) is associated with a sudden onset of renal parenchymal damage with subsequent impairment of renal function and severe injury leading to acute renal failure (ARF), which is characterised by the highest morbidity and mortality rates. Similiarly, Chronic kidney disease (CKD) associated with renal impairment for quite a prolonged period is seen more commonly in older dogs. CKD dogs are presented with a history of weight loss, inappetance, poor skin and body coat and altered micturition pattern like polyuria for more than three months. Pelander *et al.* (2015) [1] stated that Chronic kidney disease (CKD) in dogs is characterized by progressive loss of renal function, with a prevalence of up to 25% of dogs in referral institutions.

Insult to the kidneys in ailing dogs is diagnosed at large based on the history, clinical examination and based on the elevated serum creatinine values. However certain cases with renal impairment but with serum creatinine values within the normal range may be left undiagnosed. Cowgill and Langston (2011) [2] have also stated that the high mortality associated with acute renal failure (ARF) is caused by delayed detection of the condition due to insensitive diagnostic tests. Hence this article is aimed at discussing in detail the availablity of various diagnostic tests; conventional markers and clearance tests and the prospects of novel and emerging biomarkers for early diagnosis and rationale therapeutic intervention in cases of AKI and CKD which are the most common canine renal disorders.

Conventional tests used for diagnosis of renal dysfunction
For many decades, renal impairment has been diagnosed by serum biochemistry *viz.* elevated blood urea nitrogen concentration, elevated serum creatinine concentration, hyperphosphatemia, hyperkalemia or hypokalemia and hypoalbuminemia. In addition, urinalysis is also done to look for the changes in the urine specific gravity, presence of protein, RBCs and casts etc. Most of the well equipped clinics and hospitals adopt ultrasound and radiographic screening to look for the increase or decrease from normal size of the kidneys, changes in echogenicity of kidney texture etc.

Glomerular filtration rate (GFR)
GFR is the rate of blood flow to the kidneys and it serves as an indicator of the kidney's functioning capacity to filter the fluid plasma and excrete creatinine and other waste elements from the body. Kerl & Cook (2005) [3] proposed the measurement of glomerular filtration rate (GFR) as the best method for assessing renal function as it is directly proportional to functional renal mass. However, the method of estimation of GFR is little cumbersome compared to other tests like serum biochemical estimation of creatinine etc.

and hence it is less retorted in much of the practice and instituitional settings.

Clearance tests for diagnosis of renal dysfunction
The clearance tests done for diagnosis of renal dysfunction include urea clearance, inulin clearance and creatinine clearance test. However, Kerl and Cook (2005) [3] have claimed that many clearance tests are costly and time-consuming and not suitable for widespread use as a screening test.

Urine output
Though it may sound simple, vigilant watching of the voiding pattern of dog: the volume of urine in every micturition process, total volume throughout the day as well as the frequency of urination will help a longway in the diagnosis of disturbance to the kidneys in the early stages of renal insult.

Blood urea nitrogen (BUN)
Estimation of BUN is also carried out for years along with creatinine to diagnose kidney diseases. When kidneys are impaired, there is reduction in the GFR. However BUN estimation is relatively unreliable than serum creatinine, since the values of BUN are affected by non renal factors like high protein diet, pyometra etc. In dogs with healthy functioning kidneys, BUN: Creatinine ratio shall be around 10:1 (The normal BUN value is 10-15mg/dl and Creatinine value is 0.5-1.5mg/dl). In disease conditions the ratio is altered *viz.* in cases of dehydration, high protein diet and in renal hypoxia the ratio is increased to around 25:1; in cases of malnutrition, inappetance and impaired synthesis of urea by ornithine cycle as in malfunctioning of liver, the ratio is decreased to around 5:1.

Creatinine
Creatinine is a produced in the body due to the routine wear and tear of muscles in the body. In animals with healthy kidneys, creatinine is filtered and excreted out of the body. Hence the presence of serum creatinine or urine creatinine above the reference range (0.5-1.5mg/dl) is an indicator of impaired functioning of the kidneys.
Creatinine has been used as a gold standard for evaluating the renal function for decades. However it has few to many limitations which thrust the need for a better biomarker to identify the kidney involvement in early stages and to assess the severity of the condition. Though more specific to kidneys, creatinine is a late indicator of renal dysfunction. Elevation of creatinine occurs only after the GFR has impaired significantly. Polzin (2011) [4] reported creatinine as an insensitive marker as the increase in serum creatinine is mild and often remain within the reference range, until approximately 75% of nephrons become non functional. Further, creatinine levels may also be affected to a little extent by various non renal factors like age of the animal, sex, muscle mass, exercise or physical activity of the animal and to meat and other protein intake of the animal etc.
Pseudoelevation of creatinine can happen due to sample collection and processing errors like hemolysed samples, lipemic and icteric samples. Peake and Whiting (2006) [5] reported that hemolysed samples can show altered creatinine values due to the release of noncreatinine chromogens; interference of bilirubin, influence of foetal haemoglobin in neonates etc. Polzin and Cowgill (2016) [6] reported that the creatinine values may be low in lipemic and icteric samples

due to interference with the optical measurement while calibrating the creatinine values. Lowered creatinine values may also be obtained in lean, emaciated dogs and in cases with liver disorders when creatine, the precursor of creatinine synthesis is inefficient etc. This information is very important in clinical practice, as in such cases renal issues are more frequently overlooked by observing the creatinine within the normal limits.
Like creatinine, most of the conventional tests including renal clearance tests have limitations like; they are clinically relevant only in advanced stages of renal disease and not in the subclinical or early stages of renal impairment; cumbersome procedure, administration of extraneous agents by intravenous route like inulin in clearance test, insensitive to detect tubular damage etc. Hence much research is being done throughout the world for the development and employment of new renal markers to overcome the limitations of creatinine.

Renal Biomarkers
A biomarker is a characteristic that is objectively measured and evaluated as an indicator of normal biological or pathogenic processes, or pharmacologic responses to a therapeutic intervention Atkinson *et al.* (2001) [7]. Thus, the ideal biomarker for kidney injury should have the merits like: to identify the pathology in kidney earlier than the existing gold marker creatinine; should be specific to kidney; to identify the site of kidney damage whether the damage has happened in the glomeruli, renal tubules etc; should be safe and non expensive to be used in routine clinical settings.
Urine is the preferred sample for biomarker analysis as the sample collection is easy and non invasive, though certain markers have been developed such a way that can be estimated in both or either serum or urine. However, as the concentration of different analytes in urine vary based on the volume of urine in each micturition, due to differing specific gravity, it is ideal to collect a 24 hour urine output to have a correct interpretation of biomarker excretion in the urine. Futher, to be more precise and to ease the procedure, markers can also be detected as a single step urine collection instaneously by correlating the concentration of marker in urine with urine creatinine, as the creatinine values are relatively unaltered compared to other parameters excreted in urine.

Types of Renal Markers
Markers of glomerular dysfunction
Albumin, Immunoglobulin G, C-reactive protein, Proatrial natriuretic peptide etc.

Markers of tubular injury
N-acetyl-β-d-glucosaminidase, Gamma-glutamyl transpeptidase, Alkaline phosphatase, Neutrophil gelatinase-associated lipocalin, Retinol binding protein, Microglobulins, Cystatin C, Cauxin, Clusterin etc.

Markers for AKI
Serum creatinine, cystatin C, Microalbumin, Neutrophil gelatinase-associated lipocalin (NGAL), Interleukin-18 (IL-18), Kidney injury molecule 1 (KIM-1), Urinary Liver-type fatty acid–binding protein (L-FABP), Urinary insulin like growth factor–binding protein 7 (IGFBP7), Tissue inhibitor of metalloproteinases–2 (TIMP-2), β-trace protein (BTP) and enzymes like N-acetyl-β-D-glucosaminidase (NAG),

Glutathione-s-transferase (GST), Gamma-glutamyl transpeptidase (GGT) Alanineaminopeptidase (AAP) and Lactate dehydrogenase (LDH).

Markers for CKD
β-trace protein (BTP), Neutrophil gelatinase-associated lipocalin (NGAL), Liver-type fatty acid–binding protein (L-FABP), Symmetric dimethylarginine (SDMA), Fibroblast growth factor 23, uRBP4 etc.

The important attributes of the renal biomarkers enlisted above is discussed below in the light of various research findings on renal biomarkers.

Cystatin C
Cystatin C is a member of the cystatin superfamily of low-molecular-weight proteins that inhibit the activity of cysteine protease (Nakata et al. 2010) [8]. Detection of cystatin C in urine is an indicator of renal dysfunction as a result of tubular damage as in healthy animals, cystatin C is reabsorbed in the tubules. The advantages ascribed to the employment of Cystatin as a biomarker is that, it is more sensitive to detect AKI in the initial stages before elevation of serum creatinine especially in cases of drug induced AKI. Sasaki et al. (2014) [9] found urinary cystatin C to be the most sensitive index of kidney injury in dogs with gentamicin-induced acute kidney injury. The authors observed elevation of urinary cystatin C well before the elevation of creatinine and BUN values. Almy et al. (2002) [10] suggested that serum cystatin C as an alternative to serum creatinine for screening dogs with decreased GFR due to chronic renal failure. The authors carried out an immunoturbidimetric assay to detect cystatin C in 25 clinically healthy dogs and 25 dogs with renal failure. From their assay, the authors reported that the mean cystatin C concentration in dogs with renal failure was higher (4.37 +/- 1.79 mg/L) compared to the healthy dogs (1.08 +/- 0.16 mg/L).

Protein Biomarkers
Proteinuria is an indication of renal dysfunction, as protein are not excreted in significant quantities in healthy animals but they are filtered and reabsorbed back into the system. In addition to detecting renal involvement, identification of the type of protein excreted gives insight into the site of damage, whether it is in the glomeruli or tubule; which segment of the tubule and the severity of renal pathology. Jacob et al. (2005) [11] reported that an initial UP: C ≥ 1.0 in dogs with CRF was associated with greater risk of development of uremic crises and death, compared with dogs with UP: C < 1.0.

The various protein biomarkers employed in clinical and experimental studies for diagnosis of renal dysfunction and for assessment of the progression and severity of renal damage include Neutrophil gelatinase-associated lipocalin-NGAL, Albuminuria, C-reactive protein, Clusterin, and Inflammatory proteins viz. Urinary interleukin 2 (IL-2)and interleukin 8 (IL-8); Vitamin D Binding protein (VDBP); High molecular weight proteins viz. Tamm-Horsfall Protein, Immunoglobulin G; Low molecular weight proteins viz. Retinol binding protein – RBP, α1 and β2-microglobulins.

Neutrophil gelatinase-associated lipocalin - NGAL
NGAL is a protein belonging to the lipocalin superfamily. Neutrophil gelatinase-associated lipocalin (NGAL) is being used as a biomarker in much of the clinical and experimental

studies in humans. In canines, the role of NGAL has been studied by few researchers in both AKI and CKD cases. Bennett (2008) [12] reported urine NGAL as a powerful early biomarker of AKI that precedes the increase in serum creatinine by several hours to days. Lee et al. (2012) [13] showed an association between an increase in urinary NGAL and the development of AKI in dogs after surgery of different types. The significant increase of NGAL in urine was detected as early as 12 h after surgery, much earlier than the increase in serum creatinine. Palm et al. (2012) [14] also reported that urinary NGAL concentrations increased earlier than a detectable elevation of creatinine in AKI dogs.

Segev et al. (2013) [15] reported urine NGAL-creatinine ratio (UNCR) as a sensitive and specific marker of naturally occurring AKI in dogs and proposed it as a screening test for patients at risk for AKI and to diagnose AKI early in its course when the injury is mild (IRIS AKI Grade I). Hsu et al. (2014) [16] reported that death was associated with significantly higher sNGAL and uNGAL concentrations compared with survivors Among CKD dogs. Scheemaeker (2020) [17] established that uNGAL was more sensitive than the routine functional biomarkers Cr and the presence of Hb did not interfere with the measurement of NGAL.

Hence NGAL apart from its promising role as a sensitive renal marker to diagnose renal dysfunction in the initial stages, it can be well used as a prognostic indicator in CKD dogs. Further as NGAL can be estimated from urine, it can be used as an ideal non invasive marker to study the concentrations of NGAL on a large population of AKI and CKD dogs.

Clusterin
Clusterin is a glycoprotein. Zhou et al. (2014) [18] stated that clusterin was the most sensitive biomarker for detection of gentamicin-induced renal proximal tubular toxicity when used along with NGAL.

Retinol binding protein (RBP)
RBP is a protein synthesised in the liver. Excretion of this protein is suggestive of renal dysfunction as they are not significantly observed in the urine of healthy dogs. Forterre et al. (2004) [19] suggested uRBP as a sensitive marker of proximal tubule dysfunction in dogs. Smets et al. (2010) [20] stated that uRBP/c was significantly higher in dogs with CKD compared with healthy controls and the values were in correlation to sCr, BUN, UPC and IRIS-stage. Nabity et al. (2011) [21] also stated that the measurement of uRBP might be clinically useful for the early detection as well as monitoring of CKD in dogs.

Albuminuria
Albumin is a protein produced by the liver. Albumin is not normally present in large quantities in urine because of glomerular filteration and tubular reabsorption. Therefore, albuminuria is an indicator of renal dysfunction due to glomerular or tubular damage.

Proteinuria is generally detected by Chemical analysis of urine by Robert's test by observing the white ring at the interface of Robert's reagent and urine and by urine dipsticks. However in certain cases protein may be excreted in lower amounts which evade detection by urine dipstick method which is referred as microalbuminuria. Microalbuminuria is defined as an albumin concentration of 1–30 mg/dl in urine normalised to a specific gravity of 1010 (Lees 2004) [22].

Though microalbuminuria may be affected by non renal factors like cardiac involvement and urinary tract infection other than renal dysfunction, microalbuminuria can be screened in renal disorder cases as it is a sensitive indicator showing elevation even before the elevation of serum creatinine values.

C reactive protein (CRP)
CRP is a protein, serum elevation of which is an indicator of inflammation within the body. Maddens *et al.* (2010) [23] reported finding of CRP in urine as a result of glomerular dysfunction. The authors suggested CRP as a promising early biomarker for kidney injury or dysfunction as increase in serum CRP concentrations are observed even before the development of azotemia and proteinuria in dogs.

Microglobulins
α1 and β2-microglobulins are low molecular weight proteins with the potential to be used as markers of tubular injury.

Tamm-Horsfall Protein
Tamm-Horsfall protein is synthesized in the thick ascending loop of Henle and the proximal part of the collecting tubule in the kidneys of healthy dogs. But its synthesis is reduced when the nephrons are affected. Hence lowered expression of this protein in urine of dogs in renal disorders is indicative of tubular damage. As most of the AKI is caused by tubular damage, Tamm-Horsfall protein can be a promising marker to identify and assess AKI in dogs. Raila *et al.* (2014) [24] reported a dramatic reduction or even the absence of THP expression in urine with the progression of renal disease and the decrease occurred mainly in dogs with stage 3 or 4 CKD. Chacar (2017) [25] stated that urinary expression of vitamin D-binding protein (VDBP) and RBP can be used as markers of early kidney injury (stage 1 or 2 CKD), whereas low urinary expression of THP can be associated with advanced-stage renal disease (stage 3 or 4 CKD) and serve as a marker of CKD progression in dogs.

Urinary enzymes as renal biomarkers
Various enzymes like N-acetyl-β-D-glucosaminidase (NAG), γ-glutamyl transferase (GGT), alkaline phosphatase (AP), Glutathione-s-transferase (GST), alanine aminopeptidase (AAP), and lactate dehydrogenase (LDH) are probable candidates for employment as renal biomarkers. When these enzymes are excreted in significant quantities in urine they serve an indicator of damage to the renal tubules. The tubules are metabolically active portion of the renal nephrons lodged with many enzymes and hence during renal insult, the tubules are more vulnerable to get affected and subsequently the tubular enzymes are excreted in the urine. However to have a more reliable clinical significance of the excretion of these enzymes in urine, the quantity of enzyme excreted in urine is compared with the quantity of creatinine excreted in urine.
N-acetyl-β-D-glucosaminidase (NAG), is a lysosomal enzyme located in the renal tubules and the elevation of NAG is sensitive to detect renal dysfunction in early stages as it tends to be expressed even before the elevation of serum creatinine. Smets *et al.* (2010) [20] reported that the increase in uNAG was significantly higher in CKD dogs and dogs with pyelonephritis compared to dogs with uncomplicated urinary tract infection (UTI) which had unelevated uNAG values. Nabity *et al.* (2012) [26] suggested that NAG might be a useful marker of early kidney disease, as it was the only marker in

their findings that significantly increased even before elevation in urine protein:creatinine ratio. Heiene *et al.* (1991) [27] reported that increased activity of the Alkaline phosphatase enzyme in urine has been associated with proximal tubular damage in dogs.
Drug induced renal damage is an issue of grave concern in canine practice, as the various drugs used as antibiotics, pain killers and anaesthetics cause irreversible damage to the tubules and serve up to be a major cause of AKI. Greco (1985) [28] stated that measurement of Urine GGT activity was a more sensitive and reliable method of assessing acute renal tubular damage induced by gentamicin than serum creatinine concentrations or 24-hour endogenous creatinine clearance. Rivers (1996) [29] reported from their research, an increase in urine GGT-to-creatinine ratios on administration of nephrotoxic dosage of gentamicin in dogs even before the elevation in serum creatinine, urine specific gravity, and urine protein-to-creatinine ratio.

Emerging renal biomarkers
Much of the literature on renal biomarkers reveal Symmetric dimethylarginine (SDMA), Asymmetric Dimethylarginine (ADMA,) Kidney injury molecule-1 (KIM-1), Liver-type fatty acid-binding protein (LFABP), Interleukin-18 and β-Trace Protein as more promising and emerging biomarkers in human and canine renal disorders. SDMA is methylated form of arginine and is expected to be an early indicator of renal damage. Nabity *et al.* (2015) [30] reported SDMA as an earlier marker of kidney dysfunction than creatinine in dogs. The authors reported that it identified decreased renal function earlier than sCr and GFR. Polzin (2016) [31] also reported that SDMA increases earlier than creatinine and can identify a GFR decline as early as a 30%.
KIM-1 is a type 1 transmembrane protein and is an early indicator of tubular damage. As most of the AKI arise due to tubular damage than glomerular impairment, KIM can be a better marker in diagnosis of AKI than creatinine which is mostly an indicator of glomerular filteration rate. Vaidya *et al*. (2006) [32] from their research findings on experimental rodent model, proposed that urinary Kim-1 levels can serve as a noninvasive, rapid, sensitive, reproducible, and potentially high-throughput method to detect early kidney injury.

Conclusion
Research on novel renal biomarkers in experimental models and its application in clinical settings is the need of the hour in veterinary field as more and more canine cases are presented with renal issues in recent times. Serum creatinine has been used as a reliable marker for many decades by practitioners as well as institutions. However creatinine fails in identifying the early phases of renal damage. Subsequently, dogs are often presented mostly in the stages in which the damage to the kidneys has been very severe and irreversible. In such cases, therapeutic intervention becomes futile. This has created the inevitability of employing novel renal biomarkers in diagnosis of renal disorders. However, no single marker can be solely employed as a best above all as each biomarker discussed in this paper have their own advantages and limitations. Hence a panel of renal biomarkers along with other tests like renal biopsy, urine culture, imaging tools can be designed judiciously for each case definitely along with the traditional marker creatinine to have better insights into the pathology of renal damage in canine renal disorders.

References

1. Pelander L, Ljungvall I, Egenvall A, Syme H *et al*. Incidence of and mortality from kidney disease in over 600,000 insured Swedish dogs. Veterinary Record, 2015, 1-7
2. Cowgill LD, Langston C. Acute kidney insufficiency. In: Bartges J, Polzin DJ (eds.): Nephrology and Urology of Small Animals. Wiley-Blackwell, Ames, Iowa, 2011, 472-573.
3. Kerl M, Cook C. Glomerular filtration rate and renal scintigraphy. Clin Tech Small Anim Pract. 2005; 20(1):31-38.
4. Polzin DJ. Chronic kidney disease in small animals. Veterinary Clinics of North America Small Animal Practice. 2011; 41(15-30).
5. Peake M, Whiting M. Measurement of serum creatinine–current status and future goals. Clinical biochemist reviews. 2006; 27(4):173.
6. Polzin DJ, Cowgill LD. Chronic Kidney Disease. An Issue of Veterinary Clinics of North America: Small Animal Practice, E-Book. Elsevier Health Sciences. 2016; 46(6).
7. Atkinson Jr AJ, Colburn WA, DeGruttola VG, DeMets DL *et al*. Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. J Clin Pharm Ther. 2001; 69(3):89-95.
8. Nakata J, Nakahari A, Takahashi C, Chikazawa S *et al*. Molecular cloning, expression in Escherichia coli, and development of monoclonal antibodies to feline cystatin C. Veterinary immunology and immunopathology. 2010; 138(3):231-234.
9. Sasaki A, Sasaki Y, Iwama R, Shimamura S *et al*. Comparison of renal biomarkers with glomerular filtration rate in susceptibility to the detection of gentamicin-induced acute kidney injury in dogs. Journal of comparative pathology. 2014; 151(2-3):264-270.
10. Almy FS, Christopher MM, King DP, Brown SA. Evaluation of cystatin C as an endogenous marker of glomerular filtration rate in dogs. J Vet Intern Med. 2002; 16(1):45-51.
11. Jacob F, Polzin DJ, Osborne CA, Neaton JD *et al*. Evaluation of the association between initial proteinuria and morbidity rate or death in dogs with naturally occurring chronic renal failure. J Am. Vet. Med. Assoc. 2002; 226:393-400
12. Bennett M, Dent CL, Ma Q, Dastrala S *et al*. Urine NGAL predicts severity of acute kidney injury after cardiac surgery: a prospective study. Clin J Am Soc Nephrol. 2008; 3(3):665-673.
13. Lee YJ, Hu YY, Lin YS, Chang CT *et al*. Urine neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute canine kidney injury. BMC veterinary research, 2012; 8(1):248.
14. Palm C, Westropp J, LeRoy B, Segev G *et al*. Urinary NGAL: a Biomarker for Early Identification of Acute Kidney Injury in Dogs. J Vet Intern Med. 2012; 26(3):799.
15. Segev G, Palm C, LeRoy B, Cowgill LD *et al*. Evaluation of neutrophil gelatinase-associated lipocalin as a marker of kidney injury in dogs. Journal of veterinary internal medicine. 2013; 27(6):1362-1367.
16. Hsu WL, Lin YS, Hu YY, Wong ML *et al*. Neutrophil Gelatinase–Associated Lipocalin in Dogs with Naturally Occurring Renal Diseases. Journal of veterinary internal medicine. 2014; 28(2):437-442.
17. Scheemaeker S, Meyer E, Schoeman JP, Defauw P *et al*. Urinary neutrophil gelatinase-associated lipocalin as an early biomarker for acute kidney injury in dogs. The Veterinary Journal. 2020, 105423.
18. Zhou X, Ma B, Lin Z, Qu Z *et al*. Evaluation of the usefulness of novel biomarkers for drug-induced acute kidney injury in beagle dogs. Toxicology and applied pharmacology. 2014; 280(1):30-35.
19. Forterre S, Raila J, Schweigert FJ. Protein profiling of urine from dogs with renal disease using ProteinChip analysis. J Vet Diagn Invest. 2004; 16:271-277.
20. Smets PM, Meyer E, Maddens BE, Duchateau L, Daminet S. Urinary markers in healthy young and aged dogs and dogs with chronic kidney disease. Journal of Veterinary Internal Medicine. 2010; 24:65-72.
21. Nabity MB, Lees GE, Dangott LJ, Cianciolo R *et al*. Proteomic analysis of urine from male dogs during early stages of tubulointerstitial injury in canine model of progressive glomerular disease. Veterinary Clinical Pathology. 2011; 40:222-236.
22. Lees GE. Early diagnosis of renal disease and renal failure. Veterinary Clinics of North America Small Animal Practice. 2004; 34:867-885.
23. Maddens B, Daminet S, Smets P, Meyer E. Escherichia coli pyometra induces transient glomerular and tubular dysfunction in dogs. Journal of Veterinary Internal Medicine. 2010; 24:1263-1270.
24. Raila J, Schweigert FJ, Kohn B. Relationship between urinary Tamm-Horsfall protein excretion and renal function in dogs with naturally occurring renal disease. Vet. Clin. Pathol. 2014; 43:261-265.
25. Chacar F, Kogika M, Sanches TR, Caragelasco D *et al*. Urinary Tamm-Horsfall protein, albumin, vitamin D-binding protein, and retinol-binding protein as early biomarkers of chronic kidney disease in dogs, Physiol Rep. 2017; 5(11):e13262.
26. Nabity MB, Lees GE, Cianciolo R, Boggess MM *et al*. Urinary biomarkers of renal disease in dogs with X-linked hereditary nephropathy. Journal of Veterinary Internal Medicine. 2012; 26:282-293.
27. Heiene R, Biewenga WJ, Koeman JP. Urinary alkaline phosphatase and g-glutamyl transferase as indicators of acute renal damage in dogs. Journal of Small Animal Practice. 1991; 32:521-524.
28. Greco DS, Turnwald GH, Adams R, Gossett KA *et al*. Urinary gamma-glutamyl transpeptidase activity in dogs with gentamicin-induced nephrotoxicity. American Journal of Veterinary Research. 1985; 46(11):2332-2335.
29. Rivers BJ, Walter PA, O'Brien TD, King VL *et al*. Evaluation of urine gamma-glutamyl transpeptidase-to-creatinine ratio as a diagnostic tool in an experimental model of aminoglycoside-induced acute renal failure in the dog. Journal of the American Animal Hospital Association, 1996; 32(4):323-336.
30. Nabity MB, Lees GE, Bogges MM *et al*. Symmetric dimethylarginine assay validation, stability, and evaluation as a marker for the early detection of chronic kidney disease in dogs. J Vet Intern Med. 2015; 29:1036-1044.
31. Polzin DJ, Cowgill LD. Chronic Kidney Disease. An Issue of Veterinary Clinics of North America: Small Animal Practice, E-Book. Elsevier Health Sciences.

2016; 46(6).
32. Vaidya VS, Ramirez V, Ichimura T, Bobadilla NA, Bonventre JV. Urinary kidney injury molecule-1: a sensitive quantitative biomarker for early detection of kidney tubular injury. American Journal of Physiology-Renal Physiology. 2006; 290(2):F517-F529.