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Correlation between renal ultrasonography and serum cystatin C in acute kidney disease of critically ill dogs

[Correlação entre a ultrassonografia e a determinação da cistatina C sérica em cães admitidos em unidade de terapia]

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ABSTRACT

Acute kidney injury (AKI) is defined as the rapid decline in kidney function. Its development is related to critical clinical statuses, such as sepsis, complicated post-surgical recovery, and infectious diseases. Serum cystatin C (CysC) has the best correlation with the glomerular filtration rate. Ultrasonography stands out because it is highly accessible and can be done at the bedside. Twenty-eight dogs admitted to the intensive care unit with serum creatinine values <1.6 mg/dL and at-risk factors of AKI development were selected. CysC measurements and ultrasound assessments were performed daily for 72 hours. Using CysC dosage, 22/28 animals (78.6%) were considered to have AKI, and 17/22 had ultrasound compatible with AKI changes, demonstrating moderate agreement with CysC dosage. Increased cortical renal echogenicity is the most prevalent alteration in critically ill patients and is correlated with serum increases in CysC and is associated with renal structural damage.

Keywords: biomarkers, diagnosis, nephropathy, kidney, urine

RESUMO

A injúria renal aguda (IRA) é definida como o declínio rápido da função renal. Seu desenvolvimento está relacionado a quadros clínicos críticos, como sepse, pós-operatório complicado e doenças infecciosas. A cistatina C sérica (CisC) tem a melhor correlação com a taxa de filtração glomerular. A ultrassonografia se destaca por ser altamente acessível e pode ser realizada à beira do leito. Foram selecionados 28 cães, internados em unidade de terapia intensiva, com valores de creatinina sérica <1,6mg/dL e fatores de risco para o desenvolvimento de IRA. Medições de CisC e avaliações ultrassonográficas foram realizadas diariamente por 72 horas. Utilizando-se a dosagem de CisC, 22/28 animais (78,6%) foram considerados portadores de IRA e 17/22 apresentaram ultrassom compatível com alterações de IRA, demonstrando concordância moderada com a dosagem de CisC. O aumento da ecogenicidade cortical renal é a alteração mais prevalente em pacientes críticos, está correlacionado com aumentos séricos de CisC e associado a dano estrutural renal.

Palavras-chave: Biomarcador renal, diagnóstico precoce, nefropatia, rim, urina

INTRODUCTION

Acute kidney injury (AKI) is defined as the rapid decline in renal function, with a reduction in the glomerular filtration rate, which leads to serum retention of nitrogenous residues (Singh *et al.*, 2012). The IRIS (International Renal Interest Society) defines AKI as a spectrum of kidney damage, ranging from a mild loss of nephrons, clinically non apparent, to severe acute kidney failure.

Aiming to standardize the definition, facilitate early detection, assist in treatment, and provide a prognosis, IRIS (Grading…, 2016) proposed a system that classifies the injury in five stages, based on the serum concentration of creatinine,

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urine output or both. In this criterion, serum creatinine increases, changes in urinary flow, or both identify renal dysfunction. Serum creatinine values less than 1.6mg/dL characterized stage 1, along with the presence of one factor that represents AKI: altered image, urinary output less than 1mL/kg/h, or progressive increase of at least 0, 3mg/dL of creatinine in 48 hours. Various levels in serum creatinine differentiate among stages two to five.

Early diagnosis of AKI favors implementing strategies to reduce the morbidity and mortality of affected patients; however, it still represents a challenge in veterinary medicine (Coca *et al*., 2008). Serum cystatin C (CysC) stands out for having the best correlation with the Glomerular Filtration Rate (GFR) (Miyagawa *et al*., 2021), being less influenced by extra-renal factors and for being earlier in comparison to serum creatinine, being considered an excellent early marker of AKI (Souza *et al*., 2018).

Critical clinical status, iatrogenic causes, sepsis, complicated post-surgery recovery, and infectious diseases stand out among the risk factors associated with the development of AKI (Mugford *et al*., 2013).

Ultrasonography is an indispensable part of evaluating canine patients with suspected renal dysfunction (O'Neill, 2014; Pennick and D'Anjou, 2015). Several studies show the accuracy of ultrasonography in assessing renal disorders, demonstrating high sensitivity despite its low to moderate specificity (Santos *et al.*, 2013; Bragato *et al*., 2017). Therefore, it is considered the modality of image examination of choice in the emergency (Pennick and D'Anjou, 2015).

Structural chances are observed on ultrasound examination: hyperechogenicity characterizes an increase in renal cortical echogenicity. The increase in cortical thickness refers to the rise in the relationship between the two regions, cortical and medullary. Serial ultrasound examinations are necessary to determine these changes. The medullary signal reflects a hyperechogenic line's observation at the cortical medullary transition (Pennick and D'Anjou, 2015).

This work aims to identify the renal ultrasound changes and correlate them with renal dysfunction established through the CysC biomarker in critically ill dogs admitted to the Intensive Care Unit (ICU) of a Veterinary Hospital.

MATERIAL AND METHODS

This project was approved by the Committee on Animal Research and Ethics of the Federal University of Minas Gerais (CEUA UFMG) under protocol number 56/2015.

Twenty-eight dogs of different breeds, sex, and age admitted to a Veterinary Hospital ICU were evaluated, which presented serum creatinine values <1.6mg/dl and were considered at risk factors for AKI development. Animals with serum creatinine values > 1.6mg/dl or with chronic kidney disease (CKD) at the time of admission to the ICU were excluded from this study.

CysC was measured 24, 48 and 72 hours after admission to the Veterinary Hospital using an immunoturbidimetric method (Cystatin C Turbiquest Plus- Labtest®) previously calibrated with purified canine CysC (BioVendor Research and Diagnostic products). The reference range used was 0.57mg/L to 1.2mg/L according to (Souza *et al.*, 2018).

Renal ultrasound examinations were performed daily for 72 hours, always by the same examiner, using the ESAOTE MY LAB 40 (Chagrin Falls, Ohio, United States of America) device according to Pennick and D'Anjou (2015) who described the technique adopted for renal ultrasound evaluation. In the kidneys, size, renal contour, cortical echogenicity, cortical thickness, and differentiation and corticomedullary relationship were established.

The renal ultrasound changes observed were described and correlated to the different stages of AKI classified by the biomarker CysC by Kappa coefficient. Every ultrasound change observed during the 72 hours of study was considered as indicative of kidney injury. Animals that had CysC values above the reference range at any of the three times were also considered as kidney injury.

RESULTS

From the 28 dogs evaluated, 42.9% (12/28) were males and 57.1% (16/28) females. The mean age was 7.9±4.14 years, with a range of 2 to 16 years and an average weight of 12.57±11.07kg. There was no relationship between individual characteristics and AKI evelopment. There were also no significant correlations between age, sex, weight, and ultrasonographic changes (p> 0.05).

Of the 28 animals evaluated, 22 (78.5%) showed an increase in CysC values during the 72 hours of clinical follow-up. At 24 hours, nine animals showed values above the pre-established reference value. At 48 hours, another nine animals had increased values, and four animals had an increase in CysC 72 hours after admission.

Comparing the ultrasound findings with CysC, it was observed that among the 22 animals with an increase in this analyte, 17 (77.2%) presented structural ultrasound changes (Table 1).

Table 1. Results of serum CysC and ultrasound changes in critical ill dogs while in the intensive care unit of a Veterinary Hospital

	Cys C*			Ultrasound findings
Animal	24 hours	48 hours	72 hours	
1	1.27	1.32	†	hypereco
2	1.23	1.28	1.04	hypereco + loss of definition
3	0.95	0.99	0.92	normal
4	1.51	0.96	1.03	hypereco
5	1.11	1.29	1.33	increase in size + hypereco
6	1.42	1.28	1.24	increase in size + hypereco
7	1.19	1.09	1.20	normal
8	1.42	1.48	†	hypereco + loss of definition
9	1.38	1.71	1.46	hipererecoico + increase in thickness
10	1.60	1.28	†	hypereco
11	1.10	3.61	0.71	hypereco
12	0.84	0.80	1.33	normal
13	1.52	1.69	1.85	normal
14	1.19	1.37	1.42	normal
15	1.16	1.30	†	hypereco + medullary sign
16	1.40	1.34	†	hypereco + increase in thickness
17	1.18	1.28	†	hypereco
18	1.33	1.43	†	hypereco + increase in thickness
19	1.10	1.24	†	normal
20	1.39	1.42	†	hypereco + increase in thickness
21	0.98	1.35	1.10	hypereco + medullary sign
22	1.11	1.27	1.32	hypereco + medullary sign
23	1.24	1.22	†	normal
24	1.22	1.31	†	normal
25	1.28	1.40	†	increase in size + hypereco
26	0.98	1.38	1.45	hypoeco
27	0.75	0.77	1.39	hypereco
28	0.95	1.55	1.35	normal

* CysC reference range 1.29mg/L (Souza *et al*., 2018). HYPERECO- hyperechogenicity, HYPOECO- hypoechogenicity. † refers to no dosage because of death.

The renal ultrasound characteristics observed varied from hyperechogenicity (89.5%), increased cortical thickness (23.5%), change in size (17.6%), medullary sign (17.6%), reduced corticomedullary differentiation (11.8%) to cortical hypoechogenicity (10.5%). Some

animals showed more than one alteration, as described in Table 2. Nine (32%) animals had no changes during ultrasound exams.

The result obtained showed an agreement percentage of 75% and a Free-marginal kappa index of 0.50, which indicates moderate agreement between the two methodologies.

Table 2. Percentage of AKI detection due to ultrasound changes in dogs with severe diseases in an intensive care unit of a Veterinary Hospital

Ultrasound findings	Percentage (%)
Cortical Hypoechogenicity	10.5 (2/19)
Cortical Hyperechogenicity	89.5 (17/19)
Hyperechogenicity	29.4 (5/17)
Increase in cortical tickness + Hyperechogenicity	23.5 (4/17)
Increase in size+ Hyperechogenicity	17.6 (3/17)
Medullary renal sign + Hyperechogenicity	17.6 (3/17)
Loss of cortico-medullary differentiation + Hyperechogenicity	11.8 (2/17)

DISCUSSION

The comparison between ultrasound changes and CysC suggests good sensitivity of ultrasound, despite the low specificity described in other studies (Keyserling *et al*., 2002). Quaia and Bertolotto (2002) found 62 to 77% sensitivity and 58 to 73% specificity, in addition to a positive predictive value of 92% for detecting morphologic changes in the renal parenchyma.

Renal ultrasound changes are estimated to occur in approximately 10% of patients with AKI (Rivera, 2014). However, Podoll *et al*. (2013) observed changes in 38% of patients. In the present study, 17 of the 22 (77.3%) animals classified as having AKI by CysC measurement presented renal ultrasound changes.

CysC is efficient and early in the diagnosis of AKI compared to creatinine (Segev *et al*., 2016, Souza *et al.*, 2018). The present study diagnosed kidney damage in nine (32.14%) animals in the first 24 hours after admission to the ICU, with eight (89%) showing ultrasound changes. Despite the high sensitivity of the ultrasound assessment evidenced in this study, the performance of a biomarker must also analyze its specificity. In this sense, this method could not distinguish specific pathologies, making the histological evaluation necessary for a definitive diagnosis. Therefore, this technique has low specificity, although it is essential for directing the collection of histological material (Keyserling *et al*., 2002; O'Neill, 2014). Burti *et al*. (2020) demonstrated that the number and severity of ultrasound changes are correlated with the severity of kidney degeneration lesions on histopathological examination. Therefore, even if the specific diagnosis is not achieved by the method, it brings enough information to guide future procedures (Carvalho *et al*., 2010).

Different sonographic changes were evidenced in this study (Table 2). Among the morphological alterations evaluated, there was a prevalence of cortical hyperechogenicity also described as the alteration most frequently found in animals and humans with acute or chronic renal dysfunction even though they are not specific. It correlates with several pathologies such as glomerulonephritis, acute tubular necrosis, and nephrocalcinosis. The literature considers that in cases of AKI, hyp-erechogenicity is due to the presence of inflammatory infiltrate, proteins, cylinders, and calcium and is directly related to the presence of interstitial changes and loss of renal function (Pennick and D'Anjou, 2015; Garreballah *et al*., 2015). The correlation between cortical hyperechogenicity, histological abnormalities, and increase in serum creatinine suggests that high-risk patients may have kidney damage (Araujo *et al*., 2010; Carvalho *et al.*, 2010; Bragato *et al*., 2017). Therefore, due to its high prevalence, cortical hyperechogenicity may represent a sentinel sign of renal dysfunction also

in those animals with mean serum creatinine values below 1.6 mg / dl if they have risk factors for the development of kidney injury.

Cortical hypoechogenicity occurred in two animals with high CysC values. This finding reflects the presence of interstitial edema, severe inflammatory processes, or ischemia and affects mainly patients with sepsis (Faubel *et al*., 2014).

Changes in renal size, such as renomegaly, are considered typical in patients with AKI, usually due to the presence of interstitial edema, inflammation, and renal congestion (Carvalho *et al*., 2010; Rivera, 2014; Pennick and D'Anjou, 2015). However, occurrence varies with the intensity, duration of the insult, and the degree of hydration of the patient (Schmidt, 2015). The study also encountered difficulties in renal size characterization due to the extensive reference interval proposed by the veterinary literature. These factors limit its application in clinical practice (D'Anjou and Pennick, 2015), partially because of the lack of knowledge about the average renal size for that individual initially. Therefore, serial renal assessment is essential to detect slight increases in size, in addition to serving as a prognostic indicator (O'Neill, 2014, Pennink and D'Anjou, 2015, Bragato *et al.*, 2017).

In this study, among animals with renomegaly, there was no increase in renal size above the reference values proposed by Barr *et al*. (1990). In addition, to increase the reliability of kidney measurements, the average was obtained from three longitudinal kidney measurements, which became an individual reference (Santos *et al.*, 2013; Bragato *et al.*, 2017). For the diagnosis of renomegaly, the size variation is compared to the initial value obtained, as suggested by the literature (Bragato *et al*., 2017). Renomegaly was observed in only three animals in this study, a lower frequency than that reported in the literature, and can be justified by the limitations mentioned above and by the acute and initial phase of the inflammatory process, when the kidneys may be of normal size (O'Neill, 2014; Rivera, 2014). Dogs with renomegaly also showed an increase in CysC, thus justifying an association between this alteration and the presence of kidney injury, in agreement with what was observed by Bragato *et al*. (2017) when evaluating dogs with nephrotoxic injury.

Interestingly, the increase in cortical thickness occurred in four animals in this study (Table 2). Cortical hypertrophy is related to the increased relationship between the cortex and the renal medulla and indicates edema associated with inflammation. Therefore, this parameter shows a diffuse variation in the size of the cortical region without direct enlargement of the kidney. This observation suggests that this change may be a good indicator for assessing the renal size since there is still no reliable way to determine this parameter in dogs accurately. In addition, this finding confirms the literature that points to the correlation between increased cortical thickness and the presence of acute kidney injury (Pennick and D'Anjou, 2015; Yamashita *et al*., 2015).

The ultrasound change called medullary renal sign was observed in three animals, which also had increased CysC. Although its occurrence is considered typical in healthy dogs and miniature breeds, the data obtained in this study show its correlation with kidney injury although it needs to be associated with clinical and laboratory data to assess its real clinical significance (Pennick and D'Anjou, 2015).

In this study, it was observed that two animals showed decreased cortico-medullary differentiation. These animals also showed serum CysC elevation. The loss of differentiation between the cortex and the renal medulla is an ultrasound change that can occur in dogs with AKI and CKD, however, due to different mechanisms (Pennick and D'Anjou, 2015). The analysis of this differentiation depends on the presence and intensity of renal injury in the cortical and medulla. Gareeballah *et al*. (2015), in a study on renal ultrasound changes in humans, observed that 85% of patients with AKI had a reduction in the cortico medullary differentiation. It is probable that the lower prevalence of animals with this alteration in this study reflects a lower intensity of renal insult, as well as the prevalence of glomerular lesions in dogs, which predominantly affect the cortical region and do not cause a loss in the differentiation between cortex and medulla (Mugford *et al*., 2013; Pennick and D'Anjou, 2015). The prevalence of glomerular lesions in dogs also justifies that the most common finding is cortical hyperechogenicity, which was observed in most animals evaluated in this study

(Table 2). However, it should not be used in isolation, as a form of diagnosis.

CONCLUSIONS

The increase in renal cortical echogenicity is the most prevalent alteration in critically ill patients and is correlated with serum CysC increases. Increased kidney size is an indicator of AKI in serial evaluations. Cortical renal hyperechogenicity, medullary renal sign, and decreased cortica- medullary differentiation are essential elements in diagnosing kidney injury.

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