Contrast enhancement ultrasound of renal perfusion in dogs with acute kidney injury

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ABSTRACT - To evaluate the efficacy of contrast-enhanced ultrasound to assess changes in renal perfu- sion in dogs with acute kidney injury.MATERIALS AND METHODS: The left kidney of each dog in two groups was examined using contrast- enhanced ultrasound: Group A consisted of 16 healthy dogs and Group B consisted of 12 dogs with acute kidney injury. All dogs in Group A showed the same sequence of wash-in and wash-out. In Group B the distribution of contrast media showed a similar cortical phase to healthy dogs, but a faster time to maximal medullary enhancement. Group B showed increased medullary peak intensity and medullary area under the curve compared to Group A. Both qualitative and quantitative analyses showed vascu- lar changes especially in the medulla, with more rapid medullary vascularisation and increased medul- lary perfusion. These results were interpreted as medullary congestion in dogs with acute kidney injury. Contrast-enhanced ultrasound represents an easy to perform, safe, and non- invasive method to detect changes in renal perfusion in dogs with acute kidney injury.

INTRODUCTION

Acute kidney injury (AKI) is a clinical syndrome with high mor- tality in both human and veterinary patients. AKI is defined as a rapid (usually <48 hours) reduction in renal function, which can be due to prerenal, renal or postrenal causes (Cowgill & Langs- ton 2011, Legatti *et al*. 2018). According to the International Renal Interest Society (IRIS), AKI is classified into five grades of severity, based on serum creatinine concentration. The patients in IRIS Stages 1 to 2 may be non- to mildly azotaemic, and may be very difficult to diagnose, if there are no other historical, bio- chemical or imaging abnormalities. In Stages 3 to 5, azotaemia, creatinine and oliguria/anuria progressively worsen with paren- chymal damage. Kidneys affected by AKI are always characterised by a reduction of capillary

blood flow and subsequent ischaemic, inflammatory and oxidative injury, leading to cellular necrosis (Cowgill & Langston 2011, Segev *et al*. 2013). Early diagnosis, and therefore commencement of treatment, is the most important factor for therapeutic success. Unfortunately, in veterinary medicine the diagnosis of AKI is often late, and almost never in the first two stages of the disease, during which patients are still non-azotaemic. It is more common to diagnose patients at later stages of AKI, when renal damage is irreparable (Ross 2011). As serum creatinine is not a very sensitive and specific marker of AKI, current research is focused on testing new serum and urinary markers (Lippi *et al*. 2018). Among different diagnostic tools, ultrasound of the urinary system plays a fundamental role in the diagnostic approach to AKI. Common ultrasound find- ings in dogs and cats with AKI are represented by increased renal size, increased cortical echogenicity, perirenal fluid, pyelectasia, increased echogenicity of the perirenal fat and abnormal echo- genicity of urine (Mugford *et al*. 2013, Cole *et al*. 2018). Contrast-enhanced ultrasonography (CEUS) is an innova- tive method applied to the study of tissue vascularisation, which provides both qualitative and quantitative analysis. Qualitative analysis gives a subjective evaluation of vascularisation by the study of contrast distribution, while quantitative analysis, based on specific software that transforms the brightness of the signal into a time/intensity curve, can derive objective parameters of vascularisation (Hoeffel *et al*. 2010, Dong *et al*. 2013). Renal perfusion imaging with a sonographic contrast agent was first reported in 1998 (Sehgal *et al*. 1998). Since then, many clinical investigations and experimental studies have shown that the use of micro-bubble agents in colour and power Doppler sonography can improve the detection and characterisation of various renal perfusion abnormalities (Kim *et al*. 2001, Schwenger *et al*. 2006, Schneider *et al*. 2011). In human patients with AKI, CEUS showed a higher accuracy in assessing kidney vascular abnormali- ties, compared to other ultrasound techniques, such as colour, power and pulsed Doppler (Gruenewald *et al*. 1999). In veterinary medicine, there have been several studies on CEUS in normal canine kidneys (Waller *et al*. 2007, Choi *et al*. 2016), in dogs with renal diseases (Haers & Saunders 2009, Haers *et al*. 2010, 2013) and with experimentally induced dis- eases (Dong *et al*. 2013, Lee *et al*. 2017). In an experimental canine model of chronic ischaemia, CEUS was able to identify changes in blood perfusion indexes, 4 to 7 weeks earlier than serum creatinine and BUN elevation (Dong *et al*. 2013). Given the close correlation between the changes in renal per- fusion and renal function, we carried out an ultrasonographic prospective study of renal perfusion with CEUS in dogs with AKI, before starting dialysis therapy. The aim of this study was to investigate whether contrast-enhanced ultrasonography can evaluate the changes in renal perfusion in dogs with AKI.

MATERIALS AND METHODS

Case selection - The study was conducted at the Veterinary Teaching Hospital "Mario Modenato" of the Department of Veterinary Science (University of Pisa), according to the European Animal Welfare regulation (Directive 2010/63/EU). Client-owned dogs were prospectively enrolled (Ethics Committee approval number 33BIS/15), and divided into two groups: Group A consisting of healthy dogs and Group B of dogs with AKI.

Group A was a homogenous group of 16 Labrador retriev- ers, nine males and seven females, mean bodyweight 28 kg, aged between 12 and 18 months old, mean age of 14 months. Physi- cal examination, clinical laboratory analyses including complete blood counts, serum biochemistry (total protein, albumin, globulin, urea, creatinine, glucose, cholesterol, total bilirubin, alkaline phosphatase, alanine aminotransferase, calcium, phos- phorus, potassium and sodium), urinalysis, glomerular filtration rate (GFR) and abdominal ultrasound were performed in each dog to assess their health status.

Group B was composed by 12 dogs with AKI, which were referred to the haemodialysis service of the Veterinary Teaching Hospital of Pisa University between December 2016 and Decem- ber 2017. This group included 12 dogs of different breeds, nine males and three females, aged between 4 months and 10 years (mean 5.6 years), and weight ranging from 5 to 35kg (mean weigth of 20 kg). One dog was in IRIS AKI Stage 3; eight in IRIS AKI Stage 4; and three in IRIS AKI Stage 5. Leptospirosis was diagnosed in five dogs and pyelonephritis and grape toxicity in two dogs each; in three dogs the cause of AKI was unknown (Table 1).

Physical examination, clinical laboratory analyses including complete blood counts, serum biochemistry profile (total pro- tein, albumin, globulin, urea, creatinine, glucose, cholesterol, total bilirubin, alkaline phosphatase, alanine aminotransferase, calcium, phosphorus, potassium and sodium), urinalysis, and abdominal ultrasound were performed in each dog. Dogs were considered affected by leptospirosis if there was at least a fourfold rise in paired serum MAT 2 weeks apart, or a single MAT >1:800 in non-vaccinated dogs (Schuller *et al*. 2015).

Ultrasound examination procedures and image analysisBefore ultrasound examination a 24G intravenous (IV) catheter was placed in the cephalic vein for contrast agent injection. Dogs were not sedated and were placed in right lateral recumbency. Hair over the ventral and lateral portions of the abdomen was clipped. Alcohol and coupling gel were applied to the skin. CEUS images were obtained

using the Aplio 400 Toshiba® ultrasound system (Toshiba) with a convex transducer (6-8 MHz), capable of harmonic imaging. Each CEUS study was conducted only on the left kidney because it was nearer to the probe and therefore more easily studied than the right kidney (Choi *et al*. 2016). The sagittal plane of the left kidney was imaged, and the trans- ducer was not subsequently moved. The contrast agent (Sono Vue®, Bracco Imaging) was administered at a rate of 0.03mL/ kg (IV bolus) and immediately followed by a 5 mL saline flush (0.9% NaCl). All ultrasonographic examinations were performed by the same two experienced operators (T.M. and S.C.). The mechanical index (MI) was set at 0.12 for minimal destruction of the micro bubbles, the gain was set at 70 to 74%, and the dynamic range was 66 to 72%. A focal zone was placed at the distal renal cortex. The kidney was scanned continuously for 90seconds after bolus injection of the contrast agent and RAW data (good-quality video clips) were stored digitally in the hard disc of the ultrasound scanner. The acquired dynamic cine loops were analysed using integrated software (CHI-Q, Toshiba). For qualitative evaluation, three perfusion stages of renal parenchyma (early arterial, cortical and medullary phases) were evaluated. For the arterial phase, the time from the injection to the arrival of the contrast medium in the interlobar arteries was recorded; for the cortical phase, the time from the injection to the maximum homogenous enhancement of the cortex; and for the medullary phase, the time from the injection to the maximum homogenous enhancement of the medulla. Finally, the contrast medium dis- tribution in the kidney at 90 seconds was recorded and classified into three pattern types: Type 1 was characterised by both cortical and medullary residual enhancement, Type 2 by mild cortical residual enhancement and Type 3 by no residual enhancement (Fig. 1). For the quantitative evaluations, regions of interest (ROIs) were manually positioned in the middle of the renal cortex and medulla at the caudal pole, which was the closest to the probe, therefore less mobile and the easiest site to study. The ROIs were kept in position with the motion compensation option ("Motion Tracking"). The program calculated the mean echo-intensity for each ROI area. The echo-intensity data within the renal cortex and medulla were plotted over time to produce time-intensity curves (TICs). The functional renal perfusion parameters evaluated from the TICs were peak intensity (PI), times to peak enhancement (TTPs), upslope and area under the curve (AUC). PI refers to the maximum enhancement of the renal cortex and medulla. TTP was defined as the TTP from the time of the con- trast agent injection. The upslope was obtained from the data points of the TIC between 10% above the baseline and 85% of the PI. The AUC indirectly measures the mean renal blood flow and was measured from the TIC.

STATISTICAL ANALYSIS - The Mann-Whitney test was used to compare all parameters between

groups, with the exception of the proportions at the end of the study, which were compared by the Chi-squared test. For statistical analysis the software Minitab 17.2.1 was employed. A difference was considered statistically significant for $P<0.05$.

RESULTS

B-mode ultrasound of the kidney - Dogs of Group A did not show renal ultrasound alterations. Group B subjects showed renal abnormalities in 10 of 12 dogs (83.3%), which included renomegaly (n=1), hyperechoic renal cortices (n=6), hyperechoic renal cortices and medulla (n=5), pel- vic dilatation (n=3), perirenal effusion (n=5).

Contrast-enhanced ultrasound of the kidney - The contrast-enhanced ultrasound technique was easy to per- form, and contrast enhancement was clearly visible in both healthy dogs and dogs with AKI.

In Group A dogs, there was a rapid contrast enhancement in interlobar arteries (arterial phase), followed by a homogenous cortical inflow, and then by a medullary wash-in Fig. 2. The con- trast enhancement of the medulla usually remained hypoechoic compared with the renal cortex in all dogs of Group A. The wash-out phase started first in the medulla followed by a cortical wash-out. Regarding the pattern appearance of the kidney after 90seconds, two of 16 (12.5%) dogs showed Type 1, 14 of 16 dogs (87.5%) showed Type 2.

In Group B the distribution of the contrast medium was altered in two dogs: in one dog the interlobar arteries of the cra- nial pole were not visible (Fig. 3), in another patient there was an early cortical wash-out compared to the medulla, and a central portion of the medulla remained hyperechogenic. After 90sec- onds, six of 12 dogs (50%) showed Type 1 contrast pattern, one of 12 dog (8.3%) Type 2 and five of 12 (41.7%) Type 3.

Table 2 shows the quantitative analysis of Groups A and B. A statistically significant difference was found between Groups A and B for cortico-medullary time (C-M T) (11.87 ± 2.91 vs 7.16 ± 2.48; $P<0.001$), for the medullary phase (27.12 ± 3.20 vs 21.75 ± 8.95; $P<0.01$), and for the contrast distribution at 90 sec- onds ($P<0.001$).

The results of further quantitative analysis of Groups A and B are shown in Table 3. A statistically significant difference was found between groups for medullary PI (1.05 ± 0.64 vs 3.33 ± 2.85; $P<0.01$) and for medullary AUC (51.06 ± 35.69 vs 127.1 ± 85; $P<0.001$) (Fig. 4).

DISCUSSION

In dogs, AKI is often diagnosed in advanced stages of acute renal failure, when there is an accumulation of uraemic toxins, an altered regulation of hydro-electrolytic and acid-base status and oliguria/anuria (Zatelli & Cowgill 2014). In our research we studied kidney perfusion using CEUS, evaluating vascularisation and measuring various perfusion indi- ces, in order to identify the pathological patterns in dogs with AKI. In this study, CEUS was a safe and easy to perform method to study renal vascularisation in AKI dogs. All studies were per- formed in conscious patients, with no need for sedation. No adverse effects were reported in either healthy or AKI dogs, thus confirming the safety of the methodology (Seiler *et al*. 2013). Qualitative analysis showed a difference between the two groups. In all dogs of Group A, the sequence and the appear- ance of the different phases were the same as reported previ- ously (Waller *et al*. 2007). In addition, the times recorded for the different phases coincided with previous reports, with mean values of arterial phase of 11.12 seconds ±1.78 seconds, 15.25seconds±2.49seconds for the cortical phase, and 27.12 seconds ±0.68 seconds for the medullary phase (Choi *et al*. 2016). In Group B the temporal sequence and the appearance of the phases were non-homogenous. The cortical-medullary time and medullary phase were statistically lower in AKI dogs, compared to those of healthy dogs. These differences may be due to a more rapid passage of blood from the cortex to the medullary vascular system (faster cortical wash-out). In AKI dogs, vascular abnor- malities seem to refer mainly to the medullary component of the kidney, with a faster medullary perfusion. This finding may be secondary to increased microvascular congestion of the outer medullary region during AKI, as previously reported in both murine models and human patients affected by ischaemic AKI (Mason *et al*. 1984, Sutton 2009). These studies showed vascular shunting from the cortex of the kidney to the medullary area (Mason *et al*. 1984; Sutton 2009). The renal pattern obtained at 90 seconds from the beginning of the examination also showed a statistical difference between the two groups of examined dogs. In Group A 87.5% of dogs showed a Type 2 pattern with mild cortical enhancement and complete medullary wash-out, whereas this pattern occurred only in one dog from Group B (8%). The remaining dogs in Group B showed cortical and medullary residual enhancement (50%) or a complete absence of residual contrast (42%). The significant difference in the renal pattern between AKI and healthy dogs suggests vascular alterations in dogs affected by AKI. Alterations of renal vascularisation may be characterised by different disorders, such as renal infarcts (as reported in one dog of Group B). Note that renal vascularisation is influenced not only by renal factors, but also by extra-renal circulatory factors such as cardiac output, systemic pressure and

hydration status (Choi *et al*. 2016). In healthy dogs, the time-related values, obtained from the TICs, did not differ from previous reports, and were more similar to Waller *et al*.'s (2007) results than those of Choi *et al*. (2016). The intensity-related values cannot be compared with previous studies because they are very much influenced by the setting of the instrument (Choi *et al*. 2016). In the statistical analysis, Group B showed an increase in med- ullary PI and a significant increase in the area under the medul- lary curve, which suggests increased medullary perfusion in dogs with AKI, compared to those of healthy dogs. This finding may confirm the hypothesis of an increased medullary congestion in AKI dogs, and consequent slowing of the blood flow (Mason *et al*. 1984). Our study has some limitations. Firstly, we examined only the left kidney, as CEUS cannot be performed on both kidneys simultaneously. However, it has been reported that contrast agent concentrations and GFR are not significantly different between the left and the right kidney (Choi *et al*. 2016). The second limitation may be the use of manual injection of the contrast medium instead of a syringe infusion. Although manual injec- tion might potentially predispose to lower accuracy in contrast infusion, the majority of studies used manual injection. No his- tological investigation was performed in any dog; it would have been interesting to compare the histopathological findings with the results of CEUS. In conclusion, to the best of our knowledge, this is the first study investigating renal vasculature in AKI dogs using CEUS. CEUS represents an easy, safe, non-invasive method that can detect changes in renal perfusion. In dogs with AKI, the examina- tion showed changes in the physiological wash-in and wash-out phases, and the quantitative study of TCI reported an increased medullary blood congestion, similar to previous human find- ings (Mason *et al*. 1984, Sutton 2009). As ultrasound findings, such as increased renal size and cortical echogenicity, dilatation of renal pelvis and perirenal effusion, have been reported as non- specific alterations in AKI patients (Holloway & O'Brien 2007), CEUS may be a helpful tool in the complex diagnostic approach to canine AKI.

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Figure and table

Subjet	Breed	Sex	Age	Weight (kg)	IRIS AKI grade	Urine production	Plasma creatinine (mg/dL)	Diagnosis
1	Mixed-breed	M	7 years	28	4	O	9	Unknown
2	Springer spaniel	M	5 years	20	5	O	10	Leptospirosis
3	Pitbull	M	5 years	24	4	O	8	Pyelonephritis
4	Labrador retriever	M	4 years	35	5	O	20	Leptospirosis
5	Dachshund	M	5 years	5	4	O	5.1	Unknown
6	Springer spaniel	F	10 years	18	3	O	4.7	Leptospirosis
7	English setter	F	2 years	15	5	O	11	Grape toxicity
8	Mixed breed	M	4 months	7	4	O	7	Leptospirosis
9	Pinscher	M	7 years	7	4	O	6.8	Pyelonephritis
10	Mixed breed	M	6 years	11	4	NO	9	Grape toxicity
11	Golden retriever	F	2 y	24	4	O	9	Leptospirosis
12	Golden retriever	M	7 y	32	4	O	10	Unknown

Urine production (O oliguric, NO non-oliguric)

Table 1. Signalament, IRIS AKI grade, urine production, plasma sìcreatininw and diagnosis of dogs of GROUP B

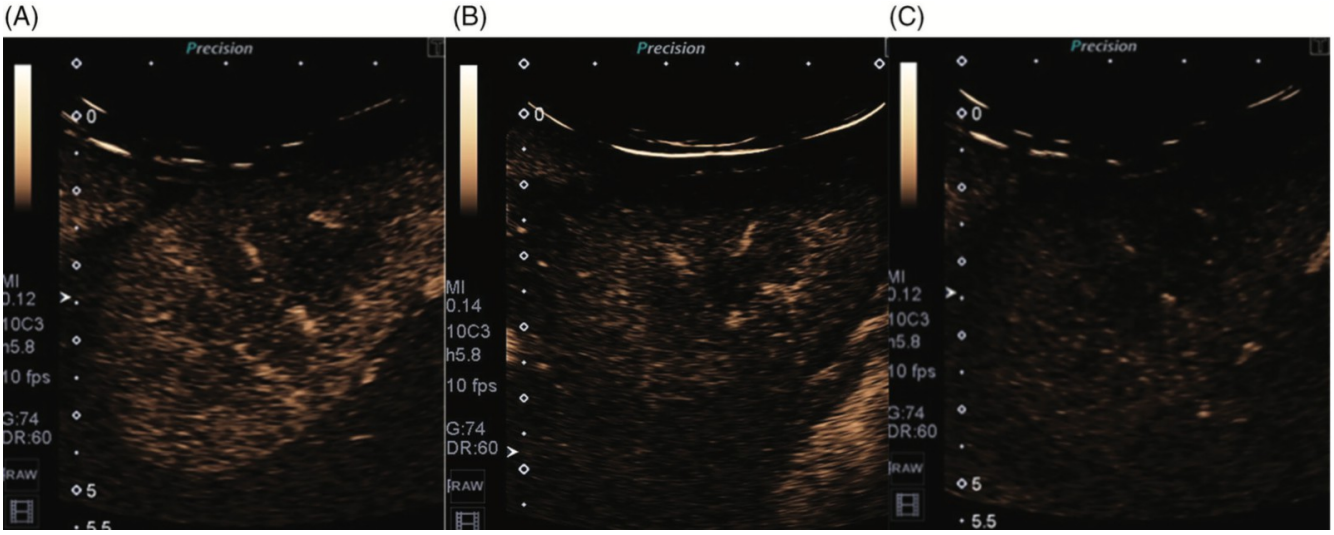

FIG 1. Longitudinal renal ultrasound scan showing the three different patterns at end study. (A) Type 1, characterised by cortical and medullary residual enhancement. (B) Type 2, only cortical enhancement. (C) Type 3, little or no residual enhancement

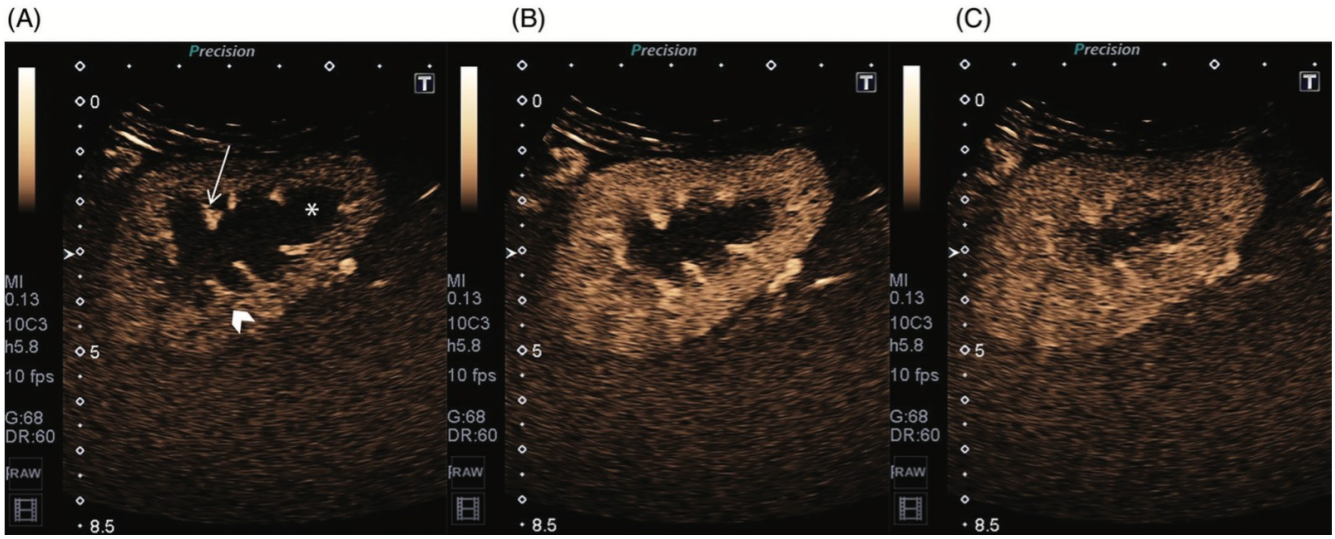

FIG 2. Contrast-enhanced ultrasonography images in a healthy dog (Group A): (A) 11 seconds postinjection, showing enhancement of the interlobar arteries (arterial phase). The interpyramidal columns with interlobar arteries (arrow), cortex (arrowhead) and the medulla (asterisk); are labelled; (B) 15seconds postinjection, showing enhancement in the renal cortex (cortical phase); (C) 27seconds postinjection showing enhancement in the renal medulla (medullary phase)

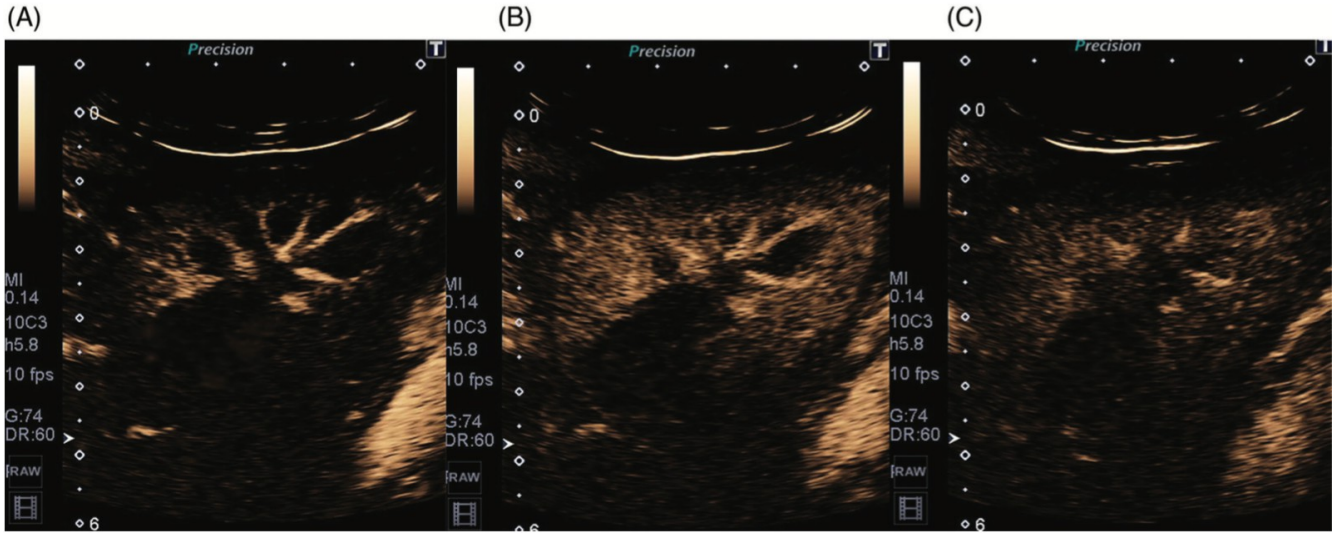

FIG 3. Contrast-enhanced ultrasound showed lack of perfusion referable to renal infarction involving a large portion of the cranial pole of a group B dog in arterial (A), cortical (B) and medullary (C) phases

	Group A (n=16)	Group B (n=12)
Arterial phase	11.12±1.78	11.33±6.10
A-C T	4.12±1.36	3.25±1.21
Cortical phase	15.25±2.49	14.58±7.05
C-M T	11.87±2.91	7.16±2.48*
Medullary phase	27.12±3.20	21.75±8.95**

A-C T time between the arterial phase and the cortical phase, C-M T time between the cortical phase and the medullary phase; *P<0.001. **P<0.01

Table 2. Mean and standard deviation of renal perfusione times in Group A (healty dogs) and Group B (dogs with AKI)

	A	B
Cortical PI	3.10±0.72	4.79±3.10
Medullary PI	1.05±0.64	3.33±2.85*
Cortical TTP	4.06±1.04	3.40±0.98
Medullary TTP	8.50±1.59	10.71±4.72
Cortical UPSLOPE	1.21±0.49	1.37±1.11
Medullary UPSLOPE	0.22±0.13	0.5±0.42
Cortical AUC	126.9±43	122.7±51.8
Medullary AUC	51.06±35.69	127.1±85**

PI Peak Intensity, TTP time to peak enhancement from contrast injection, AUC area under the curve; *P<0.001.**P<0.01

Table 3. Mean and standard deviation of renal perfusione parameters in Group A (healty dogs) and Group B (dogs with AKI)

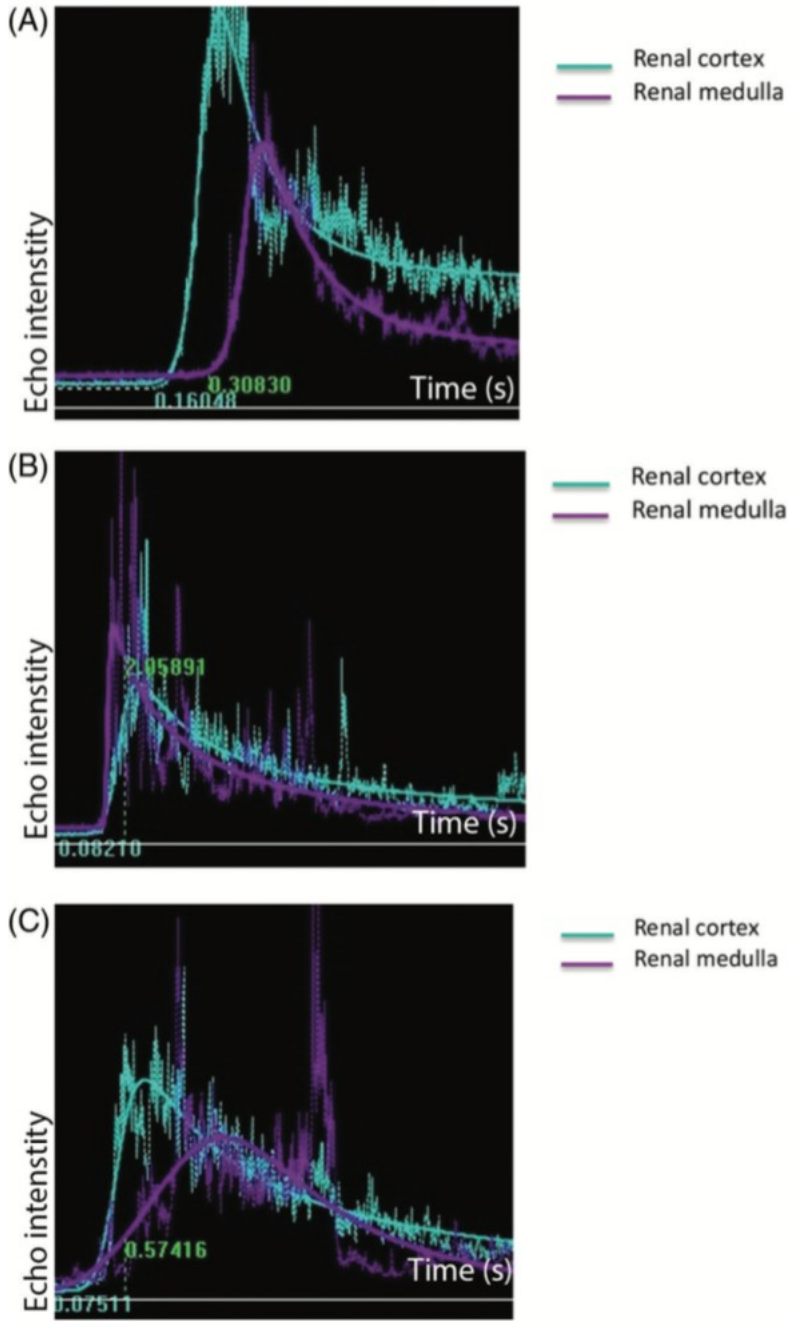

FIG 4. Time intensity curves of a healthy dog (A), dog with AKI in which is visible an important increase in medullary PI (B), dog with Acute kidney injury in which the increase of the medullary area under the curve is visible (C)