Contrast-Enhanced Ultrasound Examination for the Assessment of Renal Perfusion in Cats with Chronic Kidney Disease

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Background: Contrast-enhanced ultrasound examination (CEUS) is a functional imaging technique allowing noninvasive assessment of tissue perfusion. Studies in humans show that the technique holds great potential to be used in the diagnosis of chronic kidney disease (CKD). However, data in veterinary medicine are currently lacking.

Objectives: To evaluate renal perfusion using CEUS in cats with CKD.

Animals: Fourteen client-owned cats with CKD and 43 healthy control cats.

Methods: Prospective case-controlled clinical trial using CEUS to evaluate renal perfusion in cats with CKD compared to healthy control cats. Time-intensity curves were created, and perfusion parameters were calculated using off-line software. A linear mixed model was used to examine differences between perfusion parameters of cats with CKD and healthy cats.

Results: In cats with CKD, longer time to peak and shorter mean transit times were observed for the renal cortex. In contrast, a shorter time to peak and rise time were seen for the renal medulla. The findings for the renal cortex indicate decreased blood velocity and shorter total duration of enhancement, likely caused by increased vascular resistance in CKD. Increased blood velocity in the renal medulla has not been described before and may be because of a different response to regulatory factors in cortex and medulla.

Conclusions and Clinical Importance: Contrast-enhanced ultrasound examination was capable of detecting perfusion changes in cats with CKD. Further research is warranted to assess the diagnostic capabilities of CEUS in early stage of the disease process.

Key words: CKD; Contrast-enhanced ultrasound; Feline; Kidney.

Chronic kidney disease (CKD) is 1 of the most commonly diagnosed diseases in cats. The prevalence has been reported to be approximately 1–3% in the general feline population, even increasing to 30% in geriatric cats.1,2 Renal disease is reported to be the cause of death in 14–17% of geriatric cats.3 A primary, often unidentified trigger, initiates renal damage and nephron loss, which ultimately results in self-perpetuating renal injury and progressive renal disease.4 The most common histologic findings in cats with CKD are tubulointerstitial lesions such as tubular atrophy, interstitial inflammation, and fibrosis.5 Diagnosis at an early stage of the disease process allows timely institution of supportive treatment and slows the disease progress.6 Prompt diagnosis remains challenging. The diagnosis most often is based on increases in serum urea and creatinine concentrations combined with low urine specific gravity. These changes unfortunately are only present when a substantial portion of renal function already has been lost. Measurement of glomerular filtration rate (GFR) provides more accurate assessment of renal function, but requires multiple blood samples, limiting its use in routine clinical practice.8,9 Therefore, the search for improved noninvasive methods for the diagnosis of early renal disease is still ongoing.

Renal function is closely related to renal perfusion. Therefore, evaluation of renal perfusion could yield valuable information about kidney function and thus
facilitate earlier diagnosis. Performing an accurate and noninvasive measurement of renal perfusion is challenging. Dynamic high-field magnetic resonance imaging, contrast-enhanced computed tomography, or renal scintigraphy can be used to assess renal perfusion. In practice, clinical application of these techniques is limited by relatively high costs, limited availability, long examination times, and exposure to ionizing radiation. Doppler ultrasound examination allows calculation of perfusion indices that provide information about vascular resistance. These indices are increased in animals with renal disease, but are relatively nonspecific because they are influenced by several nonrenal factors. Moreover, Doppler ultrasound examination is limited to evaluation of macroperfusion because low-velocity flow in smaller vessels cannot be assessed.

Contrast-enhanced ultrasound examination (CEUS) is a functional imaging technique using microbubbles to enhance detection of tissue perfusion at the microvascular level. These bubbles are gas-filled spheres stabilized by an outer shell. They have a size similar to that of red blood cells and thus are restricted to the blood pool after IV injection. In cats, the literature currently is limited to the description of the normal perfusion pattern in healthy animals. In dogs, the perfusion pattern of focal mass lesions has been described, and quantitative CEUS in dogs with iatrogenically-induced hypercortisolism and ischemic renal disease has been described. Lower blood velocity was found in the dogs with ischemic renal disease, whereas an increase in renal blood volume was seen in dogs with hypercortisolism. Contrast-enhanced ultrasound examination has been shown to be a promising technique for the early diagnosis of renal disease in humans, but data on cats with current disease is lacking.

Our aim was to evaluate the efficacy of CEUS in the diagnosis of CKD in cats. Our hypothesis was that CEUS would be a practical, noninvasive technique to detect perfusion changes in cats with CKD.

Materials and Methods

The study was performed with approval of the local ethical committee of the Faculty of Veterinary Medicine, Ghent University, Belgium and the deontological committee of the Belgian Federal Agency for the Safety of the Food Chain (EC2015-68). All owners gave their full informed consent to participate in the study.

Subjects

Fourteen client-owned cats with CKD and 43 healthy control cats were included. The diagnosis of CKD was made before inclusion and was based on the presence of compatible clinical signs and laboratory findings such as increased serum creatinine concentration (>161.8 μmol/L) and decreased urine specific gravity (<1.035). Cats with clinically relevant concurrent systemic disease, hyperthyroidism, or urinary tract obstructions leading to postrenal azotemia were excluded. The cats with CKD were subdivided into 4 groups according to the classification of the International Renal Interest Society (IRIS). Cats without clinically relevant abnormalities in history, on physical examination, thoracic radiographs, routine abdominal ultrasound examination, CBC, serum biochemistry, and urinalysis were considered healthy. All medication or nutritional supplements, except for phosphorous binders in the CKD group, were withdrawn at least 14 days before inclusion.

Study Design

A thorough physical examination, including noninvasive measurement of blood pressure by Doppler ultrasonic technique and cervical palpation for scoring thyroid gland size (in cats >6 years), was performed. Blood testing consisted of CBC and serum biochemistry, including total thyroxine (TT4) serum concentration in cats >6 years of age. Urinalysis included urine specific gravity (USG), sediment analysis (as previously described), urinary protein/creatinine ratio (UPC), dipstick chemistry, and urine culture.

Thoracic radiographs (left-to-right lateral and ventrodorsal projections) and complete abdominal ultrasound examination were performed.

CEUS Procedure

A 22-gauge indwelling catheter was placed in the cephalic vein. The hair was clipped over the ventrolateral portion of the abdomen and coupling gel was applied to the skin. The ultrasound examinations were performed with the cat manually restrained in dorsal recumbency. The kidney of interest was centered on the screen and was imaged in a longitudinal plane using dual-screen (simultaneous display of conventional B-mode and contrast-mode images). The transducer was manually positioned during each imaging procedure and was maintained at the same position during CEUS.

The contrast agent, 0.05 mL/kg, was injected IV (bolus injection over approximately 3 seconds) followed by injection of a 1.5 mL saline bolus. A 3-way stopcock was used to avoid any delay between injection of contrast agent and saline. The same person performed the injection in a standardized way in all cats. Three injections of contrast were performed: 2 for the left kidney and 1 for the right kidney. The first injection was not used for further analysis, because it results in lower enhancement compared to the second and third injection. Between subsequent injections, to avoid artifacts, remnant microbubbles were completely destroyed by setting the acoustic power at the highest level and scanning the caudal aspect of the abdominal aorta for approximately 2 minutes.

All examinations were performed using a linear transducer of 12–5 MHz on a dedicated machine with contrast-specific software. Basic technical parameters were a single focus placed under the kidney, persistency off, mechanical index 0.09, high dynamic range setting (C50), timer started at the beginning of the injection, gain (85%), corresponding to a nearly dark/anechoic image before contrast agent administration. The settings were repeated during each injection. All studies were digitally registered as a movie clip at a rate of 9 frames per second, for 90 seconds.

The clips were analyzed using specialized computer software for objective quantitative analysis. Six regions-of-interest (ROIs) were manually drawn: 3 in the renal cortex, 2 in the renal medulla, and 1 on an interlobar artery. The ROIs were similar in size and drawn at the same depth for every region. For every ROI, the software determined mean pixel intensity and created a time-intensity curve. Time-intensity curves were analyzed for peak enhancement (PE), wash-in area under the curve (WiAUC), rise time (RT), mean transit time (mTT), time to peak (TTP), wash-in rate (WiR), wash-in perfusion index (WiPI), WiAUC/RT, wash-out area under the curve (WoAUC), total area under the curve (AUC), fall time (FT), and wash-out rate (WoR). Parameters related to blood volume are PE, WiAUC, WoAUC, and AUC. The PE corresponds to the maximum contrast medium signal.
intensity. The WiAUC is calculated as the sum of all amplitudes inside the range from the beginning of the curve up to the TTP. Similarly, WoAUC corresponds to the sum of all amplitudes inside the range from the TTP to the end of the descending curve. The other parameters (i.e., RT, mTT, TTP, WiR, WiPI, FT, WoR), are related to blood velocity. The WiR and WoR represent the maximum and minimum slopes of time-intensity curve. The RT corresponds to the time interval between the first arrival of contrast and the time of peak intensity. The FT, on the other hand, is the duration of contrast wash-out. Mean transit time is the mean duration of complete contrast medium perfusion. The perfusion parameters are illustrated in Figure 1. The values for the 3 ROIs in the renal cortex and the 2 ROIs in the renal medulla were averaged. Peak enhancement and WiAUC for the cortex and medulla were normalized to the values obtained for the interlobar artery.

**Statistical Analysis**

Statistical analyses were performed using statistical software. A linear mixed model with cat as random effect and health status (CKD/healthy) as categorical fixed effect was used. Age group also was incorporated in the model as a categorical fixed effect to adjust for age between CKD and healthy cats. Age groups were defined as: group 1 (1–3 years), group 2 (3–6 years), group 3 (6–10 years), and group 4 (>10 years). Correlations between perfusion parameters and renal size, IRIS stage, serum creatinine concentration (sCr), USG, and UPC were calculated for the renal cortex and medulla using Spearman correlation coefficients (r). A difference was considered statistically significant if P < 0.05.

**Results**

Breed distribution consisted of 9 domestic short- or long-haired cats and 5 pure-bred cats (2 Ragdoll cats, 2 Bengals, and 1 British Shorthair) for the group of cats with CKD and 41 domestic short- or long-haired cats and 2 pure-bred cats (2 Ragdoll cats) for the healthy cats. The mean ± standard deviation age for the CKD group was 9.3 ± 5.2 years, and 6.5 ± 3.9 years for the healthy group. The mean ± standard deviation body weight for the CKD group was 4.2 ± 1.1 kg, and for the healthy group 4.0 ± 0.7 kg. Six cats had IRIS stage 2 CKD, 1 of them was proteinuric (UPC > 0.4). Eight cats had IRIS stage 3 CKD, of which 2 cats had proteinuria.

Systolic blood pressure, sCr, serum urea concentration, USG, and UPC are summarized in Table 1.

All healthy cats had normal size and appearance of their kidneys on B-mode ultrasonography. For the CKD group, renal size was <3.0 cm for 1 or both kidneys in 6 cats, 7 cats had segmental cortical lesions in 1 or both kidneys, an irregular outline for 1 of both kidneys was observed in 10 cats, and extramedullary perfusion was decreased in all cats. One cat had mild unilateral pyelectasia, and another cat had mild bilateral pyelectasia, without signs of ureteral obstruction. A single large cyst deforming the renal cortex was present in 1 cat, whereas few small cortical cystic lesions were detected in both kidneys in another, domestic short-haired cat. None of the cats was diagnosed with polycystic kidney disease.

The contrast agent and the imaging procedure were well tolerated by all cats and no adverse effects were noticed.

Qualitative analysis of the CEUS images showed heterogeneous cortical enhancement in all cats with segmental cortical lesions (Fig 2).

Quantitative CEUS showed significant differences between CKD and healthy cats in several perfusion parameters for the renal cortex and medulla (Table 2 and Fig 2). The mTT for the renal cortex, a parameter for mean duration of complete contrast medium perfusion, was approximately 4 seconds shorter in cats with CKD compared to healthy cats (P = 0.028). The TTP for the cortex was longer in the CKD group (P = 0.003). In contrast, for the medulla, the TTP decreased for the cats with CKD (P = 0.003), associated with a shorter RT (P = 0.001) and FT (P = 0.04) (Figs 3 and 4).

For the renal cortex and medulla, significant correlations were present between the mTT and TTP and IRIS stage, and renal size. Additionally, significant correlations were identified between the RT for the renal medulla and sCr, IRIS stage, USG, and renal size. For the renal medulla, mTT and TTP also were significantly correlated to sCr, USG, and renal size. Urine specific gravity was significantly correlated to medullary RT, mTT and TTP and to cortical peak enhancement.

**Table 1.** Baseline characteristics for CKD and healthy cats presented as mean ± standard deviations.

| Variables           | Control group (n = 43) | CKD group (n = 14) | P-value |
|---------------------|------------------------|--------------------|---------|
| Blood pressure (mmHg) | 144.3 ± 13.5           | 141.0 ± 38.7       | 0.632   |
| sCr (µmol/L)       | 110.4 ± 19.5*          | 278.1 ± 61.0*      | <0.001  |
| Serum urea (mmol/L) | 8.2 ± 1.5*             | 18.8 ± 4.6*        | <0.001  |
| USG                 | 1.048 ± 0.004*         | 1.021 ± 0.013*     | <0.001  |
| UPC                 | 0.13 ± 0.11*           | 0.34 ± 0.33*       | 0.004   |

sCr, serum creatinine concentrations; USG, urine specific gravity; UPC, urinary protein:creatinine ratio. *significant difference.
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Figure 2. Representative serial contrast ultrasound images in a healthy cat (right) and a cat suffering from chronic kidney disease (left). The arrival of contrast agent in the renal cortex is delayed in cats with CKD; moreover, the duration of enhancement is shorter compared to healthy cats.

Discussion

Histologic studies in human and rodent kidneys have shown that tubulointerstitial lesions are associated with damage to the renal arterioles and arteries, and distortion and loss of peritubular capillaries. Tubulointerstitial fibrosis impairs renal blood flow and increases renal vascular resistance. Renal capillary rarefaction is considered a central mechanism in initiation and progression of CKD in people and rodents. Moreover, the renin-angiotensin system is activated in CKD leading to higher renal tissue concentrations of angiotensin II compared to plasma concentrations. Because angiotensin II is a potent vasoconstrictor, the combination of these changes is likely to decrease renal blood flow in patients with CKD, resulting in decreased blood volume and velocity. A recent study using computed tomography angiography to evaluate renal blood volume and vascular anatomy in people showed a 41% decrease in renal cortical blood volume in patients with CKD compared to healthy subjects. The patients with CKD also had decreased luminal diameters of segmental arteries. Moreover, cortical renal blood volume decreased during progression of CKD and was correlated with a decrease in GFR. Medullary blood volume was more variable and did not correlate with GFR.

The most striking findings of our study were shorter mTT time, indicating shorter enhancement, and increased TTP in the renal cortex. The longer TTP suggests decreased blood velocity. These findings correspond to previous studies in human medicine, in which decreased TTP also was described in patients with CKD of various origin and in patients with diabetic nephropathy. Decreased TTP also was an early finding in dogs in which ischemic renal disease was induced by placing an ameroid constrictor around the renal artery.

The PE, a parameter for the intensity of enhancement, reflects the blood volume entering the kidney and hence is expected to be decreased in patients with CKD. Previous studies using CEUS in humans with CKD reported a decrease in PE; however, a significant decrease in PE was not identified in our study. Relatively few patients with CKD were included in our study and larger numbers of cats are needed to determine if significance can be achieved. Moreover, PE is known to suffer from inherently high variability, because it is susceptible to many influencing factors. Variation may be attributed to the contrast agent itself (amount and physical properties of the
microbubbles), uncontrollable patient factors such as blood pressure, heart rate, filtration of the bubbles by the lungs, phagocytosis of bubbles by the reticuloendothelial system, and the manual injection procedure itself. Using controlled injection systems or continuous infusion of contrast agent, followed by flash-replenishment kinetics may improve the diagnostic accuracy of the technique.34,35

Table 2. Mean ± standard deviation values of renal perfusion parameters for cats suffering from CKD and healthy cats.

| Variable, by location | CKD                  | Healthy             | P-value |
|----------------------|----------------------|---------------------|---------|
| Renal cortex         |                      |                     |         |
| PE                   | 1635.12 ± 1008.71    | 1779.16 ± 991.75    | 0.643   |
| PE*                  | 19.24 ± 14.82        | 27.72 ± 13.18       | 0.065   |
| WiAUC                | 2751.49 ± 1442.52    | 2869.57 ± 1418.18   | 0.791   |
| RT                   | 3.01 ± 0.45          | 2.77 ± 0.39         | 0.068   |
| mTT                  | 17.63 ± 6.06         | 21.84 ± 5.97        | 0.028   |
| TTP                  | 8.75 ± 1.09          | 7.71 ± 1.05         | 0.003   |
| WiR                  | 800.61 ± 605.85      | 904.15 ± 593.68     | 0.580   |
| WiPI                 | 1000.53 ± 615.84     | 1089.36 ± 605.45    | 0.640   |
| WoAUC                | 3606.29 ± 1824.25    | 3830.47 ± 1793.46   | 0.690   |
| AUC                  | 6357.95 ± 3262.09    | 6700.00 ± 3207.05   | 0.733   |
| FT                   | 4.12 ± 0.75          | 3.83 ± 0.72         | 0.199   |
| WoR                  | 523.94 ± 444.40      | 638.30 ± 437.05     | 0.405   |
| Renal medulla        |                      |                     |         |
| PE                   | 208.71 ± 762.21      | 276.83 ± 649.58     | 0.766   |
| PE*                  | 3.65 ± 2.62          | 3.02 ± 2.36         | 0.433   |
| WiAUC                | 1653.36 ± 1307.00    | 1892.97 ± 1133.85   | 0.542   |
| RT                   | 11.23 ± 5.43         | 16.69 ± 4.66        | 0.001   |
| mTT                  | 50.48 ± 105.29       | 111.03 ± 91.08      | 0.060   |
| TTP                  | 20.16 ± 6.21         | 27.37 ± 5.38        | 0.003   |
| WiR                  | 4.69 ± 648.95        | 93.23 ± 552.73      | 0.649   |
| WiPI                 | 130.85 ± 466.66      | 172.68 ± 397.51     | 0.765   |
| WoAUC                | 2934.74 ± 1979.94    | 3519.01 ± 1970.38   | 0.387   |
| AUC                  | 4570.11 ± 3449.17    | 5446.80 ± 3065.60   | 0.404   |
| FT                   | 23.25 ± 14.07        | 32.05 ± 12.46       | 0.043   |
| WoR                  | 0.00 ± 528.36        | 70.26 ± 462.17      | 0.631   |

PE, peak enhancement; PE* normalized PE; WiAUC, wash-in area under the curve; RT, rise time; mTT, mean transit time; TTP, time to peak; WiR, wash-in rate; WiPI, wash-in perfusion index; WoAUC, wash-out area under the curve; AUC, total area under the curve; FT, fall time; WoR, wash-out rate. Values in bold represent significant differences between CKD and healthy cats.

Table 3. Correlations between IRIS stage, renal size, USG, and renal perfusion parameters for the renal cortex.

| Variable pair | r (P)     |
|---------------|----------|
| IRIS stage – mTT | −0.29 (0.03) |
| IRIS stage – TTP | 0.32 (0.02)  |
| IRIS stage – PE* | −0.37 (0.005) |
| Renal size – mTT | 0.30 (0.02)  |
| Renal size – TTP | −0.30 (0.02) |
| USG – PE*       | 0.10 (0.43)  |

Table 4. Correlations between IRIS stage, renal size, USG, and renal perfusion parameters for the renal medulla.

| Correlation | r (P)     |
|-------------|----------|
| sCr – mTT   | −0.38 (0.004) |
| sCr – TTP   | −0.43 (0.001) |
| sCr – RT    | −0.46 (0.003) |
| IRIS stage – mTT | −0.38 (0.005) |
| IRIS stage – TTP | −0.47 (0.003) |
| IRIS stage – RT | −0.41 (0.002) |
| Renal size – mTT | 0.43 (0.001)  |
| Renal size – TTP | 0.33 (0.01)  |
| Renal size – RT | 0.41 (0.001)  |
| USG – mTT   | 0.45 (0.005)  |
| USG – TTP   | 0.37 (0.006)  |
| USG – RT    | 0.31 (0.02)   |
Changes in AUC also have been described in humans with CKD, whereas no significance was obtained in our study. Both an increase and a decrease in AUC have been described in people with CKD. An increase in AUC was reported in 41 humans with CKD, whereas a decrease was reported in a study of dogs with iatrogenic ischemic renal disease. The variable effect on AUC may be explained by the fact that AUC is influenced by PE, as well as the slope of the time-intensity curve. The AUC actually is composed of 2 parts: an initial part relating to inflow of contrast and a descending part related to the outflow of contrast medium. Moreover, AUC may be influenced by the stage and progression of CKD.

The decreased TTP and RT for the renal medulla in patients with CKD in our study were not completely expected. Only 1 study has described CEUS of the medulla in people with CKD. In the study of humans, findings for the medulla paralleled those of the cortex, showing delayed time to peak. Still, the vascular anatomy and physiology of the renal medulla differ substantially from those of the cortex. The renal medulla makes up <30% of the total renal volume but only receives 10% of the total renal blood flow. Additionally, local differences are described for the outer and inner medulla, with the latter being less perfused. Vasoactive regulatory factors have different effects on cortical and medullary blood flow. The medulla is relatively insensitive to vasodilation. Therefore, locally increased blood velocity could be present in the medulla in cats with CKD. However, additional studies are needed to confirm this finding and further investigate the underlying pathophysiology.

We found significant correlations between time-based perfusion parameters and sCr, IRIS stage, USG, UPC, and renal size. However, correlation coefficients were low and did not exceed 0.50 in most cases. The highest correlation coefficient was observed for UPC and TTP for the renal medulla (r = 0.47). However, no significant changes could be detected in parameters representing the blood volume. Additional studies are necessary to evaluate the added value of CEUS in the diagnosis of early, nonazotemic CKD in cats.

Footnotes

a Sonovue®, Bracco, Milan, Italy
b iU22, Philips, Amsterdam, The Netherlands
c VueBox®, Bracco Suisse SA, Geneva, Switzerland
d SAS version 9.4, SAS Institute Inc, Cary, North Carolina

Acknowledgments

The authors thank Bracco Suisse SA (Geneva, Switzerland) for their scientific support on the use of VueBox® and Medvet (Antwerp, Belgium) for the laboratory analyses.

Conflict of Interest Declaration: Authors declare no conflict of interest.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

References

1. Lund EM, Armstrong PJ, Kirk CA, et al. Health status and population characteristics of dogs and cats examined at private veterinary practices in the United States. J Am Vet Med Assoc 1999;214:1336–1341.
2. Lulich JP, Osborne CA, Obrien TD, et al. Feline renal failure - questions, answers, questions. Comp Cont Educ Pract 1992;14:127-152.

3. Polzin DJ. Chronic kidney disease. In: Ettinger SJ, Feldman EC, eds. Textbook of Veterinary Internal Medicine: Diseases of the Dog and the Cat, 7th ed. Missouri: Saunders Elsevier; 2010:1990-2020.

4. O’Neill DG, Church DB, McGreevy PD, et al. Longevity and mortality of cats attending primary care veterinary practices in England. J Feline Med Surg 2015;17:125-133.

5. Jepson RE. Current understanding of the pathogenesis of progressive chronic kidney disease in cats. Vet Clin North Am: Small Animal Practice 2016;46:1015–1048.

6. Brown CA, Elliott J, Schmidt CW, et al. Chronic kidney disease in aged cats: Clinical features, morphology, and proposed pathogeneses. Vet Pathol 2016;53:309–326.

7. Paepe D, Daminet S. Feline CKD: Diagnosis, staging and screening - what is recommended? J Feline Med Surg 2013;15 (Suppl 1):15–27.

8. Paepe D, Lefebvre HP, Concordet D, et al. Simplified methods for estimating glomerular filtration rate in cats and for detection of cats with low or borderline glomerular filtration rate. J Feline Med Surg 2015;17:889-900.

9. Finco DR, Brown SA, Vaden SL, et al. Relationship between plasma creatinine concentration and glomerular filtration rate in dogs. J Vet Pharmacol Ther 1995;18:418–421.

10. Herget-Rosenthal S. Imaging techniques in the management of chronic kidney disease: Current developments and future perspectives. Semin Vet Pathol 2011;31:283–290.

11. Daniel GB, Mitchell SK, Mawby D, et al. Renal nuclear medicine: A review. Vet Radiol Ultrasound 1999;40:572–587.

12. d’Anjou MA, Penninck D. Kidneys and ureters. In: Penninck D, d’Anjou MA, eds. Atlas of Small Animal Ultrasonography, 2nd ed. Oxford: Wiley Backwell; 2015:331–362.

13. Tipisca V, Murino C, Cortese L, et al. Resistive index for kidney evaluation in normal and diseased cats. J Feline Med Surg 2015;18:471–475.

14. Novellas R, de Gopegui RR, Espada Y. Increased renal vascular resistance in dogs with hepatic disease. Vet J 2008;178:257–262.

15. Schweiger H, Ohlerth S, Gerber B. Contrast-enhanced ultrasound of both kidneys in healthy, non-anesthetized cats. Acta Vet Scand 2015;57:57–80.

16. Kinns J, Aronson L, Hauptman J, et al. Contrast-enhanced ultrasound of the feline kidney. Vet Radiol Ultrasound 2010;51:168–172.

17. Leinonnen MR, Raekallio MR, Vainio OM, et al. Quantitative contrast-enhanced ultrasonographic analysis of perfusion in the kidneys, liver, pancreas, small intestine, and mesenteric lymph nodes in healthy cats. Am J Vet Res 2010;71:1305-1311.

18. Haers H, Vignoli M, Paus G, et al. Contrast harmonic ultrasonographic appearance of focal space-occupying renal lesions. Vet Radiol Ultrasound 2010;51:516–522.

19. Haers H, Daminet S, Smets PMY, et al. Use of quantitative contrast-enhanced ultrasonography to detect diffuse renal changes in Beagles with iatrogenic hypercortisolism. Am J Vet Res 2013;74:70–77.

20. Dong Y, Wang WP, Cao JY, et al. Quantitative evaluation of contrast-enhanced ultrasonography in the diagnosis of chronic ischemic renal disease in a dog model. PLoS ONE 2013;8:e70377.

21. Dong Y, Wang WP, Cao J, et al. Early assessment of chronic kidney dysfunction using contrast-enhanced ultrasound: A pilot study. Brit J Radiol 2014;87:1–7.

22. Hosotani Y, Takahashi N, Kiyomoto H, et al. A new method for evaluation of split renal cortical blood flow with contrast echography. Hypertens Res 2002;25:77–83.

23. Ghys LF, Paepe D, Duchateau L, et al. Biological validation of feline serum cystatin C: The effect of breed, age and sex and establishment of a reference interval. Vet J 2015;204:168–173.

24. IRIS kidney. International renal interest society (IRIS) [Internet]. IRIS staging of CKD; c2017 [cited 2017 April 07]. Available from: http://www.iris-kidney.com.

25. Boretti FS, Sieber-Ruckstuhl NS, Gerber B, et al. Thyroid enlargement and its relationship to clinicopathological parameters and T-4 status in suspected hyperthyroid cats. J Feline Med Surg 2009;11:286–292.

26. Paepe D, Verjans G, Duchateau L, et al. Routine health screening: Findings in apparently healthy middle-aged and old cats. J Feline Med Surg 2013;15:8–19.

27. Stock E, Vanderperren K, Haers H, et al. Quantitative differences between the first and second injection of contrast agent in contrast-enhanced ultrasonography of feline kidneys and spleen. Ultrason Med Biol 2017;43:500–504.

28. Nakagaku M. Chronic hypoxia and tubulointerstitial injury: A final common pathway to end-stage renal failure. J Am Soc Nephrol 2006;17:17–25.

29. Basile DP, Donohoe D, Roethe K, et al. Renal ischemic injury results in permanent damage to peritubular capillaries and influences long-term function. Am J Physiol Renal Physiol 2001;281:F887–F899.

30. Horbelt M, Lee SY, Mang HE, et al. Acute and chronic microvascular alterations in a mouse model of ischemic acute kidney injury. Am J Physiol Renal Physiol 2007;293:F688–F695.

31. von Stillfried S, Apitzsch JC, Ehling J, et al. Contrast-enhanced CT imaging in patients with chronic kidney disease. Angiogenesis 2016;19:525–535.

32. Tsuruoka K, Yasuda T, Koitabashi K, et al. Evaluation of renal microcirculation by contrast-enhanced ultrasound with sonazoid (TM) as a contrast agent. Int Heart J 2010;51:176–182.

33. Ma F, Cang YQ, Zhao BZ, et al. Contrast-enhanced ultrasound with SonoVue could accurately assess the renal microvasculature. Nephrol Dial Transpl 2012;27:2891–2898.

34. Tang MX, Mulvana H, Gauthier T, et al. Quantitative contrast-enhanced ultrasound imaging: A review of sources of variability. Interface Focus 2011;1:520–539.

35. Hyvelin JM, Tardy I, Arbogast C, et al. Use of ultrasound contrast agent microbubbles in preclinical research recommendations for small animal imaging. Invest Radiol 2013;48:570–583.

36. Evans RG, Eppel GA, Anderson WP, et al. Mechanisms underlying the differential control of blood flow in the renal medulla and cortex. J Hypertens 2004;22:1439–1451.

37. McLeland SM, Cianciolo RE, Duncan CG, et al. Comparison of biochemical and histopathologic staging in cats with chronic kidney disease. Vet Pathol 2015;52:524–534.